# The movement of plastics through marine ecosystems and the influences on bioavailability and uptake into marine biota.

Submitted by **Adam Porter**, to the University of Exeter as a thesis for the degree of ***Doctor of Philosophy in Biological Science*** *in November 2018*.

# ABSTRACT

Microplastics are a diverse array of contaminants comprising a suite of sizes, shapes, and polymer types. Here I present a body of work investigating the distribution and movement of microplastics through the marine ecosystems via transportation and transformation pathways. First, I look at litter items of beaches of the Cornish coast, demonstrating that 41% of litter was plastics fragments unattributable to source and that this litter was continually re-stocked such that it was always present despite cleaning efforts. Then I took to the seas to conduct sea surface trawls in the North East Atlantic to investigate the floating proportion of marine plastic debris. Microplastics were found in every sample, yet were highly variable in concentration over geographic space ranging from 0.038 to 0.45 particles $m^{-3}$. Counter to the prevailing trends, plastic fragments (84 µm – 21.8 mm) were the dominant shape (63%), with fewer fibres present. The likelihood of encounter and therefore risk of plastic to plankton was calculated and it was found that for every 1 plastic particle, there were between 500 and 1000 plankton, suggesting very low risk of biological uptake for this region.

Plastics are not just found on the sea surface and are increasingly found in benthic sediments and biota. I tested whether marine snows would act as a transport mechanism of plastics from the surface to the seafloor. I demonstrate that under experimental conditions a range of plastic particle sizes, shapes, and polymer types, all readily incorporated into marine snows. This incorporation into marine snows both overcame the buoyancy of floating particles but also increased the sinking rate of dense particles. Buoyant polyethylene went from floating as a free particle to sinking at 818 m $day^{-1}$. This repackaging of plastics also increased uptake of polystyrene in the blue mussel by 300 times compared to its uptake as a free sinking particle. I then investigated another route of plastic transformation in the potential for sea urchins to act as bioeroders of plastic. Urchins generated on average 172.9 ± 62.38 plastic pieces per urchin over 10 days; creating microplastics (98.56 µm to 15.8 mm) from a macroplastic tray even when their natural food was present. Despite these generated microplastics being of a buoyant polymer type, 87% of the depurated plastics were retained at the bottom of the tanks. This demonstrates biological fragmentation and the repackaging of plastic within a benthic ecosystem setting.

Overall, my work highlights potential co-occurrence zones where plastic and plankton encounters are most likely; provides a mechanism for the transport of microplastics from the surface to the seafloor; and demonstrates two distinct mechanisms by which biological transformations of plastic can affect the behaviour of particles and their bioavailability to marine species. This all adds to our understanding of the risk that microplastics pose to marine environment.

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## ACKNOWLEDGEMENTS

Undertaking to do a PhD has been such a rewarding process; both for the work I have done of which I am proud, but also because of the people I have met along the way. As Isaac Newton put it: “If I have seen further it is by standing on the shoulders of Giants”. A PhD is a truly collaborative effort and I want to thank all those involved in my work, but also those that I have met along the way.

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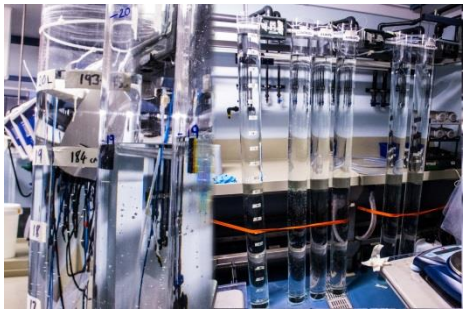













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# Chapter I: General Introduction


**Figure 1**: A water sample collected during a research cruise in the Azores, undertaken for this body of work containing a large number of microplastic fragments and planktonic organisms.

The contamination of the global environment by plastics is one of the great societal challenges of the 21st century (Galloway et al., 2017) and is an extremely complex problem both due the uncertainty about the negative effects of plastic contamination but also in the uncertainty as to how to remediate the problem (Kramm and Völker, 2018, Mendenhall, 2018). Plastic contamination of the environment has convicted the hearts and minds of society perhaps unlike any environmental issue before; likely due to its ubiquitous nature (Schulz et al., 2015a) but also due to the aesthetic dissatisfaction felt by coastal users both at home and abroad (Ryan and Jewitt, 1996, Phillips and House, 2009, Barnes et al., 2009). Plastics are a diverse group of man-made polymers that began to be used in earnest in the 1950s. Their low density, durability, barrier properties and relatively low cost have led to them being used in a

myriad of applications (Ryan, 2015) and their marketing (Life Magazine, 1955) and indeed practical use has endowed society with innumerable benefits. However, the incongruity between the rapid increase in consumption of plastic products over the last half century and the virulent public response to plastic as a pollutant has created an environment where something, and at times anything should be done to solve this problem (Santos et al., 2005).

**Plastics: An Overview**

Plastics are a useful and indeed essential part of 21st Century living. Without plastic many of both the comforts and necessities of life would be absent (e.g. plastic use in the healthcare industry, in food production, water supply etc.). The ability of plastic to `reduce the weight in transportation modes means that cutting a modern car's weight by 100 kg saves 0.2 litres per 100 km in fuel consumption and reduces CO2 emissions by around 10 g/km (Plastics Europe, 2013) and the Boeing 787 Dreamliner became the first airplane to be made primarily of composite materials (including carbon fibre reinforced polymers) making it 20% more fuel efficient than its predecessor. The latest figures on annual plastic production stand at 335 million tonnes as of 2016 (Plastics Europe, 2018). There has been a 20 fold increase in plastic production over the last half century and production is expected to double over the next 20 years (World Economic Forum et al., 2016). In Europe packaging accounts for 39.9% of plastic use, followed by building and construction (19.7%), automotive (10%), electrical and electronic (6.2%), household, leisure and sports (4.2%), agricultural (3.3%) and 16.7% others (such as furniture and medical) with the most popular polymers are polypropylene and polyethylene making up about 49% of all polymers produced in Europe (Plastics Europe, 2018). As of 2015 around 6,300 million tonnes of plastic waste have been produced of which only around 9% has been recycled (Geyer et al., 2017). It is considered that aside from plastics that have been incinerated, all of the conventional plastics ever produced still exist in either whole or fragmented form

(Thompson et al., 2005) and even if we stopped the production (or at least the leakage of plastics into the environment) of plastics they will persist for centuries (Barnes et al., 2009).

The global plastic pollution problem is of concern given the ubiquitous nature of the pollutant; with estimates (which are likely out of date already) stating that ≈93 to 236 thousand metric tonnes of plastic are afloat in our seas and oceans equating to 15 – 51 trillion particles (van Sebille et al., 2015). Nurdles, the pre-production pellets of the plastic production industry, have been regularly found in marine samples and on beaches since the 1970's (Colton et al., 1974) and are thought to mainly enter the marine environment through poor industrial regulation and practice (Ryan, 2015). In a 2016 study it was estimated that 0.95 million tonnes of "primary microplastics" (particles manufactured at a microplastic size range) will enter the marine environment annually. Primary microplastics however are a small part in a big story with the much larger inputs from land based sources (9 million tonnes) and at sea sources (from shipping and fishing of 1.75 million tonnes per annum) (Sherrington, 2016). This all equates to estimates of around 12 million metric tonnes of plastic entering the marine environment annually (Jambeck et al., 2015, Sherrington, 2016) and so a vast amount of plastic is entering the marine environment, fragmenting and being dispersed throughout ocean seascapes and ecosystems. These plastics enter the marine environment due to the mismanagement of waste; either littered or inadequately disposed of in dumps or open, uncontrolled landfills, where it is not contained (Jambeck et al., 2015). This waste can make its way into the oceans via rivers (Hurley et al., 2018, Rech et al., 2014), waste water (Browne et al., 2011) or transport by the wind (Cai et al., 2017, Enders et al., 2015). Plastics in the environment are now being found in the nano size range (Ter Halle et al., 2017) as mechanical, chemical, and biological processes continue the breakdown of plastic pieces in the ocean (Morét-Ferguson et al., 2010, Reisser et al., 2013, Law et al., 2010). Microplastic is a

ubiquitous and pervasive pollutant in the marine environment and its impact is only just beginning to emerge. It is the sources and complex nature of plastic pollution that chapter 2 of this thesis will address.

Plastic is an extremely useful material and hence calls to ban all single use plastics are potentially short-sighted given the potential knock on consequences for the environment in shifting to another product (Wagner, 2017). More than 80% of marine debris is plastic (Eriksen et al., 2014) despite comprising only 10% of municipal waste mass (Barnes et al., 2009) which clearly highlights that the plastic pollution problem is to a large extent a waste management issue (although many are single use items which could be redesigned). It has been predicted that the numbers of plastics in the marine environment will continue to increase into the future with models stating that the amount of floating plastics will increase to between 25 million and 1.3 billion ($10^8$) tonnes by 2100 (a 50 fold increase) (Everaert et al., 2018). These plastics are eventually predicted to all reach the benthic realm (sea floor) (Koelmans et al., 2017b) and pollution of the benthic realm is predicted to increase 50 fold also to maximum concentrations of 8050 particles $kg^{-1}$ on beaches (shoreline deposition environments) and 373 particles $kg^{-1}$ in deep sea sediments (Everaert et al., 2018). Plastic pollution is being reported in both macro and micro forms from remote islands in the Southern Ocean (Barnes and Milner, 2005, Eriksson and Burton, 2003) and South Atlantic (Barnes et al., 2018), the tropics (Gregory, 1999, Duhec et al., 2015), the deep sea (Taylor et al., 2016, Woodall et al., 2014, Pham et al., 2014), frozen in Arctic sea ice (Obbard et al., 2014, Munari et al., 2017), and not just in the marine environment; in rivers and lakes also (Hurley et al., 2018, Driedger et al., 2015, Fok and Cheung, 2015, Hoellein et al., 2015).

Microplastics are a complex cocktail of particles encompassing a variety of sizes, shapes, polymers, and colours (Barboza et al., 2018, Botterell et al., 2018, Hidalgo-Ruz et al., 2012), and concentrations can vary by up to 6 orders of magnitude in the marine environment (Adventure Scientists, 2018). Microplastics come in two classifications: primary and secondary microplastics. Primary microplastics are those pre-formed at the micro size classification (<5mm) (Arthur et al., 2009) such as those used in cosmetics (de Sá et al., 2018), pharmaceuticals (Cole et al., 2011), and in the well documented pre-production pellets or 'nurdles', although these seem to be decreasing in the environment as a result of better handling and transportation security (Morét-Ferguson et al., 2010). Secondary microplastics are those formed by fragmentation in the environment by photo-oxidation, mechanical transformation, and biological degradation (de Sá et al., 2018, Eriksen et al., 2014, Phuong et al., 2016). Grouping microplastics as one type of pollutant is now widely argued to be inaccurate and unhelpful, as the characteristics of a plastic particle will determine its distribution, fate in the environment and dictate to which organisms it is bioavailable to. This is beginning to be explored in the literature with Enders et al. (2015) defining large microplastics as 5 mm – 300 μm and small microplastics as <300 μm and (Koelmans et al., 2017b) making similar sub-divisions of macroplastic (> 5 mm), microplastic (5 mm – 335 μm) and nanoplastics (< 0.335 mm). The term nanoplastics is perhaps unhelpful as Nanometres are already an established measurement with 1 μm equivalent to 1000 nm and nanoplastics (defined as plastics <1000 μm throughout my thesis as per Ter Halle et al. (2017) and Hartmann et al. (2019)) are now being reported in the literature (Ter Halle et al., 2017), however the field is recognising that microplastics are not one pollutant.

Our understanding of environmental concentrations and polymer forms comes predominantly from the exploration of the sea surface utilising plankton tows to collect samples (See Fig. 1 for an example). These methods have led to a good

understanding of the size fraction above 333 μm (the standard mesh size for plankton trawls) but our understanding of the smaller particles is not so well established. In a study by Norén (2007) plastic particle concentrations in Swedish waters were up to 100,000 times greater when sampled with a 80 μm rather than a 450 μm mesh however there are trade-offs to be made as small mesh sizes clog quickly (Phuong et al., 2016) and therefore sample small volumes which makes collecting robust datasets time consuming. In Chapter 3 we use 200 μm plankton nets to try to capture some of the smaller plastic particles. Whole water sample methods have been suggested (Barrows et al., 2017, Barrows et al., 2018) along with sampling the surface microlayer using a dipped glass plate (Anderson et al., 2018). However the majority of studies still use plankton nets and so methodological development is needed to enable us to sample the smaller size fraction of plastics in the ocean.

The benthos has been identified as the major sink for plastics as 99% of plastic is predicted to eventually end up on the sea floor (Koelmans et al., 2017b). In chapter 4 we investigate a potential mechanism for the bulk transport of these microplastics. The sampling of the benthos has been fraught with complication owing mostly to the fact that sampling the benthos is costly and logistically difficult (Pham et al., 2014, Coppock et al., 2017) and plastics in sediments behave very similarly to the sediments themselves (Willis et al., 2017, Vianello et al., 2013) making separation and extraction difficult (Coppock et al., 2017). Corers are commonly used to collect deep sea samples (Van Cauwenberghe et al., 2013, Woodall et al., 2014, Fischer et al., 2015, Coppock et al., 2017, Vianello et al., 2013) as well as epibenthic sledge nets (Courtene-Jones et al., 2017) and the separation of plastics from the sediment is usually undertaken through density floatation using a variety of media (see Coppock et al. (2017)). However it is difficult to release all plastic polymers due to their density (Quinn et al., 2016). Staining techniques have purported to speed up the identification process using Nile Red to fluorescently label plastic particles (Maes et al., 2017, Erni-Cassola et al.,

2017) but the fact remains that data on benthic pollution is scare and methods either time consuming or costly.

The size classifications, forms, and polymer types of plastics found in the marine environment are a subject of much discussion at present as these will both dictate their dispersal, as well as their potential impact on organisms ingesting them (Ziajahromi et al., 2017, de Sá et al., 2018, Betts, 2008). The size of plastic particles will dictate the organisms that they are bioavailable to (Botterell et al., 2018, Vroom et al., 2017) as particles will either physically be ingestible or not, a paradigm investigated in Chapter 3 looking at the likelihood of encounter between plastic and plankton. Browne et al. (2008) showed the risk of translocation of microplastics very early on in the plastic research fields history, showing not only that plastic particles (2 μm) could be ingested by the Blue Mussel *Mytilus* edulis, but also that they could translocate into the haemolymph of the mussel. This triggered a whole suite of studies looking at the effects of microplastic particles. Our understanding of sizes of plastic particles in the environment is currently hampered by sampling methodologies (Koelmans et al., 2017b). These have historically relied on 333 μm plankton nets; thereby collecting quantitative data at sizes greater than 333 μm. This lies in stark contrast to laboratory studies which have commonly used particles less than 50 μm (44% of 169 studies). The most common size classes found in organisms taken from the environment were 400 – 800 μm (12% of studies) and 800 – 1600 μm (12% of studies) (de Sá et al., 2018). This discrepancy is likely due to the ready availability of small laboratory grade beads used in a number of other laboratory applications and the difficulty of sampling particle sizes this small in the field (Desforges et al., 2014).

Polymer type will affect the horizontal and vertical distribution of plastic particles in the marine environment (Kanhai et al., 2017, Desforges et al., 2014) as polymer type is

inextricably linked with the polymers density (explored in chapter 4 when looking at the downward transport of microplastics to the benthos). Denser polymers will sink soon after entering the marine environment whereas buoyant ones will persist and be transported towards accumulation zones (Wright et al., 2013b, Kooi et al., 2017, Barrows et al., 2018). Densities of the plastics can range from ≈ 0.9 to 1.6 g $cm^{-3}$ (Quinn et al., 2016, Claessens et al., 2013) and plastics with a density greater than seawater (≈1.02 g $cm^{-3}$) will likely become available to benthic species whereas those with a density less than seawater can ultimately be transported further by winds, waves and currents (Wright et al., 2013b, Goldstein et al., 2013) and are available to pelagic and surface feeding organisms (de Sá et al., 2018, Phuong et al., 2016). With reference to exposure studies; polystyrene has been used in 69% of effects studies despite only making up 5% of the polymers found in the water column and 12 % in the sediment. The polymers most often found in the environment are polyethylene (28% water column, 22% sediment), polyethylene terephthalate (15% water column, 18% sediment), polyamide (15% water column, 9% sediment) and polypropylene (13% water column, 16% sediment) (Burns and Boxall, 2018) which is in agreement with the statistics from Plastics Europe putting polyethylene and polypropylene as the most produced polymers at around 49% of European production.

An interesting debate is emerging when it comes to the identification of fibres in environmental samples and as with a lot of questions surrounding microplastic science it is becoming apparent that we do not know very much. Cellulose sources can be completely natural, originating from flax, hemp, sisal was well as fibres from wood and these have been used extensively for thousands of years in clothing manufacture (Comnea-Stancu et al., 2016, Barrows et al., 2018). Man-made cellulose is mainly derived from the paper and wood pulp industry but also from the production of viscose often used in tyre cord (rayon) and in the production of synthetic garments (Comnea-Stancu et al., 2016). The majority of textiles fibres are treated with dyes and chemicals

in their production and these may interact with organisms in as yet unknown ways (Barrows et al., 2018, Remy et al., 2015) meaning that despite their natural nature; the mass use of them in the production of products for human consumption may still be having an impact on the marine environment. Much like 'biodegradable plastic' these natural or semi synthetic fibres may not break down as easily in the oceans as they would do on land (Bagheri et al., 2017, Barrows et al., 2018).

Shapes of microplastics are another key metric when it comes to analysing the risk microplastics might play to marine organisms. Facial scrubs were known to contain microbeads and it is estimated that between 4,594 and 94,500 microbeads could be released in a single use of these products (Napper et al., 2015). It has been demonstrated that an average of 65% of microplastics entering waste water treatment plants are removed by primary treatment alone and with secondary and tertiary treatment up to 99.9% of what enters the waste stream at a plant will be removed (Michielssen et al., 2016, Carr et al., 2016). Yet primary microplastics are still found in the majority of marine samples (Burns and Boxall, 2018). Fibres can be released by washing clothes (up to ≈600,000 fibres per 6 kg wash) (Napper and Thompson, 2016) although a proportion of these will be subject to removal in the aforementioned waste water treatment works (dependant on the level of treatment) where installed around the world. Fibres, for context, are threadlike particles with a length ranging between 100 µm and 5 mm and a width roughly four times the diameter (Jönsson et al., 2018, Barrows et al., 2018).

The 'microbead' has been a significant focus of legislation (Burton, 2015, McDevitt et al., 2017) and social action in the last few years with the Microbead-Free Waters Act of 2015 in the USA (McDevitt et al., 2017), the addition of microbeads as a "toxin" in 2015 under the Canadian Environmental Protection Act of 1999 (CEPA) (Pettipas et al.,

2016), and bans in effect in the United Kingdom in 2016, New Zealand in 2017, and bans announced in Finland, France, Iceland, Ireland, Italy, Luxembourg, Norway and Sweden (Dauvergne, 2018). Primary microplastics however in the environment actually make up a very small percentage of those found in environmental monitoring. In Denmark it was calculated that 0.9% of the total microplastic emission into the environment comprised primary microplastics (Lassen et al., 2012) and the banning of these beads is estimated to reduce microplastics entering the North Sea basin by a mere 1.5% (Gouin et al., 2015) when it is estimated that 20000 t $y^{-1}$ of marine litter enters the North Sea (OSPAR, 1995). It is not however just in the social action and legislative field that microbeads have perhaps misdirected attention. Beads have been the predominant exposure microplastic used in laboratory studies (Lehtiniemi et al., 2018, de Sá et al., 2018) whereas Burns and Boxall (2018) by review found fibres to be the most numerous in the environment (45 – 52% of particles found) followed by fragments (29 – 33% of particles found). Similarly de Sá et al. (2018) found fibres to be reported in 23% of studies and fragments in 21% of studies reviewed but in laboratory studies these morphs were only used in 3% of studies compared to 17% of studies using beads. The lack of data indicating uptake in organisms collected alongside environmental concentrations (from water or sediment) (Burns and Boxall, 2018) as well as ecotoxicology of environmentally relevant microplastic shapes makes the assessment of both risk and harm difficult (Burns and Boxall, 2018, de Sá et al., 2018).

It is becoming increasingly clear that we don't have the knowledge we need to assess the risk of microplastics due to a mis-match between the plastics found in the ocean and what has been used to date in exposure experiments. In the first study of its kind (and admittedly in a freshwater organism) Ziajahromi et al. (2017) exposed the water flea *Ceriodaphnia dubia* to both microbeads and fibres and importantly compared the effects. The found that beads impacted up *C. dubia* through the ingestion pathway but fibres by entanglement causing reduced mobility. Concentration was also a factor as

fibres exerted a significant reduction in neonates and adult body size at a concentration of 500 μg $L^{-1}$ whereas it took a bead concentration of 1000 μg $L^{-1}$ and 2000 μg $L^{-1}$ to produce a similar effect in neonate numbers and adult body size respectively. The EC50 values for reproduction were significantly lower for fibres than bead (429 μg $L^{-1}$ with fibres compared to 958 $\mu g^{-1}$ with beads) and this illustrates that different morphs will exert different stressors and produce different responses. The polymers were however different between the two shapes and therefore may have experienced some differential distribution in the water column and indeed the concentrations required to see an effect higher than those found in the environment. The sizes were different and as yet unmeasured in the environment (1-4 μm beads and fibres ranged between 25.7 ± 10 and 1150 ± 160 μm) and this continues to highlight the need for environmental relevance and accuracy in experimental design.

**Biological Impacts**

What we do know is that macro and microplastics are abundant in the oceans. IT is well documented that macroplastics can cause biological harm (Wegner and Cartamil, 2012, Lucas, 1992, Al-Masroori et al., 2004 and Nunes et al., 2018 for example) but the evidence is less clear for microplastics. As environmental concentrations increase, it is plausible that we may reach concentrations at which organisms come to harm in the marine environment (Everaert et al., 2018, Koelmans et al., 2017a). As of 2015, 344 species have been reported to have become entangled in marine debris, and 331 species have ingested marine debris (Kühn et al., 2015). North Atlantic Right Wales (Knowlton et al., 2012), gannets (Rodríguez et al., 2013), sea lions (Raum-Suryan et al., 2009), seals (Allen et al., 2012), blue sharks (Colmenero et al., 2017), turtles (Wilcox et al., 2013, Orós et al., 2005, Casale et al., 2010). In addition, many other species have all been shown to have become entangled in marine debris; most notably what has become known as ghost fishing gear; fishing gear lost to the environment which drifts with the ocean currents (Stelfox et al., 2016). Entanglements cause

organisms to have difficulty acquiring food or avoiding predators (Laist, 1997), and even if the animal doesn't die directly; an impaired ability to move and eat will put it at a disadvantage. Entanglements can cause skin lesions, abrasions and infections or even deformations when an organism is encircled in a restrictive piece of debris (Wegner and Cartamil, 2012, Lucas, 1992) and entanglements are not reserved for marine mega fauna. Fishes (Al-Masroori et al., 2004, Nunes et al., 2018), crabs (Antonelis et al., 2011, Campbell and Sumpton, 2009), and octopuses (Erzini et al., 2008) are known to be caught in derelict traps.

These are however all interactions between relatively large fauna with macroplastics and it is predominantly organisms closer to the base of the food chain that I shall focus on throughout my thesis.

The hard surface of plastic provides many organisms an excellent substrate with which to attach itself to. From the microbial communities recently referred to as the "plastisphere" (Zettler et al., 2013) to large encrusting or fouling epibionts; the evidence of fouling and rafting of organisms on plastic debris is growing (Eriksen et al., 2019, Gregory, 2009, Goldstein and Goodwin, 2013, Winston, 1982). Deep sea anemones have been seen (through ROV footage) to extend their range by settling onto plastic bags in a muddy seafloor environment. Without the bag the anemone would not be able to settle and debris may act as a stepping stone into colonising new areas through the attachment to drifting debris (Chiba et al., 2018). Organisms have been seen using plastic as a habitat including included hydroids, anemones, asteroids, serpulid worms, crinoids, holothurians, and various other structure forming invertebrates and fishes (Schlining et al., 2013, Watters et al., 2010) and gooseneck barnacles are well known to encrust floating material (Goldstein and Goodwin, 2013). Biofouling and its impacts on species interactions with plastic are investigated in chapter 5.

Not only do plastic particles provide a habitat or substrate for marine organisms they can also be ingested. Organisms may ingest plastics actively due to misidentification as a prey item or passively through mechanisms such as filter feeding (Foley et al., 2018). Microplastics have been reported in the guts of fish (Lusher et al., 2013, Lusher et al., 2015a, Mizraji et al., 2017, Rummel et al., 2016), bivalves (Santana et al., 2016, Van Cauwenberghe et al., 2015, Davidson and Dudas, 2016) and even those ready to be sold for human consumption (De Witte et al., 2014, Van Cauwenberghe and Janssen, 2014). Microplastics have been found in decapod crustaceans (Welden and Cowie, 2016, Murray and Cowie, 2011) and microplastics have even been found in deep sea organisms such as sea cucumbers, sea stars, gastropod molluscs, hermit crabs and squat lobsters (Courtene-Jones et al., 2017, Taylor et al., 2016). There is even growing evidence that zooplankton may ingest plastic particles (Desforges et al., 2015, Sun et al., 2017, Steer et al., 2017, Sun et al., 2018b, Sun et al., 2018a). Sun et al. (2018b) demonstrated the number of plastics ingested per zooplankton and found concentrations of 0.35 particles per pteropod and 0.13 particles per copepod. However only 0.004% of the 159,000 invertebrate species have been found to have ingested microplastics (according to the review by Kühn et al. (2015)) and this is more likely a result of a lack of looking and the inherent difficulties in looking for small particles in small organisms rather there being evidence of absence in invertebrates (Lusher, 2015). By review, and in descending order of the number of studies, fish are the most commonly studied group (131 studies), followed by molluscs (40 studies), small crustaceans (39 studies), large crustaceans (22 studies), annelid worms (19 studies), mammals and birds (11 and 10 studies respectively), and echinoderms, cnidarians, reptiles, rotifers, amphibians and poriferans (9, 5, 5, 2, 1, and 1 study respectively) (de Sá et al., 2018).

Ingestion of microplastic particles resulting in any measureable effect in the small and abundant organisms of the ocean is still lacking when considering the amount of

plastics in the oceans and the relative paucity of data indicating significant harm. The majority of studies investigating harm in the laboratory have used particles sizes smaller than those well measured in the environment (Lenz et al., 2016, Burns and Boxall, 2018), have used beads in laboratory exposures when fragments and fibres are the most prevalent shapes in the environment (Hidalgo-Ruz et al., 2012, Mizraji et al., 2017, Burns and Boxall, 2018, Browne et al., 2011), polymers that are less abundant than other polymers in the environment (Burns and Boxall, 2018), and concentrations orders of magnitude higher than anything that has been found in the environment despite over 10 years of research (Vandermeersch et al., 2015, Everaert et al., 2018, Koelmans et al., 2017a). That being said there are many studies that highlight the potential for microplastics to cause harm and as the high concentrations used in exposure studies to date may well be found in specific areas of the global ocean today or indeed in the future as environmental concentrations increase (Koelmans et al., 2017a, Everaert et al., 2018) and therefore the exposure of microplastics to organisms even at high concentrations is still providing us with an understanding of what harm they could assert on marine organisms if the plastic pollution problem is left unchecked.

Laboratory studies have tried to define what the impacts of ingesting microplastics might be on smaller organisms and a wide range of effects have been demonstrated (with the caveats mentioned earlier with regards to concentration, size, shape and polymer). *Daphnia magna* have been the most commonly used organism, likely due to their widespread use in ecotoxicology and therefore the relative ease of setup and exposure monitoring in laboratories already set up for this purpose (de Sá et al., 2018). This is a freshwater species, however, and the majority of studies that focus on microplastics abundances have been done in the marine environment or when uptake is concerned the focus has been on marine species. Laboratory exposures have been done on the annelid worm *Arenicola marina* showing reduced feeding activity, reduced energy reserves (lipids), and increased inflammatory responses (Wright et al., 2013a,

Besseling et al., 2013) although other studies have not seen any response to ingesting plastics (Van Cauwenberghe et al., 2015).

Further studies have looked at mussels, and often the blue mussel *Mytilus edulis,* showing increased respiration (possibly indicating stress), oxidative stress, and inflammatory responses (Van Cauwenberghe et al., 2015, von Moos et al., 2012, Avio et al., 2015). Microplastics have also been shown to reduce the predatory performance in fishes (de Sá et al., 2015) and to influence larval growth and development of echinoderms (Kaposi et al., 2014). A number of studies have shown reduced survival and fecundity in zooplankton (Cole et al., 2013, Lee et al., 2013). Cole et al. (2015) reported predicted carbon losses to copepods of -9.1 ± 3.7 μg C copepod$^{-1}$ day$^{-1}$ when exposed to 20 μm microplastics which will have consequences for health, reproductive ability and life span (Botterell et al., 2018). Lo and Chan (2018) showed early settlement of gastropod larvae and showed a slower growth rate for as long as 65 days post exposure to 2-5 μm beads impacting post-settlement success and survival (although at environmentally relevant concentrations no effect was seen). EC50s have been reported for one study using microplastic fragments (Ogonowski et al., 2016) and two fibre studies (Au et al., 2015, Ziajahromi et al., 2018) but both of these has concentrations at least one order of magnitude higher than what is found currently in the environment and this along with the fact that the majority of papers reviewed by Burns and Boxall (2018) resulted in found no observable effect; even at the highest concentrations in the studies casts doubt on whether microplastics are truly a problem. Modelling studies suggest that in the case of floating microplastics pollution levels will remain below a 'safe' concentration until at least 2100 (Everaert et al., 2018). Overall, the documented effects range from small inflammatory responses through to death however the concentrations are often much higher than those found in the water column or sediment from which the target species is found (de Sá et al., 2018).

A somewhat controversial question in the microplastics field surrounds the potential for microplastic to act as a transfer vector for concomitant contaminants into organisms. There seem to be broadly two schools of thought on the matter. One argument is that priority pollutants such as persistent organic pollutants and metals that can sorb to plastics from the water (demonstrated by Ashton et al. (2010), Holmes et al. (2012) and Vedolin et al. (2018) on plastics collected from beaches) and create a mixture or 'cocktail' of contaminants which when ingested by an organism will be transferred into the guts of marine organisms (Rochman, 2013). These chemicals may then leach off the plastics into the surrounding tissues with the potential to cause ecotoxilogical harm (see Lee, Lee and Kwon (2019)) The counter argument is that compared to other pathways of contaminant uptake via the water and natural food sources, any transfer of chemical pollutants from ingested microplastic particles one is of small concern (Koelmans et al., 2014, Koelmans et al., 2013). Some argue that the ingestion of plastic particles may actually represent a positive outcome by removing pollutants from an organism as plastic passes through (Phuong et al., 2016). Plastics have a high sorptive capacity (Kwon et al., 2017) and the time taken for a plastic particle to reach equilibrium with the surrounding water has been shown to take months. The time to desorb for some compounds has been 14 days to 100s of years and this time frame is likely longer than the gut passage time of organisms and thus microplastics may represent a sink rather than a source or vector of pollutants in the marine environment (Burns and Boxall, 2018).

**Societal Impacts**

The societal impacts of plastic pollution are only recently being discussed especially when considering microplastics; this is a relatively young field and as such the implications and nuances are on the whole still being researched rather than discussed

and debated. In 2010 fewer than 10 peer-reviewed publications mentioned ‘microplastics’ whereas in 2017 there were around 306 publications (Burns and Boxall, 2018). The public understanding of what a microplastic is and the differences between the small pieces and those larger macroplastics seen on local beaches or the news is still lacking (GESAMP, 2015) however through social media, the print and web media, and campaign groups such as Surfers Against Sewage in the UK have created a tidal wave of response and feeling surrounding the plastic pollution problem (Gregory, 2009, Foley et al., 2018, Dauvergne, 2018, Chiba et al., 2018).

That being said the impacts of plastic pollution on society are far reaching and widespread. In a monetary sense, the cost of plastic pollution is increasing as the amount of plastic washing onto beaches increases (Nelms et al., 2017, Watts et al., 2017). In the UK the average cost of litter removal from beaches was between £6200 and £6400 per km and the total cost £3.4 million annually (Mouat et al., 2010). The mobilisation of volunteer groups has had huge success in cleaning beaches in the short term, with strategies like the Great British Beach Clean run by the Marine Conservation Society (MCS) in the UK collecting 2,376,541 items with volunteers contributing 73, 167 hours of time to cleaning beaches in 2017 alone (Nelms et al., 2017). However the long term effectiveness of beach cleans has been questioned with studies showing that without the upkeep of cleans or even in spite of cleans the litter will only return (Uneputty et al., 1998, Williams and Tudor, 2001, Fauziah et al., 2015). Floating marine debris carries a societal cost also with the Royal National Lifeboat Institution calculating their costs in response to callouts with tangled propellers ranging between £730,000 and £2,480,000 (Mouat et al., 2010).

The ubiquity of fragmented pieces, which are unattributable to their original source (Watts et al., 2017, Schulz et al., 2015b) means that whilst those engaging in beach

cleans become educated about the issue, and in the short term the beaches are clean, the long term sustainability is questionable given that up to 83% of waste is mismanaged globally and makes its way into the oceans (Jambeck et al., 2015). It is these beach clean efforts that allowed the investigation in Chapter 2 to take place and so this is discussed in more detail there. The largest attempt to clean up the oceans "The Ocean Cleanup" is set to cost 317 million euros in the removal of 42% of marine debris from the North Pacific Gyre over 10 years. However this will only remove 70,000 tonnes in 10 years (Slat, 2014) and in the face of the aforementioned 4 – 12 million tonnes estimated to be entering the marine environment every year the clean-up efforts need to start at the source not at the sink (Uneputty et al., 1998, Williams and Tudor, 2001).

The impacts of plastic pollution on human health is not well known and very little research has been done on the matter (Wright and Kelly, 2017b, Barboza et al., 2018, Wright and Kelly, 2017a). Exposure pathways have mostly been inferred through the assumption that ingestion of contaminated food and drink products may cause ingestion of plastics by humans (Wright and Kelly, 2017a). Drinking water has been shown to have microplastic contamination from several sources; in bottled mineral water (Oßmann et al., 2018), raw and treated drinking water (Pivokonsky et al., 2018), groundwater (Mintenig et al., 2019). Van Cauwenberghe and Janssen (2014) demonstrated the microplastic contamination load in mussels and oysters cultured for human consumption and inferred the amount that a top European shellfish consumer might ingest up to 11,000 microplastics per year however these particles were in the size range of 5 – 20µm. Table salts from China have been found to contain 7-681 particles $kg^{-1}$ of salt (Yang et al., 2015) and alongside this there is the question of the inhalation pathway (Wright and Kelly, 2017a) with up to 355 particles $m^2$ $d^{-1}$ being reported in the air in urban areas of Paris (Dris et al., 2016). There is even evidence emerging of humans excreting microplastics in their faeces (Liebmann et al., 2018)

however the evidence of harm is still wanting, just as it is in biological studies (Burns and Boxall, 2018, Barnes et al., 2009, Wright and Kelly, 2017b).

A few studies are attempting the close the gap between what we know now and what we would like to know; namely the *risk* of microplastics to the environment, organisms and ultimately humans. Plastic pollution can be termed a “global risk” but undertaking a risk assessment is difficult as there is a high degree of uncertainty both in terms of their non-uniform heterogeneous spread across the globe but also the broad array of particles that can be classified as a microplastic (Lehtiniemi et al., 2018, Barboza et al., 2018, Koelmans et al., 2017a). Microplastics are a by-product of our modern world and due to their global distribution by physical processes their effects can be felt far from their place of origin (Kramm and Völker, 2018) and therefore known unknowns and unknown unknowns (such as sources, sinks, and harm) confound risk analyses. Furthermore the high doses used in laboratory studies (Lenz et al., 2016, Everaert et al., 2018), and the large amount of presence/absence data not married to environmental ingestion rates in the neighbouring organisms makes predictions difficult (Burns and Boxall, 2018).

**Historical Field Development**

The microplastic problem was first identified in the 1970’s (the issues of marine debris started to be highlighted in the 1960’s by Kenyon and Kridler (1969) who found albatrosses who had ingested plastic). In 1971 Buchanan reported synthetic fibres in water samples from the North Sea and in 1972 Carpenter et al. observed polystyrene spherules ranging from 0.1 to 2 mm floating in the coastal waters of southern New England. They identified that these particles were biofouled and had absorbed concentrations of polychlorinated biphenyls (PCBs) and indeed that these were

ingested by small fish. Colton et al. (1974) demonstrated the extensive spread of floating plastics in the North Atlantic and Caribbean comprising industrial pellets and fragments and highlighting that these were concentrated close to major land-based sources. In Colton et al. there were two stark warnings made which with hindsight seem to have been almost prophetic as to how the field of microplastic science might develop and the rapid increase and understanding of mankind's pollution of the environment with plastic. They stated that once plastics are introduced into the marine environment, they are likely to remain indefinitely even though they fragment and that in our societal mismanagement of waste the abundances of plastics are likely to increase in the environment; potentially rising to levels that might cause harm.

These comments along with identified concomitant contaminants, ingestion by marine organisms leading to harm and the land based sources of these pollutants are very much what the field is still discussing today. Birds became an early focus for plastic pollution science with many studies reporting ingestion of plastics by a range of marine related birds and reporting a high proportion of industrial pellets (Bond, 1971, Baltz and Morejohn, 1976). The first post-graduate thesis on plastic pollution was published in 1980 by Bob Day again focussing on birds and the first marine debris conference occurred in 1984 focussing on ingestion by birds (Ryan, 2015). Turtles and marine mammals were seemingly the next cohort of animals to get some focus with plastics discovered in the stomachs of Loggerheads and Leatherbacks (Hughes et al., 1967, Hughes, 1974) and in the scats of fur seals (Eriksson and Burton, 2003). Entanglement also become a growth field in these larger organisms with reports of entanglements in cetaceans and sharks (Cawthorn, 1984), fur seals (Bonner and McCann, 1982, Shaughnessy, 1980). The understanding of the sources of plastics identified the plastic production (Colton et al., 1974), shipping (Scott, 1972), and fishing industries (Ryan, 2015). The first attempt to regulate plastic pollution came in the form of banning the

dumping of plastic waste at sea (in 1972) and Operation Clean Sweep targeting the industrial pellets so prevalent in the marine environment (established in 1992).

The real growth in public opinion and the creation of a growth area of research likely came however in the form of Charles Moore who coined the phrase the 'Great Pacific Garbage Patch' (Moore, 2003, Ryan, 2015). High profile studies by Thompson et al. (2004), Law et al. (2010) and Eriksen et al. (2013) were responsible for energizing the plastic pollution debate and starting to firm up the differences between macroplastic and microplastic debris as well as looking at things in greater detail and at larger spatial scales.

**Knowledge Gaps**

Over the last half century plastic pollution has become a focus for scientific research, legislative debate, and a societal cause to champion and the field seems at present to be in a state of self-evaluation with many researchers highlighting gaps in our current understanding, critiquing the work that has been done and asking big questions such as:

- Does what we see and measure in the laboratory equate to the effects that we may see in the environment? (Phuong et al., 2016).
- Do existing data on the occurrence and effects of microplastics in the environment indicate that these materials are causing harm? (Burns and Boxall, 2018, Foley et al., 2018, Everaert et al., 2018).
- Can microplastics act as a vector of persistent organic pollutants into organisms and through food chains? (Burns and Boxall, 2018, Koelmans et al., 2017a, de Sá et al., 2018, Barboza et al., 2018).

- What methods should we be using to get a more accurate picture of what is going on? (Foley et al., 2018, Koelmans et al., 2017a).

The field lies in the balance between early day hysteria and latter day complacency (Koelmans et al., 2017a) and researchers need to act with smart thinking, and well-constructed experiments (Connors et al., 2017) to navigate between the two to move towards a more rounded understanding of the issue, and to be able to provide impartial evidence towards a better future for the oceans.

A large issue in field sampling is in the standardisation of measurements and the accuracy of identification of plastics. Particle concentrations in seawater are recorded in particles per $m^2$, particles per $m^3$, and particles per litre (Phuong et al., 2016, Burns and Boxall, 2018) and concentrations in the sediment expressed in particles per unit volume of sediment or per $m^3$ or even percentage plastic by weight of sediment or particles per mass of sediment (Phuong et al., 2016, Burns and Boxall, 2018, Foley et al., 2018). These inconsistencies make it difficult to combine studies reporting the concentrations in organisms and the environment with effects based studies (Koelmans et al., 2017a). A further issue from the field is the identification or verification of 'likely microplastics' as indeed plastic polymers. The identification of plastics by size, shape, and colour should no longer be good enough (see Moore et al. (2002), Moore et al. (2001), Lattin et al. (2004), Yamashita and Tanimura (2007)), nor should the "hot needle test" (using a hot needle to melt the particle thus 'proving' it to be plastic. See Devriese et al. (2015), De Witte et al. (2014), Bellas et al. (2016)). Whilst staining techniques exist (Maes et al., 2017, Erni-Cassola et al., 2017) they do not work for all polymers and could be confounded by chitin or lipids from organisms in the water or sediment sample (Erni-Cassola et al., 2017, Shim et al., 2016) and need to be validated. One of the most common analytical techniques in the field today is the use of Fourier Transformed Infrared (FT-IR) transmission spectroscopy (encompassing Attenuated Total Reflectance (ATR) Microscopy and transmission microscopy) or

increasingly Raman Spectroscopy. In a study by Lenz et al. (2015) only 68% of visually counted microplastics were confirmed as being plastic polymers using Raman Spectroscopy. These methods do however rely on comparing spectra to a reference library and a full critique of this is provided in Comnea-Stancu et al. (2016) suffice to say the authors found that only 60% of natural fibre spectra were correctly identified by their library search (misidentified as man-made fibres) and the comparisons made are only as good as the libraries allow. It is therefore likely prudent to use a range of techniques including visual identification (or indeed staining), with subsequent confirmation using analytical methods which will only improve with time. I have attempted to fine tune much of what I have done here; in particular reporting negative results with respect to my work with the FTIR in Chapter 3 and in adjusting my data towards more realistic environmentally or biologically relevant scenarios in Chapters 3 and 5.

Most laboratory studies have also been acute studies at high concentrations; the second point of which I discussed earlier. The issue here is that organisms in the environment do not have an option to return to clean water to depurate and are potentially always exposed to low levels of microplastics throughout their life rather than high pulses over a set number of hours or days (Connors et al., 2017). A factor associated with this is egestion which is not commonly measured as this would mitigate the impact of plastics on an organism (Foley et al., 2018, Burns and Boxall, 2018) and if plastics are found in concentrations below the affect threshold for an organism (Koelmans et al., 2017a) the egestion rate may well mitigate any impact the plastic could have (Connors et al., 2017, Kaposi et al., 2014). Microplastics sizes shapes and polymers are always found in a cocktail and never as one particles size/type/shape and testing in a ‘plastic soup’ will help us understand uptake and egestion in the real world. Furthermore the distinct absence of food in exposure conditions is a concern (Lehtiniemi et al., 2018, Burns and Boxall, 2018) as the ready availability of food (or

not) may well dictate uptake based on the feeding behaviour of the organism. Food may encourage feeding or, once full with nutritious food; the organism may cease to feed (Ayukai, 1987). This may produce confounding results as even a loss in energetics may be the result of a reduced diet quality and not the microplastics themselves (Connors et al., 2017, Ayukai, 1987).

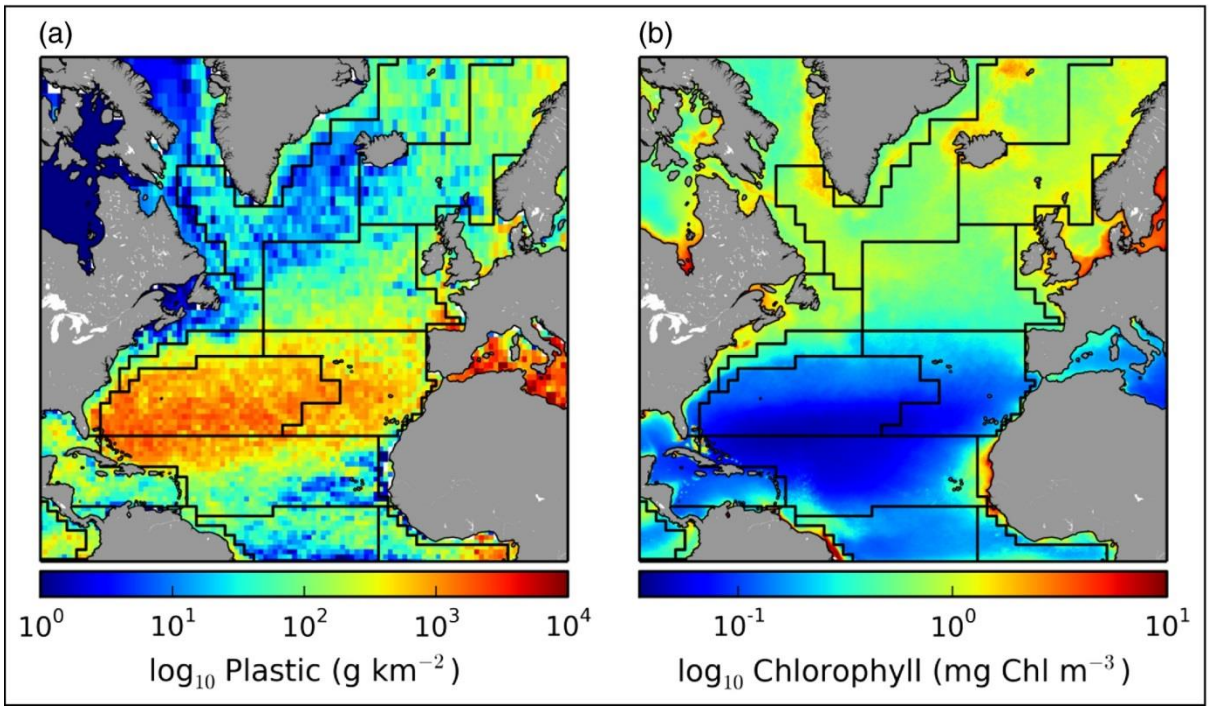


**Figure 2:** Figure taken from Clark et al. (2016) demonstrating that the biomass in the oceans is inversely proportional to the plastic concentrations meaning that high co-occurrence of plastic and plankton leading to increased ingestion may more elusive than first thought.

In this thesis I seek to explore the pathways that plastics might take through the environment (Chapters 2 and 3) and what transformations might take place to change the interaction of plastics with marine biota (Chapters 4 and 5). Due to plastics long residence time in the marine environment (Cole et al., 2014) and given 46% of plastics entering the marine environment float in seawater (U.S. Environmental Protection Agency, 2012) they are driven by wind and surface currents (Frias et al., 2014) and

plastics readily accumulate in convergent zones resulting in regions of high concentrations such as around the ocean gyres (Morét-Ferguson et al., 2010, Maximenko et al., 2012, Barboza et al., 2018). Clark et al. (2016) suggested that actually there is a spatial mismatch between where we are looking at plastics and where we are likely to see an effect of plastics in the environment (Fig. 2). This opened up one of the major lines of enquiry in this thesis; namely where are the hotspots of plastic and where might they intersect with organisms in the environment. To first get an understanding of the problem I looked at what plastics were actually in the marine environment; both through an analysis of a time series of beach litter data and also through sea surface trawls in the NE Atlantic. It was through these trawls we tried to disentangle the co-occurrence question; in what locations do relatively high abundances of plastic and plankton occur and is this co-occurrence great enough to be a risk to the zooplankton; this work is in Chapter 3.

The vertical distribution of microplastics is also paramount to understanding microplastic pollution partitioning in the marine environment and what I seek to understand in Chapters 4 and 5 in particular. Cozar et al. (2014) identified that large loads of plastic fragments are unaccounted for in surface loads collected through trawling data and as such there must be mechanisms for transport of floating debris from the surface ocean to the seafloor. I undertook to investigate whether marine snows might act as a transport vector of microplastics from the sea surface to the seafloor and how the incorporation into particulate organic matter might affect the uptake of plastic by a marine benthic filter feeder (the Blue Mussel). Once plastics have sunk and reached the benthos (as all plastics are eventually predicted to do (Koelmans et al., 2017b)) the plastics are relatively fixed in geographical space and therefore subject to interactions with species that live on the sea floor. I therefore investigated the ability of a benthic grazer, the sea urchin *Paracentrotus lividus* to transform macroplastic debris into microplastic debris and what affect the repackaging of the

plastics into faecal matter might have on the distribution of the potential fragments the urchins might create.

This thesis follows plastic from source to sink via various pathways and seeks to investigate both the likelihood of harm that plastics may pose to the marine environment but also how a dynamic pollutant in the dynamic oceans may be altered and changed, confounding conventional cause and effect based models.

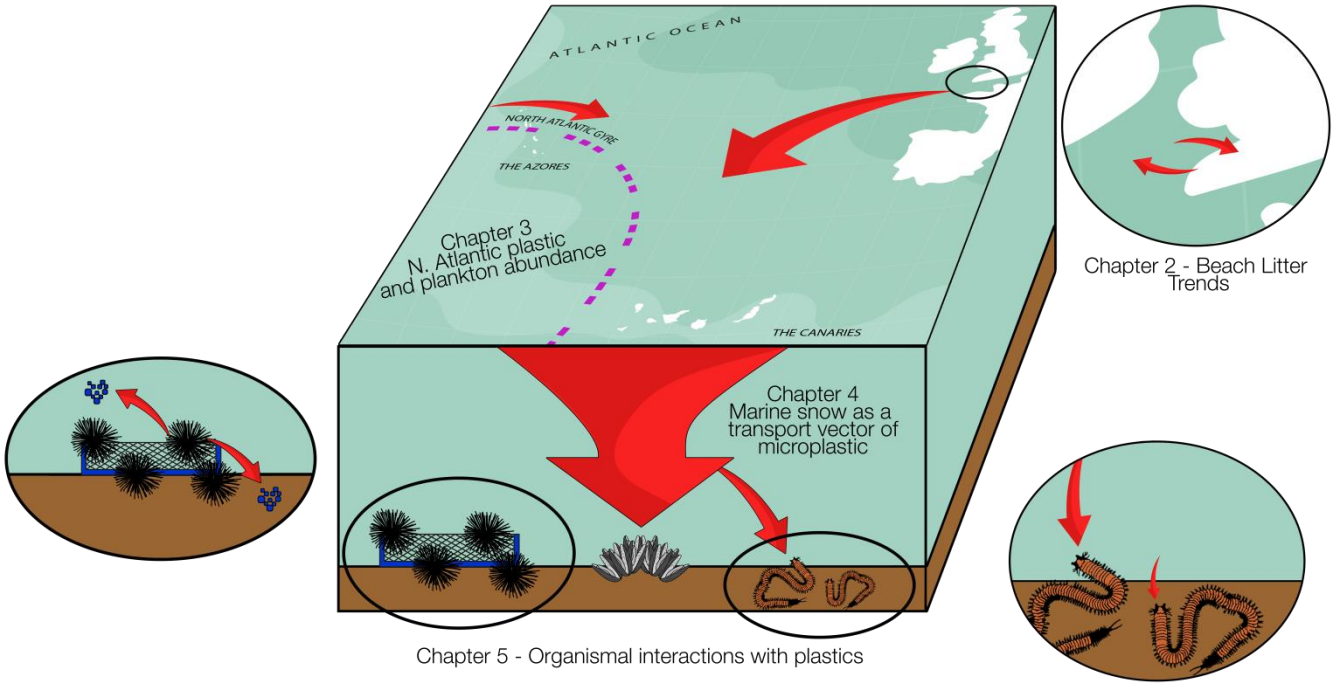


**Figure 3:** A graphical description of my PhD starting (top right) with the spatio-temporal variation of marine litter on Cornish beaches in the UK, moving to ocean scale cruises in the North East Atlantic looking for areas of microplastic and plankton co-occurrence (centre top), demonstrating the potential for marine snows to transport microplastics vertically and into marine biota (centre front), and biological transformations and interactions between microplastics and marine organisms (left and right).

In this thesis we set out to answer the following questions (summed up in Fig 3):

1. What are the sources of beach litter and can they tell us anything about the distribution and transformation of plastic pollution in the environment?

2. Can we identify hotspots of plastic and plankton co-occurrence and if so what threat does this pose to zooplankton?

3. Are marine snows a viable mechanism for the vertical transport of microplastics and will the repackaging of microplastics affect uptake?

4. Are sea urchins capable of fragmenting macroplastics into microplastics and does this fragmentation also affect the distribution of plastics in the marine environment?

# Chapter II: Through the sands of time: Beach litter trends from nine cleaned north Cornish beaches


**Figure 1:** Beach litter caught in the Strandline on a North Cornish beach

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Andrew J.R. Watts and Adam Porter undertook an equal share of the data analysis, interpretation and manuscript writing (joint first authors)

Jolyon Sharpe and employees of Cornwall Council collected the data.

Ceri Lewis edited and improved the manuscript

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# Through the sands of time: Beach litter trends from nine cleaned north cornish beaches☆

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ABSTRACT

Marine litter and its accumulation on beaches is an issue of major current concern due to its significant environmental and economic impacts. Yet our understanding of spatio-temporal trends in beach litter and the drivers of these trends are currently limited by the availability of robust long term data sets. Here we present a unique data set collected systematically once a month, every month over a six year period for nine beaches along the North Coast of Cornwall, U.K. to investigate the key drivers of beach litter in the Bude, Padstow and Porthcothan areas. Overall, an average of 0.02 litter items $m^{-2}$ per month were collected during the six year study, with Bude beaches (Summerleaze, Crooklets and Widemouth) the most impacted (0.03 ± 0.004 litter items $m^{-2}$ per month). The amount of litter collected each month decreased by 18% and 71% respectively for Padstow (Polzeath, Trevone and Harlyn) and Bude areas over the 6 years, possibly related to the regular cleaning, however litter increased by 120% despite this monthly cleaning effort on the Padstow area beaches. Importantly, at all nine beaches the litter was dominated by small, fragmented plastic pieces and rope fibres, which account for 32% and 17% of all litter items collected, respectively. The weathered nature of these plastics indicates they have been in the marine environment for an extended period of time. So, whilst classifying the original source of these plastics is not possible, it can be concluded they are not the result of recent public littering. This data highlights both the extent of the marine litter problem and that current efforts to reduce littering by beach users will only tackle a fraction of this litter. Such information is vital for developing effective management strategies for beach and marine litter at both regional and global levels.



## 1. Introduction

Marine litter is one of the most indiscriminate and pervasive pollution issues facing our seas and oceans today (Galloway and Lewis, 2016). Many recent studies have documented both the extent of marine litter throughout the world's coastal waters and open oceans (e.g. Cózar et al., 2014; van Sebille et al., 2015) and the damage that it can cause to marine wildlife via entanglement and ingestion (e.g. Wright et al., 2013; Cole et al., 2015; Watts et al., 2015). UNEP describes marine litter or marine debris as "any persistent, manufactured or processed solid material discarded, disposed of, abandoned or lost in the marine and coastal environment" (UNEP, 2005). Beach litter has two main sources; it can originate from the sea as a result of shipping, recreational boating, navigation, fisheries, aquaculture and other offshore activities, or it can originate from land-based sources such as recreational activities on the beach, rivers, from drainage systems (such as Combined Sewage Overflows (CSOs)), sewage inputs, as well as from anthropogenic activities adjacent to the beach (domestic, agricultural, landfill, shipyards, harbours, etc.) (Gabrielides et al., 1991; Semeoshenkova and Williams, 2011; Kordella et al., 2013; Thiel et al., 2013; Fauziah et al., 2015; Schulz et al., 2015). Hence, litter on beaches can comprise a wide range of litter types including various plastics, metal, timber and large items like fishing gear, and come from a variety of sources.

The issue of beach litter and marine debris has recently become

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an issue of heightened concern for both the general public and policy makers due to the increasing evidence of the harm it can cause to wildlife and the socioeconomic implications that it causes for beach users and tourism. Plastic, a large constituent of marine litter, has been found in the stomachs and entangled around stranded whales (Jacobsen et al., 2010), turtles (Tourinho et al., 2010) and sea birds (Avery-Gomm et al., 2012). Economically, up to 97% of a beach's value can be lost by a drop in cleanliness standards (Ballance et al., 2000). One study calculated that the economic benefits for Orange County, California in the United States associated with a 100% reduction in marine debris at all sites could be as much as $148 million and a reduction in only 25% could render as much as $32 million dollars to the economy (Leggett et al., 2014).

In the U.K., beach litter abundances have risen by 20% between 1994 and 2014 (Marine Conservation Society, 2015; Nelms et al., 2017). In the South West (in which our study beaches are situated), litter on beaches cleaned under the 'Great British Beach Clean' scheme was observed to be 89% higher in 2013 compared to 2014 (Marine Conservation Society, 2015) with these beaches amongst the most littered in the U.K. (Nelms et al., 2017). The cost of removing beach litter to all coastal municipalities in the U.K. is estimated to be in the region of €18–19 million per annum (Mouat et al., 2010). The costs of marine litter goes further than just that of a clean-up effort,, as demonstrated by the Royal National Lifeboat Institution (RNLI). In 2008, they made 286 rescue operations to vessels with tangled propellers costing the charity between €877,000 and €2,313,000 (Mouat et al., 2010). Furthermore, beaches provide social benefits and the presence of litter can undermine the psychological benefits of a visit to the beach (Wyles et al., 2016). Managing beach litter and reaching targets for reducing both the environmental and economic impacts of litter and its clean-up requires a much better understanding of the current trends in both the types of litter present and their sources (Unger and Harrison, 2016).

The factors affecting the accumulation of litter on beaches may vary with both location and season. To analyse beach litter trends in any detail requires long-term data sets with limited variation in the methodology applied over time and with little gaps in the data. Beach cleans have become a powerful and useful tool with which the academic community is beginning to engage with in order to gather large volumes of data about the state of our global shorelines. The U.K. has a long heritage of beach clean efforts including 21 years of Beachwatch (Marine Conservation Society, 2015) clearing 150 tonnes of litter since 1994, Surfers Against Sewage's current commitment to reduce beach litter by 50% by 2020 (Surfers Against Sewage, 2014), and Keep Britain Tidy have accrued 15,000 volunteer hours in cleaning beach in the South West (Keep Britain Tidy, 2015). The interaction of beach cleans and scientists is paramount as the data collected, if it is to be useful, is best done in a representative, systematic way with good aims and a robust standard method.

Identifying the root causes rather than just managing the consequences of marine littering is clearly of critical importance if we are to improve the state of our seas and oceans. Most of the data currently available for understanding spatio-temporal trends in the accumulation of beach litter comes from beach cleans run by local authorities or charities using volunteers (e.g. Nelms et al., 2017). As a result, data is often collected by a large number of people, with different people collecting the data at each sampling time and/or location. Whilst this is a fantastic way to get a large amount of information for a wide area, and can produce useful insight into generalised trends, this understandably also introduces a level of uncertainty and variability into any data set and often makes robust statistical analysis difficult. The litter collected is then generally categorised into a number of simplified litter types. The classical approach has just been to categorise litter by material (plastic, timber, rope etc.). However, classifying by source or original user is a much more effective way of directing management strategies towards stopping the problem at source rather than just measuring it (Schulz et al., 2015). Common litter types now used typically include sewage-related debris, fishing-related litter, shipping-related litter, beach user related litter (tourism or animal faeces), fly-tipped, and medical and then an 'uncategorisable litter items' category for those items that are too fragmented or degraded to be allocated to an original source (OSPAR, 2009; Williams et al., 2003, 2014).

The aim of this study was firstly to produce a unique data set from nine beaches around the north coast of Cornwall, U.K., using a systematic method over a six year period using consistent litter category definitions and undertaken by the same team of trained professionals on a monthly basis. The beach cleans conducted for this study were undertaken with source attribution in mind and thus work towards looking at root causes rather than just cleaning up. These beach cleans were done by hand to minimise any ecological damage associated with more mechanical methods. We then use this high quality dataset to identify the driving factors of marine litter on beaches in the South West of England, identify any seasonal or annual trends in litter type and abundance and ultimately to suggest how this knowledge can be applied to improve beach litter management.

## 2. Material and methods

### 2.1. Beach cleans

Nine beaches on the North Cornwall coast were surveyed and cleaned by the same five trained council workers during the first week of each month over a period of 6 years between January 2005 and December 2011 using the recognised OECD guidelines (OSPAR, 2010). The beaches chosen lie within a 60 km stretch of coast line

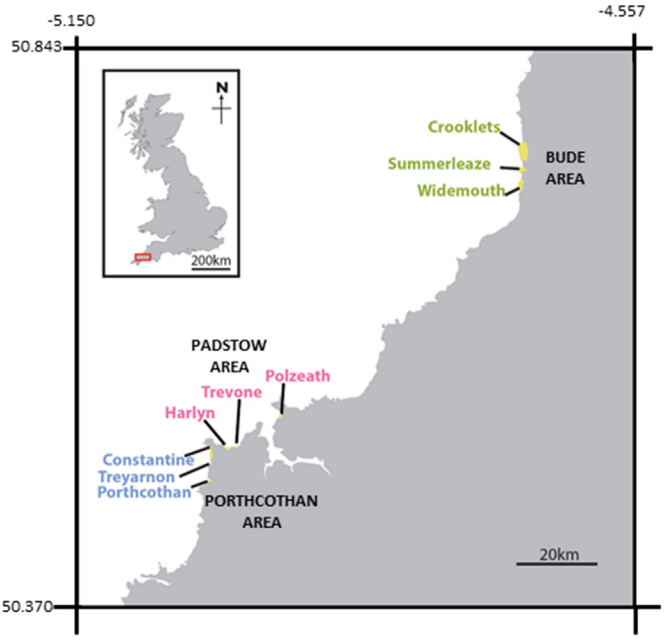


**Fig. 1.** Map of beach clean sites. North coast of the South West region of Cornwall, U.K. Three areas: Bude area (Crooklets, Summerleaze, Widemouth); Padstow area (Polzeath, Trevone, Harlyn); Porthcothan area (Constantine, Treyarnon, Porthcothan). Crooklets and Porthcothan beaches are 57 km part along the coastline.

(shown in Fig. 1) and were split into three study areas (Bude, Padstow and Porthcothan) according to their geographic location. The Bude area beaches comprised Crooklets beach (location 50.836 N, 4.550 W; sea facing 260° north), Summerleaze beach (location 50.831 N, 4.551 W; sea facing 240° north), the two main beaches of the tourist town of Bude, and Widemouth Bay (location 50.793 N, 4.557 W; sea facing 260° north) a surf and tourist beach 3 miles from the town of Bude. The Padstow area beaches comprised Polzeath (location 50.574 N, 4.915 W; sea facing 258° north) a surf beach surrounded by camping and holiday parks, Trevone (location 50.545 N, 4.977 ′W; sea facing 254° north) and Harlyn (location 50.540 N, 4.995 W; sea facing 158° north). The Porthcothan area beaches comprised Constantine Bay (location 50.537 N, 5.024 W; sea facing 265° north), Treyarnon Bay (location 50.526 N, 5.022 W; sea facing 230° north) and Porthcothan Bay (location 50.509 N, 5.022 W; sea facing 247° north).

Surveys were undertaken from the main access point of each beach and the surveys conducted along 100 m of beach; usually 50 m either side of the access point. The area surveyed was from the lowest tide line up to the uppermost extent of the beach and each survey lasted approximately one hour with one worker conducting the clean. The tide height varied at the time of sampling month by month therefore to determine an average collection area the length of beach was taken as half of the distance between the mean high tide mark and the mean low tide mark according to Ordinance Survey (2017). Total area studies each month was 159,150 $m^2$. The collection areas for individual beaches were as follows: Crooklets 16,000 $m^2$; Summerleaze 24,650 $m^2$; Widemouth 16,800 $m^2$; Polzeath 23,700 $m^2$; Trevone 12,450 $m^2$; Harlyn 12,400 $m^2$; Constantine 15,900 $m^2$; Treyarnon 13,150 $m^2$; Porthcothan 24,100 $m^2$.

The main access point was chosen as this is where most beach visitors congregate, with depreciation in numbers as distance increases from the access point. The timings of each beach clean were standardised starting early in the morning, which ensured that the litter reflected the actual load and was not affected by other beach activities. Each piece of litter collected was recorded as 1 of 111 different litter types including different plastic, timber and metal items. Based on OSPAR Marine Litter Monitoring Survey Form (OSPAR, 2010), these litter items were then categorised into source groupings (beach user, fishing, shipping, fly-tipping, sewage, and 'uncategorised source' (referred to as 'un-sourced' from here on) in order to further investigate the source of the litter found on the beaches (see S.I.1 for full categories). All of the litter was removed to be counted and categorised and it was not then returned to the beach, i.e. it was removed. Beach user items were defined as any item, which would have been left by a user of that beach and where the original nature of use was obvious. Weathered items and plastic fragments were defined as 'un-sourced' since their original entry point into the environment could not be ascertained. These items had potentially been at sea for some time meaning it could not be ascertained whether they had been deposited on that beach originally or had been transported a considerable distance. Fishing items were defined as any item of fishing equipment or any personal item that would likely to have originated from a small fishing vessel. Shipping items were those related to the business of cargo shipping including paint brushes, cleaning bottles and large disposable catering packaging. Fly tipping included as scrap metal, tires, building materials and anything else, which looked to be purposefully dumped, normally at the top of the shore. Sewage included toiletries, cotton buds and other toiletry items known to be flushed through the sewage system. Un-sourced items were those items not fitting easily into any other group including broken pieces of plastic, metal and timber (see in Supporting Information SI.1 for full list).

### 2.2. Data analysis

The average of number of litter items per metre squared per month were determined for each beach. The beaches were then grouped together as the 'Bude area' (Crooklets, Summerleaze and Widemouth beaches); the 'Padstow area' (Polzeath, Trevone and Harlyn beaches) and the 'Porthcothan area' (Constantine, Treyarnon and Porthcothan beaches). Inter annual and seasonal trends were tested via a General Liner Model (Minitab v17) with the monthly abundance being the response variable and either the year or season as the explanatory variable. Parametric assumptions of normality of residuals and homogeneity of variance were assessed visually and all data were natural log transformed to meet normality assumption. A Tukey post hoc test followed when the category of location was used as the explanatory variable. Differences were considered significant at a $p \leq 0.05$.

## 3. Results

A total of 642 beach cleans took place between January 2006 and December 2011 with 248,246 individual litter items removed from across the nine study beaches. Each beach was surveyed 72 times in total over the 6 year period. This amounts to approximately 0.02 litter items collected in every square metre each month during the study. A total area of 159,150 $m^2$ was covered each month.

### 3.1. Litter types and trends across all beaches

Across the nine beaches over the 6 year period 111 different litter types were recorded. Of these, three types dominated the litter collected at all nine locations. Plastic pieces >1–50 cm, cord (small elongated plastic fibres, either single or bundles) and plastic pieces <1 cm and made up 49% of total litter items (Table 1), the top 30 litter items can be seen in Table 1. Eighty nine percent of all litter items were plastic; 4.1% were paper, cardboard or timber; 3% metal; 0.3% glass; 3.6% were other items of mixed materials (Table 1). Forty-six percent of the litter was found to be un-sourced, followed by 32% assigned to fishing activity, 18% to beach visitors with the last 4% assigned to shipping, sewage-related and fly-tipped items. Caps and lids from drinks bottles, categorised as 'un-sourced' due to their weathered nature (raising the likelihood they had been transported from their site of original deposition prior to collection), made up 5% of litter items (a total of 13,115 were recorded, Table 1). Of the recognisable litter that could be attributed to beach users, the top items were crisps, sweets and lolly wrappers, which made up 3% of litter items (7,648 wrappers collected from the 9 beaches over 6 years), and cigarette stubs, which accounted for 2% of litter items (5,257 stubs were collected over 6 years, Table 1).

### 3.2. Inter-annual trends

There was significant variation in the amount of litter on all beaches over the 6 year period ($F_{5,71} = 3.18$ $p = 0.012$, Table 2). Litter was significantly lower in the years 2009 ($0.019 \pm 0.002$ litter items $m^{-2}$ $month^{-1}$) and 2010 ($0.019 \pm 0.001$ litter items $m^{-2}$ $month^{-1}$) than in 2006 ($0.030 \pm 0.002$ litter items $m^{-2}$ $month^{-1}$). However in 2011 the total amount of litter was found to have increased again ($0.026 \pm 0.002$ litter items $m^{-2}$ $month^{-1}$).

Further inter-annual trends in litter abundance are apparent when the data is split by study area into the three separate areas. Litter in the Bude area significantly decreased over time ($F_{5,71} = 7.42$ $p < 0.001$). Litter abundance in 2010 ($0.009 \pm 0.001$ litter items $m^{-2}$ $month^{-1}$) and 2011 ($0.017 \pm 0.004$ litter items $m^{-2}$ $month^{-1}$) was significantly lower than litter abundance in 2006 ($0.047 \pm 0.008$ litter items $m^{-2}$ $month^{-1}$, Fig. 2A). This decrease was seen in all

**Table 1**
Top 20 litter types collected from all beaches in all months. Proportion in relation to all litter items from each beach, the other 81 litter items were <0.01% of the total.

| Rank number | Litter type | Litter category[a] | Litter material[b] | Number | Proportion |
|---|---|---|---|---|---|
| 1 | Plastic pieces large > 1 cm-50 cm | Un-categorised | Plastic | 42,940 | 0.17 |
| 2 | Cord <50 cm | Fishing | Plastic | 41,011 | 0.17 |
| 3 | Plastic pieces small < 1 cm | Un-categorised | Plastic | 38,150 | 0.15 |
| 4 | Caps/lids (Drinks) | Un-categorised | Plastic | 13,115 | 0.05 |
| 5 | Rope < 50 cm | Fishing | Plastic | 12,402 | 0.05 |
| 6 | Fishing net < 50 cm | Fishing | Plastic | 10,569 | 0.04 |
| 7 | Crisp/sweet/lolly wrappers | Beach Visitors | Plastic | 7648 | 0.03 |
| 8 | Cord >50 cm | Fishing | Plastic | 7401 | 0.03 |
| 9 | Cigarette stubs | Beach Visitors | Other | 5257 | 0.02 |
| 10 | Polystyrene pieces < 50 cm | Un-categorised | Plastic | 3713 | 0.01 |
| 11 | Foam/sponge | Un-categorised | Plastic | 3682 | 0.01 |
| 12 | Drinks bottles | Beach Visitors | Plastic | 3109 | 0.01 |
| 13 | Paper pieces | Beach Visitors | Paper/card board | 2781 | 0.01 |
| 14 | Rope > 50 cm | Fishing | Plastic | 2756 | 0.01 |
| 15 | Bottle caps | Un-categorised | Plastic | 2645 | 0.01 |
| 16 | Cotton bud sticks | Sewage related | Plastic | 2509 | 0.01 |
| 17 | Bags (including supermarket) | Un-categorised | Plastic | 2264 | 0.01 |
| 18 | Drinks cans | Beach Visitors | Metal | 2154 | 0.01 |
| 19 | Caps/lids (Heavy Duty) | Shipping | Plastic | 2006 | 0.01 |
| 20 | Shotgun cartridges | Beach Visitors | Metal | 1886 | 0.01 |

[a] Total proportion of each Litter category: **Fishing** 32% (79,439 items); **Beach users** 18% 44,684; **shipping, sewage and fly-tipped combined** 4% (9930 items); **un-sourced** 46% (114,193 items).
[b] Total proportion of each Litter material: **Plastic** 88.9% (220,802 items); **Paper/cardboard and timber** 4.1% (10,269 items); **Metal** 3.0% (7475 items); **Glass** 0.3% (787 items); **Other** 3.6% (8913 items).

**Table 2**
General Liner model output. Null hypothesis: There is no significant difference in the amount of litter each month on individual beach, area and all beaches combined between each A) year or B) Season. When $p < 0.05$ the null hypothesis can be rejected and a post-hoc Tukey test was run, these are shown for each area in Figs. 2 and 4.

| | A) Year | | | B) Season | | |
|---|---|---|---|---|---|---|
| | F | df | P | F | df | P |
| Crooklets | 4.66 | 5,70 | **0.001** | 0.22 | 3,70 | 0.884 |
| Summerleaze | 7.73 | 5,70 | **<0.001** | 1.03 | 3,70 | 0.383 |
| Widemouth | 7.72 | 5,70 | **<0.001** | 1.39 | 3,70 | 0.255 |
| BUDE TOTAL | 7.42 | 5,71 | **<0.001** | 0.76 | 3,71 | 0.521 |
| Polzeath | 15.43 | 5,70 | **<0.001** | 4.91 | 3,71 | **0.004** |
| Trevone | 12.89 | 5,70 | **<0.001** | 6.83 | 3,71 | **<0.001** |
| Harlyn | 18.46 | 5,71 | **<0.001** | 3.65 | 3,71 | **0.017** |
| PADSTOW TOTAL | 19.48 | 5,71 | **<0.001** | 5.90 | 3,71 | **0.001** |
| Constantine | 3.00 | 5,71 | **0.017** | 5.14 | 3,71 | **0.003** |
| Treyarnon | 1.67 | 5,71 | 0.153 | 4.47 | 3,71 | **0.006** |
| Porthcothan | 1.54 | 5,71 | 0.190 | 3.19 | 3,71 | **0.029** |
| PORTHCOTHAN TOTAL | 2.18 | 5,71 | 0.067 | 5.50 | 3,71 | **0.002** |
| GRAND TOTAL | 3.18 | 5,71 | **0.012** | 3.36 | 3,71 | **0.024** |

categories of litter (Fig. 3A). Litter in the Padstow area increased over time, litter abundance in 2010 (0.028 ± 0.002 litter items $m^{-2}$ $month^{-1}$) and 2011 (0.034 ± 0.002 litter items $m^{-2}$ $month^{-1}$) was significantly higher than litter abundance in 2006–2009 (0.013–0.012 litter items $m^{-2}$ $month^{-1}$) (Fig. 2B). This is predominately driven by un-sourced litter items (Fig. 3Bi). There was a sustained increase in fishing related litter in the Padstow area which appears to correspond to the drop in fishing related litter in the Bude Area (Fig. 3Aiii). There was no inter-annual trend seen in the Porthcothan area comparing total monthly litter abundance ($F_{5,71} = 2.18$ $p = 0.067$, Fig. 2C).

### 3.3. Seasonal trends

When looking at the total litter collected each month across all beaches, a significant seasonal trend in the amount of litter on all beaches is observed ($F_{3,71} = 3.36$ $p = 0.024$), with the highest abundance of litter collected in the summer seasons (Tukey $p < 0.05$). The Bude area didn't follow this seasonal trend ($F_{3,71} = 0.76$ $p = 0.521$), however there was strong seasonal variation in the total litter collected in the Padstow ($F_{3,71} = 5.90$ $p = 0.001$) and Porthcothan ($F_{3,71} = 5.50$ $p = 0.002$) areas, with litter abundance being higher in the summer compared to all autumns and winters.

Fig. 5A indicates a proportional increase in tourist derived litter on the Bude area beaches throughout any given year with a peak in the summer, with a similar pattern observed for fishing litter items. In the summer months, identifiable litter items actually make up over 21% of the total litter found on the beaches (compared to 8% in the winter). The most abundant category however is un-sourced litter items (61% in winter, 47% in spring, 43% in summer and 51% in autumn).

Fig. 5B also indicates a large proportional increase in tourism derived litter in the summer months (37%) compared to 14% in the winter. Fishing holds a 44% share of the litter budget in winter; the largest proportional share across the three beach groupings.

Fig. 5C shows, as with the other beach groups, un-sourced litter is of major concern in the Porthcothan area as it holds between 40% and 46% of the total litter budget on the beaches. There is strong seasonal beach visitor use as the proportion of beach visitor litter increases from just 10% in winter to 31% in summer; almost as dramatic an increase as that seen on Bude area beaches, where summer triggered a 23% rise in beach visitor litter. Fishing litter also takes a larger proportion of the total litter budget in winter.

## 4. Discussion

This unique, systematic long-term, data set for beach litter in the South West of England has enabled us to highlight key trends in the type and amount of beach litter and its key drivers on a spatial, inter-annual and seasonal basis. We removed and recorded a total of 248,246 pieces of litter from the 9 beaches over the 6 year period which equates to an average of 0.026 ± 0.002 litter items $m^{-2}$ $month^{-1}$. Importantly, we found that the majority of this litter comprised small, fragmented, plastic pieces less than 50 cm in length, much of which had been subject to significant weathering. This weathering suggests that these items have been in the

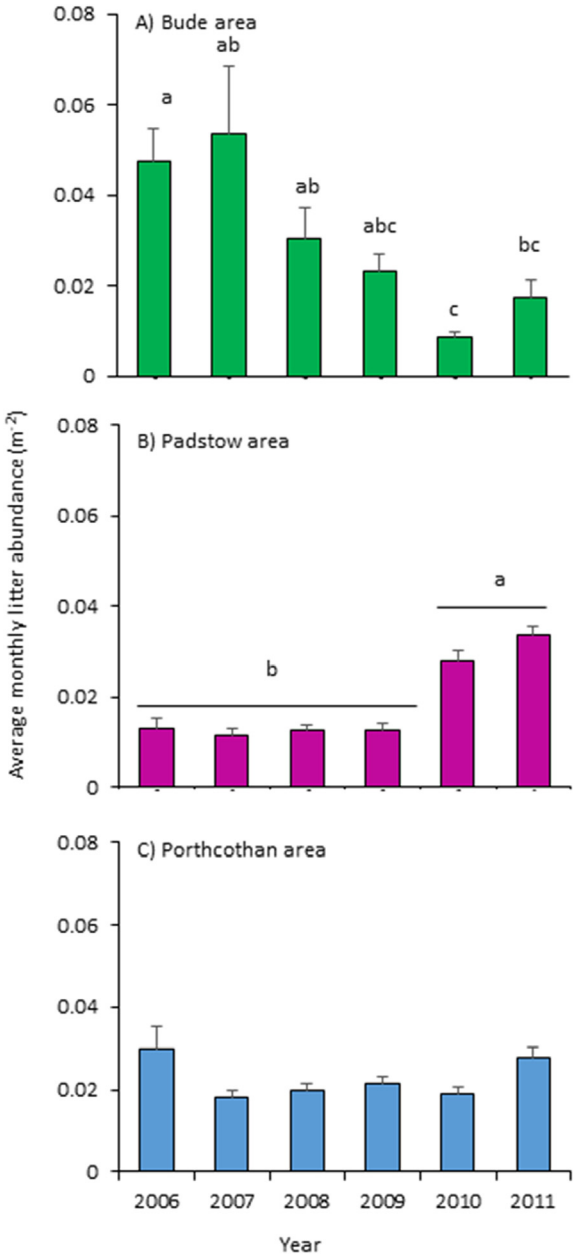


**Fig. 2.** Inter-annual trends of beach litter abundance per metre squared between 2006 and 2011. a) Bude area; b) Padstow area; c) Porthcothan area. Bars represent the average monthly litter abundance in items $m^{-2}$ for each year from 2006 to 2011 ± S.E. Means that do not share a letter are significantly different (Tukey, $p < 0.05$).

environment and/or at sea for a long period prior to being collected as part of these beach cleans. These weathered plastic pieces are therefore impossible to link to their original source due to their fragmented nature, but have clearly been present in the environment for an extended period of time prior to being removed and recorded for this study. The dominance of plastic litter is a trend seen in a number of long-term studies and our average proportion of plastic litter of 88.9% (Table 1) is in keeping with other studies. Schulz et al. (2015) reported 52–91% in a 25 year-long study and Nelms et al. (2017) reported 66% over a 10 year study). However, it is important and noteworthy to separate out the size classes and different types of plastic as we have done and find our statistic of 49% of all litter being small plastic pieces of greater significance.

Critically, our sampling method removed litter as it was counted and categorised each month, meaning that the litter counted at each subsequent sampling point was freshly deposited during the intervening month. Hence our monthly litter abundance data can also be considered the monthly 'deposition' rate for this litter. Despite this regular cleaning effort, an astonishing amount of litter was still present at all of the study beaches at each sampling time. It can be concluded, therefore, that the state of these beaches would be considerably worse were it not for the constant removal of litter by beach cleans for this area. Interestingly, litter that could be attributed to 'beach users' (including tourism based litter) only accounted for 18.7% of the overall litter loads for these Cornish beaches, despite their heavy use by tourism over the summer months (Visit Cornwall, 2011). A small but significant increase in the proportion of litter from beach users was observed during the summer months, but litter was always dominated by the weathered plastic pieces, which may have been deposited by the sea.

The total litter abundances recorded each month did show changes in abundance over the six year study period, but these changes differed in direction for the three study areas, suggesting local factors play a role. The total amount of litter observed on beaches in the Bude area showed a decrease of 17% over time, however this was not observed for the Padstow area which showed the opposite trend of a 6-fold increase. The total litter collected each month for the Porthcothan area beaches showed no change over the 6 years. The trend observed for the Bude area beaches may well have been driven by a particularly high litter count recorded in February 2007, which was 18% higher than the average for this region. There is evidence from other U.K. regions that sustained beach cleans can act to reduce the standing stock of litter over time. In South Wales, which is located just north of our study sites, a 50% reduction in marine litter abundance was observed between 1995 and 1998 due to beach cleans, with only 19% of beach litter items returning after 2 weeks without a clean (Williams and Tudor, 2001). In our study, we observed restocking of litter over the 4 weeks between samples, with only small decreases or even increases over time, indicating a continuous high input of litter onto our study beaches throughout the year. Bravo et al. (2009) calculated a global average of 1.4 litter items $m^{-2}$ from 12 studies including 149 beaches from 0.2 litter items $m^{-2}$ (Ireland, Benton, 1995) to 1–6.0 litter items $m^{-2}$ (Jorden, Abu-Hilal and Al-Najjar, 2004). Bravo et al.'s calculation excludes a beach clean from Hiroshima Bay in Japan that collected 45,000 items $m^{-2}$ at one time, since this study the reported every single fragment of Styrofoam found (Fujieda and Sasaki, 2005).

Of the total litter recorded from all beaches, only 59% could be identified by source. This comprised 32% from fishing, 21% from beach visitors, 4% from shipping, 1% from sewage and <0.1% from fly tipping and medical sources. This is similar to Nelms et al. (2017) who were able to assign 60% of the litter from the MCS data set, which surveyed around the U.K., to identifiable sources (comprising 15% from fishing, 36% from beach visitors, 3% from shipping, 5% from sewage and 0.7% from fly tipping).

The largest identifiable user group in our dataset was litter originating from fishing activities such as fishing nets, ropes and rope pieces (of varying sizes). North Cornwall is known for its fishing activity with over 400 boats registered around the north and south of Cornwall, not including boats that have come from other administrative ports (MMO, 2014). In our 6-year data set fishing gear made up 32% of all the marine litter recorded. Our findings differ to those of Unger and Harrison (2016) who attributed most beach litter in their data (derived from the MCS beach watch) to fishing activity, however, they do not provide a percentage. In their study, based on data collected by MCS volunteers, they assign many of their litter items collected, including small plastic pieces, to the fishing category without presenting any clear

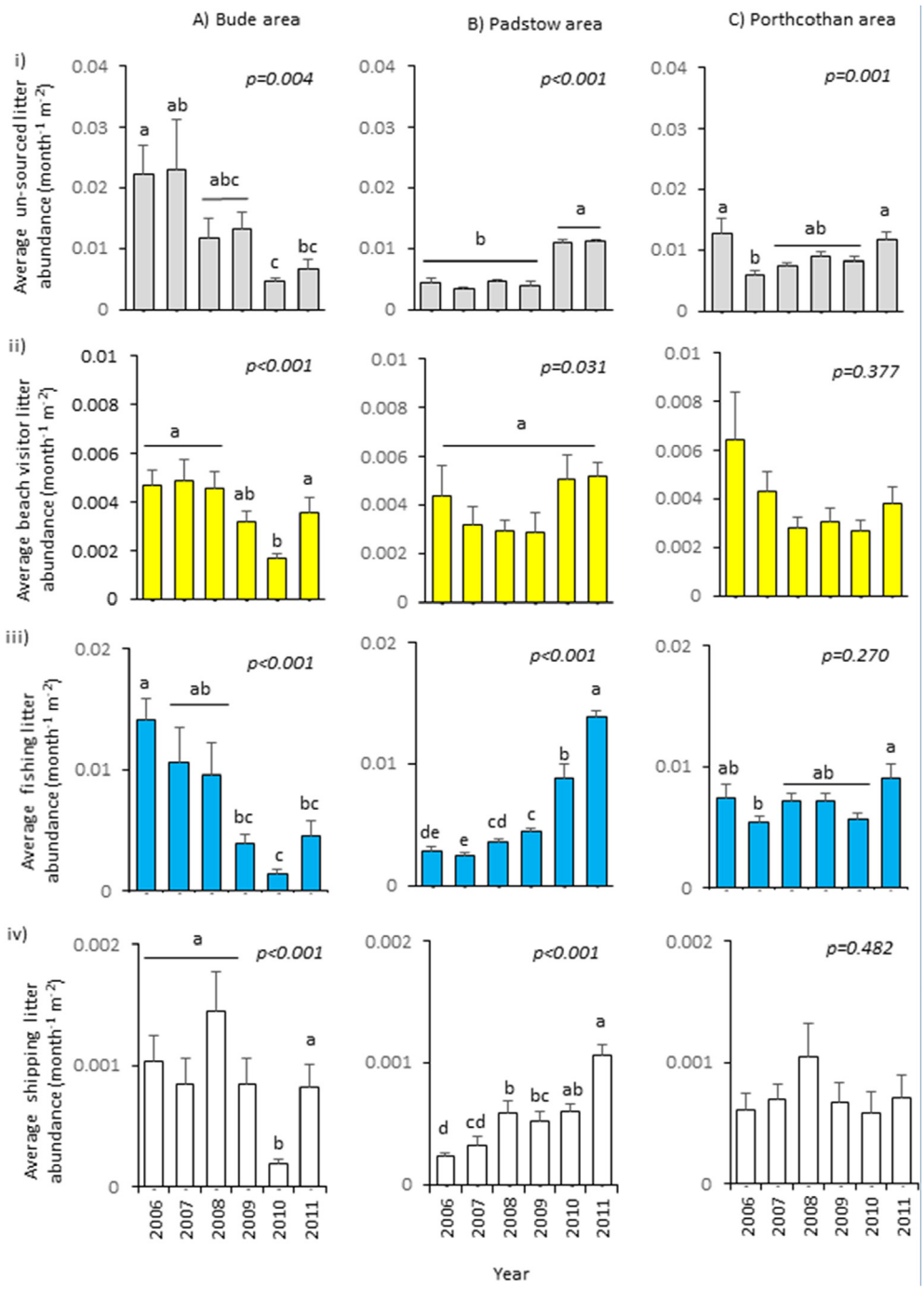


**Fig. 3.** Inter-annual trends of beach litter type 2006–2011. a) Bude area; b) Padstow area; c) Porthcothan area. Grey: un-sourced, Yellow: beach visitors, Blue: fishing White: shipping. Bars represent the average monthly litter abundance in items $m^{-2}$ for each year from 2006 to 2011 ± S.E. Means that do not share a letter are significantly different (Tukey, $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

justification for this. Unger and Harrison (2016) then conclude that fishing gear is the greatest contributor to beach litter, yet this finding is heavily driven by the unidentifiable plastic pieces in their data set. Given the fragmented nature of this type of litter it is most likely to have been at sea for a while, therefore to attribute it to any certain user (fishing, shipping, beach user) is inappropriate. This highlights a key issue with litter categorisation in studies of this nature such that the way in which litter is assigned during analysis can heavily skew the subsequent conclusions. Our use of the term 'un-sourced' for these weathered and fragmented items reflects the OECD guidelines and acknowledges that these plastic pieces are likely to originated from a complex range of sources including fishing, shipping activity and land-based sources.

We found seasonal trends in both the amount and source of litter, with the amount of litter attributed to beach visitors increasing from 8% in the winter to 21% in the summer on the Bude area beaches, from 14% to 37% on the Padstow area beaches and from 10% to 31% on Porthcothan area beaches. Importantly, 18% of the litter (total of 44,684 items in 6 years) collected during this study over the six year period could be directly attributed to beach

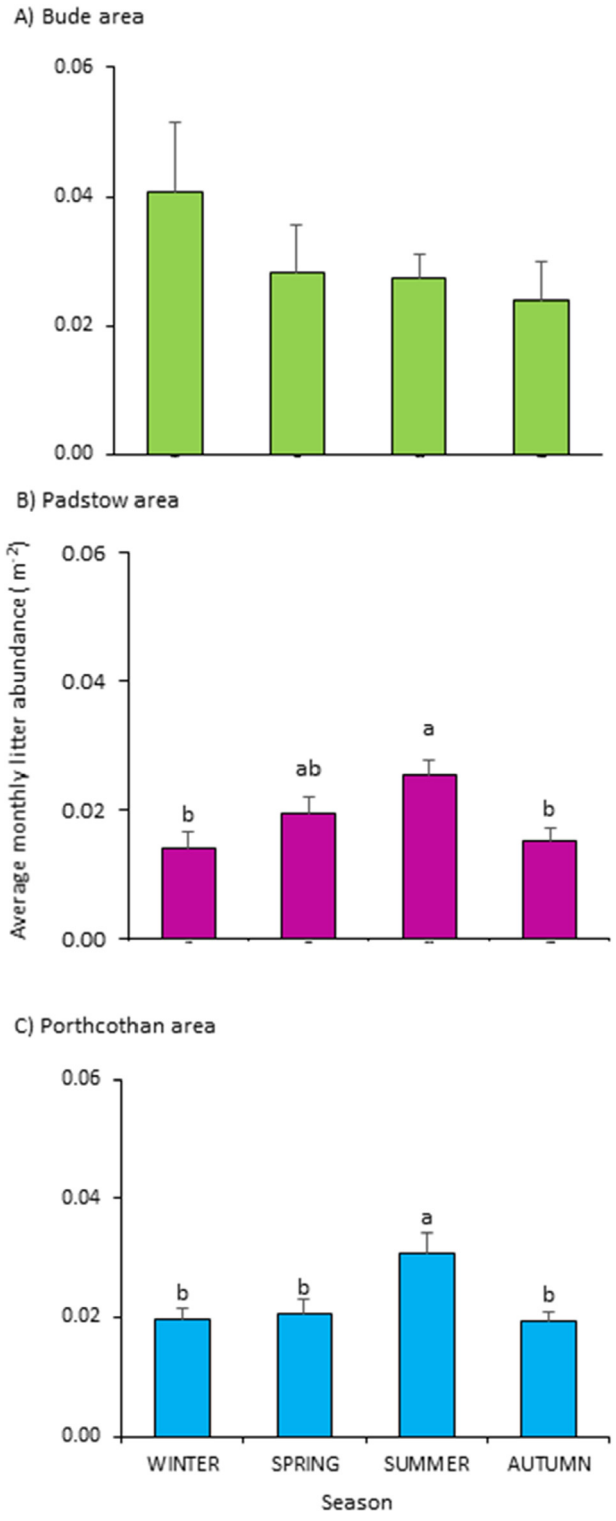


**Fig. 4.** Seasonal trends of beach litter abundance per metre squared between 2006 and 2011. A) Bude area; B) Padstow area; C) Porthcothan area. Bars represent the average monthly litter abundance in items $m^{-2}$ for each season.± S.E. Means that do not share a letter are significantly different (Tukey, $p < 0.05$).

visitors. These numbers are much lower than that reported by Bravo et al. (2009) for beaches in Chile, which were dominated by beach visitor litter with an average of 1.8 items $m^{-2}$. North Cornwall is a prime tourist destination in the summer months with the beaches being a big draw for tourists, around 774,000 people

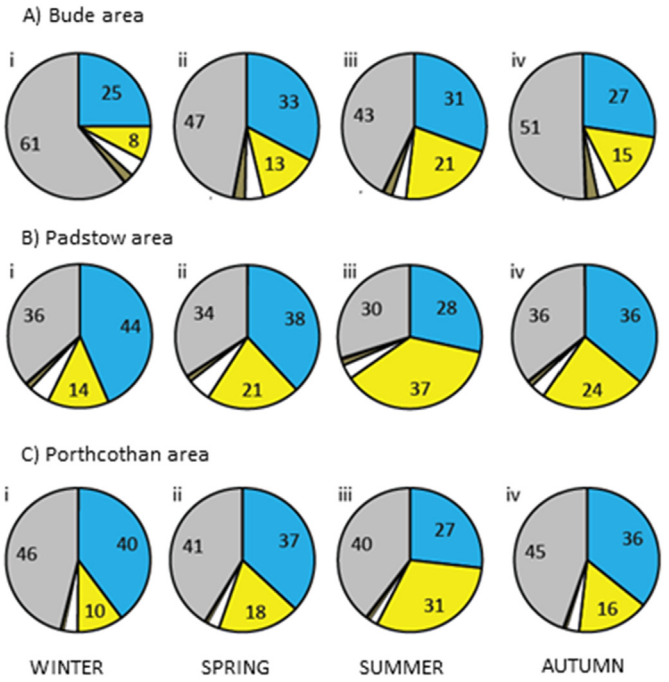


**Fig. 5.** Seasonal trends of beach litter type 2006–2011. A) Bude area; B) Padstow area; C) Porthcothan area. Grey: un-sourced, Yellow: beach visitors, Blue: fishing, White: shipping, Brown: sewage related items, Dark grey: fly tipping. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

visiting in the month of July compared to just 101,000 visiting in January (data from Visit Cornwall, 2011). Visitor numbers for our study beaches can be inferred from car parking data gathered from Cornwall Council for the time period from April 2014 – March 2015 (the data was limited in its scope therefore there was not enough to cover the entire study period). This car parking data reveals that Bude has the highest number of visitor for our study beaches (Cornwall Council, 2017) with an annual number of cars parked of 144,000, compared to 63,600 for the Polzeath area and 16,700 for the Porthcothan area. This data also shows that the number of cars are indeed higher for summer compared to winter by 5 fold at Bude, 6 fold at Porthcothan and 8 fold at Polzeath. Whilst Bude receives the most visitors of the study beaches, the difference between summer and winter is less pronounced here as Bude has a much longer 'visitor season'. This helps to explain the lack of seasonal trends seen on the Bude area beaches compared to the strong seasonal trends at both Padstow and Porthcothan beaches (Fig. 5). There are a number of other factors that will change the distribution of marine debris across our study area. Factors such as topography, near-shore water currents, prevailing wind directions and other environmental drivers (Schulz et al., 2015) will play a part, but were outside the scope of this study.

The 18% of litter we attribute directly to beach users in our study is less than that reported in the recent MCS citizen science study for the U.K. (Nelms et al., 2017), which attributed 36% of the total litter for U.K. beaches to tourism based sources. However Nelms et al. (2017) place drink bottle caps and lids in their public littering category, which is also their third largest litter type. In our analysis the caps and lids found, which made up 5% of all litter items on our study beaches, all showed signs of significant weathering, so may have come from a whole range of sources and therefore have been transported to the beach where they were collected by the action of the sea, rather than dropped directly at that location by a user of that beach. Litter of this nature is likely to be highly mobile once

released into the environment, and since our aim was to attribute the original source of litter items where possible in order to inform management practises, we made the decision to only attribute items clearly dropped at the study location as being beach-user litter. In taking this approach, our data highlights the high proportion of the litter on our beaches that cannot be attributed to a direct source and that has likely spent considerable time at sea before being deposited on the beach.

The plastic pieces which numerically dominated all of our samples probably underestimate the true amount of small plastic pieces present on the beaches, since only pieces large enough to be obvious to the eye would have been picked up. Microplastics, plastic pieces less than 5 mm in size, were not included in the study due to the nature of our sampling method, yet are often found on beaches at concentrations from 8 particles $L^{-1}$ sediment (Australia, Browne et al., 2011) to 200 particles $L^{-1}$ sediment (Brazil, Costa et al., 2010). The relationship between microplastic and larger litter items on beaches have, however, not been widely studied.

The dominance of fragmented litter items of un-sourced origin in our data set raises some important considerations. This litter is impossible to identify by source because it has been broken up by long exposures to UV damage and physical weathering, i.e. it has been in the environment for an extended period of time prior to being deposited on our study beaches and removed. Hence, stopping the release of litter into the environment at its source is clearly important. Deposition rates of marine litter on to beaches from the sea can be driven by a number of factors. For example, the physical features of the beach such as beach aspect (Gabrielides et al., 1991) and wind exposure (Thiel et al., 2013; Fauziah et al., 2015) and the type of beach use (Kordella et al., 2013) have all been shown to influence both the amount and type litter found. In general, it is the floating proportion of debris from marine based sources that gets deposited onto beaches. Van Cauwenberghe et al. (2013) surveyed sea surface, sea floor and beach litter along the Belgian coast and found that only 34% of the total litter recovered was on the beach, with 37% found floating on the sea surface (likely bound for beaches), and 29% found on the sea floor.

The constant input of fragmented (aged) litter on to our study beaches each month means that floating plastic debris is accumulating off the coast of North Cornwall and being driven on to these coasts throughout the year. Since this was the largest contributor to the beach litter recorded in our study, understanding the original sources and subsequent behaviour of this floating litter in the marine environment requires urgent further attention if the input of beach litter is to be reduced in the future. Regular beach cleans and better public awareness of beach littering, whilst being hugely important components of better beach management, do not address almost half of the amount accumulated. Beach cleans can be a contentious tool for beach litter management as they have been shown to cause ecological disturbance especially by mechanical cleaning activities such as beach raking. These activities can cause harm to the environment and the overturning of sediment then requires some level of habitat recovery before the associated assemblage can return to a steady state (Dernie et al., 2003a,b). In the U.K. 51% of municipalities clean their beaches manually, 47% use a mixture of manual and mechanical, and 2% use mechanical methods only (Mouat et al., 2010) so there is an environmental cost to be considered and as yet the relative benefits are unstudied. Despite this however our study shows that to some extent, even non-invasive methods such a litter picks can be to the benefit of beach cleanliness over time.

Given that a vast number of beaches are thought to receive a proportion of litter that is not generated in the direct vicinity, stopping litter at source is now a key target for the international community as well as local governments (Fauziah et al., 2015). It is widely acknowledged that marine litter needs to be tackled at source. The Marine Strategy Framework Directive (MSFD) directs the European Community towards the clean-up of marine litter (Williams et al., 2014). Descriptor 10 of the MSFD dictates that 'Good Environmental Status is achieved only when "properties and quantities of marine litter do not cause harm to the coastal and marine environment" (Directive, 2008/56/EC) and as such government has a responsibility for ensuring marine debris is dealt with. The implementation of laws and regulations, as well as market-based instruments such as environmental taxes and incentives (e.g. plastic bag tax or bottle refund schemes) need to be considered at all levels of governance and should be part of an integrated strategy encompassing waste management, education, outreach, laws and policies, enforcement, and adequate infrastructure (NOAA and UNEP Summary Proceedings 5th International Marine Debris Conference). Success has been seen in using financial disincentives leading the U.K. to impose a €0.06 levy on the use of single-use carrier bags; starting in Wales in 2011, reaching Northern Ireland, Scotland and England by 2013, 2014, and 2015 respectively. One year after the introduction of the Welsh charge, a ≈70–90% decrease was observed in bag use (Newman et al., 2015). It is unclear still, however, if this has any reductive effect on the amount of plastic bags ending up in our environment.

Our study has highlighted a lack of certainty in attributing sources to the majority of litter items on our beaches, with the vast majority of litter items being un-sourced due to its aged and fragmented nature. Whilst beach cleans can act to remove a large amount of litter (we removed 248,246 items over 6 years from 9 beaches), our study shows that this removed litter is rapidly replaced by items that cannot easily be attributed to source. Hence beach cleans are not tackling the problem at source and need to be considered within a wider marine litter strategy. Moves from governments to develop legislation to reduce plastic waste (such as ban on microbeads in cosmetics and the plastic bag levy) is a start, however, as we are unable to determine the source of the vast majority of litter we are picking off our beaches we need to think about this problem more holistically.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2017.05.016

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**Supporting Information**

# Through the sands of time: Beach litter trends from nine cleaned north Cornish beaches

This supporting information contains:

Table S1: Litter items within category classification

## SI.1 Litter items within category classification- to format

**Beach visitors**
4/6 pack holders
Bags (including supermarket)
Drinks bottles
Combs/hair brushes
Crisp/sweet/lolly wrappers
Cups
Cutlery/trays/straws
Pens
Shoes/sandals
Toys and party poppers
Fast food containers/cups
Surfboard/boogie board
Balloons
Clothing/shoes
Hand wipes
Towels
BBQ's
Bottle caps
Drinks cans
Foil wrappers
Nails/screws
Condoms
Nappies
Dog
Horse
Bags
Cardboard
Cigarette packets
Cigarette stubs
Cups
Fireworks
Newspapers/magazines
Tissues
Paper pieces
Ice lolly sticks
Bottles
Glass pieces

**Fishing**
Milk bottles
Cord < 50cm
Cord >50cm
Fishing boxes
Fishing line (anglers)
Fishing net < 50cm
Fishing net > 50cm
Floats
Lobster pot tags
Glow sticks
Rope < 50cm
Rope > 50cm
Buoys
Boots
Gloves (heavy duty)
Gloves (light weight)
Fishing weights
Crab pots

**Fly tipped items**
Tyres
Furnishing
Car parts
Scrap metal/appliances
Any pottery or ceramics

**Sewage related items**
Cotton bud sticks
Plastic backing strips
Tampon applicators
Tampons
Toilet fresheners
Towels/panty liners
Other sewage items (specify)

**Un-sourced**

Caps/lids (Drinks)
Cigarette lighters
Shotgun cartridges
Plastic pieces < 1cm
Plastic pieces > 1cm-50cm
Plastic pieces > 50cm
Other (specify)
Fibreglass
Foam/sponge
Polystyrene pieces < 50cm
Rubber pieces < 50cm
Other (specify) > 50cm
Cloth pieces
Sacking
Other (specify)
Metal pieces
Wire and wire mesh
Other (specify)
Cartons/tetrapak (milk etc)
Corks
Other (specify) > 50cm

**Shipping**

Cleaning bottles
Food containers
Oil bottles< 50cm
Oil bottles > 50cm
Toiletry bottles
Caps/lids (Heavy Duty)
Industrial packaging
Injection gun containers
Mesh vegetable bags
Strapping bands
Packaging
Aerosol cans
Food cans
Oil drums
Crates/pallets
Paint brushes
Wood pieces (Machined)< 50cm
Light bulbs/tubes

# Chapter III: Co-occurrence of plastic and zooplankton and the potential for microplastic encounter across ocean seascapes.

**Figure 1:** A view of Sea Dragon as we sailed across the North Atlantic Ocean trawling for plastic and plankton.

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Adam Porter and Stephanie Wright collected the samples. Adam Porter conducted the data analysis and wrote the manuscript.

Ceri Lewis edited and improved the manuscript

Brett P. Lyons and Tamara S. Galloway advised in the design and implementation of the experiments and in the interpretation of the data.

## Abstract

Microplastics have now been recorded from every part of the worlds' oceans, from the equator to the poles and from the surface to the deepest part of the ocean raising concern as the small size of these plastic particles fall within that of the normal food for many important marine species including zooplankton. Plastics and plankton are not often studied in combination however, limiting our ability to make predictions of the likelihood for ingestion occurring in the real world. To address this knowledge gap, we collected data from sea surface tows along a transect across the North Atlantic over 2 consecutive years, to assess the areas where plastics and plankton co-occur. We found evidence of microplastic contamination in every sample collected, differing in abundance and shape composition across sites. These particles were mostly fragments ranging in size from 84 μm – 21.8 mm and were mostly HDPE (≈58%). From these data we calculated that for every 1 plastic particle there were somewhere between 500 and 1000 plankton, suggesting very low risk of biological uptake across this region under current plastic contamination levels.

# Introduction

Plastic production has increased dramatically since its creation in 1907 as Bakelite and now sits at 325 million tonnes in 2016; almost 100 million tonne increase over the last 10 years (Plastics Europe, 2017) and plastic production is expected to double over the next 20 years (World Economic Forum et al., 2016). Microplastics, the smaller fraction of this plastic pollution, are now accepted as a significant classification of marine litter (European Parliament and the Council, 2010, Nuelle et al., 2014) and are so widely spread, poorly understood, and potentially impacting on our environment that they are now being considered as a planetary boundary threat alongside climate change and ocean acidification (Galloway and Lewis, 2016, Villarrubia-Gómez et al., 2018). As research continues to collate abundance data, microplastics are becoming an ever growing concern for scientists and policy makers; especially in the face of public scrutiny and demand for action (Foley et al., 2018, Dauvergne, 2018). Since Charles Moore ventured into the North Pacific (Moore et al., 2001) the race to quantify the amount of plastic afloat in the seas and oceans and its geographic distribution has intensified and yet the early questions posed by Charles Moore have gone relatively unanswered. In their 2001 paper the authors highlight that co-occurrence (where high productivity meets high plastic abundance) has not really been looked at and that encounter rates of plastic and plankton will be important in determining risk. They also highlight in early work that convergent zones are important, but that the gyres are not likely to be the places to find the impact of plastic on marine life despite their high plastic content (Moore et al., 2001) due to their low productivity.

Since then almost two decades of research has been done and a great deal more is known about the abundances and geographical distribution of plastic pollution. It is now suggested that about 12 million metric tonnes of plastic enters the oceans every year

(Jambeck et al., 2015, Sherrington, 2016) and the floating fraction is estimated to be about 15 to 51 trillion particles or 93 to 236,000 metric tonnes in the oceans (Van Sebille et al., 2015). However it is also recognised that the strong spatiotemporal variability of plastic debris can confound even very large datasets (Law et al., 2014) and that more targeted sampling is needed to interrogate the questions still being posed by the scientific community; namely what risk does plastic pollution pose to marine life and are the concentrations found in the environment large enough to have an impact (Burns and Boxall, 2018, Villarrubia-Gómez et al., 2018, Koelmans et al., 2017, Everaert et al., 2018).

A microplastics upper limit of 5 mm is relatively well defined in the scientific literature (Arthur et al., 2009) but how small they get is as yet unknown; a microplastics size is constrained only by sampling and identification methodologies (Nuelle et al., 2014, Lusher et al., 2014, Goldstein et al., 2013, Desforges et al., 2014). Norén (2007) demonstrated a 100,000 times difference in microplastic concentrations in surface seawater around the coast of Sweden when comparing the number of particles collected with a 80 µm compared to a 450 µm mesh and so the abundance of microplastics smaller than 333 µm, which is the mesh size used in most plastic surveys, may well be much greater than currently estimated. At present, and as research continues to investigate the smaller size fractions (e.g. Ter Halle et al. (2017) have identified nanoplastics in the North Atlantic Subtropical Gyre), it would seem the scale of this pollution is rapidly increasing. Thompson et al. (2004) showed a significant increase in fibre counts in the North East Atlantic from the 1960's to the 1990's. For example, Law et al. (2014) also showed a significant increase in measured concentrations between their early sampling years (2002 – 2006) and the later years (2007 – 2011). It has even been predicted that the numbers of plastics in the marine environment will continue to grow into the future with models stating that the amount of

floating plastics will increase to between 25 million and 1.3 billion ($10^8$) tonnes by 2100 (a 50 fold increase) (Everaert et al., 2018).

Studies such as the modelling work of Van Sebille et al. (2015), or the global sampling effort of groups such as 5 Gyres (Eriksen et al., 2014) have all tried to quantify how much plastic there might be in the World's oceans. Sampling constraints, a lack of global coverage of real world data, the reporting of data in the literature with inconsistent units, and model designs and assumptions all build uncertainty in these estimates (Van Sebille et al., 2015, Eriksen et al., 2014) however they all highlighted the collection of plastics in ocean gyres. In all instances, the research to date highlights a geographic unbalance in data collected on microplastic cruises; weighted heavily towards the gyres and leaves large gaps in our understanding of microplastic budgets in oceanic environments globally.

The current state of global sea surface plastic sampling is patchy at best. A vast amount of work has been done in the oceanic gyres; especially the North Pacific Gyre and yet only a small fraction of our seas and oceans have been surveyed (Van Sebille et al., 2015). The well cited model by Van Sebille et al. (2015) highlights this disparity, especially in the case of the Mediterranean where models predict 21 – 54% of the global floating mass of microplastic to be and yet only 105 surface trawls have been undertaken there (less than 1% of all the trawls used in the models). Eriksen et al. (2014) also modelled global floating plastic abundances using surface net tows and visual survey transects of large plastic debris. In collating a large dataset of 1571 field locations where plastic debris sampling had been undertaken he identified 680 locations sampled for items <200 mm. Of those 680, the North Pacific accounted for 26% of sampling locations; an area acknowledged as nutrient poor by Charles Moore

in 2001 (Moore et al., 2001). Similarly to Van Sebille et al. only 5% of studies sample locations were in the Mediterranean and 4% in the Indian Ocean.

It is increasingly being realised that many of the laboratory based studies looking at the potential for microplastics to be ingested by marine organisms are not well aligned with real world (De Sá et al., 2018, Phuong et al., 2016). For example these experiments use high dose concentrations of microplastics (Koelmans et al., 2017a, Lenz et al., 2016) and particles that are not consistent with those found in the environment in size, shape, and polymer type (Burns and Boxall, 2018). The majority of laboratory exposures have used beads whereas fibres and fragments are the most prevalent shape making up 45 – 52 % and 29 – 33% of particles found respectively (Hidalgo-Ruz et al., 2012, Burns and Boxall, 2018). Sixty-nine percent of laboratory effects studies have used Polystyrene polymers despite only making up 5% of the polymers found in the water column (Burns and Boxall, 2018) and the majority of studies have used microplastics <131 μm; smaller than those that have been confidently measured in the environment (Burns and Boxall, 2018). That being said, these endeavours are essential as to understand at what size, shape and concentration plastics might cause harm is paramount in our understanding of the future impact of microplastics as concentrations seem set to increase and these concentrations in particular may be found in site specific scenarios today (Koelmans et al., 2017a, Everaert et al., 2018). Size, shape and polymer are important as they will dictate the likelihood of ingestion, and the distribution of the plastics in the surface waters. Surface trawls will have an obvious bias toward buoyant polymer types as negative polymers will sink rapidly or soon after input into the marine environment (Kanhai et al., 2017) but the vertical distribution of plastic particles plays an important part in the assessment of risk to organisms. Finally size is of importance as an organism must be able to ingest a particle for it to potentially cause harm (Koelmans et al., 2017) (however entanglement is another issue (Kühn et al., 2015)). The issue is therefore that the harm being demonstrated in the lab

is unlikely to be seen in the environment and thus microplastic pollution may not actually pose a threat to marine life at current levels; Indeed it has been suggested that floating plastics may not pose a threat to the sea surface environment until 2100 given the lack of evidence of harm at environmentally relevant concentrations and the rate of increase of pollution in the oceans over the next century (Everaert et al., 2018). It is important therefore to find areas where particle concentrations are high in proximity to high biomass as this will determine the most likely areas where the greatest ecosystem wide risk from ingesting plastic particles may be uncovered in the environment (Koelmans et al., 2017).

The ocean gyres, despite being well surveyed are known to be oligotrophic (Morel et al., 2010, Jena et al., 2012, Jena et al., 2013) as the gyres are regions of anti-cyclonic circulation which drives downwelling and depresses the thermocline, limiting the nutrients supplied to the surface. This leads to areas of low primary productivity (Clark et al., 2016) and therefore low biomass as there is a reduced amount of food to support food webs. Much of the concerns around the threat caused by presence of microplastic in marine ecosystems stems from their size range falling within that of the natural food for many important marine biota (Galloway et al., 2017) meaning that these particles may be accidentally ingested and hence enter marine food webs. Ingestion of plastics has been widely demonstrated in laboratory and/or field studies for marine zooplankton (Cole et al., 2014, Sun et al., 2017, Desforges et al., 2015), fish (Carpenter et al., 1972, Lusher et al., 2013, Bellas et al., 2016, Lusher et al., 2015a), seabirds (Avery-Gomm et al., 2013, Baltz and Morejohn, 1976, Savoca et al., 2016), marine mammals (Fossi et al., 2012, Jacobsen et al., 2010, Nelms et al., 2018), mussels (Browne et al., 2008, Li et al., 2015, Van Cauwenberghe and Janssen, 2014), amphipods, lugworms, and barnacles (Thompson et al., 2004, Goldstein and Goodwin, 2013), and many other marine and marine-associated species. Yet the focus of research to date seems to be geographically misaligned to the important biological questions. In fact 50% of the

estimated global budget of floating microplastics are in relatively low plastic concentration regions (in relation to the gyres) according to Van Sebille et al. (2015). Hence perhaps the areas of the oceans with the greatest likelihood for biota to encounter plastics and be impacted by this encounter will be in areas outside of the oligotrophic gyres; where relatively high biomass and microplastics intersect.

Another issue limiting our understanding of the risks posed by microplastics to marine biota is a significant mismatch in the sampling effort for collecting microplastics abundance data and the areas of high biological productivity in the oceans. Clark et al. (2016) demonstrated this spatial mismatch by modelling the Chlorophyll and plastic concentrations of the North Atlantic and demonstrating this divergence of the plastic and the biomass abundances and illustrating that whilst plastic may be highly abundant, plankton are not in the NASG. What is key to understanding the impacts of microplastic in the case of this study is identifying where microplastics, at a size ingestible by zooplankton (Koelmans et al., 2017) and at concentrations high enough to make ingestion likely exist (Botterell et al., 2018). By identifying and exploring these areas, asking questions that relate to risk and likelihood of uptake, and sampling in a more targeted way it is hoped that the real impacts of plastics on marine organisms might become apparent (Kanhai et al., 2017).

We have great need in understanding what risk *environmentally relevant* microplastics pose to marine organisms in contexts and environments where exposure is likely to occur; namely in areas of co-occurrence (Collignon et al., 2012, Clark et al., 2016, Botterell et al., 2018). This sets the gyres in perspective then, given as they are oligotrophic and yet highly polluted. This is not to say ingestion could not or will not occur here; just that the likelihood of ingestion may be somewhat lower (Clark et al., 2016). There is an increasing call for microplastic science to engage in risk assessment

of plastics (Everaert et al., 2018, Koelmans et al., 2017, De Sá et al., 2018) as this will help focus our research efforts in priority organisms and locations. There are places however, where high biological productivity meet high plastic abundances; not necessarily at concentrations similar to ocean gyre plastics (although this is being seen (Desforges et al., 2014)) but certainly compared to background levels, and it is here where potential impacts might be found similar to those being described in the experimental literature on uptake and biological effects in biota (Koelmans et al., 2017).

The quantification of plastics is of course important and therefore sampling in all regions of the oceans necessary; more needs to be done to fill in the global map of plastic abundances especially with regards to marine life and sensitive ecosystems (Clark et al., 2016). The investigation of biological interactions with microplastics is crucial; laboratory studies identifying the detrimental impacts of plastics to biology, and identification and understanding of areas in the environment where plastics are likely to be ingested *in situ* by marine fauna needs to be undertaken. Areas of ocean convergence of course are the key to this (Morét-Ferguson et al., 2010, Law et al., 2014, Frias et al., 2014) but they should also be areas of co-occurrence; areas where high abundances of microplastics and high abundances of marine biota meet. This will begin then to help us understand the real world impact of microplastic on life in our seas and oceans.

To best understand where microplastics may enter marine food webs we need to consider where plastics and biota will co-occur in high levels. Microplastics are mostly generated from terrestrial sources with the exceptions being spills at sea and litter generated from commercial fishing (Sherrington, 2016). Given that areas such as rivers and estuaries are major transport networks of microplastics from the land in to the ocean (Sadri and Thompson, 2014, Hurley et al., 2018, Barboza et al., 2018) and are

among the most productive ecosystems on Earth (U.S. Environmental Protection Agency, 2012); areas of convergence and co-occurrence such as these should now take some scrutiny to uncover the microplastic problem and in likely areas of co-occurrence leading to risk of harm. The productive coastal and shelf seas are anticipated to be areas where co-occurrence of high biomass and plastic abundances intersect (Clark et al., 2016). Closed or semi-enclosed bays, gulfs or seas may be areas of accumulation also (Eriksen et al., 2014, Desforges et al., 2014) given the residence time of waters in these geographic settings. In the UK alone over 11 $km^{-3}$ of waste water is discharged into inland waters, estuaries, and the sea each year from treatment plants (Browne et al., 2011) which have the potential to be carrying microplastics to river beds and out to sea.

Other areas of interest could be areas of upwelling in proximity to centres of population. Upwelling areas are hugely productive in comparison to the gyres (Moore et al., 2001) however as plastics are seemingly depth stratified in their relative density, areas of upwelling do not preclude areas of high microplastic concentration (Desforges et al., 2014). Where an area of high plastic concentration meets an area of upwelling however, a co-occurrence front might be created which would be of interest. The work done by Desforges et al. (2014) highlights the relative abundances of microplastics between a coastal and oceanic setting and found abundances of microplastics greater than those found in the North Pacific Gyre within Queen Charlotte Sound off Vancouver, Canada; likely due to the proximity to land based sources of pollution and local oceanographic processes. The latest plastic concentration estimates by Van Sebille et. al. (2015) identify many issues with our understanding of plastic distribution and our modelling of abundances of plastic pollution but they do highlight another large potential convergence zone; the Mediterranean. The Mediterranean Sea is surrounded by large inputs of land-based plastic waste (Jambeck et al., 2015) the surface waters have a long residence time due to lack of exchange with the North Atlantic and is

proposed to be the sixth great accumulation zone (along with the 5 major ocean gyres) (Cózar et al., 2015). Abundances of microplastics will most likely mimic those seen of macroplastics with decreasing density of debris with distance from population centres (Hidalgo-Ruz et al., 2012, Gabrielides et al., 1991, Thiel et al., 2013) thus the oceanic gyres are not perhaps the priority areas to focus on in the effort to understand the risk and impacts of plastic pollution on marine life (Kanhai et al., 2017).

This study set out to investigate where plastic and biomass co-occur across an ocean seascape, using a transect across the North Atlantic, seeking to investigate a range of features comprising shelf seas, oceanic islands with western boundary upwelling, and the open ocean. The aim was to identify areas of co-occurrence where planktonic organisms might most likely encounter microplastics in the environment. To evaluate the dataset collected for this study a number of hypotheses were created based on what is known from the literature to date about microplastics and their behaviour in the marine environment.

1) Plastic abundances will differ according to location. We predict that plastic abundance will be highest in the Azorean Archipelago given its proximity to the North Atlantic Subtropical Gyre (NASG) and will be moderately high in the Canaries and the European Shelf Seas given to their proximity to the European and African Continental land masses.
2) Plankton abundances will differ according to location. We would expect plankton to be highest in the productive shelf seas as opposed to the open ocean due to nutrient limitation.
3) Most microplastics will be fibres. We expect fibres to dominate the samples as they have done in much of the research to date and fragments will be most abundant around the Azorean Archipelago given their entrainment, fragmentation, and effective isolation in the NASG over time.

4) Most microplastics will by buoyant. We would expect buoyant polymers will be more abundant than negatively buoyant polymers and the abundance of buoyant polymers will increase further away from major land masses as the negatively buoyant polymers sink out to the benthos.
5) The ratio of plastic to plankton will differ with location. We would predict that the potential encounter rates of plastic and plankton will be highest in the areas closest to major land masses given land based inputs of plastic are the greatest and the shelf seas are productive regions.

## Methods

To establish areas of co-occurrence, and to understand how microplastic abundances change over ocean seascapes sea surface trawls were undertaken across various oceanographic settings. Two cruises were undertaken aboard Sea Dragon (Pangaea Explorations, Fig. 1); a 72ft Challenger Series sailing yacht. The 2014 cruise sailed from Falmouth in the United Kingdom (50.152535, -5.061395) on the 19th September, with the first sample collected in the Celtic Sea (48.83186, -7.2798233). The cruise sampled once a day at the same time (half an hour either side of 13:00 GMT) every 300 km (± 10.6 km) reaching Horta (38.530630, -28.625258) on the island of Faial in the Azores on 25th September 2014. The cruise continued from Horta to Arrecife on the island of Lanzarote in the Canary Islands (28.964711, -13.537915) arriving on the 10th October 2014 (Fig. 2). The 2014 cruise sampled from the side of the vessel using the spinnaker pole to deploy the net 2m from the boat collecting one sample per day using a 200 µm mesh plankton net with a 0.5 m diameter. The 2015 cruise sailed from Horta in the Azores on the 31st August 2015, following a similar track and arrived in Arrecife in the Canaries on the 6th October 2015 (Fig. 3). Samples were taken between 14:00 and 16:00 in triplicate on this cruise using the same 200 µm mesh plankton nets

however samples were taken by running the net roughly 10 meters behind the vessel. The nets were then brought on board.

In all cases 200 µm neuston nets were deployed from the vessels for 20 minutes with a flowmeter (General Oceanics Mechanical Flowmeter Model 2030R) attached to the opening to record the distance travelled and thus volume filtered by the nets. GPS was used to record start and ends of each tow and the tows were undertaken at speeds between 1.5 and 2 knots (2.7 – 3.7 km $h^{-1}$). Nets were maintained at the sea surface for the duration of the tow. To remove the sample from the nets, nets were first carefully rinsed down with 50 µm filtered seawater to concentrate all particles in the cod end. The cod end was then carefully removed and the sample rinsed using the filtered seawater into 250ml screw top Nalgene bottles with pre-aliquoted formaldehyde to fix the sample at a final concentration of 4%. To control for contamination once removed from the water, the nets were kept "closed" and the opening facing downwind as the sample was processed. All personnel wore cotton clothing, gloves were worn and all water used in rinsing was filtered to 50 µm; below the mesh size of the nets. Sample bottles were kept covered with tin foil if the lids were off and all equipment was thoroughly rinsed with 50 µm filtered seawater between samples.

Tow distances were calculated by averaging distances calculated from both the mechanical flow meter attached to the plankton nets and also the GPS coordinates of the start and the end of the tow. Volumes of water filtered were calculated according to the manufacturer's conversion tables from the flowmeter data and by calculating the distance towed from the GPS data and extrapolating using the area of the net opening to calculate the cylinder of water filtered. This was done to account for the flowmeter dipping in and out of the water as the net passed through the waves as there were

times when the net was collecting water but the flowmeter was not turning; a difficulty in using neuston nets over manta trawls which have foils to help keep it submerged.

Once the samples arrived back in the laboratory, the samples were opened processed in a cell culture clean room at the University of Exeter, which is a positive pressure environment designed to keep contamination out and all sample processing was undertaken in a laminar flow hood with gloves and cotton lab coats worn at all times. Blanks were taken with upturned petri dishes open during sample processing in the fume hood but also when open in the oven and when identification was taking place at the microscope or when using the μFT-IR. Samples were filtered to 50 μm to remove formaldehyde and suspended in 250 ml of 0.2 μm filtered artificial seawater. A 5 ml syringe with the tip cut off (to allow easier passage of the sample through the nozzel) was used to take a sub-sample of the plankton.

The plankton were counted and idetified into broad taxonomic groups (e.g. copepods, caetognaths, tunicates etc. (See Fig. 14)) in a known volume (between 2.5 and 25 ml) until at least 200 individuals (where possible) had been counted per sample location and a total number of plankton per volume could be calculated. The sub-sample was then returned to the whole sample and the sample was split into quarters using the Huntsman Marine Laboratory beaker technique (Van Guelpen et al., 1982) and one quarter anylsed to find those rare species not found in the sub-sample. Once the number of plankton in the sample had been counted they were transferred back to their original Nalgene bottle and resuspended in their original formaldehyde. The plankton species data were analysed and in the 2015 data there were a huge number of fish eggs in a number of samples. As this investigation is focussed on co-occurrence and therefore potential ingestion, all non-feeding organisms are not included in the data presented or analysed of both 2014 and 2015. Diatoms, foraminifera and radiolarians

were left in the dataset as many of these do feed on plankton and the work to identify them to the level needed to separate our feeding and non-feeding organisms was beyond the scope of this study.

The samples were left to settle and the formaldehyde was poured through a 50 µm mesh filter via vacuum filtration to filter the formaldehyde liquid for plastics. The samples were then dried in a drying over at 65°C and exposed to 200 ml of 20% KOH for 48 hrs at 65°C in a drying oven to digest the plankton. The whole sample was then poured through a 50 µm filter mesh via vaccum filtration and placed under a Nikon dissecting micropcope. All particles that were not obviously organic were counted, classified by shape (fragments/films/fibres/beads) and colour, and images taken and subsequently analysed using Image-J (Schindelin et al., 2012) to gather size information and feret's diameter used to give the longest measurement for each particle. Fibres were measured by drawing a segmented line along them and length calculated in Image-J.

Particles were then analysed using a Perkin Elmer Spotlight 400 FT-IR Imaging System which has both ATR and µFT-IR functionality with a pixel resolution of 6.25 µm. For the 2014 samples a representative selection of the particles from each sample point was taken for spectral analysis meaning that on average 51% of all particles were scanned. Particles were scanned under reflectance mode on Sterlitech Silver Membrane Filters to give a good background reading that would not mask the signal of plastic polymers. Wavelengths from 4000 – 450 $cm^{-1}$ were scanned which can help remove some confusion with natural polymers (Comnea-Stancu et al., 2016) and the spectra compared to a number of libraries installed with the software as well as a library of common laboratory contaminants (e.g. blue roll, lab coat fibres etc.) which have been maually collated. Matches over 70% were accepted and the spectra were

carefully examined by eye to make sure the best and most likely match was chosen from the list of possible matches mostly based on the matching of characterisitc peaks. For the 2015 samples all representative particles from one of the three reps were analysed using the same method meaning 39% of all particles were scanned in the 2015 cruise samples. To maintain as much accuracy as possible when subsampling; particles that did not pass the 70% threshold were rejected along with those that were not plastic or gave no match at all. The shape and colour was noted and the percentage of the total count of particles with the same shape and colour were removed. For instance if three blue fibres were scanned, two producing plastic polymer scans and one a cellulose scan it was deemed that 33% of all blue fibres were to be rejected from the scanned sample and the replicate samples from the same sampling location.

Plastic polymers have inherent characteristics that can dictate their behaviour in the marine environment; particularly their density which will dictate their vertical distribution in the water column. Average densities for each polymer were taken from Quinn et al. (2016) where possible and if not the average density was found by searching plastic manufacturers websites. These densities were then plotted for both the 2014 and 2015 cruises and the relative proportions investigated in relation to the average density of seawater (1.03 g $cm^{-3}$ (Cole et al., 2016)) along the cruise track (see Results). Encounter rates were calculated to assess the likelihood of plankton encouyntering plastic fragments as a coarse analysis of risk of plastic to the plankton. These encounter rates were calculated as suggested in the literature (Moore et al., 2001, Kang et al., 2015, Sun et al., 2018b) by calculating the number of microplastics per zooplankton (Sun et al., 2017).

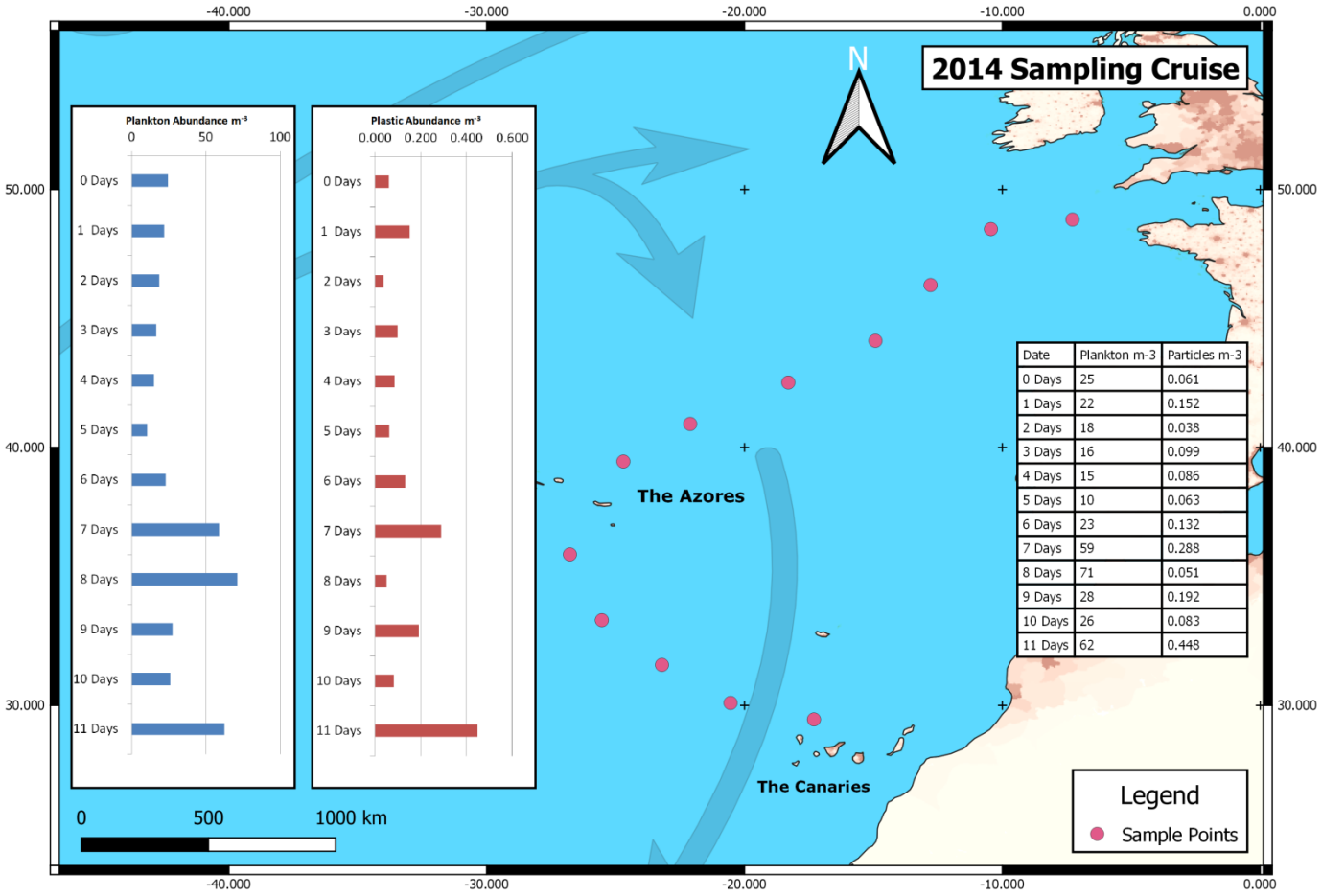
To visualise the spatial trends in the data, the data were plotted using QGIS (Qgis Development Team, 2018) according to their sampling location. Data were visualised using the WGS-84 projection and basemaps were downloaded from Natural Earth,

Population Density polygons were downloaded from ArcGIS Online by ESRI (2018), and general ocean current vector arrows were downloaded from ArcGIS Online courtesy of NOAA (2018).

## Results

Plastic fragments were found in all samples in both the 2014 and 2015 cruise years and a total of 1191 likely microplastics were identified, 971 of which were positively identified as plastic polymers. Plankton were also present in every sample and 1.8 million plankton were collected over the two cruise years. Over the two years 8190 $m^{-3}$ of water were sampled over 48.5 km of towing distance and the total ocean going distance was over 3000 nautical miles (nmi) or 5500 km. The highest concentration of plastic was found in 2014 in the Canary Islands (29.4531667, -17.3095) at 0.448 particles $m^{-3}$ equating to 123 plastic particles in the tow (Fig. 2) and the lowest concentration of plastic was found was 0.038 particles $m^{-3}$ in 2014 in the West European Basin (46.2919933, -12.78379) (2 days sail from Falmouth, Fig. 2) and the same concentration was found in 2015 in the waters around Lanzarote in the Canary Islands (29.21224, -13.5303783) (Fig. 3).

The plastic concentrations were on average 1.5 times greater across all sites in 2014 compared to 2015 although a greater distance was covered in 2014 and no replication was undertaken in the 2014 data (average particles $m^{-3}$ in 2014: 0.141 ±0.035, 2015: 0.092 ± 0.011). However, the plankton concentrations were 4.4 times greater in 2015 than in 2014.

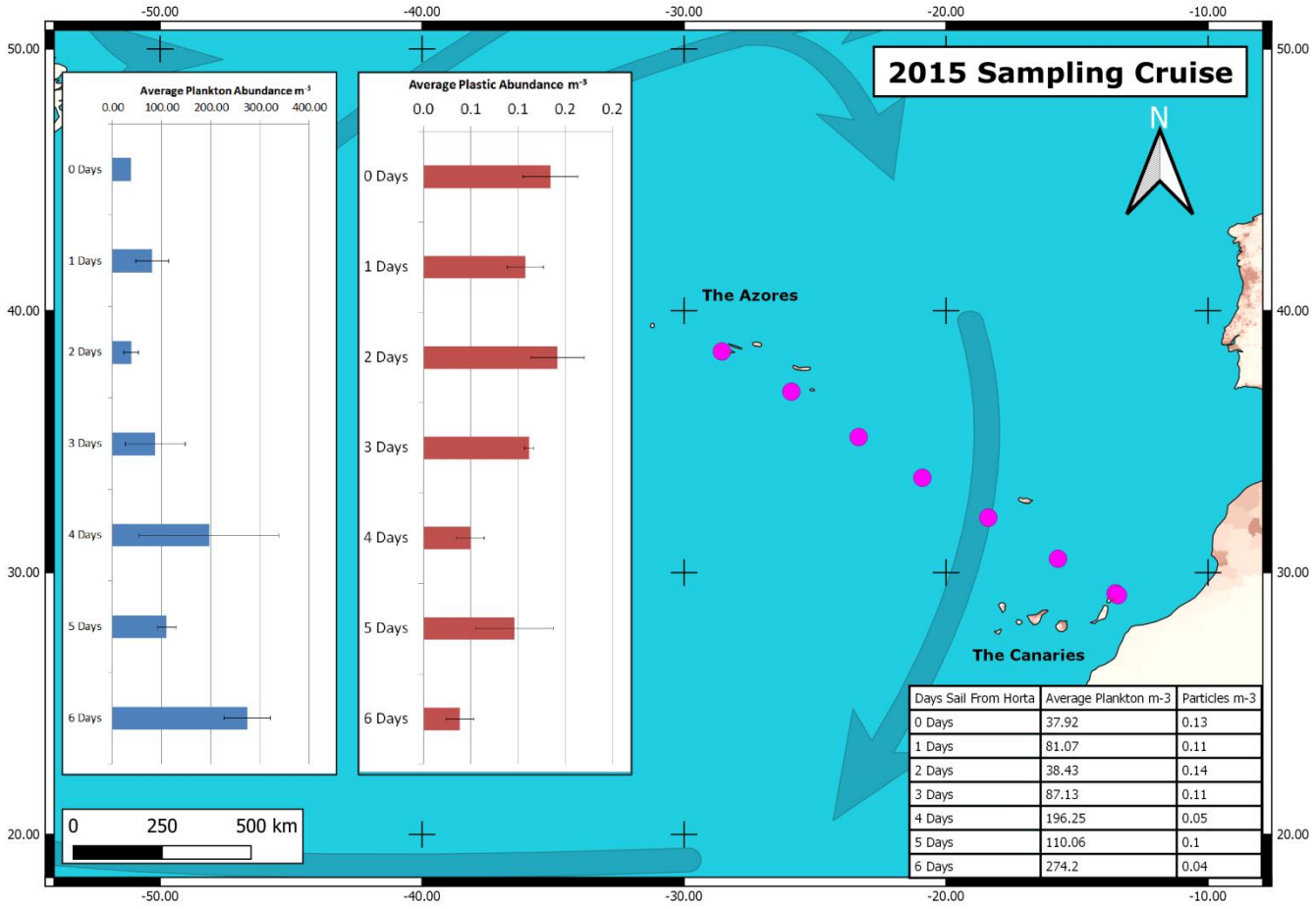



| Date | Plankton m-3 | Particles m-3 |
|---|---|---|
| 0 Days | 25 | 0.061 |
| 1 Days | 22 | 0.152 |
| 2 Days | 18 | 0.038 |
| 3 Days | 16 | 0.099 |
| 4 Days | 15 | 0.086 |
| 5 Days | 10 | 0.063 |
| 6 Days | 23 | 0.132 |
| 7 Days | 59 | 0.288 |
| 8 Days | 71 | 0.051 |
| 9 Days | 28 | 0.192 |
| 10 Days | 26 | 0.083 |
| 11 Days | 62 | 0.448 |

**Figure 2**: Abundance of plastic particles and plankton across the 2014 cruise track. Pink dots indicate sample locations and the cruise track ran from Falmouth in the UK, to the Azores and on to the Canaries. Arrows indicate major ocean currents and the colour grading of the land mass indicates relative population density.

During the 2014 sampling cruise the abundance of plastic was highest in the Canary Islands 200 nautical miles WNW of Arrecife, Lanzarote and 45 nautical miles from the Island of Palma in the Canaries and this sample contained 123 plastic particles that equated to ≈0.45 particles $m^{-3}$ (11 days sail from Falmouth, Fig. 2). The second greatest plastic abundance across the 2014 cruise track was on the first day sailing out of the Azores with 58 particles being collected in one 20 minute tow equating to ≈0.29 particles $m^{-3}$ (7 days sail from Falmouth, Fig. 2). This data whilst not having replication is in agreement with our hypothesis that plastics will be moderately high in the Canaries and UK shelf seas given their proximity to land masses. The Azorean

archipelago is not the most polluted location in this data set but is still considerably high compared to most of the track; likely due to the influence of the NASG (Fig.2).




| Days Sail From Horta | Average Plankton m-3 | Particles m-3 |
|---|---|---|
| 0 Days | 37.92 | 0.13 |
| 1 Days | 81.07 | 0.11 |
| 2 Days | 38.43 | 0.14 |
| 3 Days | 87.13 | 0.11 |
| 4 Days | 196.25 | 0.05 |
| 5 Days | 110.06 | 0.1 |
| 6 Days | 274.2 | 0.04 |

**Figure 3:** Abundance of plastic particles and plankton across the 2015 cruise track. Pink dots indicate sample locations and the cruise track ran the Azores to the Canaries. Arrows indicate major ocean currents and the colour grading of the land mass indicates relative population density.

The abundance of plankton broadly mirror that of the plastic abundance and are what might be expected with the productive areas of the Azores and upwelling around the Canaries having the most plankton followed by the shelf seas with the lowest abundances being in the open Atlantic Ocean (Fig. 2). The average plastic abundance across this track was 0.14 particles $m^{-3}$ (± 0.035 particles $m^{-3}$) or 0.00014 particles $L^{-1}$ (± 0.0000346 particles $L^{-1}$) with a total of 386 plastic particles collected over the entire

cruise. The average plankton abundance was 31.31 plankton $m^{-3}$ (± 0.0059 plankton $m^{-3}$).

The 2015 cruise only covered the area between the Azores and Canaries and saw the reverse trend of decreasing plastic abundance towards the Canaries compared to 2014. There was a significant difference in plastic abundance across the cruise track (One-way ANOVA $F_{(6,17)} = 2.84$, $p = 0.042$). However the $R^2$ (adjusted) of the ANOVA was high at 32.45% and a Tukey's Post-Hoc test could not identify a sample or samples driving the significant variance to a 95% confidence level (at 90% the first and last samples; 0 Days and 6 Days drove the variation (Fig. 3)). The average plastic abundance was lower than in 2014 with an average number of particles of 0.092 $m^{-3}$ (± 0.011 particles $m^{-3}$) and a total of 585 plastic particles were collected. The plankton abundances were higher than in 2014 however with an average of 135.71 plankton $m^{-3}$ (± 29.39 plankton $m^{-3}$). The plankton abundances were lower in the Azores in 2014 than in 2015 however, in 2015 the plankton exhibited no significant trend across the cruise track (One-way ANOVA $F_{(6,15)} = 1.79$, $p = 0.170$) likely due to the large variation in the data.

**Encounter Rates of Plastic by Plankton**

Encounter rates were calculated as per Sun et al. (2017) by calculating the number of plastic pieces per zooplankton. For the 2014 cruise the average encounter rate was 0.0048 (± 0.0006) plastic pieces per zooplankton with the greatest encounter rate of 0.0072 plastic pieces per zooplankton and the smallest encounter rate being 0.0007 plastic pieces per zooplankton (Fig. 4).

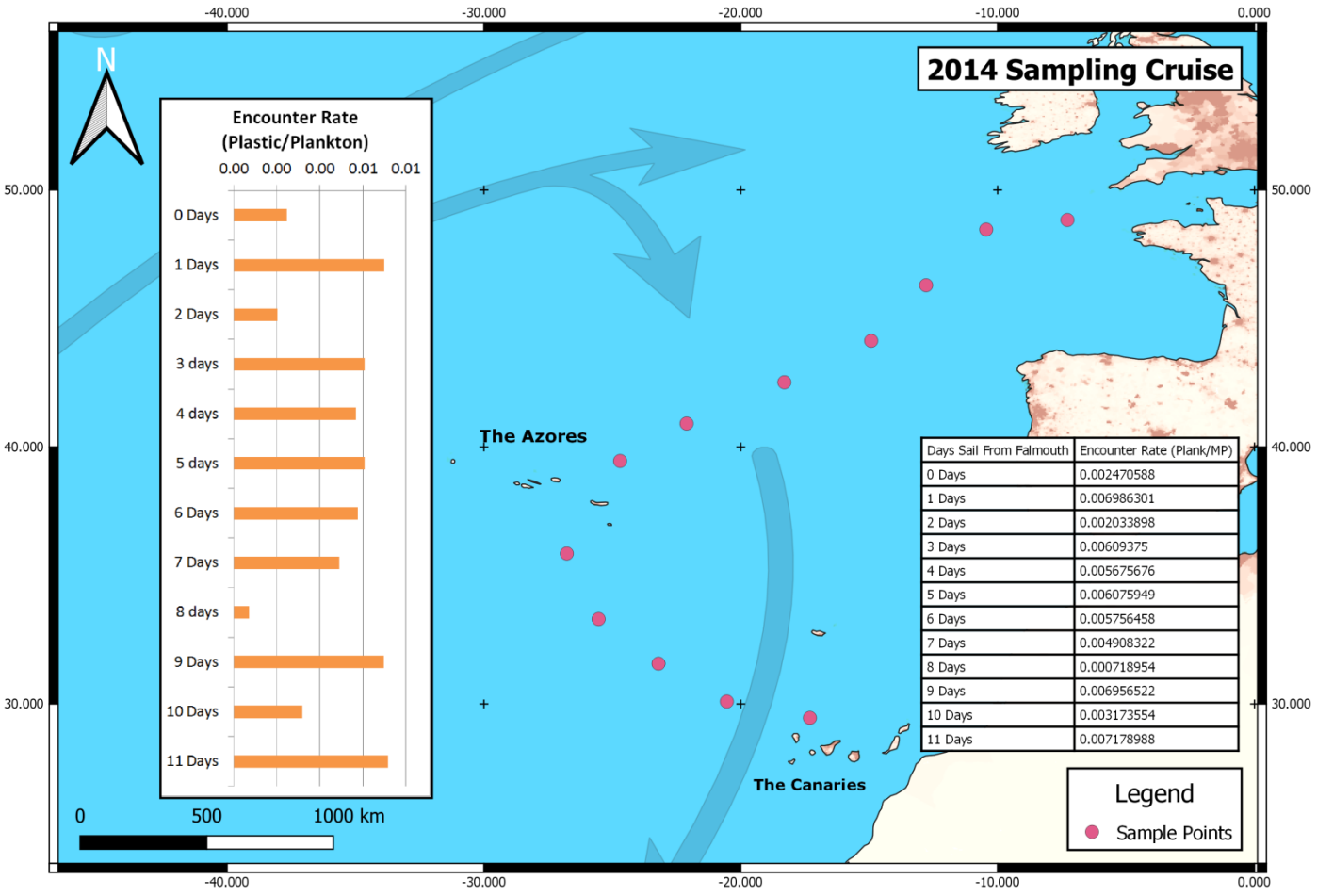
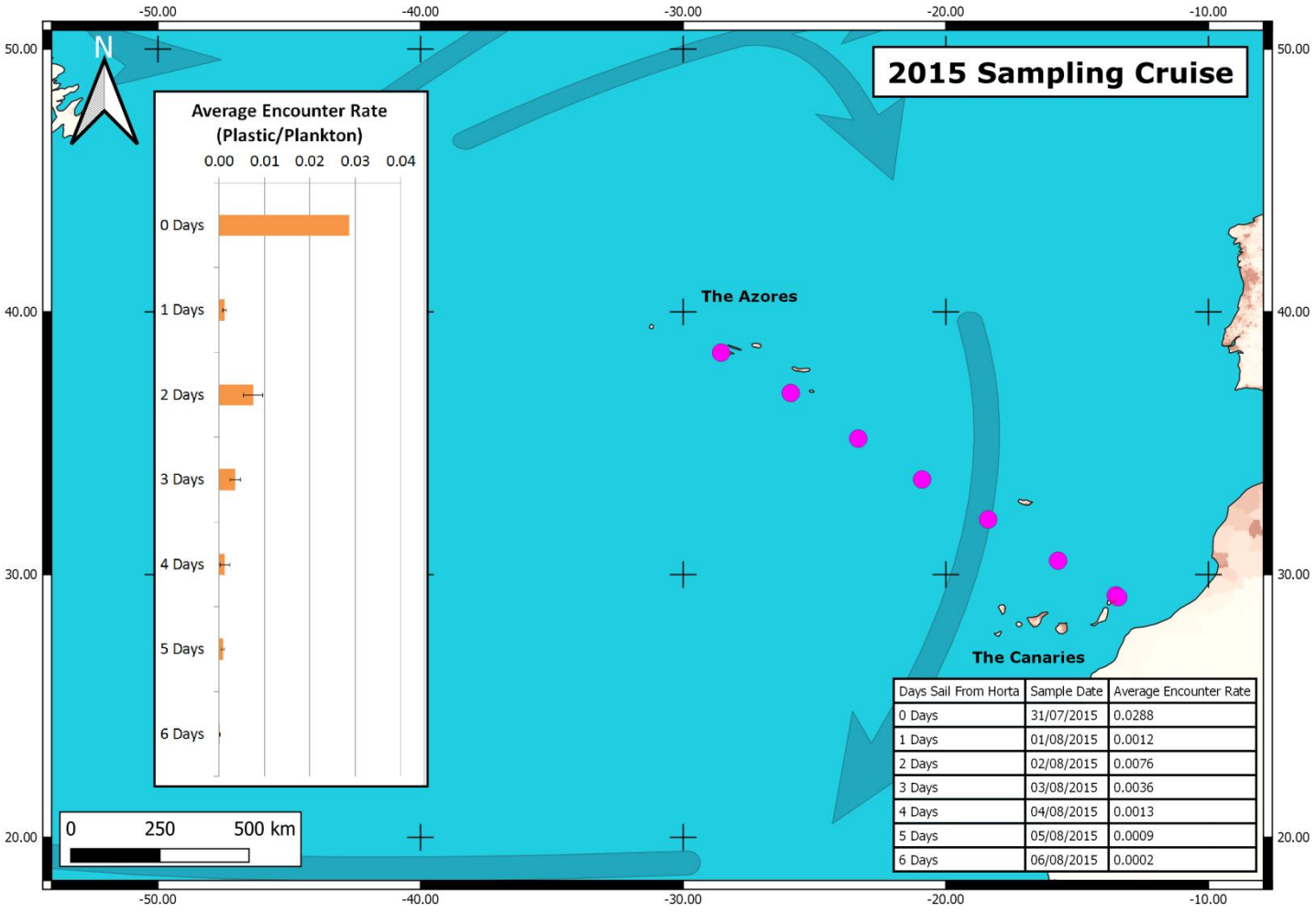



**Figure 4:** Encounter rates (number of plastic particles per zooplankton) across the 2014 cruise track. Arrows indicate major ocean currents and the colour grading of the land mass indicates relative population density.

For the 2015 cruise the average encounter rate was smaller, with an average of 0.0033 plastic pieces per zooplankton (± 0.0014 plastic pieces per zooplankton) however the variation was much greater with a maximum encounter rate of 0.029 pieces of plastic per zooplankton and the smallest encounter rate being 0.0001 plastic pieces per zooplankton (Fig. 5).




| Days Sail From Horta | Sample Date | Average Encounter Rate |
|---|---|---|
| 0 Days | 31/07/2015 | 0.0288 |
| 1 Days | 01/08/2015 | 0.0012 |
| 2 Days | 02/08/2015 | 0.0076 |
| 3 Days | 03/08/2015 | 0.0036 |
| 4 Days | 04/08/2015 | 0.0013 |
| 5 Days | 05/08/2015 | 0.0009 |
| 6 Days | 06/08/2015 | 0.0002 |

**Figure 5:** Encounter rates (number of plastic particles per zooplankton) across the 2015 cruise track. Arrows indicate major ocean currents and the colour grading of the land mass indicates relative population density.

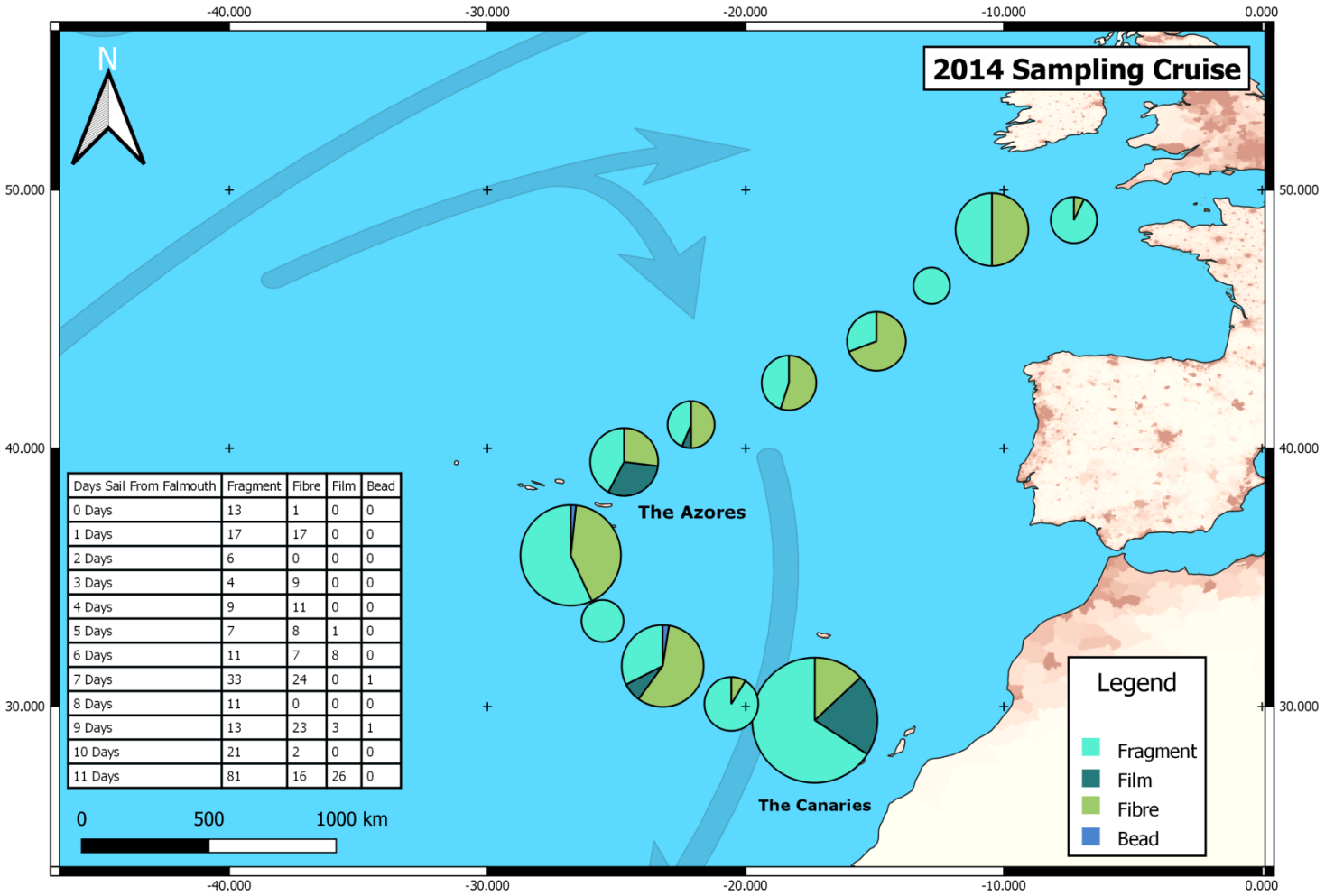
There was significant variation in the encounter rate data from the 2015 cruise (One-way ANOVA, $F_{6,15} = 45.33$, $p = 0.001$) with the sample from the Azores (0 days sail from Horta) (Fig. 6) driving most of the variation with the 2 days sail and 3 days sail making up some of the variation (Tukey's Post-Hoc Test). The 0 Days sample from the 2015 cruise was at least ten fold greater than every other sample from either 2014 or 2015.

**Plastic Particle Characteristics**

The likely microplastic particles collected during both the 2014 and 2015 cruises were inspected for shape, size, colour, and finally analysed for polymer analysis using FT-IR.

***Shape***

The shapes of the confirmed plastic particles were grouped into well-established categories: fibres, fragments, films and beads. The shapes were again plotted spatially to look at trends across ocean scales. In 2014 fragments dominated and only increased towards the Canaries (Fig. 2) making up 58.8% of the shapes identified (Fig. 6). Fibres were the second most dominant making up 30.7%, films 9.9%, and beads 0.5% of all the plastics collected. Fibres were also found throughout the cruise track but were most prevalent at sites in between the Azores and Canaries as well as sites in between the UK and Azores but were relatively low near to land masses.

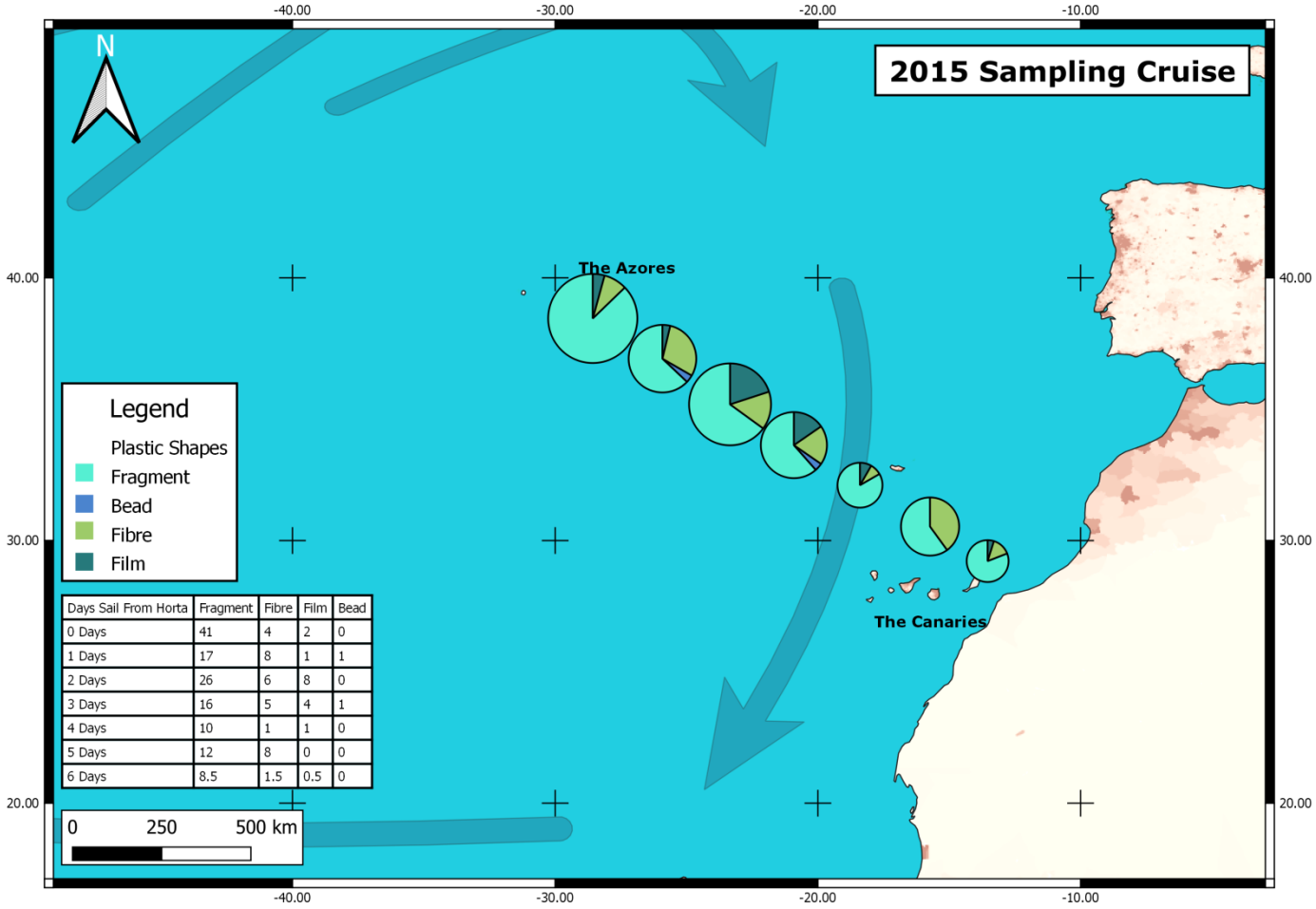



| Days Sail From Falmouth | Fragment | Fibre | Film | Bead |
|---|---|---|---|---|
| 0 Days | 13 | 1 | 0 | 0 |
| 1 Days | 17 | 17 | 0 | 0 |
| 2 Days | 6 | 0 | 0 | 0 |
| 3 Days | 4 | 9 | 0 | 0 |
| 4 Days | 9 | 11 | 0 | 0 |
| 5 Days | 7 | 8 | 1 | 0 |
| 6 Days | 11 | 7 | 8 | 0 |
| 7 Days | 33 | 24 | 0 | 1 |
| 8 Days | 11 | 0 | 0 | 0 |
| 9 Days | 13 | 23 | 3 | 1 |
| 10 Days | 21 | 2 | 0 | 0 |
| 11 Days | 81 | 16 | 26 | 0 |

**Figure 6**: Particle shapes across the 2014 cruise track showing the change in relative proportions as well as overall numbers (showed by the size of the pie charts). Arrows indicate major ocean currents and the colour grading of the land mass indicates relative population density.

In 2015, fragments made up 67% of all particles found with fibres comprising 20%, Films 12%, and beads 1% (Fig. 7). Fragments increased in number moving away from the coast (One-way ANOVA $F_{6,17}$=3.10, p = 0.01); the opposite of what was found in 2014 and fibres were most abundant close to the Azores and Canaries, again opposing that found in 2014. There were no significant differences for fibres between samples across the cruise track (One-way ANOVA $F_{6,17}$=1.81, p = 0.156)

Beads were found very infrequently in the samples showing perhaps that efforts to stop them entering the marine environment are working.




| Days Sail From Horta | Fragment | Fibre | Film | Bead |
|---|---|---|---|---|
| 0 Days | 41 | 4 | 2 | 0 |
| 1 Days | 17 | 8 | 1 | 1 |
| 2 Days | 26 | 6 | 8 | 0 |
| 3 Days | 16 | 5 | 4 | 1 |
| 4 Days | 10 | 1 | 1 | 0 |
| 5 Days | 12 | 8 | 0 | 0 |
| 6 Days | 8.5 | 1.5 | 0.5 | 0 |

**Figure 7:** Particle shapes across the 2015 cruise track showing the change in relative proportions as well as overall numbers (showed by the size of the pie charts). Arrows indicate major ocean currents and the colour grading of the land mass indicates relative population density.

***Size***

The size of particles is of interest as this heavily influences the bioavailability of plastic particles as it dictates whether an organism can physically ingest a particle but also the ease of egestion. There were no significant differences between sample sites in the sizes of fibres (One-way ANOVA $F_{(6,14)}$ = 0.37, p = 0.889) or fragments (One-way ANOVA $F_{(11,66)}$ = 0.77, p = 0.667) in the 2014 cruise track. Fibres averaged 8953 µm ± 2008 µm (Fig. 8) with the smallest fibre measured at 1057 µm and the largest fibre 60 mm. Fragments were much smaller averaging 2126.5 µm ± 271.9 µm with the smallest fragment measuring 135 µm and the largest 21.88 mm.

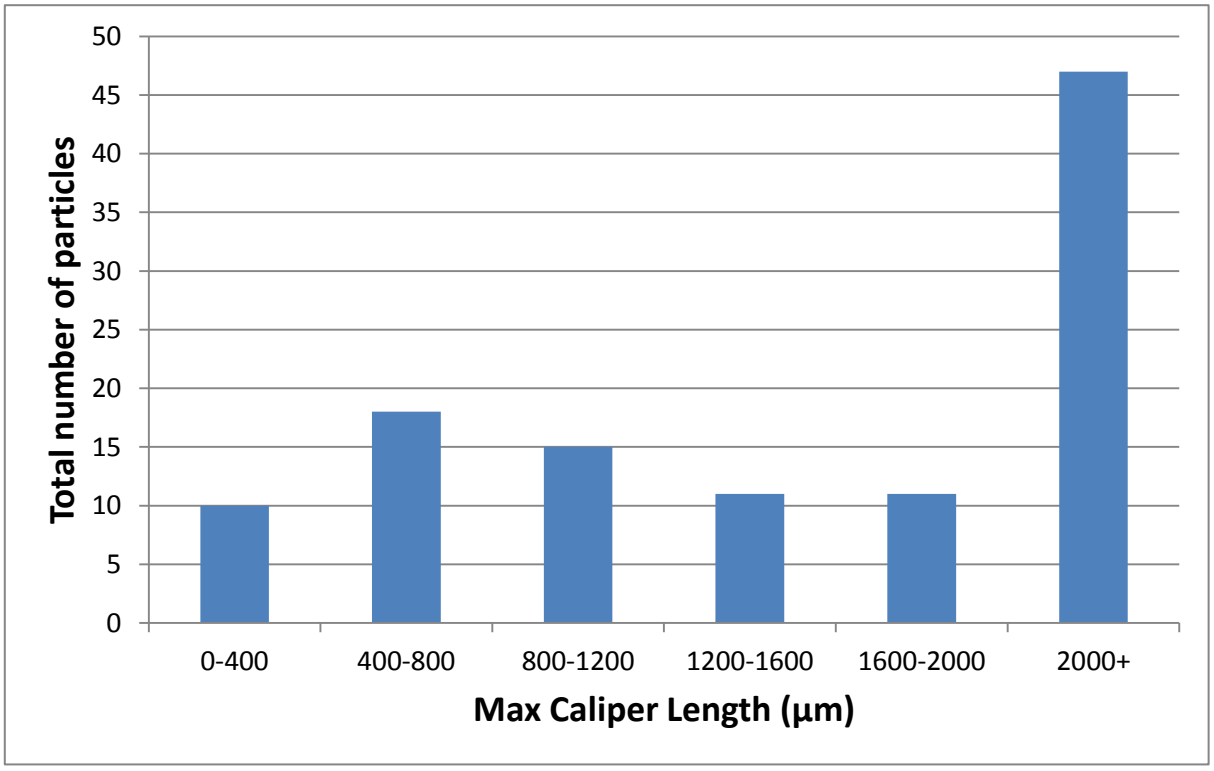



**Figure 8:** The size distribution of all plastic particles collected during the 2014 sampling cruise. The majority of plastic particles were >2000 µm (n=1).

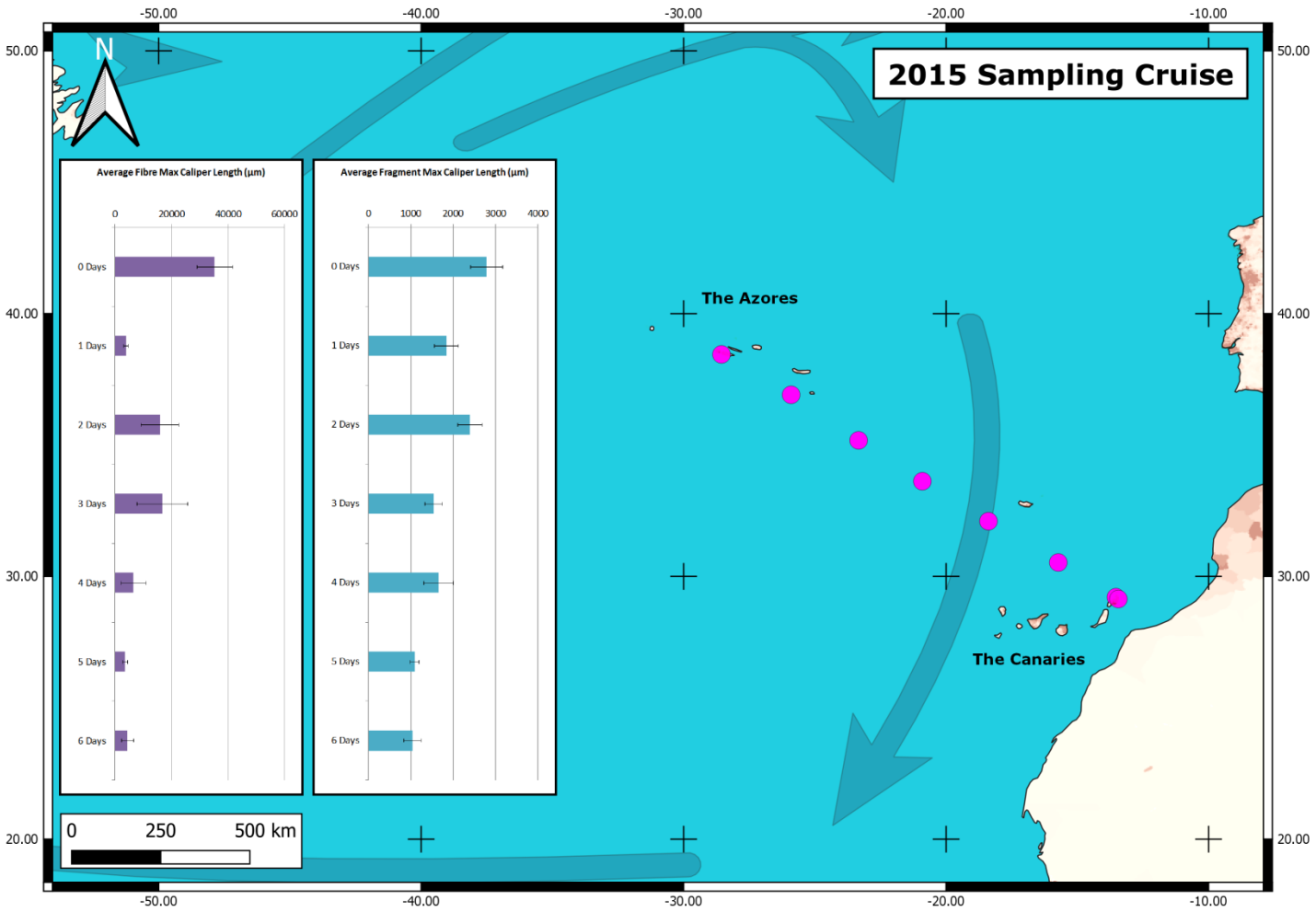


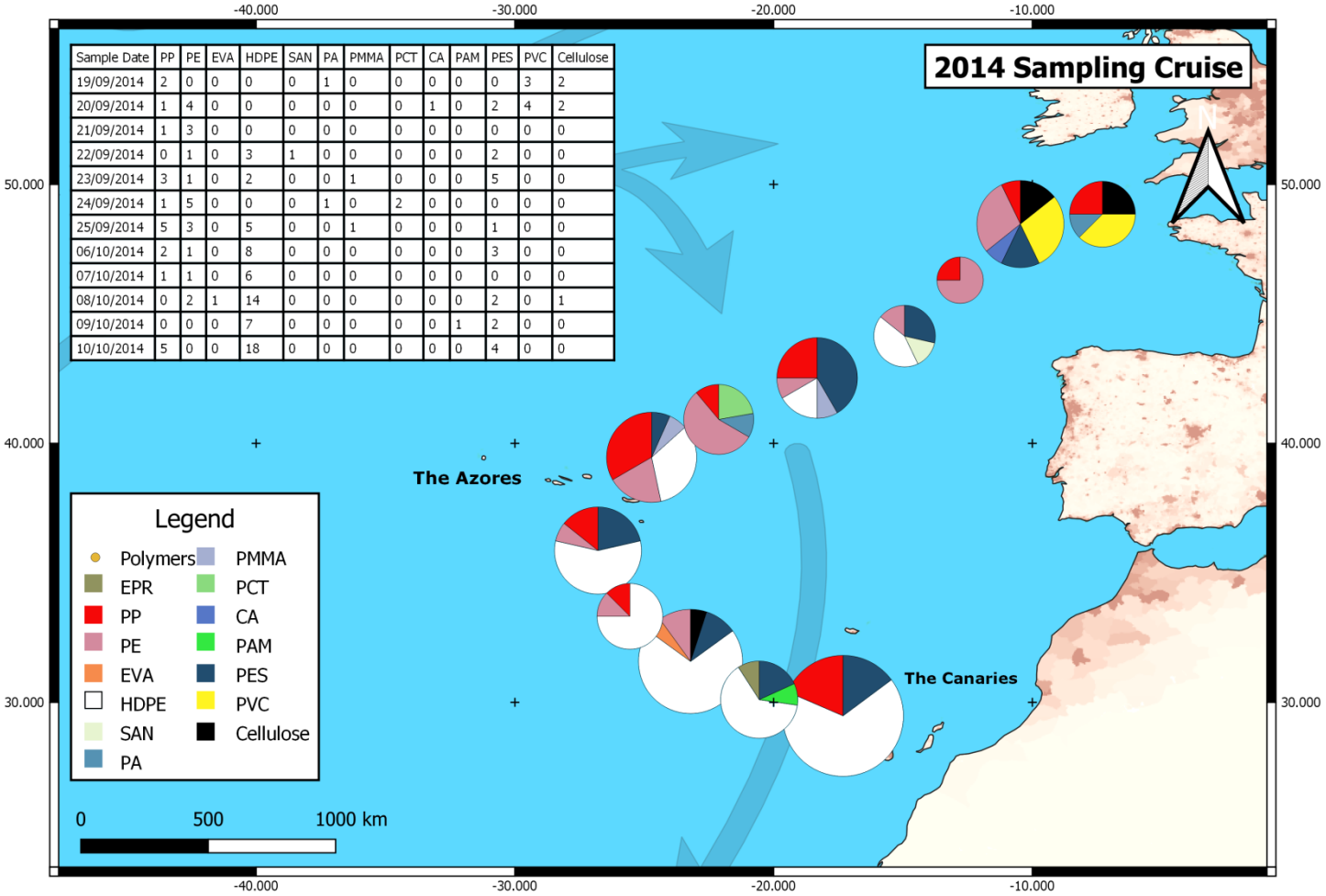

**Figure 9:** Average maximum caliper sizes of fragments and fibres collected during the 2015 sampling cruise. Arrows indicate major ocean currents and the colour grading of the land mass indicates relative population density.

In 2015 however, significant decreases in average maximum caliper size of both fragments and fibres were seen getting closer to the African continent. Fragments averaged 1763 µm ± 244 µm throughout the 2015 data set. The decrease is shown in the reduction of average fragment maximum caliper size from 2790 µm ± 380 µm in the sample 0 days from Horta to over half that in the Canaries with an average max caliper length of 1040 µm ± 207 µm. This was a significant decrease (One-way ANOVA $F_{(6,75)} = 3.99$, $p = 0.01$) with the difference being driven by large fragments found in the Azores and smaller fragments in samples 5 and 6 days away from the Azores (Tukey's Post-Hoc Test) (Fig. 9). The smallest fragment found was 84 µm and the largest fragment

10721 µm. Fibres were on average 12.33 mm ± 4.38 mm; much larger than the fragments 1982 ± 21 µm. Again there was a significant decrease in fibre size throughout the data set, with fibre sizes generally decreasing towards the African coast (One-way ANOVA $F_{(6,75)} = 3.70$, $p < 0.05$). Fibres were largest in the sample 0 days from Horta averaging 35340 µm ± 6258 µm decreasing to the smallest fibres 5 days from Horta; averaging 3573 µm ± 827 µm. The smallest fibre found was 298 µm and the largest 128.5 mm (although this was a braided length of string).

***Polymers***

During the 2014 cruise, 575 particles were collected and 51% of those particles were analysed for polymer type. After rejecting 189 particles (32%) because they had a <70% match, and two because they had no match, 386 plastic particles were identified. The most abundant polymer was High Density Polyethylene (HDPE) comprising 42% of all particles identified. Polypropylene (PP), Polyethylene (PE) and Polyester (PES) were second most abundant polymers (14% respectively) with Polyvinylchloride (PVC) making up 5% of the total. Ethylene Propylene Rubber (EPR), Ethylene vinyl Acetate (EVA), Styrene Acrylonitrile (SAN), Polyamide (Nylon 6) (PA), Polymethyl Methacrylate (PMMA), Polycyclohexanedimethylene terephthalate (PCT), Cellulose Acetate (CA), Polyacrylamide (PAM), and Cellulose made up the final 11% of particles (Fig. 10). The obvious trends show the increase in HDPE (white) moving southwards along the cruise track and the high levels of PVC (yellow) nearest to the European land mass and disappearing after the second sample. PE (pink), PP (red), and PES (dark blue) persist throughout the whole cruise track and cellulose (black) is mostly found close to the continental land mass.




| Sample Date | PP | PE | EVA | HDPE | SAN | PA | PMMA | PCT | CA | PAM | PES | PVC | Cellulose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 19/09/2014 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 3 | 2 |
| 20/09/2014 | 1 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 4 | 2 |
| 21/09/2014 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 22/09/2014 | 0 | 1 | 0 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| 23/09/2014 | 3 | 1 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 5 | 0 | 0 |
| 24/09/2014 | 1 | 5 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| 25/09/2014 | 5 | 3 | 0 | 5 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| 06/10/2014 | 2 | 1 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |
| 07/10/2014 | 1 | 1 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 08/10/2014 | 0 | 2 | 1 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 |
| 09/10/2014 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 0 |
| 10/10/2014 | 5 | 0 | 0 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 |

**Figure 10:** Proportions of the particle polymers found across the 2014 cruise track. Pie charts are scaled to the total number of polymers found to give a sense of relative abundance. Abbreviations are as follows: Ethylene Propylene Rubber (EPR), Polypropylene (PP), Polyethylene (PE), Ethylene Vinyl Acetate (EVA), High Density Polyethylene (HDPE), Styrene Acrylonitrile (SAN), Polyamide (PA), Polymethyl Methacrylate (PMMA), Polycyclohexanedimethylene terephthalate (PCT), Cellulose Acetate (CA), Polyacrylamide (PAM), Polyester (PES), Polyvinylchloride (PVC), and Cellulose. Arrows indicate major ocean currents and the colour grading of the land mass indicates relative population density.

During the 2015 cruise 616 particles were collected and 39% of particles in the samples that underwent FT-IR analysis were scanned (13% of all particles were scanned). After rejecting 22 particles because they had a <70% match and 9 with no match, 585 particles were identified as plastic. The most abundant polymer was High Density Polyethylene (HDPE, white, Fig. 11) again, comprising 48% of the polymers identified. The second most abundant polymer was Polypropylene (PP, red, 20%), followed by Polyamide (PA, light blue, 15%), Polyester (PES, dark blue, 5%), and Cellulose (black, 3%). The remaining 9% comprised Polyethylene (PE), Ethylene Vinyl Acetate (EVA), Polybutylene (PB), Polyvinyl formal, and generic adhesive. The overall trends were increasing amounts of HDPE towards the Azores, along with PP (Fig. 11). In 2015 only 10 polymers were identified compared to the 14 in 2014 (Fig. 10).

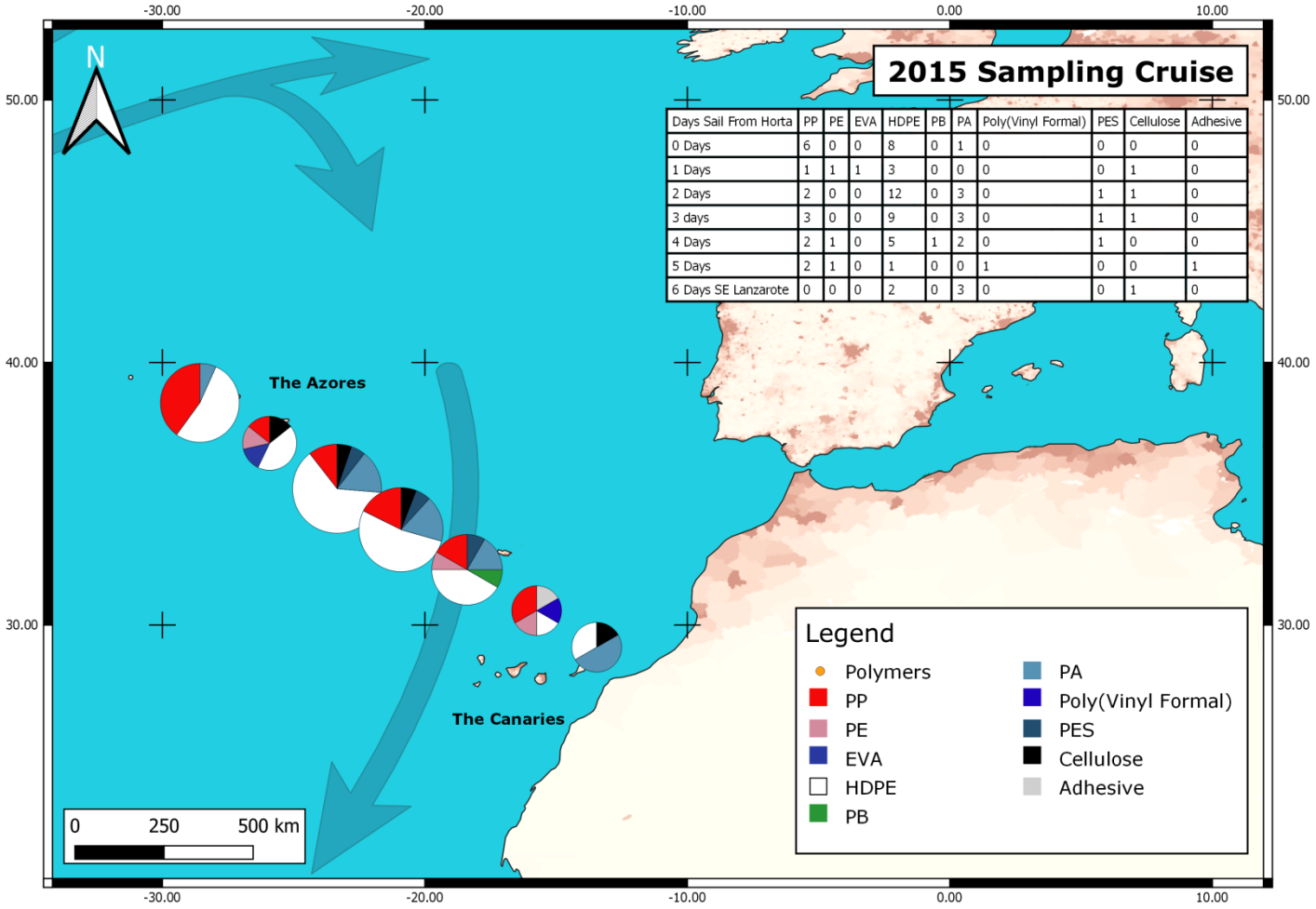



**Figure 11**: Proportions of the particle polymers found across the 2015 cruise track. Pie charts are scaled to the total number of particles found to give a sense of relative abundance. Abbreviations are as follows: Polypropylene (PP), Polyethylene (PE), Ethylene Vinyl Acetate (EVA), High Density Polyethylene (HDPE), Polybutylene (PB), Polyamide (PA), Polyvinyl Formal, Polyester (PES), Cellulose, and an unknown Adhesive. Arrows indicate major ocean currents and the colour grading of the land mass indicates relative population density.

In 2014 the number of polymers denser than seawater decreased with distance from the European continental land mass and the polymers lighter than seawater increased both in terms of number but also proportionally, indicating that dense polymers are

potentially lost as they are taken to sea from terrestrial inputs and the buoyant particles begin to dominate in the open ocean driven by surface currents (Fig. 12).

In 2015 lighter polymers dominated the cruise track as was seen in 2014. An increase in denser polymers was seen between the two archipelagos and lighter polymers increase numerically and proportionally moving away from the African continent (Fig. 13).

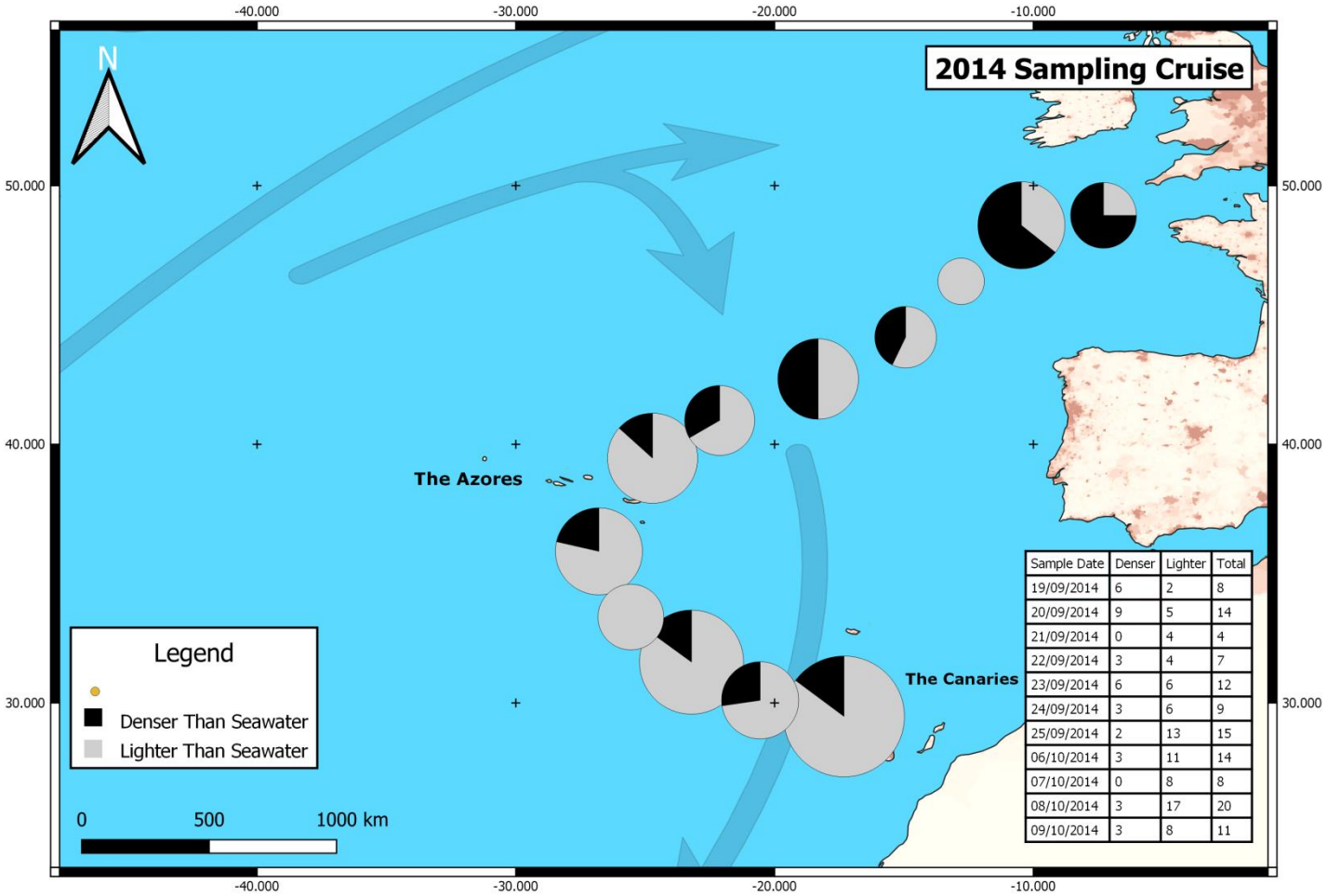
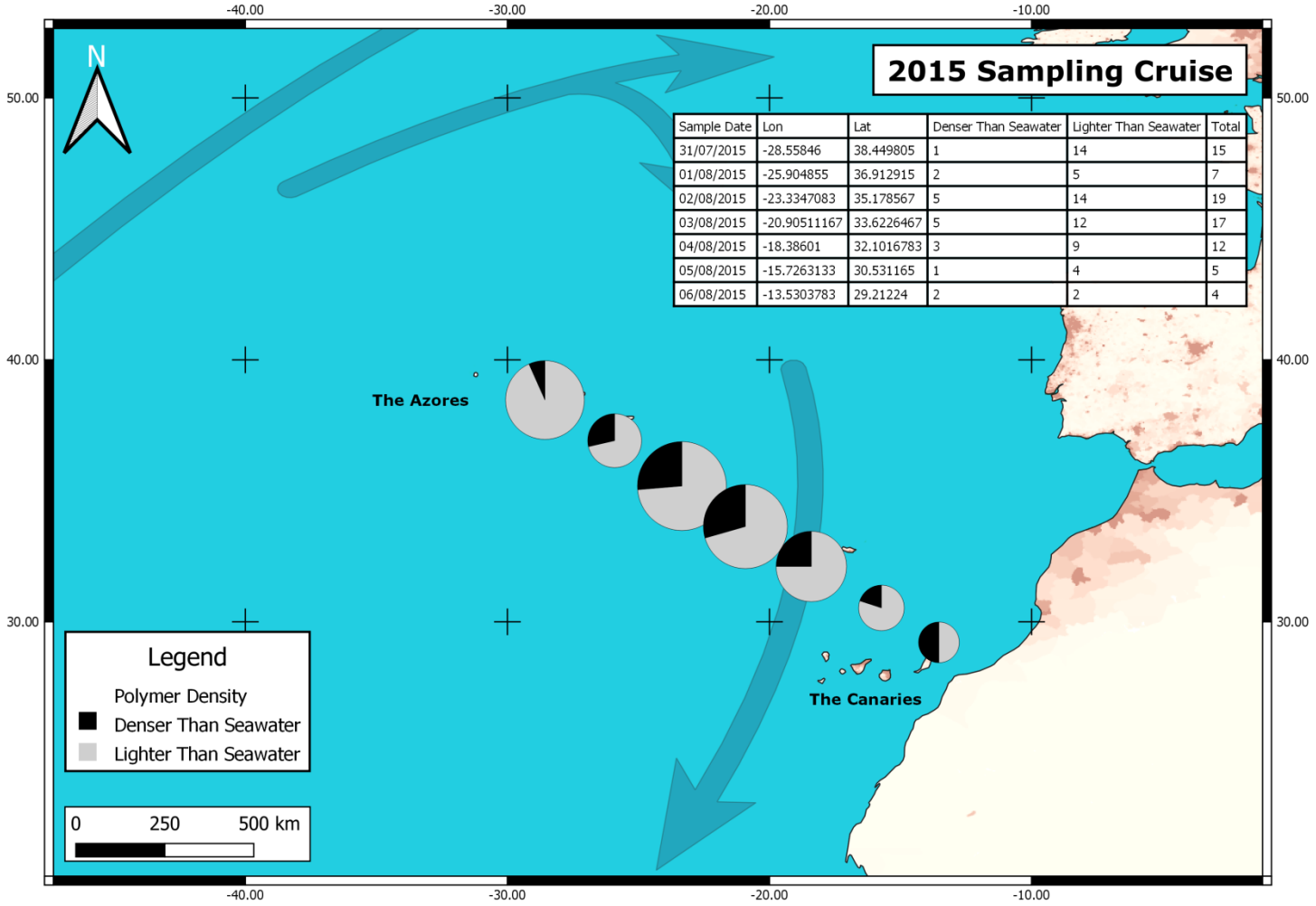



**Figure 12:** The relative densities of the polymers identified and abundances across the 2014 cruise track. Black indicates polymers denser than seawater (>1.03 g $cm^{-3}$) and grey indicates buoyant polymers (<1.03 g $cm^{-3}$). Arrows indicate major ocean currents and the colour grading of the land mass indicates relative population density.

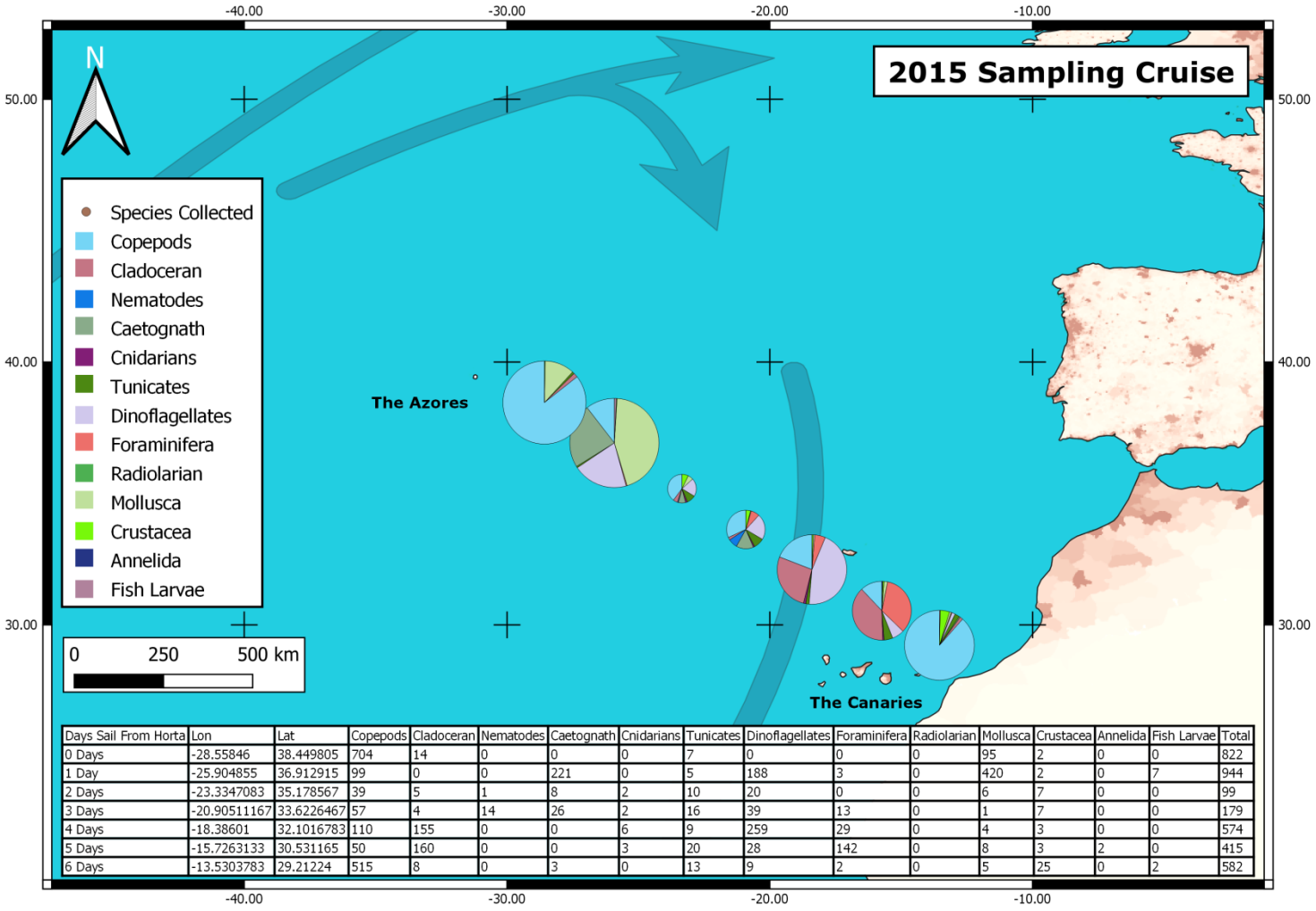



| Sample Date | Lon | Lat | Denser Than Seawater | Lighter Than Seawater | Total |
|---|---|---|---|---|---|
| 31/07/2015 | -28.55846 | 38.449805 | 1 | 14 | 15 |
| 01/08/2015 | -25.904855 | 36.912915 | 2 | 5 | 7 |
| 02/08/2015 | -23.3347083 | 35.178567 | 5 | 14 | 19 |
| 03/08/2015 | -20.90511167 | 33.6226467 | 5 | 12 | 17 |
| 04/08/2015 | -18.38601 | 32.1016783 | 3 | 9 | 12 |
| 05/08/2015 | -15.7263133 | 30.531165 | 1 | 4 | 5 |
| 06/08/2015 | -13.5303783 | 29.21224 | 2 | 2 | 4 |

**Figure 13:** The relative densities of the polymers identified and abundances across the 2015 cruise track. Black indicates polymers denser than seawater (>1.03 g $cm^{-3}$) and grey indicates buoyant polymers (<1.03 g $cm^{-3}$). Arrows indicate major ocean currents and the colour grading of the land mass indicates relative population density.

### *Plankton*

The range of species identified across the cruise track were plotted to look for trends (Fig. 14). Copepods were most abundant near the Azores and Canaries perhaps indicating nutrient richness in these waters.




| Days Sail From Horta | Lon | Lat | Copepods | Cladoceran | Nematodes | Caetognath | Cnidarians | Tunicates | Dinoflagellates | Foraminifera | Radiolarian | Mollusca | Crustacea | Annelida | Fish Larvae | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 Days | -28.55846 | 38.449805 | 704 | 14 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 95 | 2 | 0 | 0 | 822 |
| 1 Day | -25.904855 | 36.912915 | 99 | 0 | 0 | 221 | 0 | 5 | 188 | 3 | 0 | 420 | 2 | 0 | 7 | 944 |
| 2 Days | -23.3347083 | 35.178567 | 39 | 5 | 1 | 8 | 2 | 10 | 20 | 0 | 0 | 6 | 7 | 0 | 0 | 99 |
| 3 Days | -20.90511167 | 33.6226467 | 57 | 4 | 14 | 26 | 2 | 16 | 39 | 13 | 0 | 1 | 7 | 0 | 0 | 179 |
| 4 Days | -18.38601 | 32.1016783 | 110 | 155 | 0 | 0 | 6 | 9 | 259 | 29 | 0 | 4 | 3 | 0 | 0 | 574 |
| 5 Days | -15.7263133 | 30.531165 | 50 | 160 | 0 | 0 | 3 | 20 | 28 | 142 | 0 | 8 | 3 | 2 | 0 | 415 |
| 6 Days | -13.5303783 | 29.21224 | 515 | 8 | 0 | 3 | 0 | 13 | 9 | 2 | 0 | 5 | 25 | 0 | 2 | 582 |

**Figure 14:** The species found during the 2015 sampling cruise. The pies are calibrated to the total plankton abundance and thus size equates to total plankton. The most conspicuous groups are the Copepods and Mollusca at both the Azores and Canaries driving most of the abundance. Arrows indicate major ocean currents and the colour grading of the land mass indicates relative population density.

## Discussion

This data adds to the rapidly growing body of evidence that microplastic pollution of seawater is observable in the majority of sea surface tows, with microplastics found in every water sample taken in this two year study. Our plastic abundances of 0.14 particles $m^{-3}$ (±0.035) in 2014 and 0.092 particles $m^{-3}$ (± 0.01) are, however, relatively low compared to the large numbers that are often cited in the global literature. Kang et al. (2015) reported abundances of 0.64–860 particles $m^{-3}$ after the rainy season round the SE coast of Korea using a 333 µm plankton net. Norén (2007) reported of 72 – 141 particles $m^{-3}$ using a 450 µm net and abundances of 167 – 24000 particles $m^{-3}$ using a 80 µm net in Swedish coastal waters. Moore et al. (2002) reported plastic particle abundances of 7.25 particles $m^{-3}$ in Southern California using a 333 µm mesh all vastly outstripping the abundances seen in this study.

These relatively low abundances are in no way exceptional, however, as Ivar Do Sul et al. (2014) reported abundances of 0.015-0.04 particles $m^{-3}$ in the Western tropical Atlantic Ocean using a 300 µm net, Ivar Do Sul et al. (2013) found abundances of 0.01 particles $m^{-3}$ around the Saint Peter and Saint Paul Archipelago in the Equatorial Atlantic Ocean. Lusher et al. (2015b) reported values of 0.34 particles $m^{-3}$ off Svalbard in Norway using a 333 µm net and Zhao et al. (2014) reported abundances of 0.167 particles $m^{-3}$ in the East China Sea. Our work then sits in good company at the lower end of what is found in the literature but is by no means unusual. These locations reported to have lower microplastic abundances lower than found in our present study were all relatively remote, and perhaps the remote nature of our cruise track precluded low plastic numbers; especially as plastics are well known to be highly spatially and temporally variable. The Azores are 1360 km west of Portugal, 1507 km northwest of

Morocco and 1925 km from Newfoundland in Canada making them highly separated from the terrestrial inputs known to drive the plastic pollution in the world's oceans.

The abundance of microplastics recorded in each sample differed according to location along our sampling transect. The data from the 2014 cruise is in agreement with the expected spatial distribution of microplastics with peaks in microplastic abundance in the shelf seas, around the Azorean Archipelago and in the Canaries compared to the more open sections of ocean. However the Canaries are the most polluted with 123 plastic items found (11 days sail from Falmouth, Fig. 2) equating to 0.45 particles $m^{-3}$. The Azores are the second most polluted area with 58 particles collected, equating to 0.29 particles $m^{-3}$. These two samples make up 46% of the particles found during the whole cruise and represent the most polluted places. During the 2015 cruise the samples taken closer to the Azores are more polluted with plastic pieces than those taken around the Canaries perhaps supporting the 2014 dataset towards broadly agreeing with our hypothesis that the Azores will be the most polluted area due to their position on the edge of the NASG.

This study set out to look at the potential encounters of plastic by zooplankton across seascapes and hence assessing plankton data is essential in helping us understand the potential risk plastic poses to marine life, and in this case the zooplankton. A number of studies (e.g. Lusher et al. (2014), Law et al. (2010), Reisser et al. (2013) and Desforges et al. (2014) to name a few) miss this important data and indeed discard it despite having sampled using methods that would allow the quantification of plankton alongside the plastic. In our study the numbers of plankton were counted (Figs. 2 and 3) and over the 2014 cruise the plankton abundances broadly followed those of the plastic highlighting relatively abundant plankton stocks in the Azores and Canaries (7, 8 and 11 days sail from Falmouth, Fig 2.) with some productivity in the European shelf

seas and a reduction in plankton stock in open ocean environments. The average abundance of 31.31 plankton $m^{-3}$ (± 0.006 plankton $m^{-3}$) was much lower than that of the 2015 cruise with an average abundance of 135.71 (±29.39 plankton $m^{-3}$). However in the 2015 data set plankton is increasing in abundance towards the Canaries and the African continent. Certainly the shelf seas were not the most productive region sampled in this data set (only so much can be asserted from single point sampling in the 2014 dataset) and in fact it was around the Azores and the canaries that the most productivity was found. In total numbers The Canaries in 2015 were the most productive region and this data is of interest when considering the Oceanographic setting of the Azores and Canaries.

The relatively high plankton abundances around The Canaries are likely due to upwelling and although this cannot be proven as water samples were not collected for this purpose, available literature can be leant on to support this hypothesis. Coastal upwelling in the Atlantic Ocean occurs primarily at the Canary and Benguela Upwelling Ecosystems and our Canary samples fall within the region of upwelling between 26–35°N (Kanhai et al., 2017). This would explain the increase in plankton towards the Canaries in both 2014 and 2015. The Azores are well known feeding grounds for migratory baleen whales (Visser et al., 2011) and there are >460 seamounts in the region (Morato et al., 2008) which are known to be productive seascape features (Clark et al., 2012) and therefore despite the relative low abundances, The Azores are highly productive; especially during spring bloom periods (Visser et al., 2011). Upwelling has also been suggested to alter plastic abundances as deep microplastic poor water may dilute the surface water (Desforges et al., 2014, De Lucia et al., 2014) although Kanhai et al. (2017) did not find a statistical difference between microplastic abundances in upwelling and non-upwelling areas. The “low” amount of plankton in 2014 in the Azores does however need to be seen in the context of numbers and not just comparatively. In 2014 the first sample out from Horta returned 23 plankton $m^{-3}$ but in 2015 the first

sample out of Horta, which might seem “low” in Figure 3. was in fact 37.92 plankton $m^{-3}$, roughly 1.6x greater. The 2015 samples experienced much great levels of plankton than the 2014 samples and this helps to disentangle the encounter rate data in Figs. 4 and 5.

If ingestion of microplastics in the water column is to occur, co-occurrence is not enough; the shapes, sizes and polymers will dictate whether they are bioavailable for ingestion. A recent review of environmental microplastics abundance data from sampling campaigns by Burns and Boxall (2018) found fibres to be the most frequently observed microplastic shape in the environment (45 – 52% of particles found) followed by fragments (29 – 33% of particles found). This trend did not appear in our data set however. In all samples from both 2014 and 2015 fragments were the most dominant shape found making up 58.9% and 67.5% of the total plastic particles respectively (Figs. 6 and 7). The relatively small numbers of fibres; making up 30.7% and 20% in 2014 and 2015 respectively might be as a result of the lack of rayon or cellulose fibres in our samples. Only 19 cellulose fibres were found in 2014 and 13 in 2015 which is unusual given the reported prevalence of cellulose (or rayon – essentially regenerated cellulose) (Kanhai et al., 2017). Lusher et al. (2015b), Barrows et al. (2018), Woodall et al. (2014) and many other have reported a presence if not prevalence of cellulosic or ‘semi-synthetic’ fibres in their samples. Airborne contamination is also always a concern for estimates of microfibers. It has been widely proposed that fibres will dominate in coastal waters but fragments will dominate in bodies of water that have been transported long distances from shores, e.g. the gyres. There was some suggestions of this in our data as fragments were significantly more prevalent in the Azores in 2015 than the Canaries (One-way ANOVA $F_{6,17}$=3.10, p = 0.01) however a significant trend for fibres was not seen (One-way ANOVA $F_{6,17}$=1.81, p = 0.156).

Particle size is another key characteristic influencing the bioavailability of plastics to zooplankton and other marine biota. The plastic particles collected during our 2014 and 2015 transects tended to be relatively large; the average size of a plastic particle was 3188 µm (± 586 µm) in 2014 and 4486 µm (± 718 µm) in 2015 and the predominant size class in both surveys was >2000 µm making up for 47% and 48% of all the plastics found in 2014 and 2015 respectively. The smallest particle found was in the 2015 cruise measuring 84 µm. However, samples were collected using a plankton net with a 200 µm mesh size, so everything under 200 µm in our dataset is qualitative and somewhat anecdotal (there were 4 particles <200 µm). In the literature, a few studies to date have identified zooplankton ingesting plastics in the surface waters and they help shed light on the data collected here and the potential for ingestion. Steer et al. (2017) identified fish larvae with 50 – 1100 µm plastic particles (88% fibres) in their digestive system. The average size of microplastics ingested by zooplankton in Sun et al. (2017) was 90 – 200 µm. Sun et al. (2018b) also showed ingestion of fibres ranging from 18 – 3763 µm, fragments ranging from 11 – 1048 µm and beads ranging from 7 – 87 µm. Desforges et al. (2015) also showed ingestion of microplastics in the calanoid copepod *Neocalanus cristatus* and the euphausiid *Euphausia pacifia* and found the average ingested size of microplastic to be 556 ± 149 µm in the calenoid copepod and 816 ± 108 µm in the euphausiid. The literature therefore supports the possibility that a wide range of microplastics are available to marine zooplankton and whilst our average microplastic length is high; it is only just above that which has been ingested by zooplankton in the literature to date. If we set the maximum size ingestible by zooplankton as those found in Sun et al. (2018b) 47% of our fibres and 39 % of our fragments are potentially ingestible by marine zooplankton. Of the particles we sampled, 18 fragments and 47 fibres and 14 films were actually bigger than 5 mm and therefore should technically be classed as macroplastic and this provides useful insight into the potential for microplastics to be ingested and cause harm in our sample sites as the bioavailable fraction is much reduced compared to the total microplastic load.

Polymer type will act to partition plastics in the water column according to density (Porter et al., 2018, Woodall et al., 2014, Cole et al., 2013) and different polymer types may well be sampled at different depths as plastics are distributed vertically according to their inherent densities (Kanhai et al., 2017). Hence we also investigated the polymer types of the plastics sampled across our transect to look for any spatial differences. Biofouling will of course alter the densities of marine debris as the attachment of micro and macro organisms will increase the mass of the particle (Kooi et al., 2017, Gregory, 2009). We hypothesised that our samples would be dominated by buoyant polymer types and that the relative proportion of buoyant particles would increase away from the major land masses of Europe and Africa, resulting in mostly buoyant polymer types in the open ocean and around The Azores. High Density Polyethylene was the dominant polymer type found in our samples and this is in agreement with much of the available literature. Enders et al. (2015) sailed through the Azores and found 48% of their polymers to be PP and PE. Our results showed that 70% of all polymers identified were PP or PE (including HDPE). Negatively buoyant polymers behaved as expected in the 2014 data as the relative abundance and numbers of polymers denser than sweater decreased with distance from the European land mass (Fig.12) which was mostly driven by the removal of PVC from the samples moving away from the UK (Fig. 10, Yellow Pie Slices). The 2015 data also showed a proportional increase in buoyant polymers away from the African continental land mass although the denser particles had their greatest abundance in the waters between the Azores and the Canaries. Our data then broadly supports the hypothesis that buoyant polymers will dominate given our distance from terrestrial inputs even in the Celtic Sea and that dense polymers will reduce in numbers away from terrestrial sources (Figs. 12 and 13). The presence of dense polymers such as Polyester (PES) and Cellulose in Mid-Atlantic samples (Figs. 10 and 11) may well be a result of the deposition of wind driven fibres off the continental land masses (Enders et al., 2015) as these polymers

were exclusively fibrous in nature potentially suggesting that oceanographic processes are not the only drivers of plastic abundance.

Calculating an encounter rate of plastics by zooplankton has been suggested as early as 2001 (Moore et al., 2001) but has been done relatively infrequently in the literature mainly due to the aforementioned lack of plankton data collected alongside that of the plastic (or reporting plankton in terms of mass rather than individual numbers making encounter rates rather approximate (Frias et al., 2014)). Encounter rates have been reported by Frias et al. (2014) finding Microplastics:Zooplankton ratios of 0.04 – 0.14, Sun et al. (2018b) found encounter rates of 0.07 - 1.17 microplastics per zooplankton, and (Sun et al., 2018a) found encounter rates of 0.13 - 0.35 microplastics per zooplankton. Other studies have reported encounter rates with slightly different meanings such as percentage of fish ingesting plastics (they do report a microplastic:fish ratio of 1:1 – 27:1)(Steer et al., 2017), and number of specific zooplankton species to have ingested plastic from the total number (Desforges et al., 2015), or reported their encounter rates as percentages making them difficult to compare and interpret (Sun et al., 2017).

Encounter rate can mean two things in the literature at present: either calculated by the total number of microplastics ingested divided by the number of organisms processed (i.e. ratios of organisms with microplastics ingested:total number of organisms investigated) or, as used in this work as the likelihood of zooplankton encountering microplastics in the water column (i.e. number of plastic pieces:number of zooplankton) and the distinction is important (Botterell et al., 2018). The former uses measured ingestion data to approximate the impact on the group of organisms in the area sampled whereas the latter compares just the numbers of plastic and plankton collected. The difficulty with this approach is that within a litre of water a ratio of 1:1 or 10,000:10,000 return the same ratio however as we have now contrained our metric in 3D space the likelihood of encounter is obviously much greater in the 10,000:10,000

example as the space is more crowded. However this is not to say the metric is still not useful. The marine environments sampled for this work (and for others using this metric) are fluid, not constrained in space. Also in the data presented here, the actual numbers are vastly different from each other with the smallest ratio being 46 plastic particles to 1600 plankton (the plankton being 34 times more abundant) to the greatest being 24 plastic particles to 11,1900 plankton (the plankton here being 4662 times more abundant). Meaning that the acknowledge potential for confounding results is not met within these data and therefore we believe the use of encouter rate to be *indicative* of the likelihood of encounter between plastics and plankton; not necessarily meaning ingestion but external collision/adherance and ingestion combined. This is a measure of the likelihood of encounter not a measure of harm or risk and therefore is useful in framing the data in a global context and setting our horizons for where we might perceive risk to the plankton in the wider literature. In future it would be prudent, when undertaking the taxonomic identification of plankton to also scrutinise the plankton for externally adhered or ingested microplastics to allow for the more robust reporting of encounter rate that has identified ingestion as mentioned earlier.

Our average encounter rates then of 0.0048 plastic particles per zooplankton (± 0.006) in 2014 and 0.0033 plastic particles per zooplankton (± 0.0014) in 2015 are low compared to those reported in the literature. As shown in Fig. 2 the plankton numbers in 2014 are low and the as the plankton increases so too does the plastic meaning that an increase in the plastic:plankton ratio is not seen and the encounter rates are broadly similar across the cruise track (Fig. 4). Samples taken 0, 2 and 8 days away from Falmouth in 2014 have lower encounter rates (Fig. 4) because the plastic is lowest there and the plankton has not decreased with it (Fig. 2). In 2015 the largest encounter rate is seen in the sample 0 Days sail from Horta in the Azores (Fig. 5) with an encounter rate of 0.029 plastic particles per zooplankton. This however should be read alongside the actual abundances, as whilst the encounter rate is the highest for 2015

the plankton are the least abundant and therefore the likelihood of encounter is somewhat ameliorated. However, the encounter rates are greater in 2015 than in 2014 and therefore indicate a greater likelihood of plankton encountering plastic for this year's data. It would seem then that our data do not support the hypothesis that encounter rates will be highest near major land masses. This is likely due to the spatio-temporal variability of both the plastic and plankton. Our sampling of the NE Atlantic took place between July and October of 2014 and 2015 and these months occur during the low productive period for the region (Visser et al., 2011). Similarly microplastics have been found to fluctuate in concentration within short periods of time. Law et al. (2014) found a 3 order of magnitude difference between sites only 32 km and 75 km away from the net tow with the greatest microplastic abundance within a 24 hour period and therefore our snapshot does not constitute a baseline assessment.

At the present moment the literature is demanding that laboratory studies be made environmentally relevant (Lenz et al., 2016, Koelmans et al., 2017, Burns and Boxall, 2018) in order to best understand risk of microplastics to marine organisms and indeed they should but our environmental sampling also needs to be viewed in the light of risk or more accurately likelihood of uptake (as we are not measuring harm here). The encounter rates reported in this study are low, however when taking into account that only 47% of our fibres and 39 % of our fragments are potentially ingestible by marine zooplankton the likelihood of plastic ingestion by zooplankton along this seascape becomes smaller. That is not to say that entanglement or external adhesion may not impact upon zooplankton, or indeed that the presence of these larger pieces are not a problem as they can be consumed by a variety of larger species, but in answering the question of likelihood of uptake, we need to be as accurate as possible.

We aimed to understand how ocean seascapes (sailing from shelf seas through the open ocean to productive oceanic islands and on to western boundary upwelling areas) might impact upon the likelihood of zooplankton encountering microplastics and to look for hotspots of co-occurrence where ingestion might be most likely, or to put it another way where likelihood of uptake of microplastics by zooplankton might be greatest. Our understanding of risk and using targeted investigations such as that undertaken here will help narrow our lack of knowledge and uncertainty as to the impacts of plastic marine debris on life in the oceans (Koelmans et al., 2017). Hypothesis driven investigations can help us interrogate the marine environment in such a way that sampling 'to see what is there' will never do. Our findings demonstrate that actually microplastic and zooplankton numbers are highly variable; our average abundances of plastic fluctuate by an order of magnitude and our plankton abundances see a 40-fold fluctuation. If our encounter rates are coarsely adjusted for 'bioavailable plastics' to more accurately investigate the likelihood of ingestion then the encounter rates are around 0.002 in 2014 and 0.001 in 2015. This means that for every 1 plastic particle there are somewhere between 500 and 1000 plankton which makes the likelihood of plankton encountering plastic unlikely in our study. In our study the plastic concentrations would be equivalent to dosing a laboratory study with $0.92 – 1.41 \times 10^{-7}$ particles $ml^{-1}$ and the only study to date to have reported an EC50 used concentrations up to 32 particles $ml^{-1}$ and particle sizes of 1 - 4 μm (Ziajahromi et al., 2017). Abundance of microplastic particles according to size is perhaps the most important metrics when considering surface encounter and potential ingestion by zooplankton. Shape has a significant part to play as it may be that fibres are easier to ingest than fragments given the available literature investigating environmental ingestion of zooplankton however it may just be that fibres are the most prevalent particle (as found in most studies) and therefore the particles taken up by zooplankton, reflect the particles they are surrounded by leading to a greater incidence of fibre uptake over fragments or other shapes (Botterell et al., 2018, Steer et al., 2017).

This study highlights the importance of repeated time series monitoring over spatial scales as other studies have reported greater concentrations and the plankton biomass fluctuates seasonally affecting the potential for encounter via the dilution or concentration of plastic by the surrounding number of zooplankton. Whilst our abundances are perhaps low, there are hotspots of co-occurrence where relatively high plastic abundance has intersected with relatively high plastic abundances (within this study) and these are the places where encounter, and potentially uptake or entanglement may well occur. It is paramount to investigate the likelihood of encounter and to focus our analyses by using common sense and judgements constrained by the available literature to interrogate our data (as alluded to here in the difference between bioavailable fractions based on particle size and the total plastic abundance) to gain a better understanding of where the risk of microplastics to marine life is highest, and to then use that data to intensively sample those areas to understand what the actual ingestion rates are and what the concentrations of microplastic particles are like in areas where productivity is high.

# Chapter IV: Role of Marine Snows in Microplastic Fate and Bioavailability

**Figure 1:** A mussel ventilating at the bottom of our Vertical Transport Chambers

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Adam Porter designed the research, built the experimental equipment, conducted the experiment and data analysis and wrote the manuscript.

Ceri Lewis edited and improved the manuscript

Brett P. Lyons and Tamara S. Galloway advised in the design and implementation of the experiments and in the interpretation of the data.

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# Role of Marine Snows in Microplastic Fate and Bioavailability

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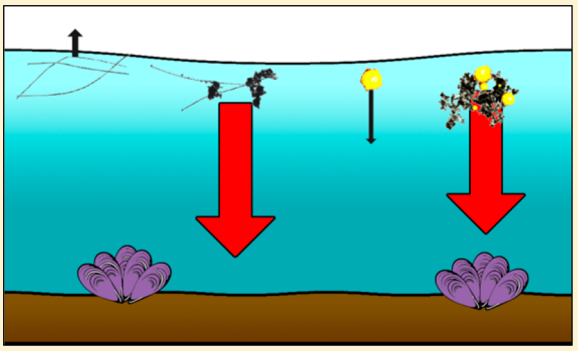
Supporting Information

**ABSTRACT:** Microplastics contaminate global oceans and are accumulating in sediments at levels thought sufficient to leave a permanent layer in the fossil record. Despite this, the processes that vertically transport buoyant polymers from surface waters to the benthos are poorly understood. Here we demonstrate that laboratory generated marine snows can transport microplastics of different shapes, sizes, and polymers away from the water surface and enhance their bioavailability to benthic organisms. Sinking rates of all tested microplastics increased when incorporated into snows, with large changes observed for the buoyant polymer polyethylene with an increase in sinking rate of 818 m day$^{-1}$ and for denser polyamide fragments of 916 m day$^{-1}$. Incorporation into snows increased microplastic bioavailability for mussels, where uptake increased from zero to 340 microplastics individual$^{-1}$ for free microplastics to up to 1.6 × 10$^5$ microplastics individual$^{-1}$ when incorporated into snows. We therefore propose that marine snow formation and fate has the potential to play a key role in the biogeochemical processing of microplastic pollution.



## INTRODUCTION

Microplastic particles (pieces of plastic <5 mm) are ubiquitous and pervasive pollutants of the marine environment globally[1,2] having been recorded from the poles to the tropics and from surface waters to the seafloor.[3] They have also been found in the guts of over 300 different marine species,[4] prompting widespread concern over their environmental impact. Global microplastics sampling efforts to date have heavily focused on the oceanic gyres and the floating portion of plastic debris, leading to a global estimate of 93−236 thousand metric tonnes[3] of microscopic plastic debris currently floating on the sea surface. However, there is a vast discrepancy between the amount of plastic estimated to enter the marine environment and what is being recorded in these surface monitoring efforts.[5,6] In 2010 alone, 4−12 million metric tonnes of plastic is thought to have entered the oceans, vastly outstripping this sea-surface data.[6]

It is becoming increasingly apparent that microplastics are not just present on the sea surface and that, somehow, these particles eventually make their way down to the seafloor. Concentrations on the deep seafloor are estimated from limited sampling efforts to be as high as 4 × 10$^9$ fibers km$^{-2}$, with an average around 1 × 10$^9$ km$^{-2}$.[7] It is even being argued that microplastics may already form part of a stratigraphic signal of the Anthropocene due to their accumulation into sediments.[8] Microplastics of buoyant polymers such as polypropylene and polyethylene, which should float as virgin (unfouled) particles, have now been reported at depths down to 5000 m in ocean sediments[9−12] and in the guts of deep sea organisms.[1] This, together with the "missing" surface plastic, suggests that environmental transformations of microplastics must occur that alter their densities and fates after entering the marine environment. Modeling approaches have started to look at how processes such as biofouling and fragmentation of plastic particles might alter particle buoyancy and hence lead to net sedimentation,[13,14] based on a series of assumptions regarding these interactions in a water column. This has led to a recent estimation that 99% of plastic entering the oceans will eventually reach the ocean floor, including buoyant polymers.[15] A key transport route for organic matter to the benthos that is not accounted for in such models is the formation of marine snows.

Marine snows are organic-rich aggregates (distinct particles >200 μm[16]) made up of fecal pellets, larvacean houses, phytoplankton, microbes, particulate organic matter (POM), and inorganics brought together by shear forces and Brownian movement.[17] Marine snows have much higher settling rates than their individual particle components (following Stokes's Law)[18,19] and are primarily responsible for the mass flux of organic material from surface waters to the deep ocean[17] forming a key component of the biological carbon pump.[20]

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Marine snow concentrations generally range from <1 to 100 aggregates $L^{-1}$[21] but can be as high as 5300 aggregates $L^{-1}$.[22] Other transport mechanisms could be in fast sinking fecal pellets, but these are usually retained in the upper few hundred meters of the water column as they are recycled by coprophagy. Much of the global oceans vertical flux of particulate material is therefore dictated by the movement of marine snow.[20] Unlike biofouling, marine snow formation is not light limited.[13] Hence it can be hypothesized that marine snows have the potential to provide an important pathway by which microplastic particles can be rapidly transported vertically downward.

Here, we investigate this potential for marine snow to form a transport mechanism to move buoyant microplastics away from the sea-surface, through the water column, and ultimately to the seafloor. As incorporation of microplastic into marine snows might increase the effective particle size of microplastics, they may also enhance their bioavailability to invertebrate consumers.[23] Here, we focus on a bioavailable fraction of what are termed "microplastics" using plastics ranging from 7−3000 $\mu$m. Using a laboratory simulation of marine snow formation, we determine whether a range of different microplastic polymers, shapes, and sizes will incorporate into marine snows, how this influences their behavior in the water column, and ultimately how it influences their uptake into a model benthic filter feeder. Given the important role that marine snow plays in the downward flux of organic material in global oceans, understanding its potential role in the movement of microplastics is key to understanding the fate of microplastics in marine ecosystems and quantifying the potential risk that they pose to marine biota and ultimately to human health via our consumption of benthic fisheries species.[24]

## MATERIALS AND METHODS

**Production of Marine Snows and Plastic Contaminated Snows.** Marine snows were produced using the modified protocol of Shanks and Edmunson[25] to include 10 $\mu$g $L^{-1}$ hyaluronic acid as recommended in Ward and Kach.[26] Natural seawater was collected 1 h before high tide from the same location for each exposure (Starcross, Devon, UK. Lat: 50.628204, Lon: −3.4477383) between February 2016 and March 2017, filtered to 200 $\mu$m to remove any large particles and plankton, and placed in 1 L Nalgene bottles. For the plastic contaminated snows, a range of plastic sizes, shapes, and polymer types was used in order to determine if shape, size, or polymer type influenced incorporation into marine snows. Test microplastics included polyamide fibers (10 × 50 $\mu$m) made according to Cole 2016,[44] polystyrene beads (7−30 $\mu$m), polyethylene beads (9−11 $\mu$m), polyvinyl chloride fragments (115−156 $\mu$m), polyamide fragments (6−30 $\mu$m), and polypropylene fibers (23 × 3000 $\mu$m) (all bar polypropylene fibers fluorescently labeled, further details in the SI, summarized in Table S1). These plastics are polymers commonly found in the environment. Polypropylene fibers are the most common fiber found in water and sediment samples with polyamide fibers being the third most common in water and sediment samples,[27] and polyethylene, polystyrene, and polyvinyl chloride are in the five major commodity plastics commonly encountered.[28] These were added to the roller bottles before rolling, to aggregate into the marine snow matrix, and the bottles with and without microplastics were placed on a roller table for 3 days at 14 rpm.

**Incorporation Index Calculations.** To establish how readily the different microplastic types and shapes incorporated into marine snows, an incorporation index was calculated according to Doyle et al.[19] Microplastics were added to the 1 L roller bottles at a concentration of 50 particles $mL^{-1}$ or 0.1 $mL^{-1}$ in the case of the polypropylene fibers (the fibers were much larger and so using their weight, the concentration was decreased by 500 times as their weight was 500 times that of the smallest bead). This affected a final concentration in our biological exposures of 2.5 particles $mL^{-1}$ and 0.05 $mL^{-1}$ respectively. This process was repeated 4 times with different seawater to account for natural variations between water collections such as variations in particulate matter and transparent extracellular polymers (TEP) concentration. The snows, once formed, were allowed to settle, and then all aggregates were pipetted into a separate falcon tube for each treatment. The snows were resuspended by gently rolling the falcon tubes, a subsample of aggregate filled seawater was then put in a Petri dish under a Leica inverted fluorescence microscope (Leica DMI4000 B using UV (360 nm), Green (515−560 nm), and Blue (450−490 nm) filters), and every aggregate >300 $\mu$m was counted to give a number of aggregates per milliliter. The total number of aggregates in each 1L bottle was then calculated with this information. Subsequently, 30−40 snows per treatment were imaged, and fluorescence was used to identify the number of microplastic particles bound into the aggregate matrix. The ImageJ software package[29] was used to measure the maximum calliper length of each snow. An average number of microplastics per snow was then calculated, and this number was multiplied by the number of snows calculated to be in the original 1 L of aggregate seawater to give a final number of microplastics in marine snows for each treatment. This then allowed the incorporation of plastics to be calculated using a modified equation from Doyle et al.:[19]

$$\text{Incorporation (\%)} = \left[\frac{\text{Concentration in snows}}{\text{Input concentration}}\right] \times 100$$

We confirmed that our calculations were not affected by the small numbers of plastics that adhered to the bottle surfaces during the snow formation step. These losses were minor and hence did not impact our results.

**Measuring Sinking Rates.** Sinking rates were calculated as "relative sinking rates" given the variety of factors including vessel size and shape (wall effects), temperature and salinity, air/water interface size, and air flow that can alter these rates.[30] The sinking rates were calculated for all marine snow polymer types with 30 individual snows being measured per replicate, all made from the same seawater, and repeated three times giving a total of 90 measurements per polymer type. To be able to compare the sinking rates of marine snows against the respective free microplastic particles, which were too small to be measured visually, the sinking rates for all plastics were calculated using Stokes's Law to calculate terminal velocity and using a modified version for cylindrical fibers.[31] To do so a 1 L measuring cylinder was filled with artificial seawater at a fixed salinity and temperature (15.6 °C, 27.1 ppt, 8.07 pH) to a water height of 360 mm and then left to settle for 30 min. A white nonreflective card was used to aid visualization, and a subsample of each plastic contaminated snow treatment was pipetted using a serological pipet (Drummond Portable Pipet-Aid XP2). The snows were allowed to sink through the pipet and released from the pipet under gravity, just under the surface of the water. Marine snows then sank for 142 mm to achieve a constant velocity and then were filmed sinking through a 36.5


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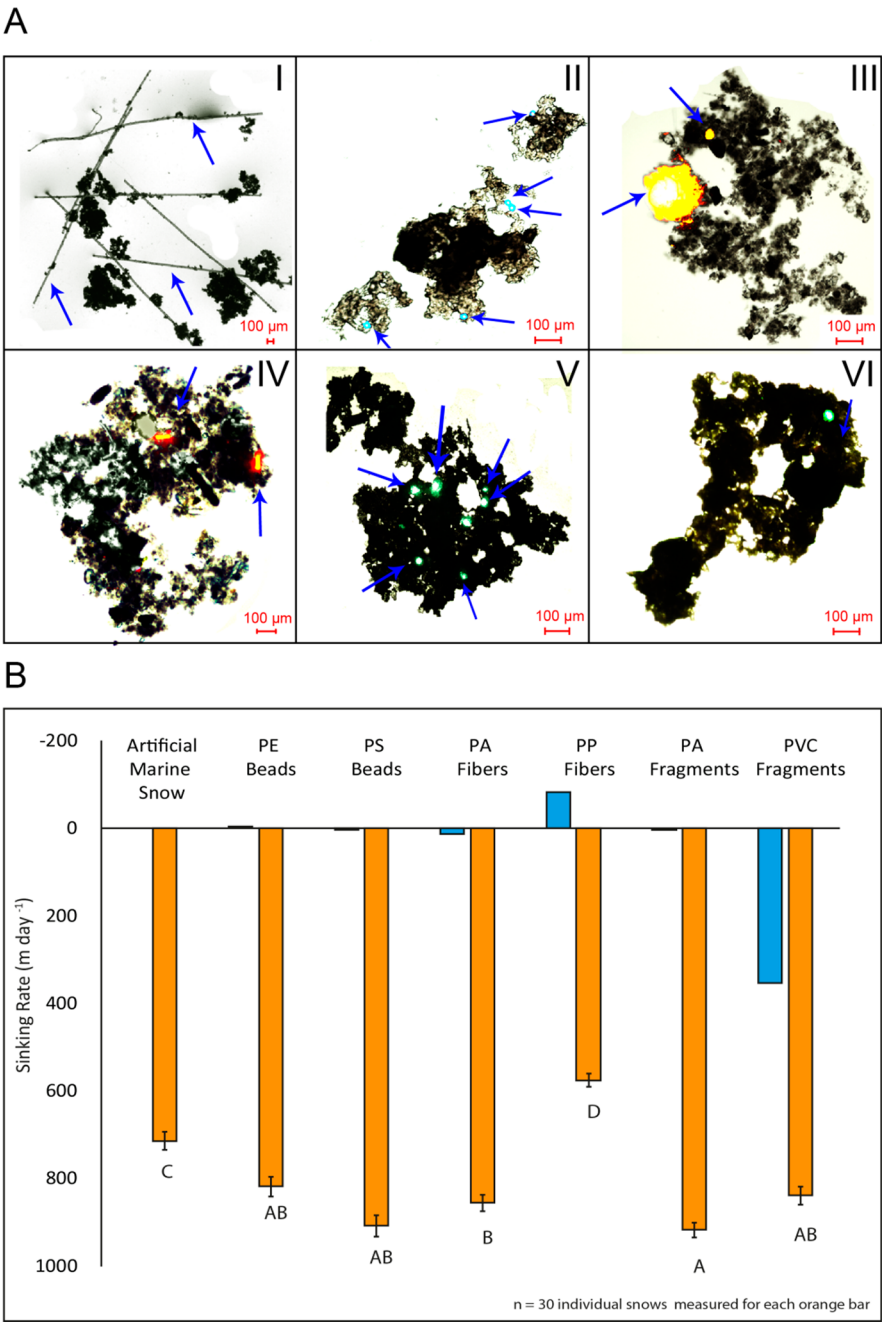


**Figure 1.** A) Images of marine snows with each of the six polymers incorporated and sinking rates plotted. I) polypropylene fibers 23 × 3000 $\mu$m (note the change in scale bar for all other microplastics); II) polyethylene beads 9−11 $\mu$m; III) polyvinyl chloride fragments 115−156 $\mu$m; IV) polyamide fibers 10 × 50 $\mu$m; V) polyamide fragments 6−30 $\mu$m; VI) polystyrene beads 7−30 $\mu$m. Blue arrows indicate incorporated microplastics that are colored by their fluorescence in the figure. B) Modeled sinking rates of microplastics (blue bars) and measured sinking rates of marine snows with plastics incorporated (orange bars). Artificial marine snow, polyethylene (PE) beads, polystyrene (PS) beads, polyamide (PA) fibers, polypropylene (PP) fibers, polyamide (PA) fragments, and polyvinyl chloride (PVC) fragments were all measured using a Canon 5D MKIII. Bars with different letters are significantly different (One-Way ANOVA, $F_{6,622} = 3001$, $p \leq 0.01$, Tukey's Post-Hoc Test).

mm window using a Canon DSLR set at 720 p, 50 fps (720 p = frame size of 1280 × 720 pixels, 50 fps = shot at 50 frames per second) . The time the snows took to sink through the window was calculated using the following equation

$$\text{Time} = \frac{\#\text{Frames}}{\text{Frame Rate}}$$

where the number of frames is derived by subtracting the frame number the marine snow entered the window from the frame number when it exited the window. Speed was then calculated using the time and distance data in m day$^{-1}$.

**Mussel Uptake Experiment.** The blue mussel, *Mytilus edulis* (shell length: 53.7 mm ±4.6 mm), was collected from a local source at Starcross, Devon adjacent to where the seawater was collected (Lat: 50.618945, Long: −3.4462054) 3 days prior to exposure. Their shells were scrubbed to remove organisms and underwent two water changes in a temperature controlled aquarium setting to allow them to depurate and were fed a concentrated blend of microalgae (Shellfish Diet 1800, Reed


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Mariculture). Mussels were then transferred to a flow-through aquarium tank and kept in treatment seawater conditions for at least 3 days to acclimate before being added to any exposure. The mussels were then starved 12 h before exposure and removed 2 h pre-exposure to ensure they ventilated promptly during the feeding exposure.

Nine mussels per treatment for polystyrene and polyethylene and 16 mussels per treatment for polypropylene (where uptake was lower therefore more replicates were required for statistical power) were exposed in individual tubes to the following for 60 min at 15 °C:

1. Controls − natural seawater that had not been rolled (i.e., no marine snow or microplastics present).
2. Marine snows with incorporated microplastics referred to as plastic contaminated snows (PCS) − Rolled seawater with microplastics incorporated into the aggregates through the rolling process.
3. Marine snows with added microplastics referred to throughout as plastic and snows (PAS) − Rolled seawater with no microplastics. Plastics were then added to the exposure at time 0 (T0) to differentiate between active feeding on the snows and passive plastic uptake from free beads in the water.
4. Seawater with microplastic spheres, referred to as a "free plastic" treatment (FP) − artificial seawater (ASW) with freely suspended plastics added. The PAS and FP treatments had PS and PE added to the 1 L bottles at a concentration of 50 particles $mL^{-1}$, and the larger PP fibers were added at a concentration of 0.1 particles $mL^{-1}$. Control mussel exposures were undertaken to assess laboratory contamination and the quality control of the protocol. No microplastics were recovered in any of the control treatments, and so controls were eliminated from the analysis.

To assess the ability of marine snows to transport plastics from the surface to the benthos, vertical transport chambers (VTCs) (Figure S2) were made to ensure a head of water above the mussel (water height of 194 cm equal to ≈20 L of water in a 116 mm interior diameter tube) to mimic a sublittoral environment and to help test sinking. Mussels were placed in the VTCs, and once every mussel was visibly ventilating each treatment was added at T0. The artificial seawater was filtered to 0.2 μm, acclimated to the aquarium temperature, and diluted to the salinity of the natural seawater taken to produce the marine snows. At T0 the aggregates making up the treatments, plastic contaminated snows (PCS) and plastic and snows (PAS), were transferred to VTCs using a serological pipet. The snows sank through the pipet and were released just under the surface of the water to ensure that the snows remained intact and that eddy formation was minimized which could prevent uniform sinking. Microplastics at the required concentrations were added to PAS and FP treatments at T0 also. The exposure was run for 60 min (T60) based on preliminary feeding trials using uncontaminated marine snows ensuring that significant uptake occurred within this time period and to ensure that slow sinking microplastics had the requisite time to reach the mussels in the experiment to be compared with the sinking marine snows.

**Microplastic Recovery and Quantification.** At T60 mussels were removed by a rapid inversion of the VTCs, causing them to cease ventilating and therefore feeding, and were then dried off with blue roll. Mussels were snap-frozen prior to dissection from their shells and dried at 60 °C for 48 h, and a dry tissue weight was calculated. Mussels were then rehydrated by placing each dried mussel in a conical flask with 20 mL of 0.2 μm filtered deionized water (DI). The mussels were left for 2 h to rehydrate, transferred to a 50 mL falcon tube with the DI water, and then homogenized using a Stuart SHM1 Homogenizer. Once a smooth homogenate was achieved, 6 × 20 μL of homogenate was viewed under an inverted fluorescent Leica microscope in a clear bottomed well plate, and the beads per well were counted. The number of beads per mussel was calculated based on the dry weight plus 20 mL of DI using the following equation

$$\frac{\text{Total Sample Volume } (\mu\text{L})}{20\ \mu\text{L}} \times \text{Well average (\#beads)} = \text{Total beads per mussel}$$

where total sample volume (μL) is the total mussel dry weight (1 g = 1 mL) added to the DI volume (mL) used to rehydrate the mussel.

**Quality Control.** Microplastics were fluorescently labeled where necessary to ensure that contamination from the laboratory environment could not be mistaken for an experimental particle. The large polypropylene fibers were not labeled as they were easily visible, but each sample was inspected fully in this case and the fibers were very distinct (see Figure S1).

Similarly the homogenization step was inspected to ensure that the plastics were not damaged in the homogenization process. Visual assessment of all plastics found no evidence of fracturing or surficial damage, even in our largest plastics, the 23 × 3000 μm polypropylene fibers.

## RESULTS AND DISCUSSION

We found that all of the microplastic polymer types, shapes, and sizes that we tested readily incorporated into laboratory made marine snows (Figure 1, details of plastics used in Table S1, images in Figure S1). Using a calculated incorporation index, we found that for the buoyant polymer (density lower than seawater) polyethylene (PE), 79% of beads incorporated into the aggregate matrix. For polyamide (PA) fibers, PA fragments, polystyrene (PS) beads, polypropylene (PP) fibers, and polyvinyl chloride (PVC) fragments, we found incorporation values of 100%.

For all polymer types and microplastic shapes tested, we measured enhanced sinking rates when these microplastics were incorporated into marine snows (Figure 1) compared to their calculated sinking rates as free particles. This relative change in sinking rate from that as a free microplastic particle to particles incorporated into snows varied according to polymer type. Critically, buoyant polymers became negatively buoyant once incorporated into marine snows and hence sank during the observation period rather than remaining on the surface. For example, buoyant PP fibers had calculated sinking rates of −82 m $day^{-1}$ and float on the surface when added to the vertical transport chamber. Once incorporated into marine snows, PP fibers became negatively buoyant, sinking at a rate of at 576 m $day^{-1}$ an increase of 658 m $day^{-1}$ (Figures 1AI and 1B). Similarly, PE beads had a negative calculated sinking rate of −0.19 m $day^{-1}$ as free particles (i.e., floated on the surface) but had sinking rates of 818 m $day^{-1}$ when incorporated into marine snows, a reversal from slightly buoyant to rapidly sinking (Figures 1AII and 1B).


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For polymers denser than seawater, sinking rates were calculated to be 0.39 m day$^{-1}$ for PS beads, 1.49 m day$^{-1}$ PA fragments, and 12.15 m day$^{-1}$ for PA fibers (Figure 1B) as free microplastic particles. Due to its high density, PVC had the greatest sinking rate as free microplastic of 354 m day$^{-1}$ and exhibited a relatively small increase to 839 m day $^{-1}$ when incorporated into snow (an increase in sinking rate of 485 m day$^{-1}$) (Figures 1AIII and 1B). PA fibers when incorporated into marine snows sank at a rate of 855 m day$^{-1}$, an increase of 843 m day$^{-1}$ compared to free PA fibers (Figures 1AIV and 1B). Marine snows contaminated with PA fragments had the fastest sinking rates of 917 m day$^{-1}$, an increase of 916 m day$^{-1}$ compared to its sinking rate as free microplastic (Figures 1AV and 1B). PS beads exhibited an increase in sinking rate of 908 m day$^{-1}$ when incorporated into marine snow from 0.39 m day$^{-1}$ as free plastic to 908 m day$^{-1}$ in marine snows (Figures 1AVI and 1B).

The sinking rates for the free microplastic particles are based on simple models for a static water column and the laboratory based observations for the plastics incorporated into marine snows made under similarly static conditions. As such these values cannot be taken as representative of true particle sinking rates under more turbulent, real-world oceanic conditions, which will vary in space and time according to a number of oceanographic processes and that act as a large force on sinking processes (although the net flux in the global ocean is downward). Our measured marine snow sinking rates are therefore higher than those generally reported for marine snows in the natural environment (reported as 1−280 m day$^{-1}$)[23] where turbulent mixing acts to slow this rate.[32] Additionally, the water used to generate the snows in this study was collected from an estuary high in lithogenic material, potentially adding denser material to the aggregate mix than might occur in open ocean conditions and which would be expected to enhance sinking.[33] Zooplankton fecal pellets have been found to sink faster than 820 m day$^{-1}$[33] however, suggesting that our rates are not beyond the realms of what is conceivable for POM in the open ocean.

The benefit of using this controlled static system is that it allows relative sinking rates to be compared for our range of test microplastics against modeled sinking rates for free plastic particles (which are also devoid of real world perturbations), without the complex confounding factors of oceanic conditions, thus allowing us to test our hypothesis. This comparative data demonstrates a clear relative increase in sinking rates of the plastic particles when they are incorporated into marine snows for all microplastics tested. Even with an obvious attenuation in sinking speed, and within a complex system of fragmentation and coagulation, the magnitude of these relative changes in sinking rates provides strong evidence that the process of incorporation into marine snows dramatically changes the behavior of the microplastic particle in a water column. Hence this data provides empirical support for the paradigm that marine snow represents an environmentally relevant, viable pathway for microplastics to be transported from the sea surface to the seafloor, including buoyant polymers which would otherwise float as virgin particles.

The aggregation of microplastics into marine snows not only altered the microplastics behavior within the water column but also altered the sinking rates of the marine snows themselves. Marine snows with PP fibers incorporated had slower sinking rates compared to uncontaminated snows (714 ± 25 m day$^{-1}$) and had the slowest sinking rate of 576 ± 16 m day$^{-1}$. This would be equivalent to a reduction of 138 m day$^{-1}$ (Figure 1), which over the average depth of the ocean (≈ 4000 m) equates to 1.3 days longer to reach the benthos. This reduction is likely due to the greatly increased size of the plastic/snow parcel formed with these large fibers (as can be seen in Figure 1AI). Polypropylene fibers formed groups of snows and fibers which would likely experience significant drag while sinking, slowing them down. All other microplastic contaminated snows sank significantly faster than the uncontaminated snows (One-Way ANOVA, $F_{6,622}$ = 3001, $p \leq 0.01$, Tukey's Post-Hoc Test) (Figure 1B), equivalent to an increase of 153 m day$^{-1}$ (SE ± 19 m day$^{-1}$). This would theoretically cause POM to reach the benthos 1 day before an uncontaminated snow might.

Altered POM sinking rates as a result of microplastic incorporation has previously been demonstrated in the laboratory using polystyrene beads for zooplankton fecal pellets[34] and for cultured algal aggregates[18] further adding to the evidence that microplastics have the potential to interact with important aspects of the oceans' biological pump. Slower sinking would potentially allow more grazing, fragmentation, and microbial degradation of marine snows[20,34] and possibly lead to rerelease of buoyant microplastics to the surface, whereas a faster sinking POM could result in higher rates of accumulation of plastic debris in the benthic realm. Of course, there are a whole suite of environmental factors that will affect marine snow sinking rates in addition to the concentration, type, and shape of microplastics, for example the amount of POM present and abiotic processes such as turbulence and homogeneity of the water column, grazing on snows as they sink potentially rereleasing plastics, temperature, salinity, and viscosity of the water.[20] However, the relative change between our plastic and marine snow sinking rates are, in most cases, orders of magnitude different (polystyrene beads increased from a free plastic sinking rate of 0.39 m day$^{-1}$ to a sinking rate of 908 m day$^{-1}$ when in marine snows and even the smallest increase was relatively large for PVC fragments from 354 m day$^{-1}$ to 839 m day$^{-1}$), and therefore even with the aforementioned attenuations in sinking rates, even sinking at environmentally measured rates, the plastics will still be traveling from the sea surface to the seafloor at a much enhanced rate than they would as individual particles and indeed the buoyant polymers would have to wait for the much slower process of biofouling to occur to overcome their positive buoyancy. A study by Zhao et al.[16] provides evidence that this process of microplastics incorporating into marine snows occurs in the field, finding marine snows with buoyant plastics incorporated in the top 2 m of the water column. Our study goes further to explore the sinking dynamics and implications of marine snows as a transport vector to the deep ocean for a range of microplastic polymers and shapes.

**Uptake of Microplastics in *Mytilus edulis*.** Finally, we demonstrate that the incorporation of microplastics into marine snow acts to increase their bioavailability to a model benthic filter feeder, the blue mussel (*Mytilus edulis*), when mussels were separated from the surface by a distance of 2 m. We selected the PS and PE beads and the PP fibers for use in the mussel exposures so as to have two size comparable beads with differing densities and the PP fibers as they were much larger particles, much less dense, and represented fibrous material, which can make up a large proportion of real world samples from microplastic trawls. We found that for all three microplastics tested, mussels ingested significantly more microplastics when they were incorporated into marine snows


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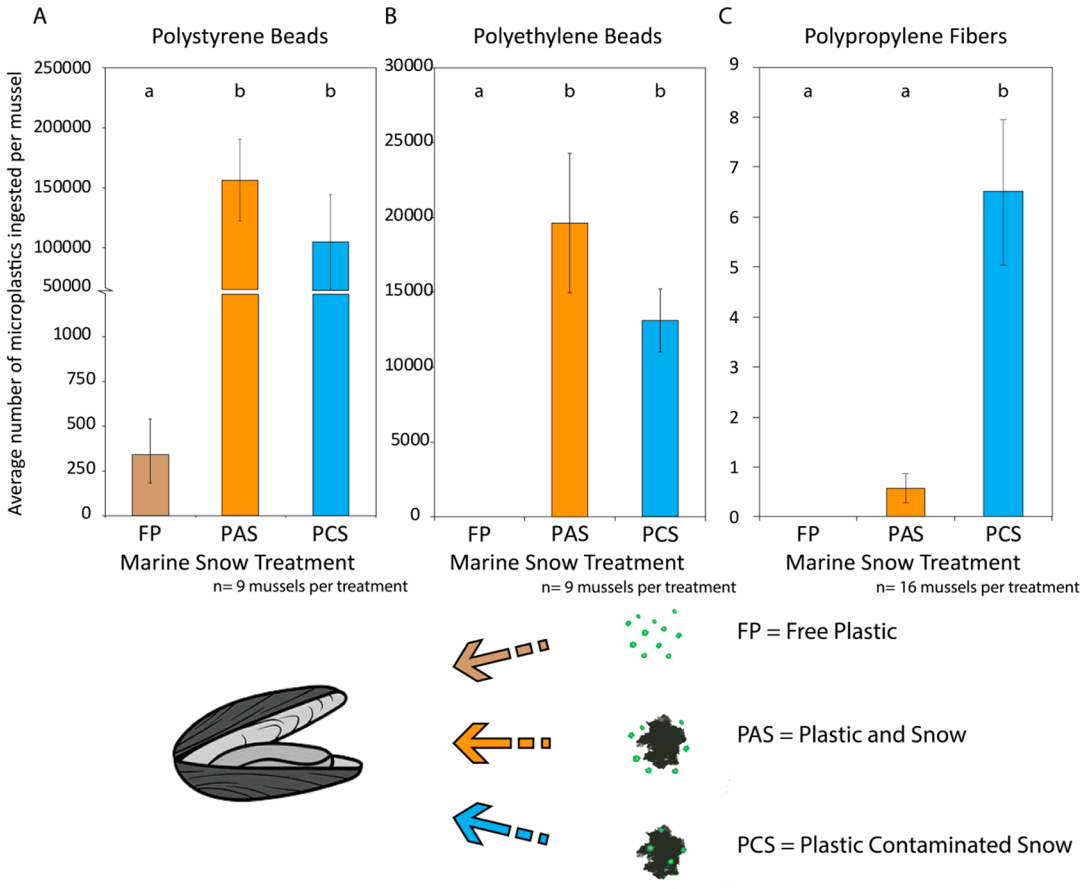


**Figure 2.** Uptake of microplastics into *Mytilus edulis* in the absence of marine snow ("free plastic" = FP), in the simultaneous presence of marine snow at the time of the uptake experiment ("plastic and snow" = PAS), and after preincorporation into marine snow matrix ("plastic-contaminated snow" = PCS) for A) polystyrene beads (7−30 $\mu$m), B) polyethylene beads (9−11 $\mu$m), and C) polypropylene fibers (23 × 3000 $\mu$m). Significant differences are highlighted by differing letters (Tukey's HSD Test). Infographic below gives a visual descriptor of the three treatment types and codes.

(plastic contaminated snows (PCS)) than when added as free plastic (FP) (Figure 2). For PS beads, uptake over an hour's exposure increased ≈300 times from an average of 340 (±158 SE) beads per mussel when freely suspended to ≈105000 (±3900) beads per mussel when incorporated into marine snows (ANOVA: $F_{3,32}$ = 13, $p \leq 0.01$) (Figure 2A). For PE, beads were only taken up by the mussels when marine snow was present (ANOVA: $F_{3,32}$ = 12.38, $p \leq 0.01$, Figure 2B). The difference in uptake of PS compared to PE when fed to mussels is likely due to the buoyant nature of the PE beads and their reduced incorporation rate into marine snows of 79% compared to 100% for PS. For the PP fibers, the ingestion rate was significantly greater (ANOVA: $F_{3,52}$ = 18.66, $p \leq 0.01$, Figure 2C) when incorporated into the marine snow (6.5 ± 1.5 fibers per mussel) compared to when fibers were mixed with snows at the start of the exposure (0.6 ± 0.3 fibers per mussel). As with PE no fibers were ingested when input as free plastics (Figure 2).

An increased uptake of freely suspended microplastics by the mussels was also detected when they were added to the vertical transport chambers at the same time as previously formed marine snow (plastic and snow treatment (PAS)) (Figure 2B). This is likely due to a combination of factors; first, that the process of incorporation is happening *in situ* as the plastic and marine snows are mixed at the start of the experiment such that some plastics are collected as the snows fall. Second, the downdraft of the sinking snows is likely to be enough to carry down plastics in their wake as a large body of particulate matter with high densities sink. Even with buoyant PE this is plausible as the polymer is only just less dense than the surrounding water and so an energy flow moving downward may be enough to overcome the buoyancy of the particle by itself. Indeed a number of oceanographic processes including saline subduction, offshore convection, and dense shelf water cascading have been hypothesized as routes of microplastic transport to deeper waters.[7]

Interactions between microplastics and biota are observable throughout marine ecosystems globally.[35] Plastic debris has been documented to have entangled or been ingested by at least 557 species, including marine mammals, seabirds, and many benthic organisms.[4] Mussels, used here as representative filter feeders since this is a common feeding mode in benthic ecosystems, are efficient at the capture of small particulate matter,[26] readily ingest microplastics,[36] and obtain 5 to 10 times more nitrogen from marine snows than from dissolved organic matter and particulate detritus.[37] Our findings suggest that not only do marine snows redistribute microplastics by drawing them downward but they also, potentially increase the uptake of microplastics via a bioconcentration process. This concentration process has been recognized in studies looking at marine snow and pathogen interactions[38] whereby organisms have increased exposure to a pathogen due to their aggregation


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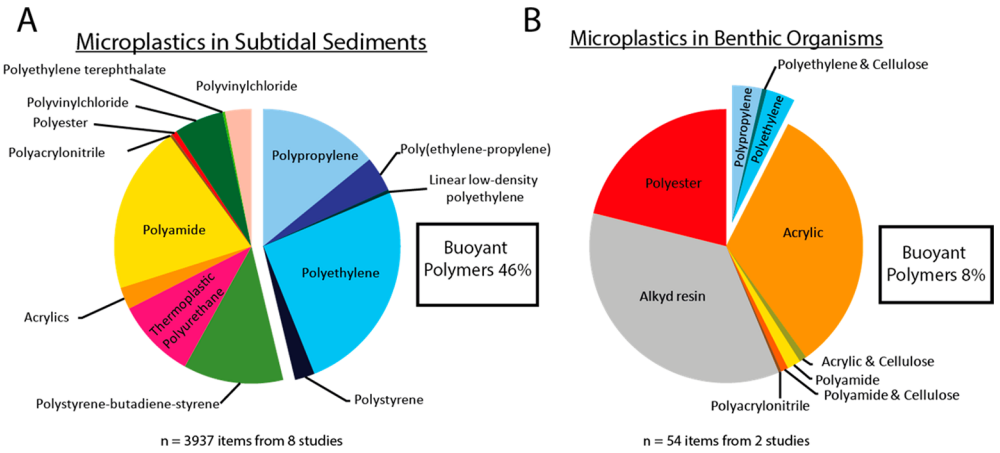


**Figure 3.** Microplastic polymers identified in subtidal environmental samples from 1−2700 m. Data was collected from a literature search of microplastic studies that both listed and quantified the polymer types found in A) subtidal sediments (8 studies) and B) deep sea organisms (2 studies) (see Table S2).

within a food source. Marine snows may well be enhancing the bioavailability of microplastics to invertebrate consumers, causing bioaccumulation in mussels on an as yet unknown scale and potentially causing biomagnification through the food chain and potentially to humans also.[38]

To examine the wider relevance of our findings, we conducted a review of the existing literature on microplastic pollution in benthic samples (see Table S2 for references and details). This reveals that a variety of different polymer types, shapes, and sizes of microplastics, including buoyant polymers, have been found in sediments and the guts of benthic species. Microplastics have been found in benthic sediments at depths ranging from 1 to 2700 m[7,10] (Figure 3A) and in the guts of organisms at depths of 334−2200 m[1,7] (Figure 3B). Buoyant polymers PE and PP are reported in deep sea organisms at similar concentrations to those ingested in our study (2 to 5 fibers per individual in Taylor et al.)[1] compared with 0 to 21 fibers per individual (mean ± SE of 6.5 ± 1.45 per individual) in the present study. Of all the plastics recovered from these studies buoyant polymer types comprised 46% of all plastics in sediments. In benthic organisms buoyant polymers made up 8% of the total number of plastics recorded.

Overall, our results demonstrate that the formation of marine snow represents an environmentally relevant, viable pathway for microplastics to be transported from the sea surface to the seafloor and into benthic fauna by ingestion. This mechanism has the potential to fill in the gap between what we know is entering the marine environment and the relative fraction found in sea surface trawls and adds further evidence to the prediction that plastic contamination of the benthic habitat is occurring at much greater volumes than first thought. The soft sediments that cover much of the ocean floor are dynamic and productive habitats, supporting many ecologically and economically important species and playing key roles in ecosystem functioning,[39] raising questions as to the impact that microplastic pollution is having on these important communities.[40−43] There is however a multifaceted issue to disentangle: first, that marine snows have the potential to be a highway for plastic transport to the benthic realm and second, that plastics, in theory, have the ability to influence the fluxes of POM in the marine water column. We also show that mussels ingest more plastics when they are incorporated into marine snows potentially leading to adverse effects as yet unseen with standard feeding models based on free plastics. This mechanism of plastic delivery to benthic organisms is also potentially important as plastics have been repackaged into a food source and concentrated, opening questions regarding whether this will influence the effects of plastics on these organisms.[16] Addressing the paucity of data relating to the presence of midwater and benthic microplastics, evidence of the transport pathways, and the understanding of their fate upon reaching the benthos is of paramount importance when taking a global ocean view of microplastic pollution. The transformations of plastics that occur during their journey from source to sink will be critical in their distribution and thus our understanding of risk of microplastics to marine systems. This study is an important step in understanding the fate and sinking dynamics of microplastics in global oceans and highlights the potential for microplastics to affect more than just the sea surface.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b01000.

Additional Materials and Methods, Figures S1 and S2, and Tables S1 and S2 (PDF)

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A.P., B.L., T.G., and C.L. wrote the manuscript.

### Notes

The authors declare no competing financial interest.

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## Supporting Information

# Role of Marine Snows in Microplastic Fate and Bioavailability

This supporting information contains:

Additional Materials and Methods

**Fig S1:** Images of microplastics exposed to the marine snow formation process.

**Fig S2:** Design of the Vertical Transport Chambers (VTCs).

**Table S1:** Microplastics used in marine snow incorporation experiments detailing their concentration, size, source, fluorescent signature and densities.

**Table S2:** Microplastics found in benthic sediments and deep sea organisms.

## Materials and Methods

### *Microplastic preparation*.

Six different plastics were used; polyamide fibres (PA), polystyrene beads (PS), polyethylene beads (PE), polyamide fragments (PA), polyvinylchloride fragments (PVC), and polypropylene fibres (PP). PS and PE beads were bought from Spherotech and Cospheric respectively and were thoroughly washed by dilution in 15 ml of MilliQ water and centrifugation. These beads have fluorescent dyes incorporated into the polymer and so did not require dyeing. The supernatant was removed and the beads were re-diluted, transferred to a new 15 ml falcon tube and underwent centrifugation. This was repeated three times to ensure the removal of surfactants and anti-microbials. Polyamide and polyvinylchloride fragments were produced using a Freezer/Mill (SPEX Sample Prep Freezer/Mill 6870) to grind down pellets into microplastic fragments. Polyamide polyfilament line was purchased from Goodfellow Cambridge Ltd. and microfibres were produced following Cole[44] using a cryotome (LEICA CM1950) to section the fibres to 50 μm lengths. Polypropylene fibres were purchased from Goonvean Ltd.

To enable their quantification after uptake by the mussel *Mytilus edulis*, these plastics were fluorescently labelled using Nile Red (technical grade, N3013, Sigma Aldrich) or Radglo or were bought as fluorescent beads (Table S1). For PA fibres and PVC fragments a solution of 500 μg $mL^{-1}$ Nile Red was prepared, and the plastics and Nile Red mixed in a 15 ml Falcon tube, vortexed and left to stand for 10 minutes. The plastics were then filtered out onto 5 μm polycarbonate filters (Whatman Cyclopore),

rinsed with acetone to remove excess dye, washed with ultrapure water and allowed to dry before being suspended in 0.2 µm filtered artificial seawater (ASW)[44]. To dye the PA fragments with Radglo a similar technique was used. Plastics and Radglo EA – 30 were mixed in air (pigment: particles = 1: 20 wt/wt) causing electrostatic adhesion of the fluorescent particles to the plastic surface. The plastics were then submerged in acetone and the acetone allowed to evaporate until dry. The plastics were then washed in acetone over a 5 µm polycarbonate filter, followed by an ultrapure wash and then suspended in ASW[44]. PP fibres were large enough (3000 µm x 23 µm) to not necessitate dyeing as they were easily visible in the mussel homogenate under a microscope.

**Figure S1.**

Images of microplastics exposed to the marine snow formation process. A) polyamide fibres 10 x 50 µm; B) polystyrene beads 7–30 µm; C) polyethylene beads 9 – 11 µm; D) polyamide fragments 6 – 30 µm; E) polyvinylchloride fragments 115 – 156 µm; F) polypropylene fibres 23 x 3000 µm (note the changing scale bars).

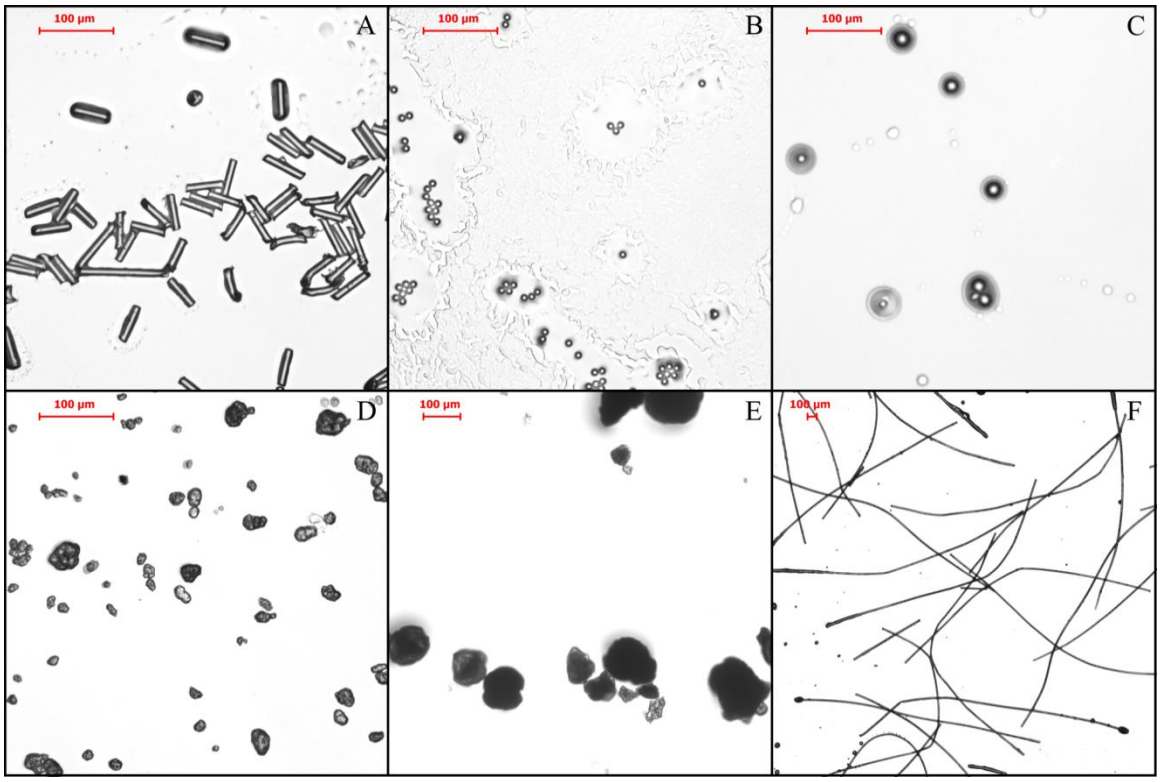

**Figure S2.**

Vertical Transport Chambers (VTCs) designed to create a head of water above the mussel so as to separate ‘sea surface’ from ‘sea floor’ and to inhibit drawdown of aggregates and microplastics by the mussels siphoning action.

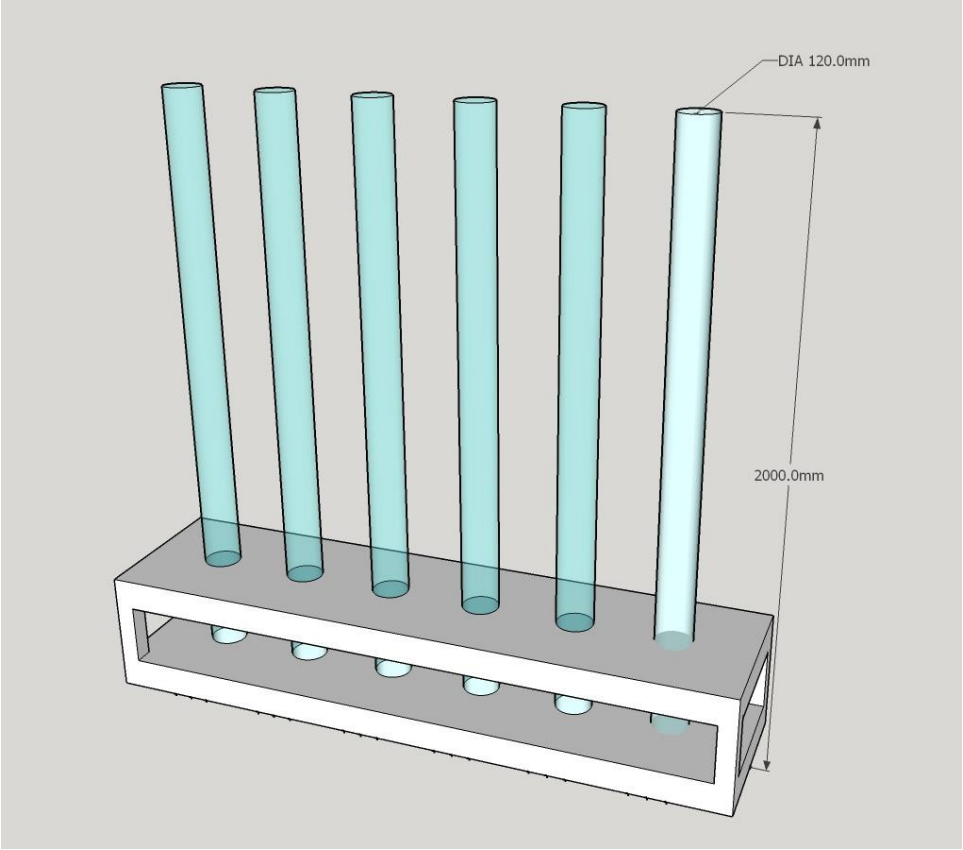

**Table S1.**
Microplastics used in marine snow incorporation experiments detailing their concentration, size, source, fluorescent signature and densities.

| | **Polystyrene Beads (PS)** | **Polyethylene Beads (PE)** | **Polyamide Fibres (PA)** | **Polypropylene Fibres (PP)** | **Polyamide Fragments (PA)** | **Polyvinylchloride Fragments (PVC)** |
|---|---|---|---|---|---|---|
| **Exposure Concentration (particles ml$^{-1}$)** | 50 | 50 | 50 | 0.1 | 50 | 50 |
| **Size (µm)** | 7 - 30 | 9 - 11 | 10 x 50 | 23 x 3000 | 6 - 30 | 115 - 156 (±1.88 - 2.84) |
| **Source** | Spherotech | Cospheric | Goodfellow | Goonvean | Laboratory Made | Laboratory Made |
| **Fluorescence** | 400−500 nm excitation, 450−550 nm emission | 365 nm excitation, 450-650 nm emission | Nile Red: 552 nm excitation, 636 nm emission | Not Required | Radglo EA-30: 465 nm excitation, 510 emission | Nile Red: 552 nm excitation, 636 nm emission |
| **Density (g cm$^{-3}$)** | 1.05 | 0.97 | 1.13 - 1.15 | 0.855 - 0.946 | 1.13 - 1.15 | 1.35 - 1.45 |

**Table S2.**

Microplastics found in benthic sediments and deep sea organisms. Plastic item units have been removed as they were inconsistent and data used to generate a proportional representation of polymers as found in Figure 3.

| Location | Depth (m) | Compartment | Size (µm) (max length) | Type | Polymer | Number | Reference |
|---|---|---|---|---|---|---|---|
| Equatorial Mid-Atlantic | 334 | Organism | Not Reported | Fibre | Modified Acrylic (1), Polypropylene (1) | 2 | 1 |
| Equatorial Mid-Atlantic | 611 | | | | Viscose (2), Polyester (1) | 3 | |
| SW Indian Ocean | 954 | | | | Viscose (1), Polyester (1) | 2 | |
| SW Indian Ocean | 1062 | | | | Acrylic (2), Nylon/Polyethylene (1), Polyester (1), Polypropylene (1) | 5 | |
| SW Indian Ocean | 1062 | | | | Modified Acrylic (1), Polyester (1) | 2 | |
| Rockall Trough, UK | 2200 | Organism | 23-6250 | 87% Fibres, 13% Fragments | Polyester | 7.83 | 40 |
| | | | | | Polyamide and Cellulose | 0.48 | |
| | | | | | Polyacrylonitrile | 0.18 | |
| | | | | | Alkyd resin | 19.7 | |
| | | | | | Polyamide | 0.9 | |
| | | | | | Acrylic and Cellulose | 0.48 | |
| | | | | | Polyethylene and Cellulose | 0.3 | |
| | | | | | Acrylic | 14.25 | |
| | | | | | Polyethylene | 0.9 | |

| Location | Depth (m) | Compartment | Size (µm) (max length) | Type | Polymer | Number | Reference |
|---|---|---|---|---|---|---|---|
| Venice Lagoon | 1 | Sediment | 42-445 | Not Reported | Polyethylene - 48.4% | 696.7 | 10 |
| | | | 15-1660 | | Polypropylene - 34.1% | 491.4 | |
| | | | 45-224 | | Poly(ethylene-propylene) - 5.2% | 74.5 | |
| | | | 15-2413 | | Polyester - 3.6% | 51.4 | |
| | | | 42-259 | | Polystyrene - 3.5% | 49.8 | |
| | | | 18-950 | | Polyacryylonitrile - 2.6% | 37 | |
| | | | 55-203 | | Alkyd resin - 1.4% | 20.5 | |
| | | | 60-163 | | Polyvinylchloride - 0.5% | 8 | |
| | | | 93 | | Polyvinyl alcohol - 0.4% | 6.1 | |
| | | | 715 | | Polyamide - 0.3% | 4.7 | |
| NE Atlantic Ocean | 1400 | Sediment | 2.4 - 14.5 | Fibre | Polyethylene (12), Cellulosic (3) (n=2) | 15 | 41 |
| Equatorial Atlantic Ocean | 2700 | | | | Polyethylene (13), Cellulosic (8), Acrylic (2), Modified Acrylic (2) | 25 | |
| Polish coast of the Baltic Sea | 11 - 400m | Sediment | 100 - 5000 | 86% Fibres, 7% Films, 7% Fragments | Poly(ethylene-propylene) - 12% | 10.8 | 11 |
| | | | | | Polyacrylonitrile - 13% | 11.7 | |
| | | | | | Polyvinyl Alcohol - 25% | 22.5 | |
| | | | | | Polyester - 50% | 45 | |
| Changjiang Estuary, China | Sublittoral 0-36m | Sediment | 46.8 to 4968.7 | 93% Fibres, 6% Fragments, 1% Pellets | Rayon - 63.1% | 359.67 | 12 |
| | | | | | Polyester - 18.5% | 105.45 | |
| | | | | | Acrylic - 13.9% | 79.23 | |
| | | | | | Poly(ethylene-propylene) - 1.5% | 8.55 | |
| | | | | | Polyethylene terephthalate - 1.5% | 8.55 | |
| | | | | | Polystyrene - 1.5% | 8.55 | |

| Location | Depth (m) | Compartment | Size (µm) (max length) | Type | Polymer | Number | Reference |
|---|---|---|---|---|---|---|---|
| Terra Nova Bay (Ross Sea, Antarctica) | 25-140m | Sediment | 300 to 22000 | 42.8% Fibres, 35% Film, 22.2% Fragments | Polyamide - 29.9% | 498.3 | 9 |
| | | | | | Polystyrene-butadiene-styrene 28.4% | 465.08 | |
| | | | | | Polystyrene - 1.9% | 33.22 | |
| | | | | | Thermoplastic Polyurethane - 20.5% | 348.81 | |
| | | | | | Ethylene-propylene rubber - 4.1% | 67.95 | |
| | | | | | Polyvinyl Alcohol - 0.1% | 1.51 | |
| | | | | | Polyvinylchloride - 6.8% | 113.25 | |
| | | | | | Polypropylene - 1.5% | 24.915 | |
| | | | | | Polyethylene - 6.5% | 107.965 | |
| Plym Estuary Entrance | 10m | Sediment | 80-5000 | Fibres | Polyester - 13% | 8.2 | 42 |
| | | | | | PET - 7% | 4.4 | |
| Plymouth Sound Breakwater | 10m | Sediment | 400-5000 | Fibres & Fragments | Polyester - 25% | 18.5 | |
| | | | | | Acrylic - 25% | 18.5 | |
| | | | | | Ethylene-propylene - 12.5% | 9.3 | |
| | | | | | Polypropylene - 12.5% | 12.5 | |
| Central Adriatic Sea | 7 - 119m | Sediment | 1000 - 30000 µm | Fibres 69.3% | Polyamide - 46.7% | 276 | 43 |
| | | | | | Polyethylene - 28.1% | 166 | |
| | | | | Fragments 16.4% | Polypropylene - 4.6% | 27 | |
| | | | | Films 14.3% | Thermoplastic polyurethane - 3.9% | 23 | |
| | | | | | Linear low-density polyethylene-octene - 2.7% | 16 | |

## 5.1 Chapter V: Sea urchins as bioeroders of plastics.


**Figure 1:** Sea urchin (*Paracentrotus lividus*) feeding on polyethylene mushroom tray and kelp (*Saccharina latissima*).

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Adam Porter and Katie Smith collected the Sea Urchins. Adam Porter designed the setup. Adam Porter and Katie Smith ran the experiment. Adam Porter analysed the data and wrote the manuscript.

Katie Smith, Brett Lyons and Ceri Lewis helped edit the manuscript.

## Abstract

It is increasingly recognised that plastic pollution of the marine environment is highly dynamic in nature. Larger plastic items are fragmented or eroded into smaller and smaller pieces as its moves through marine ecosystems and small particles can be fouled or flocculate into larger aggregates. Whilst physical processes play a major part in photo- and oxidative degradation of plastic debris, biological process may also contribute to the breakdown of larger plastic items into smaller particulates, yet this has not been studied well to date. Here, we demonstrate the potential for the sea urchin Paracentrotus lividus to act as bioeroders of macroplastics. We found that urchins readily graze on macroplastic surfaces, with this grazing activity generating microplastics, when held in experimental systems together. On average each urchin produced 172.9 (± 62.38 pieces) smaller plastic pieces (118 – 15797 µm) from one macroplastic item over a ten day period. This plastic fragmentation by the urchins grazing activity was strongly influenced by the additional availability of natural food and by the presence of fouling of the macroplastic surface. Fragmentation of macroplastic by urchins dropped by 97% when urchins were exposed to virgin plastic in the presence of natural food (kelp). However, when macroplastic was biofouled urchins acted to fragment this plastic irrespective of the presence of additional food. The majority of fragments produced were negatively buoyant due to both the biofouling process and indeed the fouling by faecal matter, sinking to the bottom of the exposure systems and this poses risks to a wider variety of organisms as these smaller fragments are in the bioavailable size range of a much wider suite of species than the original macroplastic item potentially transferring the fragments through the food web.

# Introduction

Plastic is acknowledged as a global contaminant affecting the majority, if not all, of marine and freshwater systems as well as the terrestrial landscape and even the air we breathe and water we drink (Wright and Kelly, 2017a, Wright and Kelly, 2017b, Mintenig et al., 2019, Oßmann et al., 2018, Pivokonsky et al., 2018). As a result, plastic pollution is currently receiving unprecedented attention in the media, from government, and society in calls to clean it up and yet its impacts on an ecosystem scale are still poorly understood (Koelmans et al., 2017, Burns and Boxall, 2018, Everaert et al., 2018). Plastics are found in a range of different shapes and sizes (Cole et al., 2011) and these are altered by both physical and biological process that influence shape, size and surface properties, all of which are thought to influence their fate and behaviour (de Sá et al., 2018, Thompson et al., 2009, Everaert et al., 2018). Plastic pollution has received considerable attention due to its visible nature; spoiling both the aesthetics of coastlines worldwide (Watts et al., 2016, Ryan and Jewitt, 1996) but also the broad size ranges that plastics are both manufactured at (from 600 m long gas pipes to microbeads in face washes) and broken down into, mean that the effects are far reaching. These effects can be seen in all size ranges of animals from entanglement of large organisms such as fur seals (Waluda and Staniland, 2013) and turtles (Duncan et al., 2017), to ingestion by sperm whales (Jacobsen et al., 2010), marine fishes (Lusher et al., 2013, Rummel et al., 2016) and worms (Van Cauwenberghe et al., 2015), to the translocation of tiny particles into the tissues of mussels (Browne et al., 2008).

The size of any plastic debris item will influence which species might accidentally ingest it or become entangled by it,and hence is likely to be an important factor determining risk (Betts, 2008, Karami, 2017). As plastic breaks down into smaller and

smaller fragments it will become more bioavailable to a wider range of smaller organisms (Wright et al., 2013) with plastic are now be recoded on a nano-scale (<1000 μm) in the environment (Ter Halle et al., 2017). Macro and microplastics, once in the marine environment will also start to biofoul; accumulating biofilms and encrusting organisms on their submerged surfaces. These processes will affect the hydrophobicity and buoyancy of plastic and once the density of the plastic exceeds that of the surrounding water it will sink (Kooi et al., 2017, Gregory, 2009). In the case of plastics denser than seawater, they will sink out to the benthos and become colonised at depth by benthic organisms. In a recent modelling exercise Koelmans et al. (2017) indicated that 99.8% of plastics that entered the marine environment between 1950 and 2016 had sunk below the ocean's surface. Hypothesised transport mechanisms taking plastics from the surface include biofouling (Kooi et al., 2017, Auta et al., 2017), faecal pellet transport (Cole et al., 2016), and via marine snows (Galloway et al., 2017, Porter et al., 2018, Zhao et al., 2018).

Microplastics are of concern because the smaller the fragments are, the more bioavailable they become to a wider range of aquatic organisms, which subsequently have the potential to cause harm. These microplastic particles have been shown to be ingested by very small zooplankton ingesting 500 – 800 μm sized microplastics (Desforges et al., 2015), 11% of mesopelagic fish (n=761) were found to have ingested plastic particles ranging from 500 μm to 11.7 mm (median 1.9 mm) (Lusher et al., 2015). Captive grey seals and their feed were investigated for microplastic ingestion by Nelms et al. (2018). They found that half of the seal scats produced by seals fed wild caught Atlantic mackerel (and one third of the mackerel) were found to contain microplastics ranging from 50 μm to 6000 μm; likely impacting more upon the fish than the seals. Therefore understanding the processes which influence the breakdown of larger plastic items into microplastic and the dynamics of these plastics as they move through marine ecosystems are key to understanding their impact.

Plastics are highly recalcitrant due to their high molecular weight and strong bonds within the polymer and are often designed to be highly durable and to resist degradation (Palmisano and Pettigrew, 1992, Zheng et al., 2005). As a result, they can persist for a long time resulting in a build-up in terrestrial and marine environments (Welden and Cowie, 2017, Fauziah et al., 2015). They are, however, susceptible to fragmentation that results in the creation of secondary microplastics; smaller pieces that are less than 5 mm in size (primary microplastics are those manufactured to <5 mm) (Arthur et al., 2009). This fracturing is mostly caused by photochemical degradation that results in embrittlement, creating cracks and ultimately degrading plastic items into many pieces (Welden and Cowie, 2017, Cózar et al., 2014). The degradation of plastic is however a complex process influenced by a suite of environmental factors when plastic enters the marine environment; light levels, the environmental compartment the plastic is situated in (beach, sea surface, deep sea), temperature, and the chemical and physical properties that affect the distribution of the plastic (density) and its persistence in the environment (polymer type, shape, and structure) which all affect degradation (Andrady, 2015, Phuong et al., 2016). As a result, degradation is highly dependent on location and environmental conditions (Welden and Cowie, 2017) with length of exposure to UV irradiation a key factor (Signor et al., 2003, O'Brine and Thompson, 2010) and thus at the very least degradation can be influenced by latitude.

The process of photodegredation increases the susceptibility of plastics to biofouling (Kerr and Cowling, 2003) and colonising organisms such as crustaceans (Davidson, 2012), polychaetes (Jang et al., 2018) and even microbes (Zettler et al., 2013) have been shown to further fragment plastics, in a process known as bioerosion. Bioerosion is where substrates and structures are broken down by living organisms and represents an important, landscape altering process as organisms remove inorganic

particles or weaken structures, directly or indirectly increasing the weathering of a surface (Pappalardo et al., 2018, Carter and Viles, 2005). Modern surfaces include biogenic (shells, wood and bones), abiogenic (rocks) and now anthropogenic structures, including plastic debris (Svane and Petersen, 2001).

Microbial degradation has been suggested as a potential mechanism for solving the global plastic pollution problem, allowing microbes to utilise the polymers as a source of carbon (Auta et al., 2017). For example, the fungi Aspergillus clavatus has been shown to degrade low density polyethylene (LDPE) films, resulting in a 35% weight loss after 90 days of incubation (Gajendiran et al., 2016). Similarly, Penicillium simplicissimum has been used to degrade HDPE with the fungus utilising intact polyethylene as its carbon source for growth (Yamada-Onodera et al., 2001), and Comamonas acidovorans is able to utilise polyurethane (PU) as its sole carbon and nitrogen source, breaking down this polymer also (Nakajima-Kambe et al., 1995). It has even been hypothesised that bacterial and fungal assemblages adapted to decomposing natural polymers in a salt marsh system may also secrete enzymes that degrade synthetic polymers (Weinstein et al., 2016). These are primarily terrestrial organisms but research on marine organisms that might be capable of doing this in the oceans is beginning. Zettler et al. (2013) demonstrated by rRNA gene sequencing that there are a number of microorganisms to be found on microplastic debris that are capable of hydrolysing plastics and may play a part in the breakdown of plastics. These micro-organismal biodegradation processes need to be seen in the wider context of the plastic pollution issue however, as plastics are dynamic in the ocean, driven by meteorological and oceanographic processes and by their physical properties which dictate their partitioning in the ocean and thus micro-organismal degradation will only play a part in the overall fragmentation of plastic debris..

Larger organisms too have been shown to breakdown plastics via their feeding and burrowing behaviour. A 1 x 1 m2 colony of boring crustaceans Sphaeroma quoianum (100,000 individuals) is estimated to produce up to 630 million polystyrene fragments during the boring process in colonising a polystyrene dock float (Davidson, 2012). The grazing of biofilms formed on plastics in marine and aquatic environments, by macro and micro-organisms, has also been suggested to play a part in the delamination and fragmentation of plastics resulting in microplastic formation (Rummel et al., 2017). Hodgson et al. (2018) demonstrated fragmentation of both clean and biofouled high density polyethylene (HDPE) plastic bags by the amphipod Orchestia gammarellus in its feeding behaviour. In their experiments biofouled plastic bags were shredded four-fold more than non-fouled bags suggesting that biofouling can alter the palatability of plastics and increase their ingestion. Seabirds (Order: Procellariiformes) also seem to favour biofouled plastics over non-fouled because the fouling organisms give off a particular scent related to zooplankton grazing, making the plastic particles smell like food and resulting in increased uptake (Savoca et al., 2016).

The transport of microplastics to the benthos whether in shallow or deep-sea environments is becoming of significant interest with all sizes and types of litter items being recorded on the seafloor (Maes et al., 2018, Pham et al., 2014, Taylor et al., 2016, Vianello et al., 2013) having sunk to the benthos or having been fouled or fragmented enough to sink. Large litter items may not present a threat to the majority of benthic species given their relative sizes but, once present on the seafloor, they can become aggregation devices; offering 3D structure in areas of little rugosity (Chiba et al., 2018) and therefore attracting organisms as the plastics foul and give off olfactory cues leading to benthic grazers encountering and grazing upon these plastic items.

Here we investigate a benthic sea urchin as a bioeroder of macroplastics leading to fragmentation in a variety of environmental scenarios to attempt to better understand how bio-fragmentation might occur in the marine environment and to start to understand the complex interrelationships plastics have once they enter the benthic realm. Sea urchins are keystone species in many benthic marine ecosystems; directing the structure of their communities, both as grazers and prey, and are economically valuable in fisheries (Pearse, 2006). In the same way that sea urchins are capable of grazing algae and altering the physical structure of their substrate in the natural environment we investigated if urchins had the ability to do so when set in the plastic pollution context.

## Methods

### Collection and maintenance of animals

Adult purple sea urchins (Paracentrotus lividus) were collected from Dunmanus Seafoods, Bantry, County Cork, Ireland and shipped to the University of Exeter in March 2017. The urchins were maintained in 500 L tanks on a recirculating system (35 ppt, 15 oC, artificial seawater [ASW]) for the duration of the experiment until allocated to an exposure tank. The urchins were fed by adding large pieces of the kelp, Saccharina latissima (collected from Shaldon, Devon, UK and frozen until used) ad libitum three times per week. The kelp was collected and frozen to kill off any epibionts and make feeding easier.

### Experiment 1: Does Sea Urchin Grazing Activity Produce Microplastics?

We first tested the hypothesis that urchin grazing activity would act to fragment plastics; turning macroplastics into microplastics. To test this, sea urchins were placed into aquarium tanks with a virgin (unfouled) plastic tray. We used polyethylene (PE) “mushroom trays” (Fig. 1), commonly used in the catering industry to simulate a large

macroplastic litter item. PE is a buoyant polymer (0.926–0.940 g cm-3 (Quinn et al., 2016)) affording an advantage in recovering any fragments created by the urchins, as if released unfouled by the urchin, they would be expected to float. Prior to experiments, mushroom trays were soaked in clean ASW for 48 h (virgin plastic) to allow the plastics both to soak clean, and for any external chemicals to leach off before the experiments started.

Three glass aquarium tanks were filled with 42 L of 0.2 µm filtered artificial seawater each (15oC, 35ppt) and three urchins added to each of the three tanks. One PE mushroom tray was placed into each tank and was weighted down using aquarium plant weights to ensure it remained negatively buoyant. Three urchins (average size 43.6 mm (±0.86 mm), average weight 35.08 g (±1.97 g)) were then transferred into each tank giving nine urchins in the initial treatment. The tanks were aerated continuously. Urchins were then left for 9 days with 3 water changes. Water changes were conducted by siphoning water from the top of the tank, being careful not to disturb the faecal detritus at the bottom, and all water was passed through a 20 µm mesh cable tied to the end of the siphon hose. Siphoning was halted as soon as the suction started to disturb the detritus at the bottom of the tank. At the end of day 9, urchins were transferred to individual 2 L beakers for 24 hrs to depurate. To determine whether sea urchins were producing microplastics via their grazing activity, and whether any microplastic produced became positively buoyant (floated) or became negatively buoyant due to fouling during the grazing process or by faecal pellet encasing and egestion, we then processed the water from the main exposure tanks collecting the water and detritus at the bottom of the tank separately.

**Experiment 2: Does the Presence of Natural Food influence Plastic Fragmentation.**

To determine whether bioerosion by sea urchins would happen under more natural scenarios where food would be present, we exposed adult sea urchins to different environmental treatments. Three treatments were used with an increasing level of complexity between each treatment. The three treatments were: (1) Kelp and macroplastic – sea urchins placed in a tank with virgin macroplastic PE trays and S. latissima. (2) Biofouled plastic – sea urchins placed in a tank with biofouled macroplastic trays and (3) Biofouled plastic and kelp – sea urchins placed in a tank with biofouled macroplastic trays and S. latissima to best simulate the environment in the laboratory. The setup and time points for these exposures were exactly the same as Experiment one described above, with three sea urchins per replicate tank. To foul the trays they were tied to a dock piling weighted at the low water mark in the Teign estuary (Devon, UK, 50° 37' 36.6276"N, 3° 26' 43.3536" W) for one month. The trays were fouled with barnacles, other small invertebrates and mainly green filamentous algae. The urchins were, as in Experiment 1, left for 9 days in their exposure tanks with 3 water changes undertaken. Those exposures that included kelp, the same amount of kelp was introduced alongside water changes and the old kelp removed and rinsed back in to the tank to ensure no plastics had adhered to the kelp.

**Seawater and Sea Urchin Analysis**

Following each experiment, the bulk of the water from each tank was filtered to 20 µm and the remaining water (a thin film left after pouring off water) and any faecal matter was then filtered over a separate 20 µm mesh. After 24 hrs depurating in the smaller beakers, the urchins were removed and frozen for gut contents analysis. The water from these depuration beakers was again filtered to 20 µm and any detritus and faecal pellets on the bottom of the beaker collected on another filter mesh. Urchins were dissected in a fume hood and their entire internal cavity flushed into individual 50 ml flacon tubes with 20% KOH and placed in the oven at 65oC for 48 hours to digest the biological material and the remaining liquid filtered onto a 20 µm mesh.

### Plastic Analysis

All filters for all partitions (water and detritus) under all conditions were then examined under a Nikon dissecting microscope for the number of plastic fragments and images taken to gather size and shape information. Plastic fragments collected from the seawater, faecal samples and gut content samples for all treatments were confirmed as plastic by ATR and μ-FTIR spectral analyses. Particles were analysed using a Perkin Elmer Spotlight 400 FT-IR Imaging System which has both ATR and μFT-IR functionality with a pixel resolution of 6.25 μm. The spectra produced of the plastic fragments collected were then compared to both spectra taken from the original plastic trays and from industry spectral libraries (Fig. S2). Fragments were then measured for the size and lengths using the Simple Interactive Object Extraction (SIOX) plugin in FIJI (a version of Image J with image analysis plugins included) (Schindelin et al., 2012, Rueden et al., 2017).

Whilst the urchins grew significantly in holding tanks throughout the experiment (increasing by 17.4 g on average over the experiment (One-way ANOVA, $F_{3,32} = 7.91$, $p = 0.001$)) there was no correlation between the urchin size and the plastic pieces produced by each urchin (One-way ANOVA, $F_{1,34} = 0.51$, $p = 0.479$) and the weight increases may have been compounded by the increased food availability throughout the study and therefore fullness of gut.

### Statistical analysis

Treatments were compared using analysis of variance in Minitab (Version 16 (Minitab Inc., 2003)) and a Tukey's post-hoc test used to identify means that are significantly different.

## Results

**Experiment 1: Does Sea Urchin Grazing Activity Produce Microplastics?**

All Paracentrotus lividus individuals from each of the three experimental tanks were observed grazing on the submerged clean polyethylene (PE) macroplastic trays throughout the experimental period. Following the 9 days experiment, microplastic were found to have been produced in all three replicate tanks, with an average of 257 pieces of plastic fragment (± 105.8 pieces) per tank present in the seawater (Fig. 2). This is equivalent to an average 85.67 microplastics per urchin over the 9 day tank exposure. During the subsequent 24 hour depuration period in clean seawater, the sea urchins further released an average of 109 pieces of plastic fragment (± 28.38 pieces) per tank (equivalent to 36.33 pieces per urchin). Dissection of the sea urchins at the end of this 24 hours depuration period revealed a further average of 55.67 (±40.22 pieces) still within the gut tissues of the urchins (Fig. 2). Hence, over 10 days (9 days in the main tank and 24 hrs in a depuration beaker) one urchin produced on average 172.9 (± 62.38 pieces) plastic pieces and in total our urchins produced 3024 pieces across all exposures and in all stages.

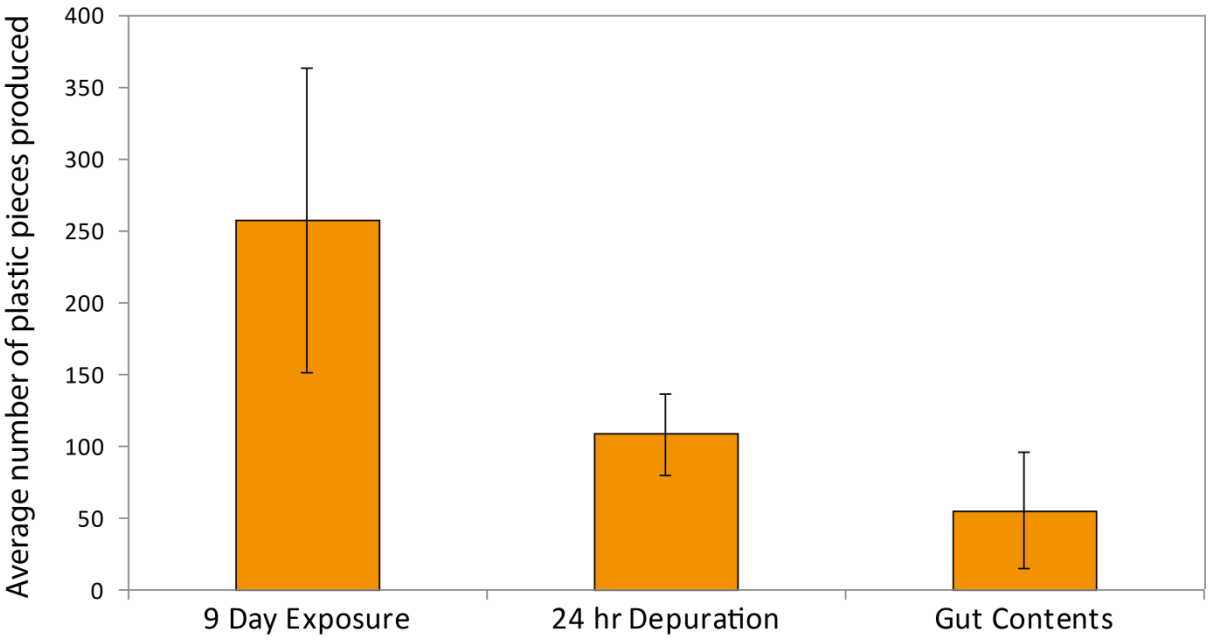



**Figure 2:** Average plastic fragments created per tank by the sea urchin *Paracentrotus lividus* when exposed to clean polyethylene plastic trays. Data as mean ± standard

error. Nine day exposure numbers were produced by three urchins in three tanks and an average made for the tanks (n=3). The number of microplastics generated during 24 hr depuration were gathered by placing an urchin in a beaker of filtered seawater and allowing the urchin to depurate for 24 hrs. Gut contents plastics were those removed from the urchin after depuration and both 24 hr depuration and gut contents data were summed according to the tank they came from and averaged accordingly (n=3) to make the data comparable.

**Experiment 2: Experiment 2: Does the Presence of Natural Food influence Plastic Fragmentation.**

When sea urchins were exposed to 'clean' PE trays in the presence of a natural food source in the form of kelp, microplastic could still be found in their tanks following the 9 days exposure. The number of plastic fragments generated by their grazing activity was lower than observed in the first experiment with no food added, with an average of 7 pieces (± 4.04 pieces) of plastic per tank generated over the 9 days (or 2.33 fragments per urchin) (Fig. 3a). During the subsequent depuration period in their individual beakers the sea urchins released an average of 1.11 pieces of plastic fragment per urchin (± 0.31 pieces) over 24 hrs (Fig. 3b). When dissected following the 24 hr depuration, only three of the nine urchins were found to contain any plastics at all within their guts, and those that did contained one microplastic fragment each (Fig. 3C), averaging 0.33 pieces per urchin (± 0.33 pieces). sea urchins in the presence of plastic trays that had been allowed to become naturally biofouled, but with no additional kelp, a total of 290 plastic fragments were recovered from the exposure tanks following the 9 day exposure, averaging 96.67 pieces (± 21.67 pieces) per tank (equivalent to 32.22 fragments per urchin) (Fig 3a). The sea urchins exposed to the biofouled trays only, depurated 202 plastic fragments over 24 hrs with an average of 22.44 fragments per urchin (± 5.21 pieces) (Fig. 3b). When dissected following the 24 hr depuration, 257 pieces were found in the guts of the sea urchins, with an average of 28.56 pieces (±12.42) per urchin (Fig. 3c).

In the final treatment with biofouled macroplastic trays and kelp present, urchins produced significantly more plastics than in the other two treatments (One-way ANOVA, $F_{2,6} = 24.30$, p-value = 0.001) producing a total of 976 pieces of plastic averaging 159.67 fragments (± 15.51 pieces) per tank (although over 9 days the differences between biofouled plastic and biofouled plastic with kelp was non-significant (Tukey's Post-Hoc Test) but both were significantly different from the clean plastic with kelp treatment) (Fig. 3a).

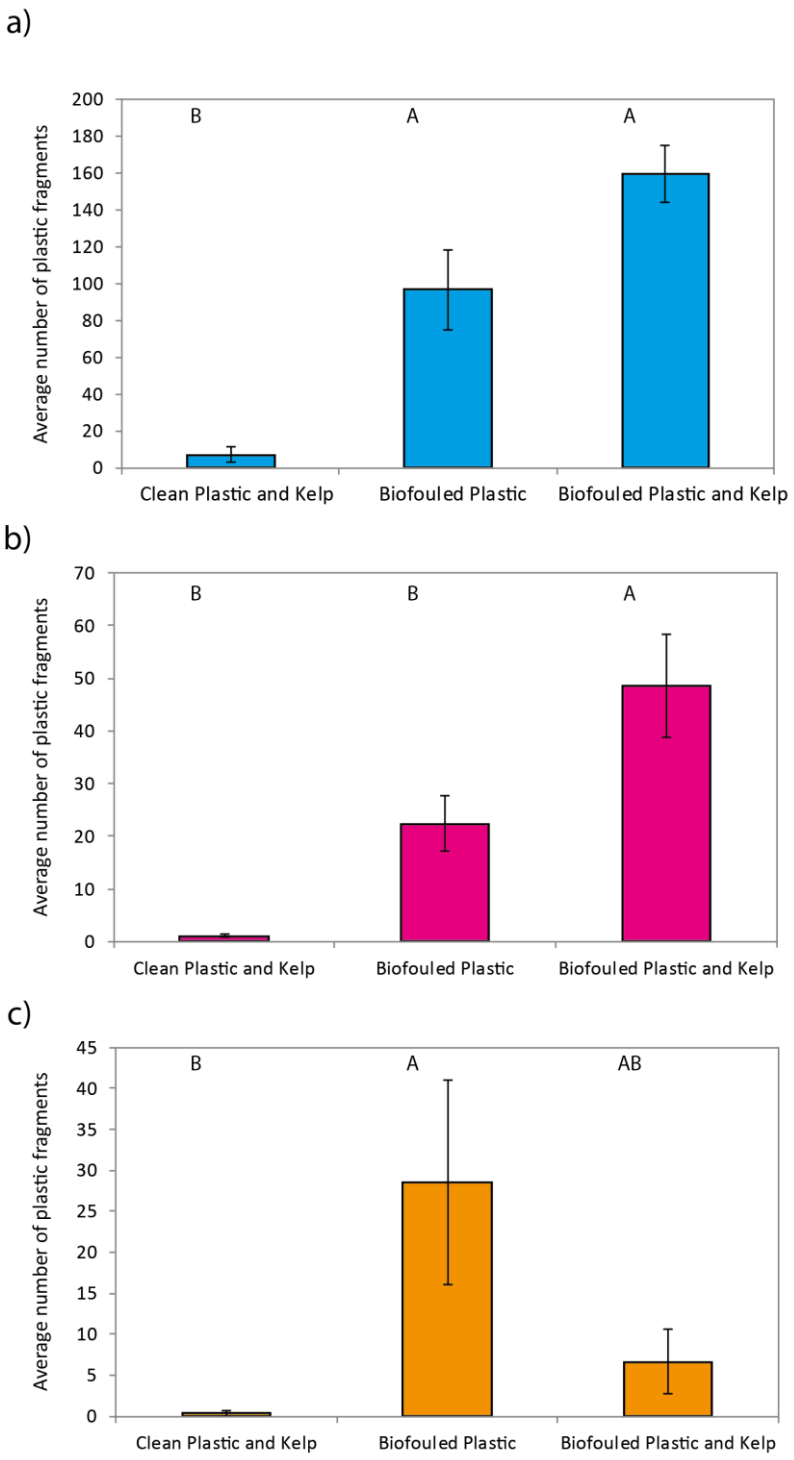



**Fig.3:** Amount of plastic fragments produced under the three treatment conditions. The plastic have been separated into fragments found in a) the tank over the 9 day exposure period, b) in the 24hr depuration beakers, and c) in the gut contents after 24hrs of depuration. Bars that do not share a letter are significantly different.

The plastic fragments produced by the grazing activity of the urchins comprised both meso- and micro-plastics sized fragments (Fig 4a-f), ranging from the smallest measured as 98.56 µm to the largest fragment of 15.8 mm (or 15,797 µm), averaging 1,024 µm (± 29.48) in maximum width (Feret's Diameter) (See Fig. S2 for full size distribution). There were small and relatively rounded fragments (Fig. 4) as well as large shredded fragments (Fig. 4 b, c and d). On the smaller fragments it was easy to identify characteristic bite patterns such as the double indented grooves on the left hand side of the fragment in Fig. 4c. The size of the fragments generated by the urchins differed between food treatments (Fig. 5). Urchins exposed to biofouled plastic with and without kelp generated significantly larger plastic fragments than the other treatments and those with clean plastic and kelp generated significantly smaller fragments (One-way ANOVA, $F_{3,776} = 9.85$, p-value = 0.031, Fig. 5). Fragments formed from biofouled plastic trays with kelp averaged 1145 µm (±49.25 µm) and biofouled trays without kelp averaged 1050 µm (±48.48 µm). Fragments created from the clean plastic trays with kelp had the smallest average fragment size of 444 µm (±63.13 µm), and the crates from the original proof of concept experiment had an average fragment size of 843 µm (±57.88 µm).

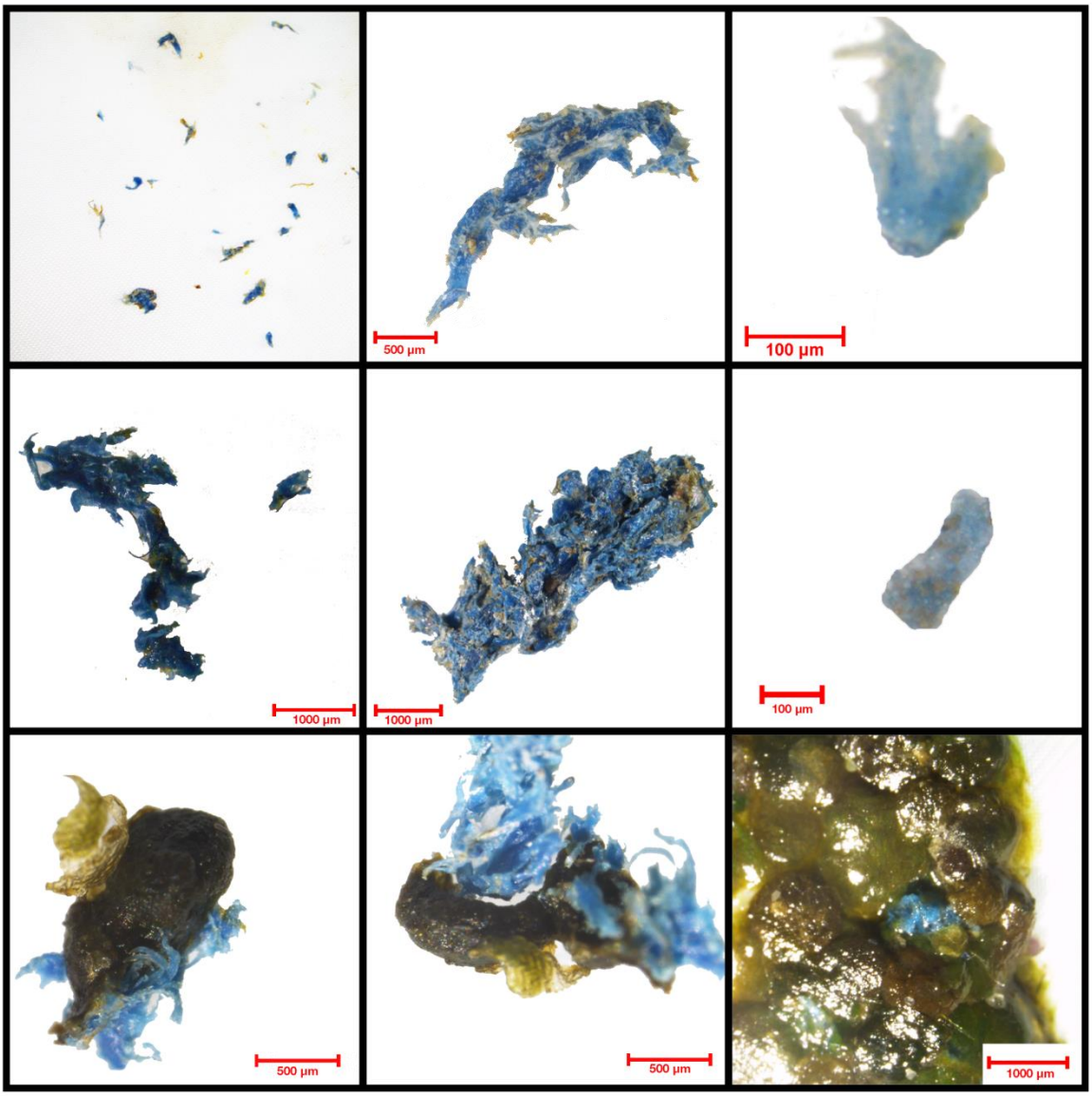



**Fig. 4:** A selection of fragments made from the plastic mushroom trays by sea urchins. A) Collection of fragments from one urchin. B – F) fragments of varying sizes and shapes produced by the sea urchins. G) Fragment protruding from a faecal pellet, H) Faecal pellet broken apart, I) Mass of faecal pellet with plastic fragment entrained in the mass.

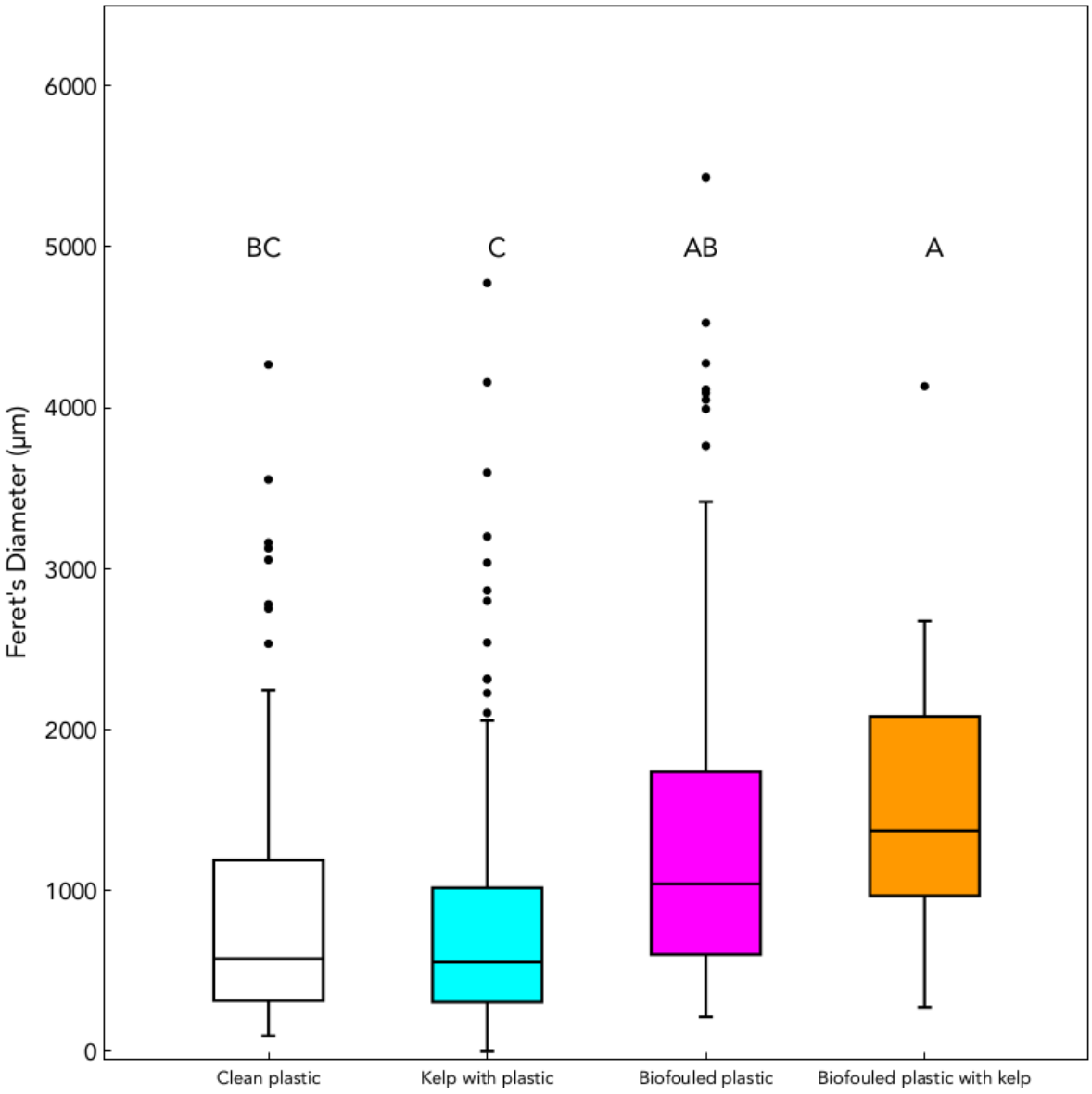



**Fig. 5:** Sizes of plastic fragmented generated by the sea urchins under the different treatments over 9 days. Urchins exposed to biofouled plastic with kelp generated significantly larger plastic fragments than the other treatments and those with clean plastic and kelp generated significantly smaller fragments (One-way ANOVA, $F_{3,776}$ = 9.85, *p*-value = 0.031). Bars that do not share a letter are significantly different from each other (Tukey's post-hoc test).

**Partitioning of Microplastics by Paracentrotus lividus.**

To investigate the partitioning of plastics caused by the urchins repackaging plastics into faecal material the number of particles created by urchins found floating on the surface of depuration beakers and the plastics entrained in faecal matter at the bottom of the depuration beakers were calculated. Fig. 6a shows the number of fragments released to the surface between our three treatments and Fig. 6b shows those found to be redistributed to the sea floor within our experiment. Urchins produced very few plastics in the clean plastic and kelp treatment overall and released no plastics to the surface and only 1.11 fragments (± 0.30 fragments) per urchin to the bottom of the beaker in faecal material. Under the biofouled plastic with kelp exposure, urchins released the most plastics to the surface out of the three experimental treatments. Urchins under this scenario released an average of 6.77 fragments (±2.33 fragments) per urchin to the surface of the beaker in 24 hrs. The urchins however also released over twice as much to the bottom of the beaker in the same 24 hr period releasing on average 15.66 fragments (±3.17 fragments) per urchin. Finally, the urchins exposed to the biofouled trays with kelp released an average of 4.56 fragments (±1.86 fragments) per urchin to the surface of the beaker in 24 hrs but released the most plastics to the bottom of the beaker, releasing 44 fragments (±9.79 fragments) per urchin.

Across all treatments 86.8% of fragments were released to the bottom of the beaker with 100% released in the clean plastic and kelp treatment, 70% in the biofouled tray with no kelp treatment, and 91% in the biofouled tray with kelp treatment.

In the floating fraction of plastics the treatments were significantly different from each other (One-way ANOVA, $F_{2,24} = 4.03$, p-value = 0.03) with a post-hoc Tukey's test identifying the clean plastic and kelp treatment and the biofouled plastic with no kelp being significantly different from each other (Fig. 6a see letters). In the faecal fraction the biofouled plastic with kelp treatment was significantly different from the other two treatments (One-way ANOVA, $F_{2,24} = 13.45$, p-value = 0.001) (Fig. 6b. see letters).

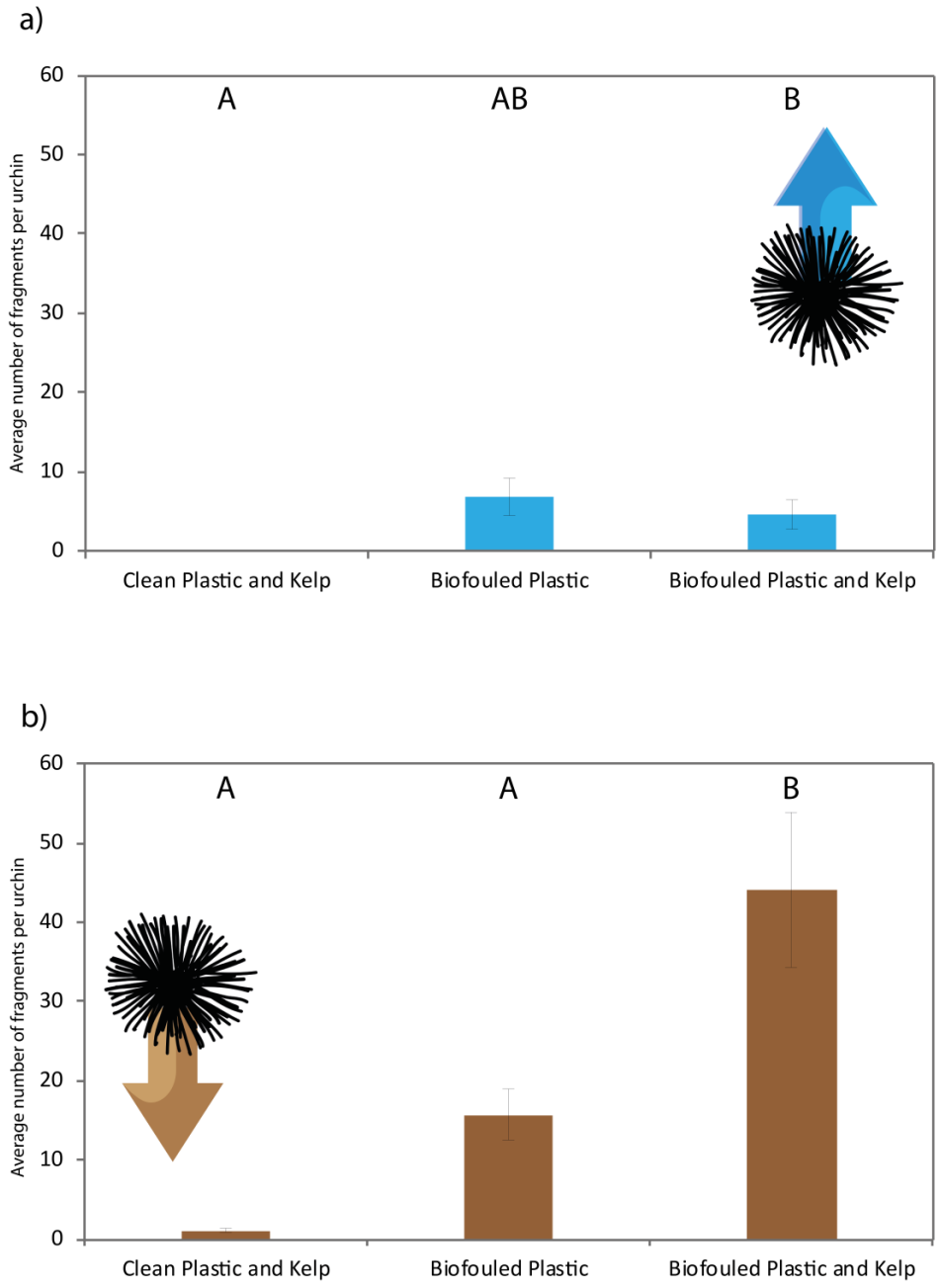



**Fig. 6:** Partitioning of plastic fragments by the sea urchin *Paracentrotus lividus*. After grazing on plastic trays for 9 days the urchins were placed into individual beakers to depurate for 24 hours. The water was filtered from the beaker after this time and the faecal pellets collected allowing us to look at how the urchin may be redistributing the plastic fragments it creates within the environment (n = 9). a) shows the number of plastic fragments that were recovered over the overlying water and b) shows the

number of plastic fragments recovered from the faecal detritus at the bottom of the beaker. Bars that do not share a letter are significantly different.

## Discussion

Our data clearly demonstrate that the sea urchin Paracentrotus lividus will readily graze on macroplastic surfaces under a range of different conditions and that their grazing activity results in the generation of microplastics. Over 10 days (9 days in the main tank and 24 hrs in a depuration beaker) one urchin produced on average 172.9 (± 62.38 pieces) plastic pieces and in total our urchins produced 3024 pieces across all exposures and in all stages. The presence of food significantly altered the number of plastic particles produced depending on the exposure scenario and biofouling increased the number of plastic fragments produced significantly (Fig. 3).

Hence, we propose that sea urchins have the potential to bioerode plastics when they encounter them, adding to the literature that feeding behaviours of benthic invertebrates can generate large amounts of plastic fragments.

Our sea urchin data is similar to that of Jang et al. (2018) who found that individuals of the burrowing polychaete Marphysa sanguinea found naturally inhabiting Expanded Polystyrene (EPS) buoys in the field contained small polystyrene pieces (average 131 ± 131 pieces) within their guts having generated these from the larger plastic item into which they had burrowed and could produce up to 1600 particles per day in laboratory exposure (Jang et al., 2018). Similar results have also been demonstrated for the boring crustacean, Sphaeroma quoianum, which was found to produce a highly variable number of microplastic particles ranging from a minimum of 89 particles per burrow for smaller individuals (i.e. from small burrows 1.6 mm long) to a maximum of

4630 particles from a burrow 17.4 mm long from a larger individual Davidson (2012). The author suggested that in the field (outside of experimental conditions) these crustaceans may produce as many as 4900 (±1.1) to 6300 (±2801) particles per isopod. The detritivore amphipod Orchestia gammarellus has also been demonstrated to produce microplastics when feeding on plastic debris producing 2.04 to 8.23 fragments per amphipod per day (Hodgson et al., 2018) (compared to our 17.29 pieces per urchin per day). These previous studies highlight that the difference between the laboratory and the field can be highly variable. The study by Davidson (2012) suggests that boring crustaceans will produce more plastics in the field than in the lab whereas Jang et al. (2018) show the opposite that polychaetes produce more plastic fragments under laboratory conditions than in the field.

All of these previous studies have, however, stopped short of a crucial part of the story; what happens when a natural food source is present? Organisms encountering plastics on the benthos under natural scenarios will be highly likely to have natural food sources available to them at the time. Plastics in the marine environment will quickly become; indeed biofilms of biopolymers and bacteria have been shown to form on surfaces within hours of marine exposure and within weeks develop algal fouling (Ye and Andrady, 1991). Buoyant plastics will, under certain scenarios, begin to sink having developed a specific gravity greater than seawater in 2 - 10 weeks (Ye and Andrady, 1991, Fazey and Ryan, 2016) and this coupled with the movement of negatively buoyant macro plastics will transport these large items (and indeed smaller microplastics (Kaiser et al., 2017)) to the benthos.

The gut retention times of a number of urchin species have been recorded as being between 8-40 hours and in Paracentrotus lividus 21-33 hrs (but up to 56 hrs) (Lawrence et al., 1989) and in the study by Lawrence et al. (1989) the addition of non-nutritious sawdust at a 1:1 ratio with food did not alter the feeding rate and so we assume at the outset that plastics have a similar affect. This, and the large deviations

from the mean highlights that the feeding rates of individuals were likely highly varied and that feeding is not a constant process for these urchins.

We found that plastic fragmentation by the urchins was strongly influenced by the additional availability of natural food and by the fouling of the macroplastic crates. First we provided the urchins with virgin plastic crates (i.e. unfouled) in the presence of their natural food in the form of kelp (Boudouresque and Verlaque, 2007). We found that whilst the urchins did still graze on the clean plastic crates when kelp was available for consumption, this was reduced by 97% compared to when no natural food was present. Hence, it appears that in the presence of a natural food source, the urchins choose only to graze on food items and disregard the trays, leading to incidental fragmentation and uptake only. This highlights the importance of incorporating natural food into microplastic feeding studies, as organisms will almost always have a choice of substrates and food sources in the marine environment.

When the plastic trays were allowed to foul naturally prior to their addition to the experimental tanks, but no additional food was included, urchins created an average of 96.67 pieces per tank. This is most likely due to the adherence of food items on the plastic of the biofouled tray. By biofouling, the palatability of the tray has effectively been altered, as seen in previous studies (Hodgson et al., 2018, Rummel et al., 2017), and the urchins therefore graze upon the trays as they endeavour to consume the biological material growing on the tray, generating and consuming plastic fragments in the process under the biofouled tray without kelp scenario. Importantly, in the final scenario where the plastic is biofouled and kelp is present, sea urchins still readily grazed the macroplastic trays producing the highest amount of microplastic per tank of the three food scenarios tested here.

Starving urchins have been shown to have longer gut residence times than continuously feeding echinoids as they have poor musculature and so require a constant influx of food and water to drive out that which has already been processed in the gut (Frantzis et al., 1993). This then may explain the significantly greater proportion of plastics in the guts of the biofouled plastic (with no kelp) fed urchins (Fig. 3c). This exposure had none of the preferred food source and only that which had fouled on to the tray, thus being somewhat nutrient limited. This is corroborated by the numbers of fragments found in the guts of the urchins under the initial plastic only exposure (on average 18.5 fragments per individual urchin (±9.51)) as these were higher than the biofouled plastic with kelp and clean plastic with kelp exposures.

Plastics were covered in varying forms of detritus dependant on the exposure scenario and the fraction from which the fragments were extracted. Those that were floating or taken from the initial clean plastic trial were relatively clean but once food was introduced plastic fragments could be found fouled with faecal matter or encased within individual faecal pellets (Fig. 4g-i). This is in contrast to Hodgson et al. (2018) who found no fragments within the faecal pellets of the amphipods studied, but rather in the egested fluid surrounding the pellets. The urchin generated fragments also had both a larger size range and were roughly twice as large as those made by amphipods meaning that the amount, and size of fragments being produced by the urchins, as well as their encapsulation into faecal matter would make them much more bioavailable to a lager range of organisms with potentially greater impacts given the size of some of the fragments.

Food selection can be complex in sea urchins and their feeding is largely driven by nutrient content (Tomas et al., 2015). If the food quality is low this may increase grazing activity (Rodríguez et al., 2018) which is likely what caused the large amount of

fragmentation in the initial clean plastic investigation. However, urchins also increase their grazing when nutritional content is increased and when there are a diversity of food sources (Jiménez-Ramos et al., 2018, Tomas et al., 2015, Rodríguez et al., 2018). This therefore may explain why the feeding on the biofouled trays with kelp received the greatest grazing (Fig. 3) as the olfactory cues, and mere food availability encouraged the urchins to graze at an increased rate compared to the other treatments. This likely also explains the size of fragments produced by the urchins in that at the greatest grazing pressure under the biofouled plastic with kelp scenario (understood here by the number of fragments produced; 479 fragments produced in total under the biofouled tray with kelp scenario) the greatest variety of fragments were produced.

Urchins generated more sinking particles than floating particles in all treatments, and we found significant impacts of the different food treatments on the number of floating particles produced such that the biofouled plastic with kelp treatment produced the greatest number of fragments and the clean plastic with kelp treatment produced the fewest fragments. For the sinking faecal fraction of particles produced, the biofouled plastic with kelp treatment was significantly different from the other two treatments. This is of significance as the plastic trays were made from polyethylene (which has a density of 0.926–0.940 g cm-3 (Quinn et al., 2016)) which is a buoyant polymer, however through the interaction with the urchin and the biofouling process the physical properties of the particles generated by the bioerosion have been altered and these fragments have become available to the benthos, coated in a film of, or even encapsulated within biological material.

Understanding the partitioning of microplastics between those floating on the sea surface or within the water column and the sinking particles that will reach the benthos is important for understanding the so called ‘missing plastics’ within the world’s oceans. The amount of plastic entering the environment every year (4-12 million metric tonnes

in 2010 (Jambeck et al., 2015)) does not tally with the amounts being found in the environment through sea surface trawls. Therefore there must be mechanisms, both biological and physical, at work that act to redistribute plastics and microplastics away from the sea surface and through the rest of the marine environment (Koelmans et al., 2017, Kooi et al., 2017, Porter et al., 2018). These mechanisms are key to a better understanding of both the fate of plastics within the ocean and their risk to the whole environment. Small plastic fragments generated by the sea urchins feeding on the macroplastic debris has the potential to be bioavailable for other marine organisms, depending on how these fragments partition between water and benthos, i.e. whether they sink or float once released. Hence any biological interaction with plastics that may change the shape or behaviour of plastic particles in the environment will likely influence the movement of plastics through marine ecosystems. Hence, in addition to determining whether the feeding behaviour of urchins acts to fragment larger plastic items into smaller ones, we also wanted to determine whether this fragmentation process might alter the partitioning of plastic within a water column, moving plastic particles either to the water's surface or towards the sea floor.

With the plastics made more palatable, broken down into small pieces, and released both into the water column and to the sediment there is then an increased potential for additional species to encounter and potentially ingesting these plastic fragments. The fragmenting action of bioeroders therefore has the potential to influence the distribution and palatability of plastic pollution. Bioeroders come in all shapes and sizes from microborers such as cyanobacteria and fungi to macroborers such as bivalves, worms, sponges and barnacles through to the grazers such as urchins and even fishes (Glynn and Manzello, 2015). Microboring organisms have been shown to generate between 18 and 30% of the sediment influx to the sea floor on Davies Reef, Australia through the boring and fragmentation of coral reefs there, producing 0.35kg m-2 y-1 of calcium carbonate sand (Tudhope and Risk, 1985). Boring crustaceans have been known to

destroy wooden research vessels and infrastructure (Scott, 1991), in 1965 estimates of marine borer damage by the U.S. Navy were approximated at $500 million dollars annually (Goodell et al., 2007), and damage has been seen in South African power supply services, Canadian Nuclear Power systems, UK gas mains and many other systems by microbial degradation . Furthermore boring organisms shape the marine landscapes which can effect biodiversity (both positively and negatively through controlling rugosity), control abiotic processes by altering the hydrodynamics of an area, and in breaking down rocks and other materials can release essential elements into the water to be available for other organisms (Davidson et al., 2018).

With the ever growing use of plastics in construction due to its lightweight, highly flexible, cheap and formable nature, instances of bioerosion damaging structures are likely to increase. In the plastics world they have damaged aquaculture systems (Davidson, 2012, Scott, 1991) and broken the cooling pipes for power plants (Jenner et al., 2003) however, bioerosion as shown above is a bigger process than the degradation of anthropogenic structures, it is a major structuring force in natural communities (Davidson et al., 2018) and occurs globally. So alongside these instances of the destruction of purpose built structures we can overlay the global plastic pollution problem and the ever increasing instances of organismal encounters with plastics in the environment as the rate of plastic inputs increases (Kühn et al., 2015, Jambeck et al., 2015, Everaert et al., 2018). Indeed a 50 fold increase is predicted from present day concentrations and despite a prediction that floating microplastics may pose little threat until beyond 2100 it has been suggested that adverse ecological effects will be felt in the benthic environment in the second half of the 21st century (Everaert et al., 2018).

It must be acknowledged here that urchins tend to have a small home range, moving 379.2 cm ±22.3 cm over three months in one study (Hereu, 2005) which may reduce the likelihood of them encountering benthic plastic debris often. However, whilst they

have a small range they feed at high intensity (Elmqvist et al., 2003). Urchins are responsible for barren seascapes when conditions (removal of predators, or the formation of fronts) allow them to remove the algal structures in an ecosystem quickly and completely and therefore it is conceivable, even likely, that urchins may encounter plastics in their environments and may choose to graze on them, especially as refuges provided by the 3D nature of most plastic products may also help attenuate predation (Sala et al., 1998). This may indeed play into the larger plastic pollution paradigm; that plastics may transport or provide a settling platform for biofouling organism; providing structure and food and therefore encouraging colonisation. This in turn would provide ample opportunity for urchins as well as other bioeroding organisms such as worms, polychaetes, and even fishes to fragment these artificial habitats due to their nutritional value and their acting as shelter. Other bioeroding organisms with larger ranges may also be part of this fragmentation process such as large grazing fishes (parrotfishes and surgeon fishes) and possibly even larger organisms such as green turtles although the grazing pressure they might exert is low as they travel large distances compared to small organisms and so may move on from a biofouled plastic item after a number of bites.

Our data highlights the potential of bioeroders to exacerbate the microplastic pollution problem and to not only ingest plastic particles themselves, but also potentially transform litter items too large for many organisms to ingest into more bioavailable microplastic fragments (Figs. 4 & 5). The effect of biofouling only seems to increase the rate of plastic fragment production (Fig. 3) and these fragments are on the whole retained in the benthic realm (Fig. 6). Furthermore we hope our results might encourage a more environmentally relevant approach to experimental design as our initial exposure using a clean plastic tray produced more fragments than any other treatment (non-significant) and yet was highly unrealistic and the addition of food effectively “switched off” the plastic fragmenting behaviour. Once the trays were fouled

however the urchins began producing plastic fragments again and interestingly the addition of food in this scenario (biofouled plastic with kelp) seemed to further enhance the biofragmentation. This is likely due to the fact that urchin grazing is controlled by predation, water flow and food availability (Hereu, 2005) and we altered one of these when fouling the trays.

Our work provides new insight into the dynamic nature of plastic pollution in marine ecosystems, demonstrating that biological processes will act to both alter the shape and size of plastic debris items but also influence its partitioning between water column and benthos. Our data also demonstrates the complexities of processes surrounding bioerosion in the marine environment; that food availability plays an important part in the interaction of species with plastics and that the fragmentation process of macroplastics by organisms may well be producing large amounts of microplastics and delivering them to the local environment in sizes that are bioavailable to a much larger range of species than just those that might eat the macroplastic item whole.

## Supporting Information

# Sea urchins as bioeroders of plastics.

This supporting information contains:

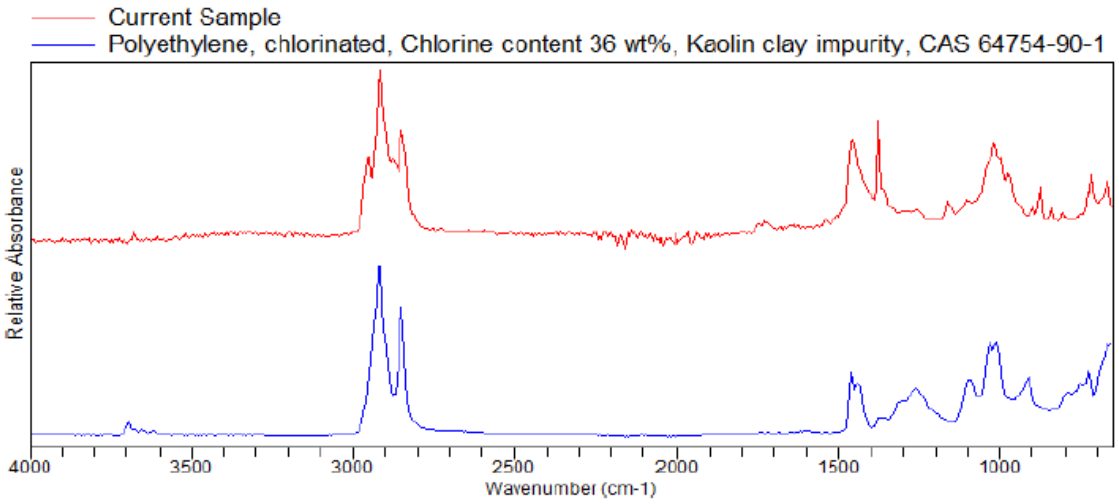
Additional Materials and Methods

**Fig S1:** An ATR FTIR Scan of the Blue Trays confirming them as polyethylene trays taken using a Agilent Cary 630

**Fig S2:** Design of the Vertical Transport Chambers (VTCs).

**Table S1:** Microplastics used in marine snow incorporation experiments detailing their concentration, size, source, fluorescent signature and densities.

**Table S2:** Microplastics found in benthic sediments and deep sea organisms.




| Quality | Library | CAS# | Name |
|---|---|---|---|
| 0.78444 | Agilent Polymer Handheld ATR Library (190) | | Blue Tray Urchin Exp - PE |
| 0.75847 | Agilent Polymer Handheld ATR Library (107) | | Polyethylene, chlorinated, Chlorine content 36 wt%, Kaolin clay impurity, CAS 64754-90-1 |

**Fig. S1** An ATR FTIR Scan of the Blue Trays confirming them as polyethylene trays taken using a Agilent Cary 630

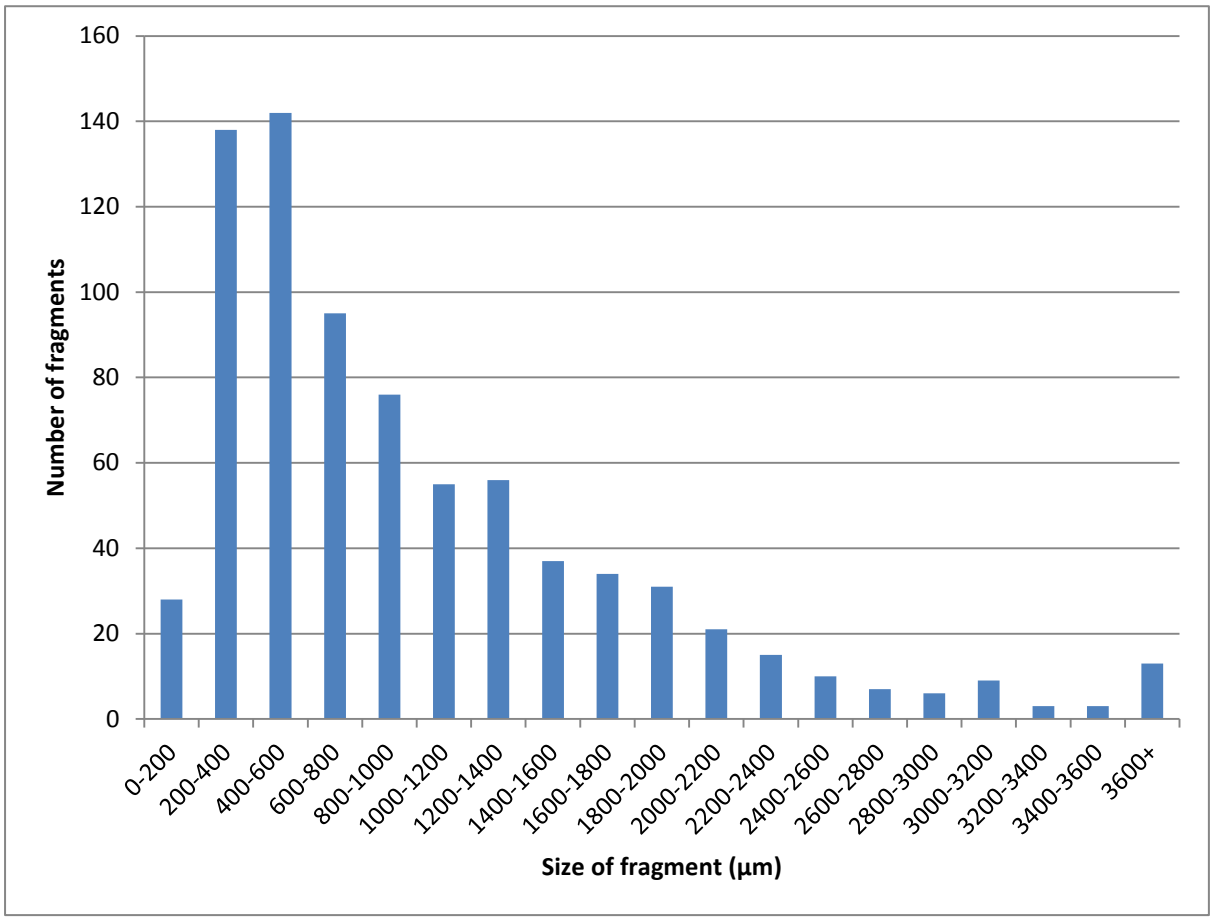



**Fig S2**. Size distribution of plastic fragments created by all urchins under all exposures.

# Chapter IV: General Discussion


**Figure 1:** A multidisciplinary team of scientists that I was fortunate to work with in the Galapagos during my PhD. This image summarises the broad scale approaches we need, the expertise required, but also how much I have enjoyed the last 4 years.

This thesis set out to understand the movement of plastics through marine ecosystems, what interactions microplastic might have with marine organisms, and what transformations might occur when plastics enter the oceans. The body of work I have generated for this thesis adds to the growing evidence surrounding the dynamic nature of plastic, revealing its variability over spatial and temporal scales (chapters 2 and 3), its vertical movement through the water column (chapters 4 and 5), and the potential for interactions with biota to alter its size, shape and distribution (chapters 4 and 5).

The spatial and temporal dynamics of plastic pollution are evident in the field-based components of my thesis in both beach macroplastic litter (Chapter 2) and plastic particles, including micro and macro litter collected from sea surface trawls in the North Atlantic (Chapter 3). Understanding spatial and temporal variability in plastic pollution is key to predictions of future contamination scenarios if plastic production and waste mismanagement continues at current rates (Jambeck et al., 2015, Koelmans et al., 2017a*,* Everaert et al., 2018). Our ability to determine the impacts of any mitigation strategy tackling the amount of plastic in our oceans similarly relies on these data and hence Chapter 2 and 3 provide further evidence to help support and direct future research and policy. In the 6 year data set for beach litter items found on beaches in the South West of England in Chapter 2, I revealed the variability in abundance, in time and space, of plastics in the beach environment. Significant variations were found between years in beach litter abundances across all sites, however, the increases in litter abundances were not contiguous. Litter was significantly lower in 2009 and 2010 than in 2006 however in 2011 the litter abundances had increased significantly once more, despite the beaches being cleaned monthly. Similarly the beaches did not experience a consistent spatial trend despite being in close proximity to each other (all 9 beaches lying within 60 km of coastline). The most northern beaches, in the Bude area, experienced a decrease in litter over time, the central beaches, in the Padstow area, experienced a marked increased over time and the southern beaches, in the Porthcothan area, experience no real change over time. This highlights the difficulties faced by managers attempt to deal with the problem of plastic pollution as its delivery to the shores of the UK is not uniform and will be driven by global ocean circulation processes as well as local actions by local individuals. A strong spatial and temporal variability was also evident in the data collected for Chapter 3. Interestingly, the spatial patterns of plastic distribution observed over these two cruises did not always conform to the current paradigms within the literature. I hypothesised that the shelf seas and oceanic islands we were visiting (The Azores and Canaries) would be hotspots of plastic compared to the open ocean and this was broadly born out to be the case.

However, in the 2014 cruise year the most contaminated samples were found in the Canaries (123 plastic items found) and in the 2015 year were found in the Azores (47 plastic items found (±14.45 pieces)). This again demonstrates both the dynamic nature of the pollutant but also that there are exceptions to the norm, both in the way that my plastic abundances were comparatively low in this region, but also that The Azores, hypothesised to be the most contaminated area due to its proximity to the NASG was not always the most contaminated meaning that there are other forcings on plastics to be considered.

The expected patterns of microplastic shapes in the ocean did not conform to what I first expected either. I hypothesised that fibres would be more abundant in coastal waters given their land based inputs through wastewater, their prevalence in the literature, and that fragments would be dominant in proximity to the North Atlantic Subtropical Gyre due to the accumulation and fragmentation of plastic over time. Fragments, in fact, dominated throughout the dataset making up 58.9% and 67.5% of the plastic items found in the 2014 and 2015 cruises and fibres only making up 30.7% and 20% for the 2014 and 2015 cruises. Our cellulose fibre counts were also low compared to the literature (e.g. Kanhai et al., 2017, Lusher et al. 2015b and Barrows et al., 2018) with only 19 cellulose fibres found in 2014 and 13 in 2015. The trends in the polymer types fitted with the idea that denser polymers sink out of the surface oceans before making it out into the open ocean and that buoyant polymers dominate the open oceans. Interestingly I hypothesised that the presence of denser polymer fibres may have been driven by the wind off the continental land mass, adding further evidence to the dynamic and complex nature of understanding the spatio-temporal dynamics of plastic pollution. We did not find any recognisable items in our samples (we did spot some bottles whilst under sail) and this mirrored the plastics found on beaches in Chapter 2. The majority of items found on the beaches were items which we could not attribute to source (46% of the total items) including plastic fragments and rope

fragments, alongside a variety of bottle tops. The fragmented nature of plastics highlights the important point made both in Chapter 2 and 3, and one discussed in the literature when considering management or mitigation of the problem, in that plastic waste must be stopped at source rather than collected and “cleaned-up”. Beach cleans are not a cost effective way of mitigating the problem and indeed Chapter 2 showed that the litter is continually restocked despite cleaning. These data add further weight to the calls for better waste management, and tighter controls on the production and use of single use plastics.

The data on macro and microplastics collected from my two field studies additionally highlights the range of shapes, size, and polymer types that comprised plastic pollution of the surface ocean and clearly demonstrate that microplastics are not one pollutant but in fact a complex mixture of items with different properties (Galloway et al., 2017). Chapter 2 identified 42,940 plastic fragments between 1 and 50 cm and 38,150 items smaller than 1 cm along with 41,011 pieces of plastic cord less than 50 cm (microplastic were not specifically sought out in their smaller sizes as the litter items were collected by hand and eye, picked off the beach) and these data compliment the data in Chapter 3 in recognising plastic contamination of the marine environment as a dynamic and complex mixture and highlight a number of important questions. Fundamentally important is our definition of what a microplastic is in terms of size and shape in particular (Hartmann et al., 2019). A few of my particles in Chapter 3, as well as fragments generated by my sea urchins in Chapter 5 fall above the range of a ‘microplastic’, defined as a particle <5mm, and this highlights the arbitrary nature of the definition given that larger particles can be found to behave in similar ways and potentially exerting similar risks to organisms in the oceans. Attributing definitions or broad classifications to plastic items contaminating the marine environment is difficult as it is profoundly affected by what question is being asked. As in Chapter 3 when considering what plankton might eat, we reduced our dataset to the largest size of

plastic item recorded within the zooplankton literature, 3.76 mm meaning that only 47% of our fibres and 39 % of our fragments were potentially ingestible by marine zooplankton (based on the available literature - Sun et al., 2018b). Attributing different items to source in Chapter 2 was similarly fraught with issues as various papers have assigned things like bottle tops and rope to tourists and fisherman respectively. I felt that this was a step too far as bottle tops can be lost as sea from fishing, or wash thousands of miles from their point of entry and thus blaming local tourism would not help support what we now understand, that the problem is a global waste management issue not a localised one. Similarly rope could be used in many industries and so to point the finger at the fishing industry is an attempt to pass the buck on dealing with the problem and distracts from the need for better regulation, and governance over our waste disposal and our generation of waste in the first place.

The laboratory based experiments for Chapters 4 and 5 then demonstrate mechanisms by which interactions with biota further add to the dynamic nature of plastic pollution. I demonstrated that microplastics can effectively be made 'larger' and less buoyant via interactions with particulate organic matter which led to an increase in their sinking rates and hence fate and behaviour within the water column (Chapter 4). I then demonstrated a mechanism by which the grazing activity of sea urchins generates both positively and negatively buoyant smaller microplastic fragments from a larger macroplastics item, again influencing the fate and behaviour of plastic pollution (Chapter 5). Urchins created 976 fragments even under environmentally relevant scenarios with biofouled plastics and when food was available, and this repackaging of plastics into faecal pellets changed the physical properties of the plastic polymer. Both of these mechanisms alter the relative size and behaviour of the plastic particle and can explain the benthic accumulation of plastic. Again this demonstrates the dynamic nature of plastic pollution, as it is also moving vertically through the oceans and is not just a sea surface problem. This fragmenting and then incorporation faecal matter

made a buoyant macroplastic tray accumulate as microplastic fragments on the bottom of the exposure system (on average 87% were retained on the bottom of the exposure tank). It also coated the plastic in organic matter which has been shown to make plastics more bioavailable (Hodgson et al. 2018). Similarly marine snows (Chapter 4) caused plastics to have their physical characteristics altered by the incorporation within organic matter derived from natural seawater. The sinking rates of all the tested microplastics increased when incorporated into snows, and importantly with large changes observed for the buoyant polymer polyethylene with an increase in sinking rate of 818 m $day^{-1}$ mirroring that of the capture and retention of microplastic fragments in the benthic realm by the fragmentation by sea urchins and encapsulation in organic matter.

Since publishing this work this idea that marine snows transport plastics downwards has further been supported by newly published field based measurements of plastic in marine snow from other researchers (Zhao et al., 2018) and the growing body of literature demonstrating both buoyant and negatively buoyant polymers in benthic sediment and organisms. Marine snow formation will always be highest in productive areas of the ocean (Turner, 2015) and therefore the ideas explored in Chapter 3; looking for areas where high abundance of plastic and plankton co-occur will also be areas where the marine snow pathway is strongest. Hence, these areas are where microplastics are most likely to be incorporated into marine snow and transported to the benthos. In 2014, Cozar et al. identified a missing fraction of plastics from sea surface trawls, a fraction that should have been present according to fragmentation models. He hypothesised that there must be mechanisms transporting floating plastics away from the sea surface. Cole et al. in 2016 demonstrated that zooplankton can excrete microplastic in faecal pellets, packaging plastics into detrital material and causing it to sink rapidly to the benthos. My work fills in the identified gap from Cozar et al. (2014) and builds on Cole et al. (2016) as faecal pellets are part of the marine snow

system. This work has therefore filled in missing links in our understanding of the vertical transport dynamics of microplastic, built on previous work, and identified a viable, globally occurring mass transport mechanism for the downward transport of microplastics as hypothesised by Cozar et al. (2014), Ward and Kach (2009) and Zhao et al. (2017) amongst others. This study supports the latest modelling data showing that 99% of all plastics will eventually end up in the benthic realm (Koelmans et al., 2017b) as the marine snows help overcome the physical characteristics of plastic particles (primarily density) which ordinarily would keep them at the ocean surface (46% of polymers are considered buoyant (U.S. Environmental Protection Agency, 2012)).

Not only did being incorporated into marine snow increase the sinking rates of these plastic particles, but also their uptake into a marine benthic filter feeder. Uptake increased in mussels, where uptake increased from zero particles (in the case of buoyant polymers) to 340 microplastics individual$^{-1}$ for free floating microplastics to up to $1.6 \times 10^5$ microplastics individual$^{-1}$ when incorporated into snows. Similarly, in Chapter 5 the sea urchins released fragmented microplastics generated by the urchin grazing activity to the benthos by incorporating the fragments into faecal matter which might also be predicted to influence subsequent uptake by other organisms, but was not tested here. Hence these chapters also address an important question as yet rarely touched on in laboratory studies, that of the changing palatability of plastics when they come into aggregation with organic matter, potentially increasing the likelihood of ingestion as the plastics may well be sought out as food, with the plastic masked by edible detritus. This idea that that surface coatings of plastic particles may play in influencing the outcomes of encounters between plastics and biota is an emerging area of research (Galloway et al., 2017).

It has been shown that once plastic particles enter the marine environment they will begin to biofoul rapidly, developing biofilms within hours of marine exposure and within

weeks develop algal fouling (Ye and Andrady, 1991). Buoyant plastics will, under certain scenarios, begin to sink in 2 - 10 weeks (Ye and Andrady, 1991, Fazey and Ryan, 2016) and so coating plays an important part in the distribution story but also in the way organisms may interact with plastics. These coatings have been shown to increase the palatability of marine plastics, for example Hodgson et al. (2018) showed a four-fold increase of shredding of biofouled bags by the amphipod *Orchestia gammarellus* in its feeding behaviour compared to clean bags. Savoca et al. (2016) have also suggested that marine related seabirds may preferentially ingest biofouled plastics over non-fouled plastics as the fouling can elicit olfactory cues for food. Perhaps more importantly it's also been shown to influence the uptake and retention of plastic particles once ingested. This has been demonstrated by Cole and Galloway (2015) showing a significant increase in the uptake and retention of aminated particles (those coated with amino acids) compared to 'clean' microplastic.

A key finding of Chapter 5 was the role of natural food in determining the selectivity of the sea urchins when it comes to their interactions with or ingestion of plastics. The addition of food dramatically altered the urchins interactions with the plastic as when the choice between clean plastic or kelp was presented they fed on the kelp with plastic fragment production dropping significantly from 771 fragments produced when no food was present to only 21 fragments produced over 9 days by 9 urchins. This poses the question of whether organisms really will encounter, interact, and possibly ingest plastics in the real world when their preferred food source may well be available. However, in taking this a step further by fouling the plastic crates, the number of fragments produced by the sea urchins was significantly affected. It is important therefore to think about the question being asked in microplastic science as if we are measuring interaction of uptake it is important to think about the biology and ecology of the organism being exposed as well as the environment within which they find themselves as these will have implications for the likelihood of interaction (as in

Chapter 3 when considering the bioavailable fraction of plastics) which, as previously stated, will help steer our understanding of the risk plastics posed to individual organisms and whole ecosystems.

The movement of microplastics through marine ecosystems is a complex pathway forced not only by the physical processes at work in the seas and oceans but also by the broad spectrum of properties the microplastic contaminant class encompasses and by the organismal interactions with those plastics altering their fate. Plastics are highly spatio-temporally variable and it has been shown that the abundance of plastic can vary significantly (3 orders of magnitude) between sample sites in close proximity within a 24 hour period (Law et al., 2014). Plastic pollution is not just a sea surface problem as roughly half of the polymers produced sink in seawater and the benthos is therefore affected. Woodall et al. (2014) demonstrated fibre concentrations of $4 \times 10^9$ fibres $km^{-2}$ in the deep sea and buoyant polymers are being found in the benthic environment and within deep sea organisms (Taylor et al., 2016, Woodall et al., 2015). The distribution of microplastics is not constrained only by their physiochemical properties, but by organismal interactions through biofouling (Peter, 2015, Fazey and Ryan, 2016), incorporation into faecal matter and marine snows causing vertical transport (Cole et al., 2016, Porter et al., 2018), oceanographic processes such as upwelling, salinity, water temperature and wind (Kanhai et al., 2017, Desforges et al., 2014). Atmospheric deposition is even a factor that can confuse trends of plastics as the leave land and move towards the ocean gyres (Enders et al., 2015). In this thesis I demonstrate some of these processes both through laboratory study and field sampling and highlight the dynamic and complex nature of microplastic as a pollutant.

In this thesis I also demonstrate how incorporation into marine snows and biofouling processes can significantly increase the uptake of microplastic particles into marine

biota. This makes microplastics much more complex that dissolved pollutants in the water as not only are they non-uniform in their distribution but they are also always changing in size and shape, and undergoing transformations as they become not one particle but a particle with inherent chemicals, and attached organisms. This concept of an 'ecocorona' suggests that microplastic may well keep a record of its environmental progress through ocean ecosystems and when interacting with organisms (primarily through ingestion) may not behave as expected (Galloway et al., 2017).

The heterogeneous distribution, forms, and characteristics of microplastics create a diverse pollutant and their movements and transformations will affect the risk plastics pose to the marine environment. Risk is likely to be the next horizon for plastic science given the aforementioned variability in all drivers and descriptors of plastic and in this thesis I have explored how environmental transformations might influence the risk of plastics to marine organisms (especially in Chapters 3 and 4). It should now be recognised that to assess only one characteristic of a plastic and its impact on an organism is not very relevant (Koelmans et al., 2017a) and we can use what we already know about microplastics in the ocean to frame future sampling efforts and experiments in terms of likelihood of ingestion or impact on a marine organisms or ecosystem. The identification of co-occurrence areas identified in this thesis (Chapter 2) is paramount to this work as it is in these areas that we are most likely to be able to see an impact of plastic pollution in environmental samples, if indeed one exists at all. The tuning of laboratory exposure to environmentally relevant scenarios is key (as demonstrated in Chapter 3 and 4) as building experiments that reflect real processes in the ocean may in fact *increase* the risk of plastics at environmentally relevant concentrations as opposed to single particle exposures at high concentrations (Burns and Boxall, 2018), designed to find effect thresholds.

This thesis demonstrates the dynamic nature of plastics and follows their movement from source to sink via oceanographic distribution, biological interactions, and transformations that create new paradigms in our understanding of plastics in the ocean. The data herein reinforce the complex nature of plastic as a pollutant and demonstrate that microplastics are not just one pollutant but encompass a range of pollutants that exert different risks to different ecosystems and organisms. Future work should seek to explore these models both looking to see if they hold true in the marine environment (in the case of Chapters 3 and 4) and to seek to better understand the dynamics of distribution, co-occurrence, encounter rate and ultimately risk to the health of our seas and oceans.

I would also hope that this thesis might provide some solutions to the plastic pollution problem. It would overwhelmingly seem that once plastic items enter the marine environment they are subsequently to innumerable interactions, modifications, and transformations. As in Chapters 4 and 5 once plastics are in the ocean they become much more dynamic as there are physical, chemical, and in the case of this thesis, biological interactions that will start to occur almost immediately. Chapters 2 and 3 highlight the myriad of shapes, sizes, and types of plastic items in the marine environment, which would all have, at some point, been a recognisable item with a purpose and an owner. It is therefore apparent that if we allow plastic to enter the marine environment it is effectively out of our control and releases a whole new force into the marine environment allowing habitat expansion, altering competition, and of course, being ingested potentially causing harm. Therefore it is paramount that we as a society: at the individual, national, and international level work together across all sectors (as in Figure 1) to understand our own connection with our waste, transform our thinking around how to deal with it, engineer new products that better suit our purposes and think about the end of life for products and not just the start. To pursue such things as biodegradable plastic does acknowledge that we might never be able to stop litter entering the marine environment and falls short, in my mind of a total solution

as it should be within our grasp to stop generating such quantities of disposable material, but also to contain, manage, recycle or repurpose items to secure a better future for our planet.

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**To all those who know…you know**