Assessing the potential for trophic transfer of microplastics through the Thames food web

By

Alexandra R. McGoran

Thesis submitted in fulfilment of the requirements of a Doctor of Philosophy.

20th December 2021

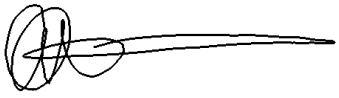
Revisions submitted December 2022

School of Life Science and the Environment, Department of Biological Sciences, Royal Holloway, University of London

Life Sciences, Natural History Museum, London

London NERC DTP, Cohort 4

I, Alexandra R. McGoran, hereby declare that this thesis and the work presented in it is entirely my own. Where I have consulted the work of others, this is always clearly stated.

Signed: 

Date: 20th December 2021

Contents

List of tables and figures

Figure 6.13. Length of particles divided by size fractions between trophic levels, where trophic level 1 is *Corophium volutator* and *Hediste diversicolor*, level 2 is *Crangon crangon*, level 3 is Solea solea and Platichthys flesus, level 4 is *Merlangius merlangus* and *Trisopterus luscus* and level 5 is *Halichoerus grypus and Phocoena phocoena*. **163**

Acknowledgements

There are many, many people who need to be acknowledged for their support over the past four years. I hope these acknowledgements go some way to thanking you all for what has been some of the best experiences of my life!

I would like to thank my supervisors Prof Dave Morritt and Dr Paul Clark for their tremendous support and advice during my PhD; their guidance and encouragement made this thesis possible. I would also like to thank my co-supervisor Dr Helene Burningham, whose insight allowed me to research sediment cores. Unfortunately, this line of research was set aside due to time constraints, but the field work was a valuable experience which I will take with me going forward. I am incredibly grateful to Dr Eleanor Adamson and the Fishmongers' Company who have supported this project both financially and by sharing their many connections. Speaking to the Company's members and joining their networking events has been both rewarding and enjoyable. Thank you to Dave Pearce for assisting with access to field sites. Thank you to the Port of London Authority (PLA), Environment Agency (EA) and Marine Management Organisation (MMO) for granting permissions to trawl in the Thames. Thank you to Rod Bingham and Ben Ferris of the *Boy Daniel* SD4 for collecting samples for the present thesis. All my field work and a lot of my lab work would not have been possible without the incredible support of Brian Smith. Thank you for always answering my stupid questions, for joining every field session and making sure my samples arrived safely at the Museum the following day and for always being there to listen to my rants when things hadn't turned out quite right. Without you, there would have been no top predators in this food chain analysis, you made this PhD what it is. To everyone who helped with the seal and porpoise dissections, Andrew Baillie, Ollie Crimmen, Dr Natalia Fraija, Roberto Portela Miguez, Dr Anne-Claire Fabre, Dr Travis Park: thank you. Additional thanks to Ollie and Natalia. Ollie, you came to the Thames with me and helped me identify fish remains from the seal stomachs. Natalia, you walked me through the first seal dissection which allowed me to take the lead in the next dissection. Thank you to Jacqueline Mackenzie-Dodds and everyone in the Molecular Collections Facility – Dr Fiona Allan, Kirsty Lloyd, Muriel Rabone, Heather Allen - you let me take over a section of your lab and office space and were always ready to help when I needed it. Thank you to Dr Wren Montgomery, without whom FTIR analysis would not have been possible. You answered my questions even on your days off and went out of

your way to ensure I could meet a tight deadline when I was moving house. Thank you to Dr Emma Humphreys-Williams, Dr Stanislav Strekopytov and Mark Underhill for giving me access and training on FTIR. Thank you to Dr Eileen Cox for always supporting PhD students during Doctoral Training Partnership training and at the Museum. A huge thank you to James Maclaine for trusting me with some amazing mesopelagic specimens that, whilst not part of this thesis, is one of the most memorable experiences of my PhD. Thank you to Dr Ralf Britz for helping me clear and stain specimens and answering my questions about fish anatomy. Thank you to Dr Michael Spence and Dr Christopher Lynam (Cefas) for their assistance with the EcoSIM models. Thanks to Luis Moliner Cachazo for identifying a damselfly nymph in the diet of one of my fish simply using an image on Twitter. Thank you to the microplastics Twitter community for helping when I was stuck or just needed to discuss an idea through. Thank you to the press offices, communications team and learning programme at Natural History Museum and Royal Holloway University of London for giving me opportunities that made this time so memorable. Lastly and probably most importantly, thank you to my support network and my family, especially Daniel Brooks and Danny Hodgson. You kept me going even when the PhD was tough or the situation seemed impossible. You believed in me and talked me through all my problems. Thank you!

This thesis was funded by Natural Environment Research Council (NERC), as part of the London NERC Doctoral Training Partnership (Grant Number NE/L002485/1), and a case partnership with Fishmongers' Company. This research would not have been possible without their contributions. Thank you.

Lastly, I would like to thank Dr Amy Lusher (NIVA) and Dr Rudiger Riesch (RHUL) for reviewing this thesis as the external and internal examiner, respectively. The quality of the document greatly increased due to their feedback. Thank you!

Author declaration

I, Alexandra R. McGoran, declare that I am the sole author the work contained in this thesis and that I performed all the research myself. This thesis is submitted to Royal Holloway, University of London for the award of PhD in biological sciences and none of this work has been submitted to any other degree. Chapter 5 of this thesis has been published in a peer-reviewed academic journal. I am the lead author of this paper and all contributions from my co-authors are included at the start of the chapter. Chapter 6 contains mention of samples collected during my Masters at Royal Holloway University of London, however, the research is novel to this thesis.

Abstract

Monitoring of plastic pollution focuses on macroplastics (>2.5 cm), with microplastics (<5 mm) only quantified in ad-hoc research studies. When microplastics are studied, it is only in flagship species and little is known about food web interactions with microplastics. Using the River Thames as a case study, this thesis establishes an ecosystem-wide assessment of macro- and microplastics. Such an approach has yet to be utilised elsewhere. This thesis provides evidence for the trophic transfer of microplastics and, through examination of macroplastics, highlights potential sources of microplastics. Furthermore, the present research demonstrates that macroplastic monitoring alone is insufficient to identify all major sources of plastic in the environment. Macroplastic is dominated by single-use products, in particular food packaging with rainfall contributing to inputs of sewage-related debris. Microplastic concentrations in sediment range from 0.2 to 2.6 particles per gram, with fibres being most abundant both in biota and sediment. Microplastics are readily ingested, with marine mammals as top predators (e.g., *Halichoerus grypus* and *Phocoena phoceona*) consuming the most (102 items on average). When organism size is accounted for, however, *Corophium volutator* and *Hediste diversicolor* ingests higher concentrations. Therefore, benthic infauna are exposed to the greatest microplastic concentrations. *Eriocheir sinensis* (Chinese mitten crab) are also highly contaminated, accumulating large tangles of plastic in the gastric mill. Over 90% of individuals contain tangles, many of which were made of over 100 plastic fibres. Microplastic colour and polymer type varies between the environment and biota with a greater proportion of coloured fibres and cellulosic particles present in the digestive tract. The results suggest that microplastics can be transferred to predators, but biomagnification is unlikely. The evidence provided supports the use of an ecosystem approach to plastic monitoring in addition to the use of model species and can provide insight into products posing the greatest risk. This evidence can be used to influence policy, product design and disposal.

Chapter 1

Background

1.1 Terminology

Plastic litter is typically divided into four main fractions based on size: macroplastic, mesoplastic, microplastic and nanoplastic. There are no strict guidelines on what size limits apply to these categories. Macroplastics are typically >25 mm with mesoplastics occupying the size fraction between microplastics and macroplastics. But the definition of the term microplastics has been controversial, leading to differing definitions of the term (Verschoor, 2015). Originally microplastics were categorised as all items smaller than 5 mm, which makes mesoplastics between 5 mm and 25 mm in length. The upper limit of 5 mm was selected so that nurdles were included in analysis of microplastics. This definition is the most widely used and is supported by the National Oceanic and Atmospheric Administration (NOAA) (Arthur et al., 2009) and the EU Marine Strategy Framework Directive (MSFD) Technical Subgroup on Marine Litter (Hanke et al., 2013). Since then, it has been argued that microplastics should only include plastic on a microscale (< 1 mm). Prior to the development of a nanoplastic category, no lower limit for microplastics was defined (Masura et al., 2015; Verschoor, 2015; Rochman et al., 2019). Instead reporting varied with sampling technique between publications. Hanke et al. (2013) proposed a lower limit of 20 µm, whilst GESAMP (2015) proposed 1 nm. Typically, identification of microplastics smaller than 100 µm is limited, with 20 µm being the around lower limit of identification for micro Fourier Transform Infrared (µ-FTIR) spectrometers. Nanoplastics are now considered to be all plastic particles smaller than 1 nm, which makes this the accepted lower limit of microplastics. It is, however, still vital to report the lower limit of detection to allow the accurate comparison between studies. Some organisations further divide these categories with the MSFD Technical Subgroup on Marine Litter recommending the following limits. Small microplastics: 0.020–1 mm, large microplastics: 1–5 mm, mesoplastics: 5–25 mm, with each group being divided into 100 µm intervals (Hanke et al., 2013). For the present thesis, microplastics are considered to be between 5 mm and 1 nm. The detection limit, however, prevents the identification of particles at the lower boundary.

Microplastics are further divided by their origin, with two categories: primary and secondary microplastics. Primary microplastics are those manufactured as a microplastic. This includes microbeads and nurdles. Secondary microplastics were originally part of a larger item that has broken up over time. For instance, UV degradation has been demonstrated to result in the formation of microplastics and affects plastic properties (Ainali et al., 2021). Depending on the polymer, fragmentation of plastic can be either fast or slow (Min et al., 2020). Secondary microplastics are typically more common in the environment (e.g., Egger et al., 2020; Rowley et al., 2020). Fibres shed from textiles can be defined as both primary and secondary particles. They are manufactured with a diameter small enough to be considered a microplastic and can also fragment from clothing during washing. In the present thesis, primary and secondary microplastics are not considered separately.

Microplastics as a category are a complicated pollutant consisting of many polymers with many plastic additives. Polymer type can affect the density and properties of microplastics. In addition, microplastics vary by shape (e.g., pellets, microbeads, fibres, films and fragments), size and colour. Nor and Obbard (2014) proposed that to visually identify plastics, particles must contain no cellular structures, must not be segmented, should be a uniform thickness, homogenously coloured and are not shiny. Horton et al. (2017), however, argued that plastic could be shiny and not homogeneously coloured and recommended that particles also meet at least two of six additional criteria: pieces are unnaturally coloured, have an unnatural coating, have an unnatural shape, are robust when pulled with forceps, are shiny or glassy and are flexible. Due to the great diversity of microplastics, some researchers are recommending a dichotomous key or guide to the standardised identification of microplastics (Rochman et al., 2019; Lusher et al., 2020a). Categories are as follows: fibres, bundles of knotted fibres, fragments, films, foams, and beads. Fibres have a length significantly greater than width and are typically a uniform thickness. Bundles of knotted fibres, sometimes referred to as tangles, cannot be separated and counted or contain more than 20 items. Fragments are angular items originating from large plastic items and are often rigid. Whilst films are fragments with two dimensions significantly greater than the third, often flexible (except for items such as polymer-based paints). Foams are expanded fragments that contain bubbles or have been aerated. Finally, beads are specifically engineered to be this size and shape can be spherical, ovoid or cylindrical pellets (Lusher et al., 2020a). In the present thesis,

particles are described by shape and colour. A representative subset of 10% were analysed by FTIR. All reported concentrations include the confirmed plastics in addition to those identified visually. As such, it is possible that the results are an overestimate. They represent the worst-case scenario.

In addition to microplastics, microlitter includes microfibres and other anthropogenic materials that are not plastic (e.g., cellulosic and cotton fibres, rubber fragments). In the present thesis, cotton and cellulosic fibres were reported separately. The NHM FTIR library is extensive and includes several variations of cotton and cellulosic polymers from different manufacturers. As such, a microfibre identified as cotton would match several cotton spectra before having a lesser match with cellulosic semi-synthetic items, such as Rayon. Cellulosic fibres were grouped as the distinguishing between Rayon and Avicel etc., was more difficult. Details of libraries used for FTIR can be found in the Supplementary Material (S1).

Definitions of bioaccumulation and biomagnification in respect to microplastics can vary. In the present thesis bioaccumulation was defined as the retention of microplastics by biota longer than natural items, such as food, that has the potential for greater concentrations of microplastics to be found inside biota than the environment. Biomagnification was considered to be the retention of microplastics by predators from contaminated prey resulting in higher concentrations of microplastics at the top of the food web, as occurs with some chemical pollutants such as DDT. The two terms are distinguished by the risk to organisms at different trophic levels. For instance, bioaccumulation can affect all biota regardless of their position in the food web, whilst biomagnification sees an increased risk to biota at higher trophic levels.

1.2 Plastic production and pollution

The term plastic encompasses many synthetic and semi-synthetic polymers, such as polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC), polyester (PES) and polyamide (PA), more commonly known as nylon. Plastic is a flexible and durable resource with a diverse range of uses. As such, it is an increasingly popular material in high demand. In 2018, 359 million tonnes of plastic (not including some synthetic fibres) were produced globally (PlasticsEurope, 2019b), peaking at 368 million tonnes in 2019 (PlasticsEurope, 2021). Production has increased every year and, for over a decade, global plastic production (not

including synthetic fibres) has increased by at least 9 million tonnes a year (PlasticsEurope, 2016, 2018a). Production only decreased in 2021 to 367 million tonnes (PlasticsEurope, 2021). It is estimated that if resin from fibre production were included, plastic production would be 63 million tonnes more in 2016 (Lebreton and Andrady, 2019). By 2018, fibre production had risen to 107 million tonnes (Tobin and Urban-Rich, 2022). Packaging demands the greatest proportion of plastic resources and PP, which is used in food packaging, is the most commonly produced polymer followed by PE (PlasticsEurope, 2018a, b). Demand for packaging has driven the increase in PP production, but production has increased for all polymers (PlasticsEurope, 2018a). Production of plastic material is greatest in China, followed by Europe. Furthermore, plastic is exported across the globe, creating many opportunities for it to litter the environment (PlasticsEurope, 2018a).

Plastic pollution has been reported across the world (Shim et al., 2018), with 15 million tonnes of marine plastic waste generated per year, mostly from coastal areas; fishing, shipping and aquaculture; in-land sources and the exporting of waste (Forrest et al., 2019). In 2015, up to 99 million tonnes of mismanaged plastic waste was generated and this could rise to nearly 300 million tonnes by 2060 (Lebreton and Andrady, 2019). Similar to trends in plastic production, PP and PE are the most commonly recovered plastics in the marine environment (Hidalgo-Ruz et al., 2012; Wright et al., 2013; Almroth and Eggert, 2019).

Plastic affects both terrestrial (e.g., Duis and Coors, 2016; Nizzetto et al., 2016; Zhao et al., 2016; Lwanga et al., 2017; Kumar et al., 2020; Li et al., 2020) and aquatic environments, including marine (e.g., Barnes et al., 2009; Cózar et al., 2014; Dai et al., 2018; Peng et al., 2018) and freshwater ecosystems (e.g., Horton et al., 2017; Blettler et al., 2019; Turner et al., 2019; Uurasjärvi et al., 2020), with plastic being recovered from all seas and oceans (Bhuyan et al., 2021). Plastic is even prevalent in remote locations, such as Antarctica (Reed et al., 2018), the Arctic (Tekman et al., 2020) and the deep sea (Van Cauwenberghe et al., 2013; Woodall et al., 2014; Courtene-Jones et al., 2017; Chiba et al., 2018; Peng et al., 2018; Amon et al., 2020; Zhang D., 2020; McGoran et al., 2021), where some of the greatest concentrations of microplastics have been reported (Kane et al., 2020).

In aquatic systems, plastics are present in all components (Wright et al., 2013; Cowger et al., 2021): surface water (e.g., Carpenter and Smith, 1972; Eriksen M. et al., 2013; Cózar et

al., 2014; Eriksen et al., 2014; Takarina et al., 2022; Yu et al., 2022), water column (e.g., Thompson et al., 2004; Reisser et al., 2015; Cera et al., 2022), benthos (e.g., Claessens et al., 2011; Horton et al., 2017; Peng et al., 2018; Bhuyan et al., 2021, Arturo and Corcoran. 2022; Yu et al., 2022) and shore (e.g., Thompson et al., 2004; Browne et al., 2011; Fred-Ahmadu et al., 2022). Barnes et al. (2009) estimated that 10% of household waste is plastic but found that a much higher proportion of litter recovered from the shore is plastic. The study suggested this was possibly due to its longevity in the environment compared to other debris. Oceanographic models predicted that there were a minimum 5.25 trillion pieces of plastic on the ocean's surface alone (Eriksen et al., 2014). Although, marine plastic concentrations are likely to be much higher as it is estimated that 94% of marine plastic sinks to the seafloor (Almroth and Eggert, 2019). Past literature has focused on the marine environment (Kallenbach et al., 2021), with more studies concentrating on marine organisms than freshwater species (Lusher et al., 2017). Additionally, working groups and international bodies (e.g., GESAMP (Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection), ICES (International Council for the Exploration of the Sea) and OSPAR (named after the Oslo Convention in 1972 and the Paris Convention in 1974)) all focus on marine areas. OSPAR consists of 15 Governments and the EU who co-operate to protect the Northeast Atlantic Ocean also focus on the marine environment (Kallenbach et al., 2021). Freshwater plastic debris was first reported in Swiss lakes by Faure et al. (2012). Since then, research into rivers (e.g., Morritt et al., 2014; Horton et al., 2017; Tibbetts et al., 2018; Huang et al., 2021; Xia et al., 2021; Youngblood et al., 2022) and lakes (e.g., Free et al., 2014; Corcoran et al., 2015; Bertoldi et al., 2021; Felismino et al., 2021) has increased. Rivers transport plastic from terrestrial sources, the origin of an estimated 80% of marine microplastics (Rochman, 2018), into the sea (Cole et al., 2011; Lechner et al., 2014; Jambeck et al., 2015; Rochman, 2018) and lakes (Corcoran et al., 2015; Petersen and Hubbart, 2021). Globally, up to 2.7 million tonnes of macroplastic is discharged from riverine sources per year (Meijek et al., 2021), with rivers and watersheds larger than 100 km^2 accounting for the transport of 91% of mismanaged waste (Lebreton and Andrady, 2019). Indeed, plastic debris in rivers correlates with the proportion of mismanaged plastic waste on the surrounding land (Seo and Park, 2020). Similarly, in the Ganges River Basin littering is more common in urban areas with smaller populations leading to inputs to the river (Youngblood et al., 2022). Modelling estimates have previously designated 10 rivers, predominantly in Asia, as the most

polluting, responsible for between 50% and 94% of riverine macroplastic discharges (Lebreton et al., 2017; Schmidt et al., 2017). These models, however, neglected many factors, such as spatial variation in plastic sources, and more recent estimates find that, in fact, over 1,000 rivers are responsible for 80% of riverine discharges (Meijek et al., 2021). Furthermore, rivers can act as hotspots for plastic pollution (Windsor et al., 2019a).

1.3 Fragmentation of macroplastic and microplastic inputs to aquatic systems

Whilst plastic does not biodegrade, it can fragment through mechanical and oxidative processes (Weinstein et al., 2016; Andrady, 2017; Andrady, 2022; Sipe et al., 2022). Eriksen et al. (2014) reported that the abundance of macroplastic debris in the environment was increasing at a slower rate whilst microplastic debris was becoming increasingly common as large items break up. Similar to the quantification of plastic in the environment, research into weathering has focussed on the marine environment. Evidence demonstrates that fragmentation occurs more commonly on beaches than in floating litter and, as such, it has been suggested that microplastics in the water column fragmented on land prior to entering the sea (Andrady, 2022). The review by Andrady (2022) also noted that further study of weathering in natural conditions was lacking and necessary to confirm experimental observations. As microplastic concentrations rapidly increase through macroplastic break up, it is therefore imperative that microplastic concentrations are monitored in addition to macroplastics.

Microplastic research also focusses on the marine environment, and this is where they were first reported (Carpenter and Smith, 1972). Microplastics have since been studied with increasing frequency. The number of papers published on microplastics since 2010 has increased 50- to 100-fold (Connors et al., 2017; Periyasamy and Tehrani-Bagha, 2022). Plastic in aquatic systems can be transported to the open ocean, deposited in the sediment, ingested or washed onto the shore (Shim et al., 2018). Van Sebille et al. (2015) estimated that between 15 and 51 trillion pieces of microplastic had accumulated in the ocean to date. Whilst models have predicted that even with considerable mitigation strategies, between 2016 and 2040 over 700 million tonnes of plastic could accumulate in terrestrial and aquatic environments (Lau et al., 2020). A review by Hidalgo-Ruz et al. (2012) revealed that estimates of microplastic contamination in the marine environment were inconsistent amongst studies and between

sediment and water samples. Indeed, Maes et al. (2017b) reported higher concentrations of microplastics in the sediment of the North Atlantic compared to in the surface water. Furthermore, a review by Hidalgo-Ruz et al. (2012) also highlighted that microplastic abundance has been estimated as between less than one piece per m^2 (8×10^{-5} per m^2) and thousands per m^2 (77,000 per m^2), with less plastic being recorded at the sea surface than on the shore. Khuyen at al. (2021) also reported finding greater concentrations of microplastics in beach sand compared to seawater. In addition, microplastics also accumulate closer to the shore in urban lakes (Vaughan et al., 2017). Plastic is also known to accumulate in high-pressure systems, gyres, in the ocean (Moore, 2008). In the North Pacific central gyre, the largest of these systems, surface water contained six times more plastic than plankton (Moore et al., 2001). High plastic to biota ratios are still recorded in more recent studies (Egger et al., 2021). Microplastics do not remain in the surface waters of gyres indefinitely and have been found to migrate vertically (Egger et al., 2020; Vega-Moreno et al., 2021). A recent study reported concentrations as high as 1.9×10^6 per m^2 in the seabed of the Tyrrhenian Sea (Kane et al., 2020).

As with macroplastics, microplastics can be transported great distances from land-based sources out to sea, with rivers acting as a major pathway (Schmidt et al., 2017; Wagner et al., 2019; Liu et al., 2022). Indeed, microplastics are most abundant at the mouths of estuaries (Browne et al., 2010; Wright et al., 2013) and are common in wetlands bordering estuaries (Qian et al., 2020). Indeed, the Firth of Clyde had some of the highest surface water concentrations of microplastics across the whole of the Scottish coast (Russell and Webster, 2021). Similarly, the plastic load in the surface water of rivers is reportedly higher than that of lakes, up to nearly 3,000 particles per cubic metre (Dris et al., 2018).

1.4 Sources of plastic pollution in aquatic environments

Plastic pollution can originate from a variety of sources, including atmospheric fallout, landfills, agricultural runoff, wastewater, littering and sewer overflows (Dris et al., 2018). As plastic is often lightweight, it can easily be blown or washed from the land into aquatic environments (Moore, 2008). Jambeck et al. (2015) estimated that between 4.8 and 12.7 million tons of macroplastic debris entered the marine environment globally each year.

Fibres are by far the most abundant type of microplastic in the environment (Wright et al., 2013; Shim et al., 2018), appearing most commonly in sediment (e.g., Claessens et al., 2011; Vaughan et al., 2017; Turner et al., 2019), and water (e.g., Napper et al., 2021; Tanentzap et al., 2021; Aytan et al., 2022), as well as in biota (e.g., Rochman et al., 2015; McGoran et al., 2017, 2018; Atici et al., 2021; Zaki et al., 2021). Turner et al. (2019) found up to 882 plastic particles per kg of dried sediment in a British urban lake and these were almost exclusively fibres. It is believed that many of these fibres originate from the textiles industry (Hartline et al., 2016). In the environment PES (including Polyethylene terephthalate, PET) and cellulosic fibres (including cotton and semi-synthetic polymers, such as rayon) are commonly recovered (Miller et al., 2017; Sillanpää and Sainio, 2017; Napper et al., 2021; Zaki et al., 2021; Periyasamy and Tehrani-Bagha, 2022), reflecting the use of textile materials. Washing machines have been proposed as a major source of plastic pollution even in remote areas (e.g., Antartica, Reed et al., 2018). Various studies have estimated the volume of fibres released by domestic washing machines, with Browne et al. (2011) first calculating that washing a fleece released up to 1,900 fibres per wash. Evidence suggests that polymer type, garment structure and washing conditions such as temperature and the use of detergent can influence fibre release (Periyasamy and Tehrani-Bagha, 2022). Napper and Thompson (2016) reported that an average 6 kg wash of acrylic garments could release 700,000 fibres. PES has, however, been found to shed a greater abundance of fibres, with estimates of 6,000,000 fibres per 5 kg washed (De Falco et al., 2018b) and 13,000,000 per kg washed (Sillanpää and Sainio, 2017). There is now also evidence that laundry dryers emit fibres into the air (O'Brien et al., 2020). In comparison, the number of fibres released in effluent from wastewater treatment plants (WWTPs) is far less than these estimates, with Browne et al. (2011) reporting that on average only one fibre per litre was discharged in a tertiary plant. Similarly, Ziajahromi et al. (2017b) found that effluent from WWTPs released between 0.2 and 1.5 microplastics per litre. Talvitie et al. (2017a) tested the efficiency of four different wastewater treatment systems including membrane bioreactor, discfilter, rapid sand filtration and dissolved air flotation. The study found that all could achieve an extraction of over 95% of particles, however, the discfilter treatment resulted in just 40% recovery in some samples. After secondary treatment in a tertiary WWTP, over 99% of microdebris had been removed from the affluent, suggesting that the third treatment step offered little additional benefit (Talvitie et al., 2017b). Similarly, Chinese WWTPs could remove between 89% and 94% of microplastics

(Zhang et al., 2021). Yet, a survey of over 100 Greek WWTPs showed that 94% lacked a filtration system with a fine enough mesh to recover microplastics (Mourgkogiannis et al., 2018). Despite the effective removal of microplastics, the sheer volume of wastewater processed results in a large input of fibres to the environment (Talvitie et al., 2017b; Mourgkogiannis et al., 2018; Zhang et al., 2021). Estimates of daily microplastic release in effluent are frequently in the millions (Mourgkogiannis et al., 2018; Zhang et al., 2021), but have been recorded as high as 1.4×10^8 particles per day (Talvitie et al., 2017b). Additionally, large quantities of plastics present in sludge will enter the environment through its application on agricultural land (Talvitie et al., 2017b; Koutnik et al., 2021). Nizzetto et al. (2016) estimated that in Europe between 63,000 and 430,000 tonnes of microplastics could be added to farmlands annually, exceeding estimates of surface water contamination globally. Whilst in the U.S., annual release of microplastics from biosolids could be as great as 785–1,080 items a year (Koutnik et al., 2021).

Microplastics can also enter aquatic systems through runoff. The origins of these plastics could be from various sources including agriculture (Hurley et al., 2018; Koutnik et al., 2021), roads (Sundt et al., 2014; Kole et al., 2017; Tamis et al., 2021; Worek et al., 2022) and shipyards (Sundt et al., 2014). At present there is little evidence for the quantity of plastic entering aquatic systems through runoff (Dris et al., 2018). In biosolids applied to agricultural land fragments dominate (Hurley et al., 2018). This contrasts with the microplastics found in WWTP effluents and water samples where fibres dominate (Wright et al., 2013; Talvitie et al., 2017b; Zhang et al., 2021). In a semi-arid environment, an estimated 136,000 microplastics could runoff per hectare in one rainfall event, with fragments preferentially transported whilst fibres are retained in the soil (Hurley et al., 2018).

Suspension of particles in the air is another pathway in which microplastics can enter aquatic environments as well as being a source of contamination during laboratory and field work. This source of microplastics has only recently been documented with a growing number of studies on the topic (e.g., Dris et al., 2016, 2017). Fibres are extremely abundant in atmospheric fallout and it was estimated that 3–10 tons of fibres could be deposited annually in the Paris Metropolitan Area (Dris et al., 2016). In London, up to 1,008 microplastics fall per m^2 in a day (Wright et al., 2020). Additionally, microplastics can be transported by snow (Bergmann et al., 2019) and rain, with increased abundance during storm events (Hitchcock,

2020). Abundance is also affected by sampling location with a greater number of fibres in urban environments (Wright et al., 2020) and indoors (Dris et al., 2017) and, therefore, there is a greater risk of contamination in the laboratory compared to the field. Indoor microplastic abundance is influenced by the amount of usage of space and airflow, with air-conditioning resulting in the resuspension of particles (Zhang Q. et al., 2020). Fibres typically originate from textiles in the indoor environment (Zhang Q. et al., 2020), with about two-thirds of fibres in the environment and indoors being organic (Dris et al., 2016, 2017).

1.5 Impacts of macro- and microplastic pollution

In the 1970s, the plastic industry claimed that the only negative effect of plastic pollution was that it was an eyesore (Derraik, 2002). Evidence is mounting, however, for the detrimental impacts of macroplastic pollution. Early work on plastic pollution noted that it contributed to at least eight negative outcomes: 1) litter on beaches impacting enjoyment, tourism and potentially public health; 2) plastic entangles animals; 3) plastic is ingested; 4) plastic does not biodegrade and is therefore persistent in the environment; 5) plastics can accumulate chemical pollutants; 6) plastic on the benthos can inhibit gaseous exchange of bottom-dwelling organisms if they are covered; 7) plastic damages nursery grounds and; 8) plastic can entangle and damage vessels and put crew removing the debris and the vessel at risk (Moore, 2008). Similarly, Gregory (2009) highlighted reduced aesthetic value, entanglement, ingestion, smothering, ghost fishing and the cost of beach cleans as major negative impacts of marine plastic pollution. Additionally, the study documented invasive species using floating plastic as a vector to colonise new habitats. Recent reviews have since highlighted that plastic can contribute to human health conditions, such as diabetes and obesity; can affect the gut microbiome; can cause genotoxicity; can cause inflammation of the lung if inhaled; can accumulate and leach chemicals which act as endocrine disruptors and reduce reproductive success (Iroegbu et al., 2021). Furthermore, plastic degradation releases CO_2 which contributes to greenhouse gas emissions and plastic in the environment can affect nutrient cycling and can change sediment properties (MacLoed et al., 2021). Production of plastic requires a greater amount of energy to produce than other materials (Lebreton and Andrady, 2019) and rely on fossil fuels for their production. It has been estimated that greenhouse emissions from global plastic pollution cost $695 billion per annum (Forrest et al., 2019). More sustainable products are therefore likely to pollute the environment less in

addition to having a reduced impact on the global climate crisis. Furthermore, plastic pollution reduces the climate resilience of marine ecosystems and increases the threat of climate change than climate change alone (Lincoln et al., 2022).

As well as the environmental costs of such pollution, there are financial implications to litter on beaches and shorelines. According to Williams and Simmons (1996) Kent County Council spent $15 million to keep local beaches clean. The timeframe of this expenditure is not, however, mentioned in the study. Indeed, the charitable organisation Thames21 (2017) spent over £2 million between 2016 and 2017 removing litter from the Thames foreshore. Although Williams and Simmons (1996) argued that beach cleans are ineffective at keeping waste off the shore, a study in the Forth of Firth reported that daily and weekly beach cleans significantly reduced shoreline litter and even annual cleans reduced rubbish abundance (Storrier et al., 2007).

The impact plastic has on the environment varies by the particles shape and size. On a large scale, macroplastic is less bioavailable than micro- or nanoplastics. But, even within the category of microplastics, size and shape can affect if negative impacts of exposure are detected (Earn et al., 2021). Macrodebris can result in entanglement and ingestion (Laist, 1987; Wright et al., 2013; Provencher et al., 2017; MacLeod et al., 2021). A review by Kühn and Van Franeker (2020) highlighted that 914 species are known to ingest or entangle in plastic. Just five years earlier, records only reported 550 marine species being affected, yet this was still more than twice the number documented in 1997 (Kühn et al., 2015). It is estimated that 100,000 marine mammals die annually in the North Pacific due to entanglement in fishing gear (Moore, 2008). There is, however, a bias in studies towards fish and seabirds, with only 75 accounts of ingestion by marine mammals in the review by Kühn and Van Franeker (2020). Moore (2008) estimated that millions of animals had been entangled in abandoned and lost 'ghost' fishing nets and lines. Entanglement has even been reported in the deep sea (Chiba et al., 2018; Amon et al., 2020), with primnoid and isidid coral colonies at depths greater than 1,000 m entangled in fishing gear, ropes and plastic bags (Amon et al., 2020) which may reduce filter feeding capabilities. Large plastic items can also resemble prey to large sea creatures, such as turtles (Gramentz, 1988). Over 701 species are known to ingest plastic (Kühn and Van Franeker, 2020) and it is likely many more species are affected but have not been recorded. Similarly, Provencher et al. (2017) calculated that

upwards of 690 species could be affected by plastic ingestion. Figure 1.1 highlights examples of microplastic ingestion evidenced in the literature and how affected species fit into food webs.

Due to their small size, microplastics are widely bioavailable (Wright et al., 2013). Galloway et al. (2017) illustrated that microplastic contamination can have effects at different levels in an ecosystem, from cellular impacts and individual mortality to population-wide effects. Ingestion of microplastics is perhaps the most commonly reported negative impact of plastic pollution. There are many pathways that enable microplastics to enter the food web, including mistaken identity of plastic for prey, incidental capture, inhalation and trophic transfer (Wright et al., 2013; Au et al., 2017; Setälä et al., 2018). Non-selective filter feeders are at particular risk of consuming microplastics (Moore, 2008) with, for example, baleen whales ingesting more plastic than toothed whales (Kühn and Van Franeker, 2020). Moore (2008) proposed that plastic was capable of breaking down into fragments that could mimic any natural food source.

Reviews by Lusher at al. (2017) and Foley et al. (2018) highlighted that microplastic ingestion can negatively impact growth, immune response, food consumption, fecundity and energy levels as well as having generational effects. Microplastic ingestion does not affect all animals in the same way nor to the same degree. A meta-analysis by Foley et al. (2018) demonstrated that zooplankton growth, survival and reproduction were all negatively impacted by microplastics. By comparison, molluscs and echinoderms were largely unaffected with some suggestion of impacts on reproduction, consumption and growth. The meta-analysis also revealed that whilst juvenile fish demonstrate reduced feeding in the presence of microplastics, adult fish are unaffected. Critchell and Hoogenboom (2018) noted, however, that the size of microplastics affected the likelihood they would be ingested with only small microplastics negatively impacting juvenile planktivorous fish (*Acanthochromis polyacanthus*). There is a lack of studies investigating the impacts of microplastic on fish health which hinders our understanding of impacts in this group (Foley et al., 2018). Size of microplastics was also found to have an effect in zooplankton. According to Cole et al. (2015), when in the presence of microplastics, copepods exhibited reduced feeding and selectively fed on smaller particle sizes. This study demonstrated that reduced carbon consumption

ultimately led to increased mortality and reduced fecundity, including smaller egg sizes and lower hatching success.

Despite the abundance of fibres in the environment, studies into the effects of microplastic ingestion have primarily focussed on microbeads (Rebelein et al., 2021), primarily due to their availability from suppliers (Ziajahromi et al., 2017a). In addition to their abundance, a review by Wright et al. (2013) noted that fibres were the most harmful form of microplastic. Evidence shows that microfibres could have greater negative effects when ingested compared to microbeads (Qiao et al., 2019; Daniella Hodgson, pers. comm.), especially affecting organisms at the base of the food web (Rebelein et al., 2021). For example, Ziajahromi et al. (2017a) demonstrated that *Ceriodaphnia dubia* showed greater detrimental effects after exposure to microfibres compared to microbeads and that deformities in the carapace only developed when this waterflea was exposed to microfibres. Cole et al. (2019) did not find that fibres resulted in greater negative effects than granules of plastic but did report different impacts of exposure to the two shapes. A review by Foley et al. (2018), however, found more evidence of negative effects in studies which used spherical microplastics compared to fibres. This may be driven by the larger number of studies using the former.

The most common impact of microplastic ingestion is reduced food consumption (Foley et al., 2018). Microplastic ingestion is rarely lethal, unlike macrodebris ingestion, which particularly affects seabirds, turtles and some cetaceans (e.g., Carey, 2011; Santos et al., 2015; Ryan et al., 2016; Alexiadou et al., 2019; Roman et al., 2019). Evidence does suggest, however, that some seabirds can regurgitate plastic in boluses (Bond et al., 2021; Grant et al., 2021). Additionally, sub-lethal effects are often reported to a lesser degree in higher order vertebrates (Puskic et al., 2020). The risk of fatality from ingestion increases as more items are consumed (Roman et al., 2019). Microplastics can negatively impact feeding and energy stores which in turn adversely affect fitness and reproductive success (Galloway et al., 2017). Moreover, plastic ingestion may have knock-on effects in the food web, with mortality in low trophic organisms impacting prey abundance for predator species (Foley et al., 2018; Nelms et al., 2018). Additionally, the impacts of microplastics on organisms varies with environmental factors such as temperature (Foley et al., 2018).

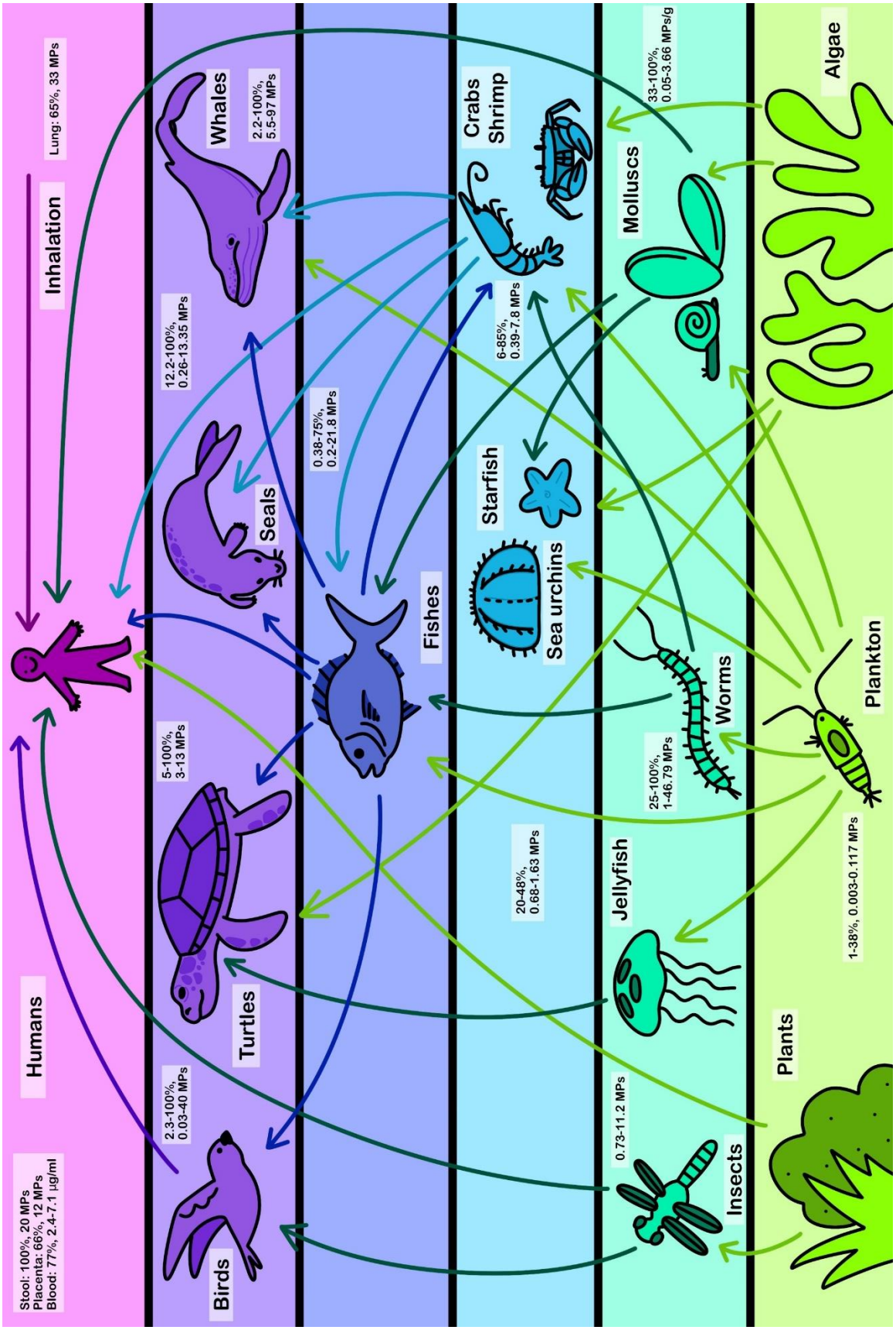


Figure 1.1. Microplastic contamination across food webs. For the full list of references used to create this illustration see the supplementary material (S2).

Despite mounting evidence of the negative effects associated with microplastics, it is not known whether the effects observed in laboratory studies also occur in nature. It has been suggested that plastics are unlikely to have negative chemical impacts once ingested. For instance, Koelmans et al. (2016) proposed that plastics were probably not a key source of hazardous chemicals and that prey items are more likely the vectors of pollutants. Lenz et al. (2016) reviewed studies investigating the effects of microplastics and determined that the concentrations of plastics used were unrealistic when compared to recorded environmental levels, with studies using concentrations up to seven orders of magnitude higher. Although levels of environmental contamination have been considered in some studies and an effort has been made to test smaller volumes of plastic (Sussarellu et al., 2016), it has been noted that using local hotspots to determine contamination could result in studies not having a wider relevance and that more studies should test appropriate concentrations (Lenz et al., 2016). In addition, laboratory studies have often relied on virgin plastic rather than 'weathered' plastics (e.g., Besseling et al., 2013; Sussarellu et al., 2016). Given these limitations, more experiments are being conducted on a wider range of plastics under more realistic conditions. Parker et al. (2021) reviewed microplastic ingestion by freshwater fishes, reporting that most studies used concentrations within the limits recorded in the environment. The same review noted that physiological effects were the most common response to microplastic exposure, but that effects were concentration dependent.

1.6 Plastic pollution and associated chemicals

Once ingested, microplastics can cause physical damage with chemical additives and sorbed persistent organic pollutants (POPs) able to leach into the tissue (Moore, 2008; Wright et al., 2013). It has been proposed that chemicals associated with plastics can have additional negative effects such as endocrine disruption (Wright et al., 2013; Lusher et al., 2017). Although it is noted that further research is needed to understand whether microplastics act as vectors for POPs to organisms (Rodrigues et al., 2019; Tang, 2021). There is a lack of studies investigating the impacts of plastic ingestion when other contaminants, such as POPs, are present (Foley et al., 2018). Microplastics can accumulate a greater load of chemical pollutants compared to seawater, up to 10^6 times greater (Mato et al., 2001). Indeed, several studies have demonstrated this (Rodrigues et al., 2019), but the concentrations accumulated can vary with environmental factors (e.g., pH, salinity, temperature) and the physical

properties of the plastic (e.g., aging, polymer type, shape) (Tang, 2021). A review by Tang (2021), however, reported that typically concentrations of POPs on microplastics resulted in insignificant or low impacts on organism health. Ultimately, microplastics are only one contaminant present in aquatic systems. A variety of pollutants would be impacting organisms in these systems and studies exploring the combined effects of these are necessary to fully understand the role microplastics play in transferring chemicals and causing negative effects. An early study by Browne et al. (2013) reported that laboratory exposure of *Arenicola marina* (lugworm) to microplastics and chemical contaminants resulted in the transfer of the additive to the animal tissues. This resulted in reduced ability to defend against pathogens, negatively impacted burrowing rates and caused increased mortality. Browne et al. (2013) did also note that sand was more effective than PVC at transferring chemical additives to tissue when ingested. Diepens and Koelmans (2018) used model food webs to demonstrate that chemical accumulation in the presence of plastic depends on the type of chemical, with polycyclic aromatic hydrocarbons (PAHs) accumulation but no such trend is reported for polychlorinated biphenyls (PCBs). Studies have also demonstrated that microplastics can act as vectors of heavy metals in aquatic (Brennecke et al., 2016; Wang et al., 2017; Sarkar et al., 2021) and terrestrial environments (Hodson et al., 2017; Sarkar et al., 2021), with some polymers having a greater affinity for metal adsorption (Godoy et al., 2019). Furthermore, evidence suggests that biofilm formation on the surface of microplastics increases the adsorption capability of the particles (Wu et al., 2022). It must be noted, however, that although plastics can accrue heavy metals, many are inherent (Wang et al., 2017) and there is evidence that ingestion may not result in negative effects (Hodson et al., 2017; Foley et al., 2018). Brennecke et al. (2016) proposed that ingestion of heavy metal-loaded microplastics could result in biomagnification. In addition to the sorption of heavy metals, biofilms can form on microplastics and the bacteria present in these communities can pose risks to the health of organisms which ingest plastic (Kirstein et al., 2016; Rummel et al., 2017). Some researchers note, that whilst bacteria can be recovered from microplastics, their virulence is not well understood. Having said this, the scientific consensus is that microplastics can be vectors for pathogens and antimicrobial-resistance genes (Bowley et al., 2021). It has been demonstrated that heavy metals, bacteria and microplastics all interact, with microplastics acting as hotspots for bacteria with antibacterial resistance genes which are co-selected for in the presence of some heavy metals (Imran et al., 2019). Moreover, the impacts of

microplastic exposure may vary as environmental stressors change and there is a call for more research on microplastics and other anthropogenic stressors (e.g., ocean warming, acidification, eutrophication) (Parker et al., 2021).

1.7 Aims

With high concentrations of macro- and microplastics polluting aquatic environments, monitoring plastic distribution is therefore key to developing risk assessment strategies (Everaert et al., 2018). Monitoring provides robust data which can be used to inform mitigation strategies. Presently, monitoring of plastic pollution focusses solely on macroplastic (e.g., OSPAR Commission, 2022) and often identifies the most common items in the environment as targets for mitigation. Evidence suggests, however, that monitoring macroplastic alone is insufficient and investigation into meso- and microplastics is also required to understand risks and sources of pollution (Blettler et al., 2019). Indeed, Schmidt et al. (2017) reported that 95% of items recovered from rivers were microplastics compared to macroplastics. Though it should be noted that some studies are designed to only investigate microplastics and exclude macroplastics. Consequently, some data, taken in isolation, portrays an incomplete picture and are biased to one size or the other. When considering risk, it is essential to assess interactions with biota and, as such, monitoring of microplastic ingestion is required. At present, few species have been proposed as sentinels for microplastic ingestion. Therefore, the aim of the present thesis is to develop an ecosystem-wide assessment of plastic waste with a focus on food webs using the River Thames as a case study.

The impacts of plastic pollution on the environment have started to become more apparent and concern over the removal and prevention of such contamination has increased. To better understand the pathways through which plastics move and improve management of such waste, the disparities in knowledge must be addressed. Building on the information already gathered, this thesis aims to develop an ecosystem-wide assessment of plastic pollution with the focus of providing environmental evidence for the movement of plastic through the food web (Figure 1.2). Harmonised methodology is lacking in the field and as such, prior to investigating the food web, a degree of method development was required (Chapter 2). It was suspected that potassium hydroxide (KOH) would be the most likely

solution for digestion of organic material as it is widely used in the literature and low cost makes it highly suitable for harmonisation and comparability. Enzymes, however, were proposed to be faster and more effective. Since microplastics can be difficult to trace back to source, macroplastic debris will also be addressed as this can be used to highlight sources of waste production and pathways of pollution (Chapter 3). The present thesis aims to determine whether macroplastic concentrations and product types differed in the water column and the riverbed. This was deemed critical to understanding plastic pollution as it would determine its longevity in river system, the transport of plastic to the foreshore and out to sea as well as the likelihood of biota interacting with the litter. Macroplastic in the environment can degrade and fragment, contributing to microplastic concentrations. Thus, in Chapter 4 microplastic abundance in the Thames riverbed was investigated. It was expected that microplastics would accumulate in the sediment since microplastics likely follow similar transport mechanisms as fine silty/muddy sediment. If microplastics present in the sediment are retained for a greater duration than those in the water column, sediment samples can be used to estimate the highest concentration biota are exposed to over long periods. Additionally, benthic species and infauna are likely to be the most exposed to microplastics compared to species in the midwater. Brachyuran crabs were selected as at-risk species for ingesting microplastics due to their close association with the sediment, burrowing behaviour and past evidence in the literature. Their ingestion of microplastics was quantified in Chapter 5 where it was proposed that most individuals would contain microplastics, with tangles of fibres forming in the gastric mill (GM). It was expected that all brachyuran crabs would be affected equally. Building on this knowledge, the Thames Estuary food web was developed using analyse the diet of fish and marine mammals (Chapter 6). The simplified food web was used to determine the likelihood of bioaccumulation and biomagnification. It was hypothesised that bioaccumulation, trophic transfer and biomagnification were possible in an estuarine food web. To this end, it was expected that similar types of plastic would be recovered in all studied species (i.e., any preferential ingestion of plastic at lower trophic levels would be reflected in the plastic present in their predators). Further to this, seasonal temporal changes in plastic abundance were explored in both the environment and biota (Chapter 6). Plastic concentrations were expected to be greater in winter and autumn when rainfall would be high, resulting in larger inputs of plastic into the environment.

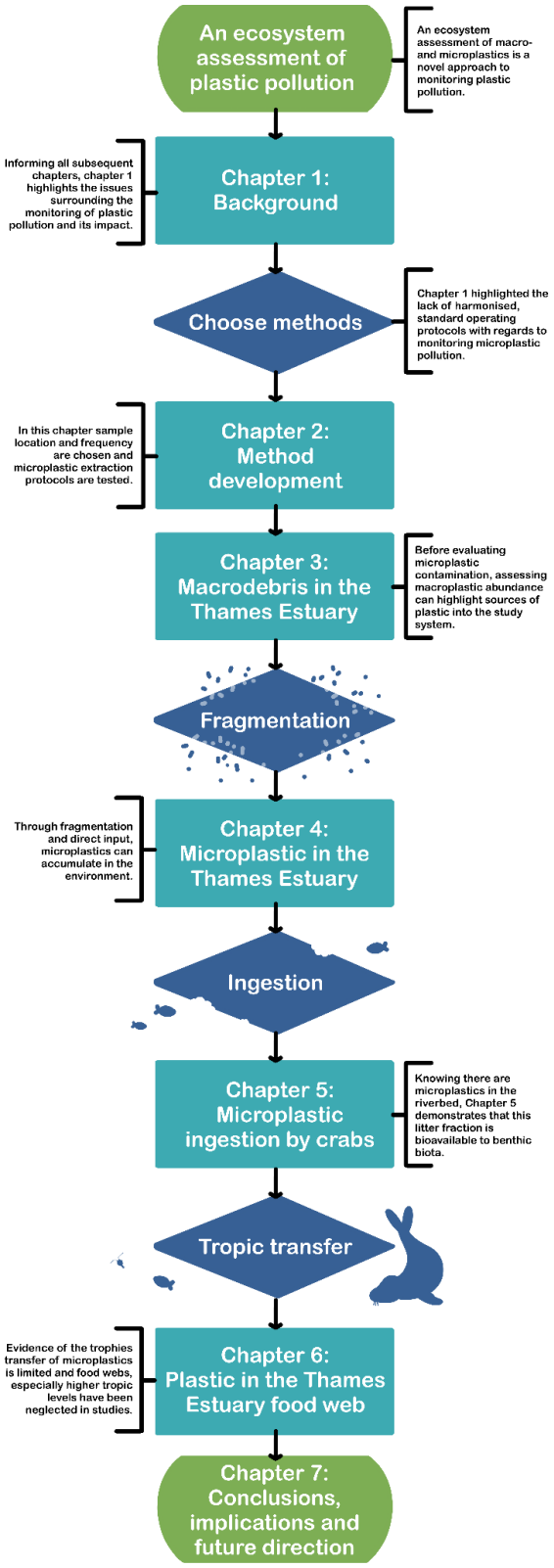


Figure 1.2. The structure of this thesis was based around an ecosystem assessment of plastic contamination in the Thames Estuary. This illustration demonstrates the interconnectedness between chapters.

Chapter 2

The River Thames and plastic pollution: sample site, methodology and the development of a digestion extraction protocol

2.1 Background and study area

Estuaries are some of the most productive ecosystems in the world, are important habitats for commercial fish stocks and provide several ecological services to the animals that inhabit them, including habitat, spawning grounds, nursery grounds, food and protection from predation (Beck et al., 2001; Selleslagh and Amara, 2008; Amara et al., 2009). Strong environmental gradients, as well as seasonal variations, produce a variety of niches to support fish at several life stages and this additionally impacts microplastic availability (Ferreira et al., 2019). But estuaries are at risk from a variety of anthropogenic pressures with an estimated 60% of the global population living on the coast (Amara et al., 2009). Indeed, in the UK most major urban centres are situated on rivers and estuaries (Kirby et al., 2004). Globally, estuaries are one of the marine ecosystems most affected by anthropogenic change (Halpern et al., 2008) and are hotspots and transport pathways for plastic (Browne et al., 2010; Wright et al., 2013; Schmidt et al., 2017; Wagner et al., 2019; Liu et al., 2022). Despite this, a limited number of studies into estuarine plastic pollution have been conducted and most focus on tropical estuaries (Kazour et al., 2018).

The River Thames, situated in southeast UK (Figure 2.1), has a catchment of 16,000 km^2. This encompasses ca. 15 million people concentrated in many different towns and cities, including Greater London (Environment Agency, 2016). Land-use in the catchment varies but is mostly rural. Overall, 17% of the river basin is urbanised, mostly in the East (Environment Agency, 2016; Hutchins et al., 2018). The Thames Estuary is turbid and highly tidal (Uncles and Mitchell, 2011). The average flow rate of the Thames ranges from 0.33 m^3s^{-1} at Ewen to 78.17 m^3s^{-1} at Kingston and the catchment has a mean annual rainfall of 710 mm (Marsh and Hannaford, 2008). Freshwater has an average residence time of two months (Uncles and Mitchell, 2011). The riverbed consists of a mixture of coarse sediment, sand and mud, with some of these sediments categorised as near pristine by the Environment Agency (EA) and representing important habitats for fish (ABP Marine Environmental Research Ltd, 2013).

The Thames catchment supports a diverse range of species, with some of national and global importance occupying key habitats such as the estuary and saltmarshes (Environment Agency, 2016). Over 950 species have been reported in the Thames Estuary (Thomas, 1998), including benthic invertebrates, fishes and marine mammals. The inner estuary is dominated by freshwater species with increased salinity to the outer estuary where more marine species reside (e.g., European flounder, *Platichthys flesus*). The mid estuary contains species such as the ragworm *Hediste diversicolor* (ABP Marine Environmental Research Ltd., 2013).

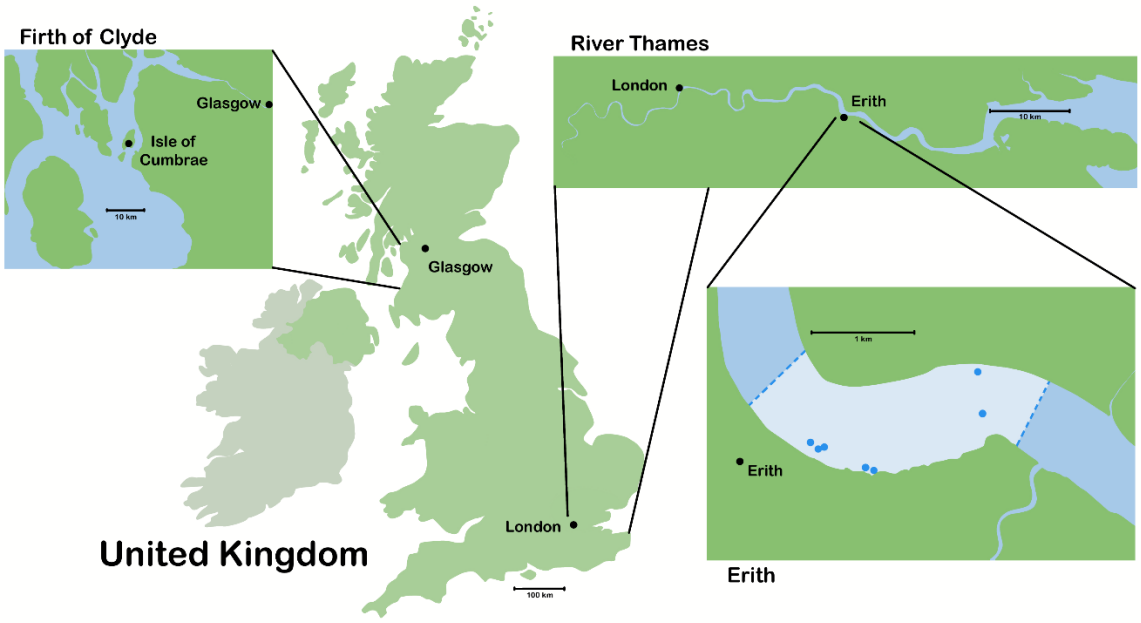


Figure 2.1. UK map with pull-outs for the Firth of Clyde (fish samples collected for digestion protocol development) and River Thames (biota and sediment samples collected for microplastics analysis). Black dots mark urban centres and blue dots mark the location of sediment grab samples. The reach marked in pale blue on the map of Erith denotes the region where trawls were conducted. Map drawn by A. McGoran.

Over 125 species of fish have been reported in the Thames Estuary (Zoological Society of London, 2018) which is an important spawning and nursery habitat for Atlantic herring (*Clupea harengus*) and Dover sole (*Solea solea*), and nursery habitat for whiting (*Merlangius merlangus*), sprat (*Sprattus sprattus*) and European bass (*Dicentrarchus labrax*) (ABP Marine Environmental Research Ltd., 2013).

Cetaceans, including harbour porpoise (*Phocoena phocoena*) and white-beaked dolphin (*Lagenorhynchus albirostris*), are common in the southern North Sea. Harbour porpoise and bottlenose dolphin (*Tursiops truncatus*) have been observed in the Thames Estuary and it is home to two pinniped species: grey seal (*Halichoerus grypus*) and harbour seal (*Phoca vitulina*; ABP Marine Environmental Research Ltd., 2013; Castello y Tickell and Barker, 2015). Marine mammals are protected by the Wildlife and Countryside Act 1981, EU Habitats Directive, Bern Convention and Seal Act of 170. Additionally, the harbour porpoise is listed as an OSPAR threatened species. Harbour porpoise abundance peaks in early spring and their diet consists of sandeels, gadoids and clupeids (ABP Marine Environmental Research Ltd., 2013).

Grey and harbour seals can be found hauled out on sandbanks in the estuary. Harbour seals travel widely within the Thames but move shorter distances when foraging compared to grey seals who can travel hundreds of kilometres to forage. Most foraging occurs with 100 km of the haulout site, with the main prey items consisting of sandeels (Ammodytidae), cod (*Gadus morhua*) and *Solea solea*. Plaice (*Pleuronectes platessa*), *Platichthys flesus* and dab (*Limanda limanda*) also present in the diet (ABP Marine Environmental Research Ltd, 2013).

The rich biodiversity supported by the Thames represents a dramatic improvement from its state in 1957. At this time the River Thames was declared biologically dead due to a lack of oxygenation (Wheeler, 1979; McConville et al., 2020). Indeed, present trends in marine mammal and bird populations are improving in the estuary. However, fish populations are on the decline (Zoological Society of London, 2021).

The Thames basin has historically been exposed to many environmental threats from human activities (Tinsley, 1998). Current trends indicate that the tidal Thames is improving (Zoological Society of London, 2021), though for many contaminants there are insufficient data available. Today, nearly half of the waterbodies in the catchment are affected by pollution from wastewater and 17% are affected by pollution from urban centres and transport (Environment Agency, 2016). Over 200 tonnes of waste are removed from the river annually (McConville et al., 2020). Plastic pollution decreases with distance from urban areas (Barnes et al., 2009; Corcoran, 2015) and WWTPs fail to remove all plastic pollution (see section 1.4 Sources of microplastic pollution in aquatic environments). Macro-, meso- and

microplastics have been recovered from the bottom water, surface water, sediment and shores of the Thames and its tributaries (Morritt et al., 2014; Horton et al., 2017; Fischer, 2019; McConville et al., 2020; Rowley et al., 2020). The foreshore is a mosaic of floating and sinking litter hotspots (McConville et al., 2020) with these accumulations having detrimental effects on local biota (McCoy et al., 2020). During three-months of fyke-net sampling, Morritt et al. (2014) recovered 8,490 pieces of litter, predominantly plastic from the Thames Estuary riverbed. The litter sample was dominated by food packaging, sanitary products and cigarette wrappers. Similar findings were reported during Thames21 and Marine Conservation Society beach cleans (Fischer, 2019; McConville et al., 2020). The foreshore of the Thames is dramatically changing due to deposited plastic, with wet wipes mounds accumulating rapidly, with some accumulations as large as four tennis courts. Over 50,000 wipes were recovered in just four trips to the foreshore (McConville et al., 2020). Erith is one of 20 recognised hotspots for litter, with marsh reeds retaining floating litter (McConville et al., 2020).

Fauna, such as fish, have long been used as a proxy to monitor the health of estuaries (Selleslagh and Amara, 2008; Amara et al., 2009; Selleslagh et al., 2009). There is an abundance of fish in the Thames Estuary with over 125 species present (Thomas, 1998; Coclough et al., 2002; Zoological Society of London, 2018). Estuaries are dominated by a few common species (Selleslagh and Amara, 2008) and, in the Thames, *P. flesus* is one of these (Clark et al., 2017). Environmental monitoring has therefore often studied *P. flesus* (Kirby et al., 2004; Amara et al., 2009; Marchand et al., 2010). Fish have frequently been included in microplastic studies with reports of between 0.38% and 75% (S2, supplementary material) of individuals containing plastic in the gastrointestinal tract (GIT). Plastic may have entered the GIT during feeding either as misidentification as food, incidental ingestion during prey capture or trophic transfer (Wright et al., 2013; Au et al., 2017; Setälä et al., 2018). There is a lack of studies into the effects of plastic ingestion on fish health (Foley et al., 2018), so it is not possible to determine the impacts of its consumption, although it is likely to reduce feeding activity (Foley et al., 2018).

Estuarine and freshwater fish in the Thames have also been shown to ingest plastic, both in the tidal reach of the Thames and upstream (McGoran et al., 2017, 2018; Horton et al., 2018). These studies found that between one- and three-quarters of fish contained microplastics after visual inspection of the gut contents (McGoran et al., 2017; Horton et al.,

2018; McGoran et al., 2018). McGoran et al. (2017) provided the first evidence of plastic ingestion by fish in the Thames, revealing that up to 75% of *Platichthys flesus*, a benthic species, had ingested plastic. Only 20% of sampled European smelt (*Osmerus eperlanus*), a pelagic species contained plastic in the gut. McGoran et al. (2017) proposed that bottom-dwelling species were exposed to more plastic than those in the water column. Indeed, a greater proportion (33%) of sampled roach (*Rutilus rutilus*) (Horton et al., 2018), a benthopelagic species which feeds both on the riverbed and in the water column (CABI, 2012) in the Thames freshwater reaches ingested plastic compared to the smelt analysed by McGoran et al. (2017). McGoran et al. (2018), however, found that both pelagic fish and flatfish ingested more plastic on average than other benthic fish. Thus, it is possible that flatfish are at particular risk of plastic ingestion rather than benthic species. Only 33% of Thames flatfish sampled by McGoran et al. (2018) had plastic in the alimentary canal, compared to 75% in McGoran et al. (2017). As dissections are just a snapshot of plastic ingestion, providing an insight only in the instance the samples were collected, it could be the case that plastic prevalence in fish is fluctuating as it passes through the digestive system and more is ingested. It seems unlikely that plastic accumulates in the gut. Otherwise, a greater proportion of fish would be expected to ingest plastic in the study by McGoran et al. (2018).

The predominant form of plastic ingested by fish in the Thames was fibres as opposed to fragments of the macrodebris present in the Thames (Morritt et al., 2014). The most abundant polymers in the river are PP, PET and PE (Horton et al., 2018; McGoran et al., 2018). Microbeads and glitter have been recovered from the surface water of the Thames, as well as larger fragments of plastic, including food packaging, stationery items and industrial debris (Rowley et al., 2020). Sediment samples have been collected from three tributaries of the Thames and the microplastic abundance estimated (Horton et al., 2017). The study revealed that debris was present in all sampled tributaries, with anthropogenic debris being more abundant at sites with a higher number of sewage inputs. Additionally, smaller pieces (1–2 mm) were more common that larger items (2–4 mm). Raman spectroscopy identified most analysed particles as anthropogenic due to the presence of dyes in the particles (Horton et al., 2017).

In the River Thames there are numerous wastewater outfall and combined sewer overflows (CSOs). For example, there are nine outfalls on a ca. 6 km stretch of the river around

Erith (Figure 2.2). Of these nine outfalls, two discharged raw sewage in 2020. Each outfall released untreated discharge between 73 and 113 times, resulting in a cumulative 3,000 hours of discharge (The Rivers Trust, 2022). In the immediate vicinity of the sampling sites used in the present thesis there are two outfalls, neither of which released untreated sewage in 2020 (The Rivers Trust, 2022).

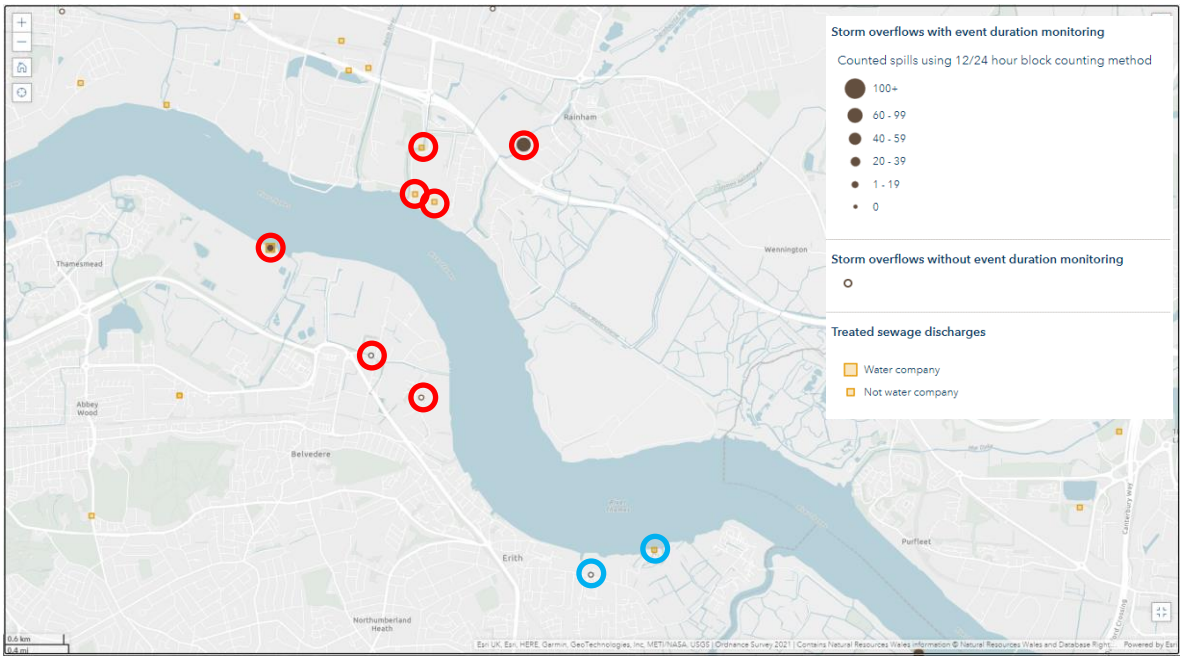


Figure 2.2. Wastewater outfalls and combined sewer overflows in the vicinity of Erith, River Thames. Nine outfalls (circled) are present on a ca. 6 km stretch of the river, with two outfalls (circled in blue) at the sampling site for the present study. Map generated by The Rivers Trust (2022) with support from public donations, The Fishmongers' Company's Fisheries Charitable Trust and The Prince of Wales's Charitable Fund. Scale bar = 0.6 km/0.4 miles.

In addition to wastewater outfalls, three landfill sites border the Thames Estuary in the vicinity of Erith: Rainham Landfill, Rainham Marshes and Camas Building Materials (Figure 2.3; Environment Agency, 2022a, b). Rainham Landfill/Veolia Ltd. has been in operation since 1998 and collects industrial, commercial and household waste; Camas Building Materials was operational between 1977 and 2019; and Rainham Marshes is still operational.

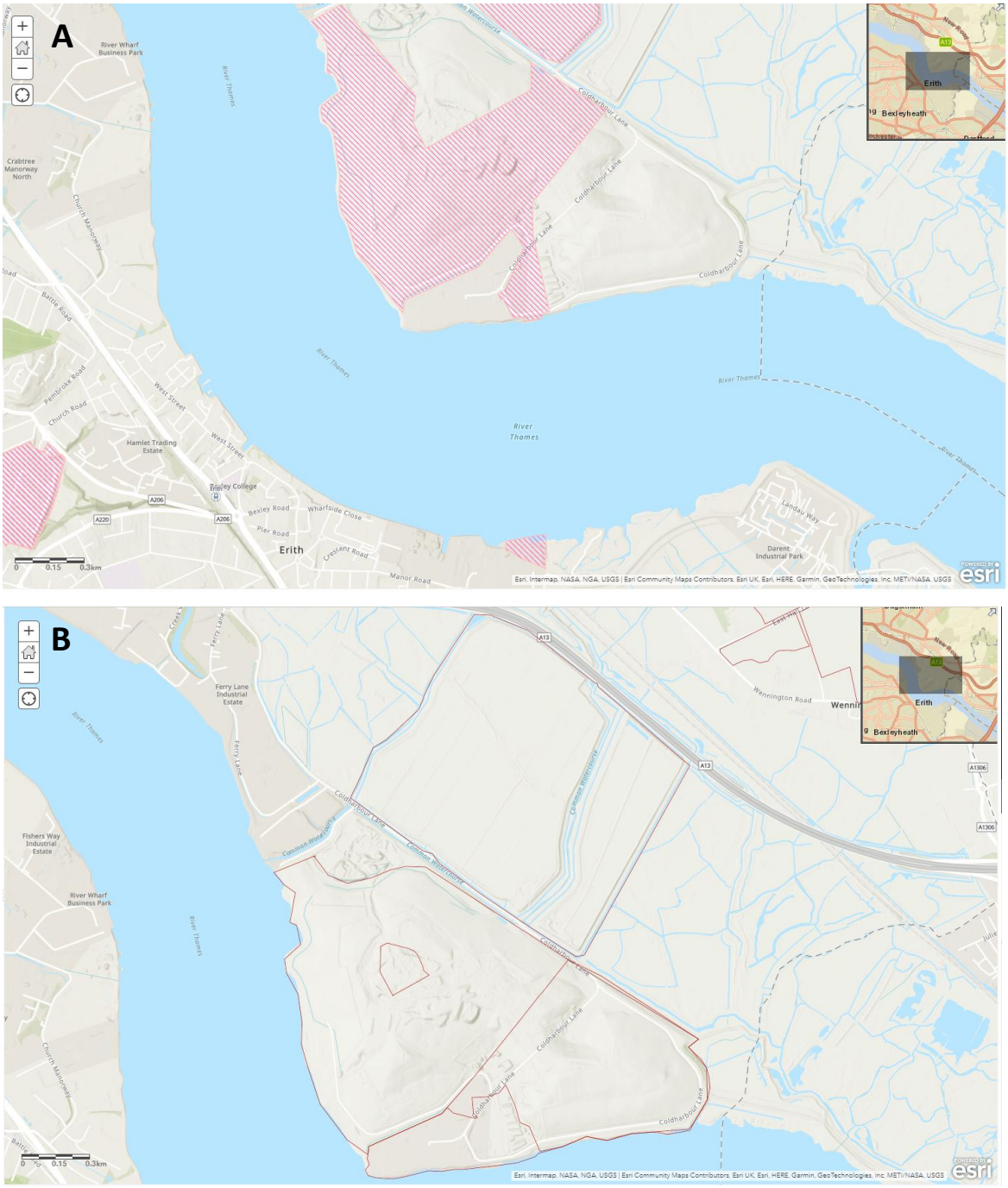


Figure 2.3. Three landfill sites (in red) border the Thames Estuary in the vicinity of Erith. A) Historic landfill sites on the Thames Estuary; B) Current landfill sites. Maps produced by Environment Agency (2022a, b). Scale bars = 0.3 km.

2.2 Sample collection

All samples, including macro- and microplastic, were collected as part of one survey spanning over two years (eight visits over the survey ca. 3 months apart). This is a novel ecosystem-wide approach to monitoring both macro- and microplastics.

2.2.1 Sediment

An Ekman grab was deployed from the vessel *Boy Daniel* SD4 at Erith, Thames Estuary (Figure 2.1). Three ca. 3 L sediment samples on each sampling session. Grab samples were collected at slack water near to the shore at a depth of ca. 12 m (Supplementary Material, S3). Sediment, covered by estuarine water, was stored in a sealed plastic container at room temperature.

2.2.2 Biota

Fish and shrimp were collected from The Thames in the Erith area, during 15-minute midwater and benthic trawls from fishing vessel *Boy Daniel* SD4. The trawl comprised a standard mesh size of 80 mm with a fine 16 mm mesh insert. A maximum of ten trawls (five midwater and five on the riverbed; depth of ca. 9–12 m) were collected each sampling session (Supplementary Material, S3). On some occasions sampling quotas set by the MMO were met prior to completion of 10 trawls. In these instances, no further trawls were conducted to limit the negative impact on the ecosystem. Biota were identified onboard and stored on ice overnight until they could be frozen the following morning at -20 °C. Fish were stored in plastic bags, whilst shrimp and crabs were stored in plastic boxes.

Stranded marine mammals, including cetaceans and pinnipeds are reported to the UK Cetacean Strandings Investigation Programme (CSIP), based at the Institute of Zoology, Zoological Society of London with partners across the country, including at the Natural History Museum (NHM). On an ad hoc basis, when marine mammals stranded in the broader Thames area and were accessible for collection or dissection, they were included in the present study. Individuals were only included if they were fresh and in good condition (ideally collected within 48 hours of death).

Sediment containing meiofauna was allowed to settle and left for ca. one hour so that amphipods could create burrows and leave tracks on the surface. Amphipods were then removed with forceps. This step was repeated until no obvious signs of amphipods remained. To recover polychaetes from the sediment, a small piece of frozen tissue collected from the fishes in the present study (post-dissection) was placed on the sediment surface near burrow entrances. Polychaetes were collected with forceps when they emerged from the burrows. Amphipods and polychaetes were stored in individual glass vials with plastic stoppers and coated in filtered industrial methylated spirit (IMS). Polychaetes were identified using Fauvel (1923) and Chambers and Garwood (1992). Based on the information available, the Nereididae worms were assumed to be *Hediste diversicolor*. The species is common in the estuary (Attrill, 1992) and Nereididae jaws thought to be *H. diversicolor* were found during dietary analysis. Microplastic extraction was undertaken after the search for infauna was completed.

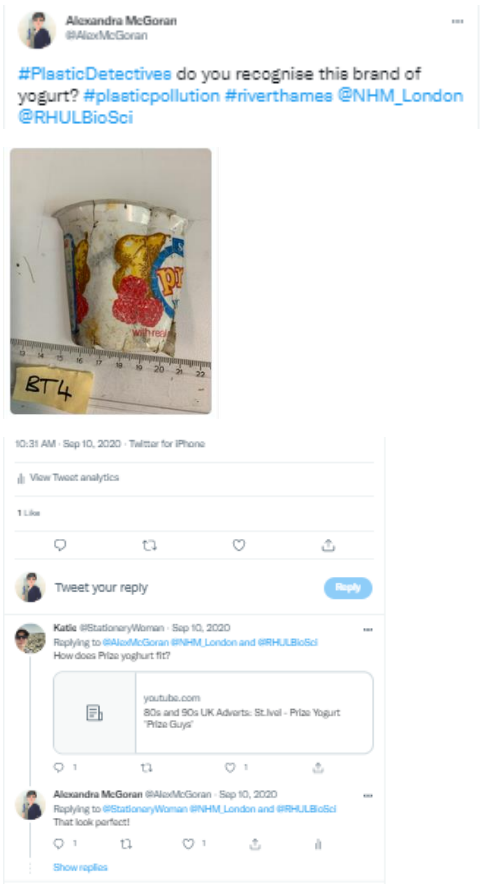
2.2.3 Macroplastic

Large plastic items were collected from the Thames in the Erith area during the midwater and benthic trawls described above. Debris items were stored in sealed plastic bags and transported to the NHM for examination. The plastic was rinsed, measured along the longest dimension (to the nearest cm), photographed and its morphology described (e.g., shape, colour). Where possible, the product (e.g., food packaging, plastic bottle), brand and age of the item were identified. This was assisted by brand websites and Wikipedia. Where logos were unfamiliar or damaged, outreach through social media was used to help identify products. This is a novel citizen science approach to plastic identification and enables the classification of a wider range of items and allows for the discovery of an increased variety of sources (e.g., international sources). Images of the plastic item were shared on Twitter with the hashtag #PlasticDetectives and tagging NHM and Royal Holloway University of London (RHUL) accounts to maximise reach (Figure 2.4). This technique was used by other researchers (Figure 2.4D) and the Plastic Detectives hashtag has been applied by National Geographic Explorers.

A

B

C



D

Figure 2.4. Examples of the Twitter citizen science initiative. Plastic items were identified by members of the public using social media and verified by comparing the item with images online. A) Nabeghlavi healthy water (Georgia); B) Colombina (Colombia); C) Prize Yoghurt (ca. 1980–1990s); D) Plastic Detectives used by other researchers to ID plastic.

The following equation was used to calculate catch per unit effort (CPUE).

$$CPUE = N / (15 \times n)$$

CPUE = number of items recovered per minute of sampling; *N* = total number of items recovered in benthic or midwater trawls; *n* = number of trawls. For each sampling date, CPUE was calculated for benthic and midwater trawls.

2.3 Material collected

During sampling, 1,115 fishes (17 species), 235 crabs (3 species), 1,206 shrimp (only *Crangon crangon* identified), 384 amphipods (1 species), 76 polychaetes (1 species) and 24 ca. 3 L sediment samples were collected. Of the collected biota, 816 fishes (ten species), 384 *Corophium volutator*, 367 *Crangon crangon* and 76 *Hediste diversicolor* were dissected. CSIP recovered six grey seals (*Halichoerus grypus*), five of which were suitable for dissection, one harbour porpoise (*Phocoena phocoena*) and two species of Mysticeti.

2.4 Dietary analysis

2.4.1 Fish and shrimp

Fish and shrimp were caught during a previous study (McGoran et al., 2018) from the Thames Estuary between Thamesmead and the Isle of Sheppey in November 2015. A total of 118 fish and 116 shrimp were collected using beam trawls (mesh size 80 mm) as well as trammel and fyke nets. After inspection for plastics, gut contents were stored in 70% ethanol. Dietary analysis was conducted on *Platichthys flesus*, *Merlangius merlangus*, *Solea solea* and pouting (*Trisopterus luscus*). Key prey items were identified in 85 *P. flesus*, 29 *M. merlangus*, 17 *S. solea* and 6 *T. luscus*. Fish were measured for standard length (to peduncle), greatest depth and weighed as defined by FAO (1974). The diet of sampled individuals collected for the present study were also examined for prey items during microplastic extraction. After digestion, soft prey was destroyed but hard items, such as chitin, persisted. Identifiable prey items were separated for identification.

2.4.2 Seals and porpoises

When filtering marine mammal stomach contents, parasites, bone fragments, otoliths and other hard prey items were removed for identification. For one *H. grypus* individual, a sample of the stomach contents was removed and frozen until dietary analysis could be completed with the help of Prof Dave Morritt (RHUL) and Ollie Crimmen (Senior Fish Curator, NHM). This sample was collected as the stomach was still partially frozen during dissection and contained partially digested fish. Removing this sample allowed for species identification. Furthermore, if the fish stomach was still intact separate analysis of prey contamination was possible. If no fish stomachs were intact, the fish remains were then soaked in boiling water and cleaned with a brush. The fish bones were fixed in isopropanol for 48 hours and dried at room temperature. The bones were then examined under the microscope and compared to specimens from the NHM fish skeleton reference collection, images accumulated by the senior fish curator (O. Crimmen) and Watt et al. (1997). The size of fish prey items was estimated from otoliths using Härkönen (1986) and Camphuysen and Henderson (2017).

2.4.3 EcoPath with EcoSim Model comparison

The trophic levels assigned in the present study through qualitative assessment of abundant prey in the diet was compared to the numerical trophic levels assigned by the EcoPath with EcoSim Model (v6.6.16540.0 North Sea 1991–2013) (ICES, 2017; Mackinson et al., 2018).

2.5 Sample processing

2.5.1 Digestion protocol development

With a growing interest in the monitoring of plastic pollution, it is necessary to develop a standard approach to the extraction of microplastics (Lusher and Hernandez-Millian, 2018; Lusher et al., 2020b; Lu et al., 2021; Morgado et al., 2022). Nevertheless, the methods currently used to extract microplastics from the digestive tract of organisms vary greatly (Lusher et al., 2017), except for standardised seabird monitoring by OSPAR and proposed monitoring in the Arctic (Provencher et al., 2022). It has been noted for a long time that there

is a lack of consistent protocols (Arthur et al., 2009). It is often agreed that digestion is a beneficial step for isolating microplastics (Lusher et al., 2017). Lusher and Hernandez-Millian (2018) proposed that extraction protocols, specifically focussed on marine mammals, must be easy to use and able to be adapted to answer different research questions and allow cross-disciplinary research. The aim of the present study was to determine an efficient and simple protocol for the extraction of plastics from the GIT of fish and other fauna. The hypotheses tested were that 1) one or more protocols will be more effective at recovering microplastics from organic samples and; 2) one or more tests will be more cost effective than the other trials. The most effective protocol, both in terms of microplastic recovery and cost, would then be implemented.

Phase I: digestion of organic matter using chemicals

KOH, 10% is one of the most widely used chemicals for the digestion of organic matter when extracting microplastics. The technique was first described by Foekema et al. (2013) and has been used in recent studies (e.g., Zhao et al., 2016). In some instances, it was reported as successfully digesting material in as little as two days (Karami et al., 2017; Kühn et al., 2017). Organic matter (gut contents from fish) was covered with KOH three times the volume of the organic matter and left to digest at room temperature for 2–3 weeks. Dehaut et al. (2016) tested the procedure and concluded that the digestion time was not suitable for large scale studies. Instead, Dehaut et al. (2016) recommended digesting material (fish, crab and mussel tissues) at 60 °C for 24 hours. Similar adaptations have commonly been adopted (Rochman et al., 2015; Phuong et al., 2018). Phuong et al. (2018) reported that less than 1% of mussel tissue remained after digestion in 10% KOH. One distinct benefit is that, unlike many chemical digestions, KOH does not damage most polymers (Dehaut et al., 2016; Karami et al., 2017; Kühn et al., 2017; Lusher et al., 2017). It has been noted, however, to cause discolouration to PA and degrade cellulose acetate, PES and PE (Dehaut et al., 2016; Karami et al., 2017; Kühn et al., 2017; Lusher et al., 2017). To reduce the damage to polymers, lower molarity and reduced temperatures have been used (Karami et al., 2017; Kühn et al., 2017). It has also been observed that digestion of lipid-rich tissues in KOH can produce an oily residue (Lusher et al., 2017).

Nitric acid (HNO_3) has also been trialled in multiple studies to digest organic matter for the extraction of microplastics, including digestions of zooplankton (Desforges et al., 2015), crustaceans (Devriese et al., 2015), bivalves (Claessens et al., 2013; Van Cauwenberghe and Janssen, 2014; De Witte et al., 2014; Van Cauwenberghe et al., 2015) and fish (Karami et al., 2017). Claessens et al. (2013) used HNO_3 to digest samples overnight at room temperature, followed by two hours of boiling. The results demonstrated that the digestion could recover both PS microbeads and PA fibres (Claessens et al., 2013). Desforges et al. (2015) modified the protocol to reduce risk of plastic destruction and concluded that HNO_3 was the most effective digestive agent for the removal of organic matter. At room temperature, some tissue remained, but when heated to 80 °C all tissue was digested in 1 hour. Phuong et al. (2018) also reported that almost all organic matter was digested by HNO_3 (less than 1% of tissue remained), but demonstrated that the digestion was successful at room temperature as well as when heated to 50 °C. In contrast, Karami et al. (2017) digested fish tissue in 5% and 69% HNO_3 and found that 10% KOH at 40 °C was a more suitable digestive agent. Nevertheless, HNO_3 proved to successfully digest almost all organic matter (69% at room temperature; Karami et al., 2017). Desforges et al. (2015) hypothesised that HNO_3 heated to 80 °C for even as little as 30 minutes could result in the destruction of some polymers (PA and PET) and biopolymers (acetal and polyetheretherketone). HNO_3 has been shown to damage some polymers, such as PE, PS and PA (Catarino et al., 2016; Dehaut et al., 2016; Karami et al., 2017; Lusher et al., 2017), and can leave a residue on recovered plastics that hinders identification (Desforges et al., 2015; Lusher et al., 2017).

Phase II: digestion of organic matter using enzymes

Enzyme digestions are becoming an increasingly more popular approach to microplastic extraction (e.g., Cole et al., 2014; Nelms et al., 2018). Some enzymes, such as Proteinase K, are costly and although effective are not always efficient. Museums have used enzyme macerations to prepare skeletons and a similar approach can be applied to the extraction of plastics. Concentrations of 2.3% to 13.3% savinase and lipex have been used to digest rodent flesh (Simonsen et al., 2011; Eriksen A.M. et al., 2013). This will be the first application of these enzymes to microplastic analysis.

2.5.1.1 Materials and methods

To optimise the extraction of microplastics from the digestive tracts of estuarine biota, it was necessary to compare common digestive protocols. The contents of the alimentary canal from fish were used in all digestions. Fish were sampled using a beam trawl (mesh: 50 mm) in the Firth of Clyde in November 2015 and May 2016. Overall, 16 species of fish were collected, totalling 565 individuals. The material collected from the fish was visually examined for plastics in a previous study (McGoran et al., 2018). The gut contents were stored in 70% ethanol in 50 ml Falcon™ tubes sealed with Parafilm® until use. A subset of 405 pooled individuals (15 species) was used for the digestion analysis (Table 2.1).

Table 2.1. Fish collected from the Firth of Clyde, Scotland in November 2015 and May 2016 (McGoran et al., 2018) used in the present thesis for the development of a digestion protocol. Full breakdown between samples found in supplementary material (S4).

Species	**Frequency**
Limanda limanda	199
Pleuronectes platessa	68
Hippoglossoides platessoides	54
Platichthys flesus	39
Glyptocephalus cynoglossus	20
Myoxocephalus scorpius	5
Melanogrammus aeglefinus	4
Taurulus bubalis	3
Agonus cataphractus	3
Scyliorhinus canicula	2
Merlangius merlangus	1
Solea solea	1
Chelidonichthys cuculus	1
Phrynorhombus norvegicus	1
Trisopterus minutus	1
Pholis gunnellus	1
Gadus morhua	1

Reference materials were collected from Barnet Europe for the spiked recovery tests. Three polymers were selected: PP (Barnet Europe, FIL HIGH TENACITY, dtex 466 f 72, Type: FZEK040); PES (Barnet Europe, FIL HIGH TENACITY, dtex 110 f 24 Type 390A, Type: XNDK009) and PA (Barnet Europe, nylon 66 FIL HIGH TENACITY, dtex 000470 f 140, Type: AICK028). PP and PES were selected as these polymers are some of the most common in the environment. PA was chosen as it is a less robust polymer and more likely to degrade with chemical treatment. It was proposed that if PA could be recovered, then all sensitive polymers would also likely be persistent. It was decided that the reference materials would be fibres rather than pellets or microbeads as fibres are most commonly recouped from biota and are more sensitive to digestions.

Phase I digestions

Phase I digestions (KOH, 10% at 60 °C; HNO_3, 69% at room temperature) were conducted on 15 ml of gut contents with 15–45 ml of the respective digestive agent. It took between 8 and 53 fish to make 15 ml of material. On average, each 15 ml sample contained the gut contents of 47.5 fish. Each sample was spiked with ten fibres of PP, PES and PA for a total of 30 fibres cut to ca. 1 mm in length. Five replicates were run for each digestion protocol.

The addition of HNO_3 to organic matter resulted in the formation of a foam. The volume of foam produced by sample one was sufficient to cause the entire sample to overflow. Sample one was therefore removed from analysis. Claessens et al. (2013) reported a similar loss of material when using a combination of HNO_3, hydrogen peroxide and sodium hydroxide. To reduce foam production in the remaining samples, HNO_3 was added in small volumes (500 µl to 2 ml every 0.5–1.25 hours).

Organic material was allowed to digest in KOH or HNO_3 for two days (46 hours) in a water bath. After digestion, contents were diluted with 100 ml of filtered distilled water and vacuum filtered through glass fibre filters (Whatman™, grade GF/C, size 4.7 cm). Filters were then searched and microfibre counts recorded.

Phase II digestions

Additional tests were run using a dry weight of 1 g of gut contents to maximise sample size. This volume is a more realistic estimation of fish gut size and therefore provided more applicable results than tests in phase I. Gut content samples were dried in an oven (Raven 100L, LO/RAV/09/1) overnight at 50 °C for a minimum of 12 hours. Once dried, the samples were transferred to a desiccator (Secador) for a minimum of two hours. Samples were spiked with ten fibres of PP, PES and PA (30 in total) cut to ca. 5 mm in length. Four digestion protocols were compared: 10% KOH, 10% protease and lipase (enzyme solution 1: 5 ml savinase, 5 ml lipex, 90 ml filtered distilled water), 5% protease (enzyme solution 2: 5 ml savinase, 95 ml filtered distilled water) and 2% protease (enzyme solution 3: 5 ml savinase, 295 ml filtered distilled water). Five replicates were run for each digestion protocol. These digestions were filtered and searched using the methodology previously described.

The KOH samples were checked after 24 and 48 hours. Digestion continued for an additional 3–148 hours (8-day/196-hour maximum digestion). Samples were checked at several time intervals to determine the optimum time for digestion. Phase I digestions demonstrated that 48 hours was sufficient to digest organic material but did not provide information on whether a shorter period would achieve the same level of digestion or whether a longer period would be more successful. As enzyme digestions were expected to be faster, optimum time for digestion by KOH was key for comparison. Samples digesting in enzyme solution 1 were checked after 1 hour and allowed to digest for a further 1.5–162 hours (1 week/163 hour maximum digestion). Enzyme solutions 2 and 3 were allowed to digest for 2–26 hours (1 day).

A subset of fibres was analysed using micro-FTIR on an AutoIMAGE FT-IR Microscope System from PerkinElmer. Fibres from the longest digestion of each treatment were analysed to confirm that spiked polymers were recovered. Polymer identity was confirmed by comparing scanned spectra with a library of spectra from known materials built internally by NHM Scientific Associate Mark Underhill.

Statistical analysis was conducted using R version 3.4.2 (R Core Team, 2017). The two most efficient digestion protocols were compared with a t-test. Two tests were run which

compared fibre recovery and digestion effectiveness. Normality was tested with a Shapiro-Wilk test.

A qualitative test was also run after digestions. For PP and PES, 10 cm of yarn (collective of fibres wound together and not separated) were immersed in 10% KOH and placed in a 60 °C oven for 2 days. Controls were also set up where yarn was immersed in water and heated for 48 hours. The yarn was then observed under a binocular light microscope, followed by FTIR analysis.

2.5.1.2 Results

Phase I digestions

Digestion with 10% KOH resulted in 26–51% reduction in material (average: 36.6%, Table 2.2). The water level in the bath, however, dropped overnight due to evaporation, despite a foil cover being in place. It was noted that this would result in an inconsistent temperature and therefore an unpredictable digestion. As such, the KOH digestion was repeated in phase II.

Table 2.2. The proportion of fish stomach contents digested by phase I protocol tests during a 46-hour period. The digestion efficiency could not be calculated for all samples in test 2 due to foam production by HNO_3.

	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**
Test 1- KOH 60°C	51%	67%	36%	38%	26%
Test 2 – HNO_3 at room temperature	N/A	N/A	N/A	N/A	N/A

HNO_3 proved to be an ineffective digestive agent, producing a high volume of foam during digestion. All samples experienced partial overflow during the addition of HNO_3. The digestive agent had to be added in such small quantities that it took over a day to reach 15 ml

of solution. This was much less than the recommended 45 ml (three times the volume of organic material; Foekema et al., 2013). The foam produced during the digestion resulted in samples being contaminated by plastics in the laboratory and prevented the digestion from being quantified. As such, it was not possible to record the proportion of material successfully digested by HNO_3. Claessens et al. (2013) noted that foam was only produced when HNO_3 was cold. But, HNO_3 was observed to be destructive to some polymers at room temperature in this present study. Filtering the acid resulted in the mesh dissolving immediately. It was decided that heating the acid would only increase the degradation of plastics. Furthermore, HNO_3 was deemed too destructive for microplastic analysis.

Quantification of these protocols was difficult due to foam production by HNO_3 and the imprecise measure of volume using Falcon™ tubes. Organic matter was also insufficiently digested, often blocking filters. It took over four hours to search half of one filter from a HNO_3 digestion. Due to the time taken to search one filter and the potential for contamination by foam, no other filters were searched in the phase I digestions. To overcome this problem, phase II digestions used a smaller volume of material and did not include HNO_3.

Phase II digestions

The results of the digestion protocols are summarised in Table 2.3. KOH digested between 26% and 67% of organic matter (average: 43.6%) and resulted in the recovery of 67–93% of fibres (average: 83.4%). Samples in enzyme solution 1 were digested 43–54% of material (average: 46.6%) and 77–100% of fibres were recovered (average: 90.8%). Enzyme solution 2 resulted in the digestion of between 3% and 21% of material (average: 11.6%). Between 40% and 87% of fibres were recovered (average: 73.4%). Enzyme solution 3 digested between 3% and 22% of material (average: 7.5%) and 67–107% of fibres were recovered (average: 84.8%).

Table 2.3. The proportion of fish stomach contents digested by phase II protocol tests and the time taken for each digestion to occur. The digestion efficiency for test 3 sample 5 could not be calculated due to an error in initial mass recording.

	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**
Test 1- KOH 60 °C	2 days	2 days	1 week	1 week	1 week
	51%	67%	36%	38%	26%
Test 2 – enzyme solution 1 50 °C	2.5 hours	2.5 hours	1 day	1 week	1 week
	43%	45%	44%	54%	47%
Test 3 – enzyme solution 2 50 °C	2 hours	2 hours	2 hours	1 day	1 day
	8%	10%	16%	3%	21%
Test 4 – enzyme solution 3 50 °C	2 hours	2 hours	2 hours	1 day	1 day
	3%	3%	2%	22%	N/A

Comparison of phase I and II digestions

The Shapiro-Wilk test found that digestion effectiveness was not significantly different from a normal distribution (w = 0.97495; p = 0.9325). Similarly, fibre recovery was normally distributed (w = 0.94565; p = 0.6174). T-tests revealed no significant difference between fibre recover (t = -1.1989; df = 7.874; p = 0.2654) or digestion effectiveness (-0.40855; df = 4.6132; p = 0.7011) between KOH digestion and enzyme digestion with solution 1. Mean digestion effectiveness was 43% and 46% with KOH and enzyme solution 1 respectively. Fibre recovery was 83% and 91% respectively. The results of digestions in KOH and enzyme solution 1 are summarised in Table 2.4.

FTIR analysis identified recovered fibres from KOH 2-week digestions as PP and PA. PES was not recovered and PP showed signs of clumping and possible melting (Figure 2.5).

PES was recovered from 2-day digestions. All polymers were recovered from enzyme digestions and showed no signs of damage.

Visual examination revealed no discolouration, clumping or degradation of PP or PES fibres from yarns placed in water or KOH at 60 °C for 2 days. It was observed that a residue was present on all samples stored in KOH (Figure 2.6). To test whether when dried this residue caused fibres to clump together, the yarn was removed from the solution, placed in a Petri dish and dried overnight at 60 °C. Studying the dried fibres under the microscope demonstrated that the residue, when dry, caused fibres to adhere to one another and made it difficult to separate them. The fibres, however, were not melted together by the KOH digestion. Analysis with FTIR showed that the spectrum produced by each polymer did not differ between the control and digested fibres. All fibres could be accurately identified by comparison to the library of spectra. The spectrum produced by the dried residue did not match PES or PP.

Table 2.4 compares the remaining phase II digestions to determine the optimal technique for the extraction of microplastics from stomach content.

Table 2.4. A comparison of digestion efficiency, optimal duration, polymer recovery and cost for KOH and enzyme solution 1 digestions of fish stomach contents.

	KOH	**Enzyme solution**	**Optimal choice**
Greatest proportion of sample digested	67%	54%	KOH
Average proportion of sample digested	43.6%	46.6%	Not significantly different
Optimal duration of digestion	2 days	2 hours	Enzyme solution
Average proportion of fibres recovered	83.4%	90.8%	Not significantly different
Cost per 100ml of prepared solution	Ca. £0.14	Ca. £1.70	KOH

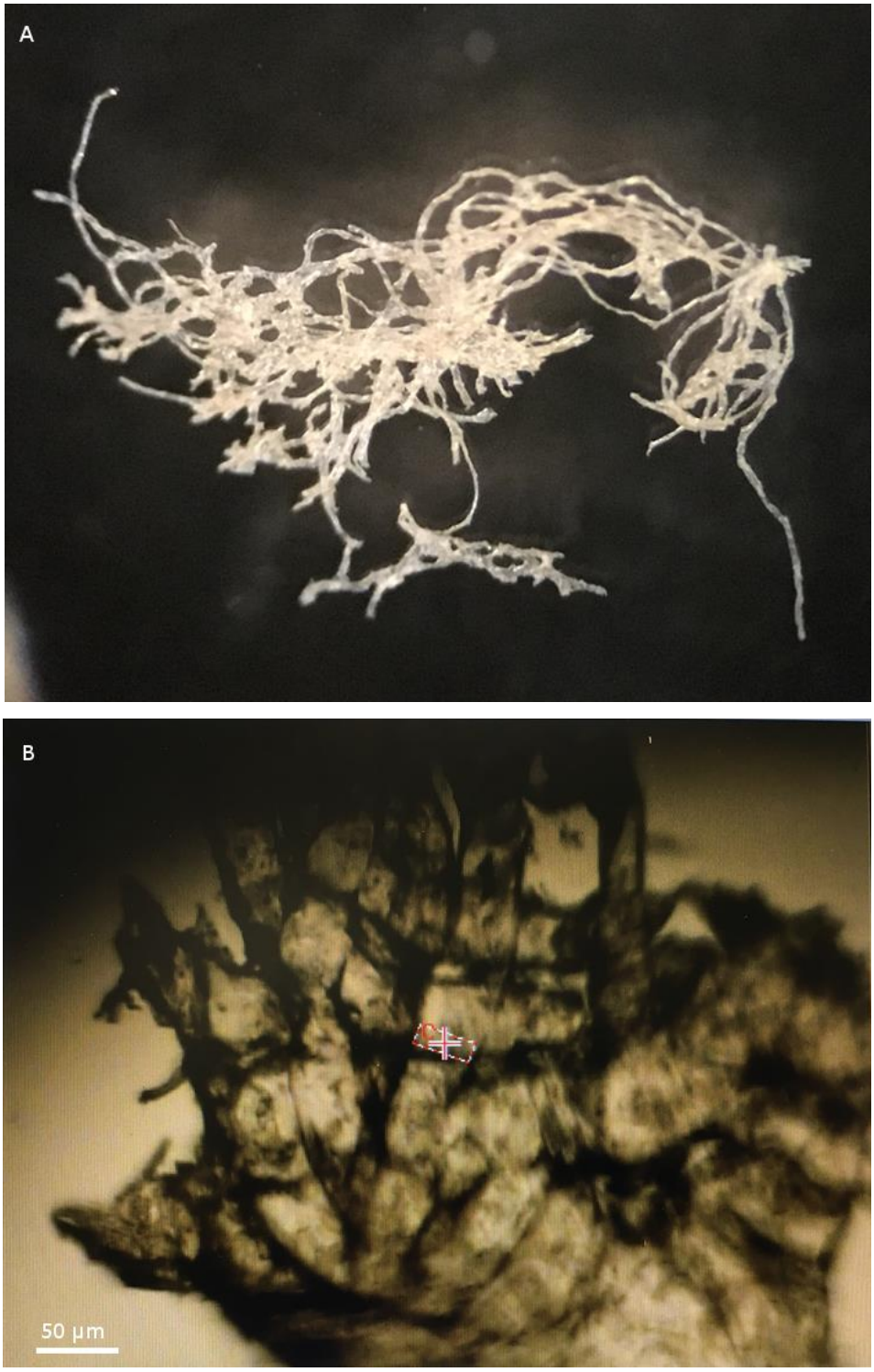


Figure 2.5. PP fibres recovered from 10% KOH digestion after 2 weeks. Fibres appear to be clumped and broke when attempted to separate. A) Observed under a binocular microscope, scale unavailable; B) Observed with micro-FTIR.

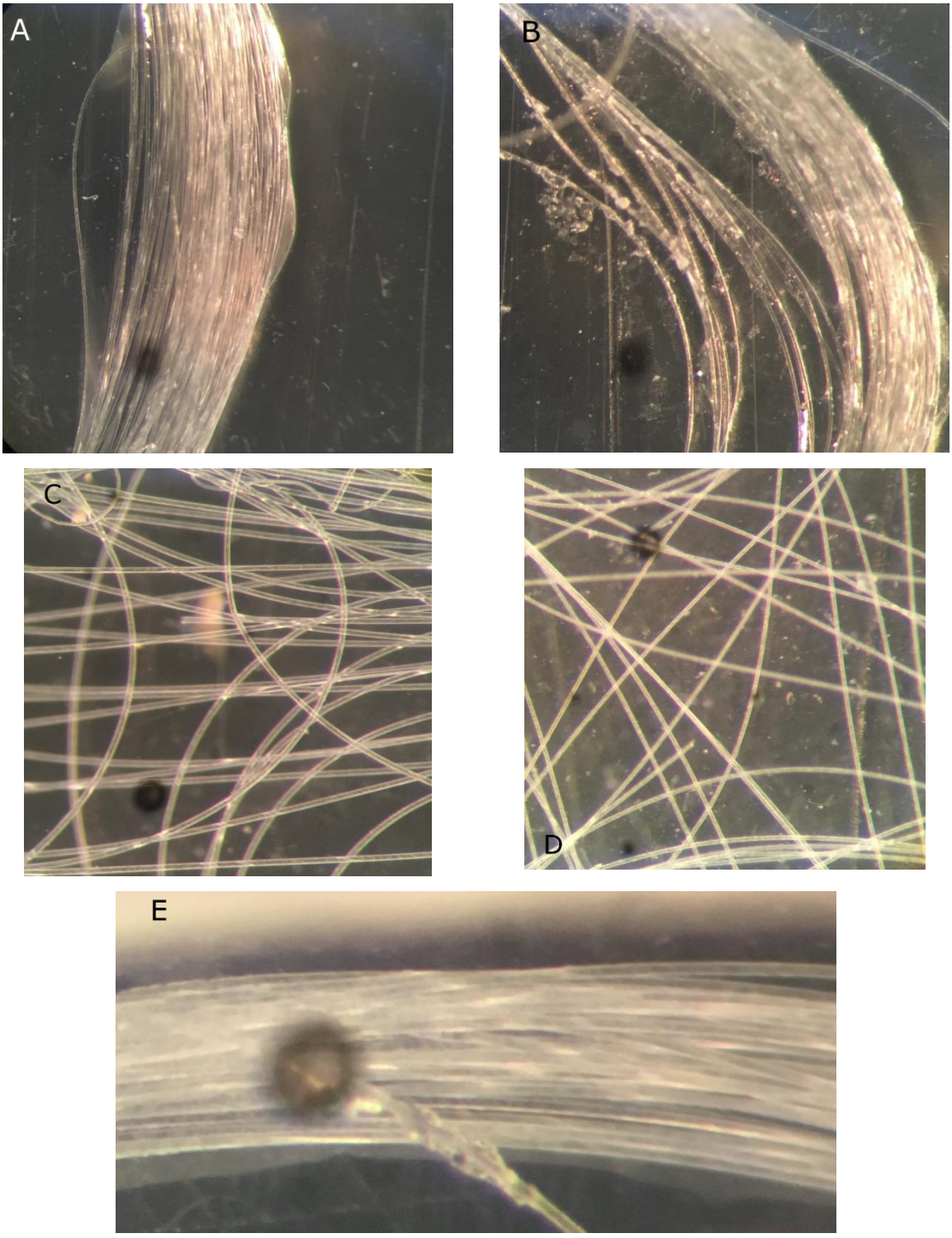


Figure 2.6. A residue was present on all samples stored in KOH. A) PP fibres from control sample; B) PP fibres from KOH digestion with residue present; C) PES fibres from control sample; D) and E) PES fibres from KOH digestion with residue on fibres and suspended in KOH. Scale unavailable.

2.5.1.3 Discussion and conclusion

A limitation of the protocols tested is that smaller particles are probably overlooked and therefore published values are likely to be an underestimate of plastic abundance (Hidalgo-Ruz et al., 2012; Connors et al., 2017). It is important to note that plastic abundance increases with decreasing size of particles (Shim et al., 2018). To quantify these lower size fractions, Eriksen M. et al. (2013) used scanning electron microscopy. Their study reported that, on average, in the surface water of a freshwater system there were 43,000 particles per km^2 and a maximum of 466,000 particles per km^2. Another method for recovering smaller particles is the use of pyrolisis-gas chromatography/mass spectrometry (Py-GC/MS). By combining Py-GC/MS with microwave-assisted extraction, Hermabessiere and Rochman (2021) were able to detect particles down to between 0.002 μg and 0.18 μg. These methods could not be implemented in the present study but could be explored in the future.

HNO_3 is an unsuitable reagent for microplastic extraction from biota as it is highly likely to damage the polymers before recovery. Indeed, Gulizia et al. (2022) found HNO_3 to be the most damaging of several commonly used chemical digestions. Equally, enzyme solutions 2 and 3 are not recommended. These solutions are ineffective at removing organic matter during digestion and therefore do not aid microplastic recovery.

KOH and enzyme solution 1 digestions produced comparable results with respect to digestion effectiveness and plastic recovery. Duration of digestion and cost, however, differ greatly between the two. The present study provides evidence that KOH, especially if used for a week, could degrade PES. Lusher et al. (2017) reported that KOH heated to 20 °C could damage PES even at concentrations as low as 1%. Despite this, Lusher and Hernandez-Millian (2018) recommend KOH as the most effective technique currently available for digesting biological material, with a focus on marine mammals. Their study highlighted its low cost, ease of use and availability. Additionally, it is the most implemented approach allowing for a comparison with a range of studies (Lusher and Hernandez-Millian, 2018). Initial tests suggested that PP might be partially digested by KOH causing fibres to melt together into clumps. Lusher et al. (2017) reported that PP should be resistant to KOH even at 50% heated to 50 °C. Drying the fibres after KOH digestion, however, revealed that the clumping effect was the result of dry residue. FTIR analysis confirmed that during a 2-day digestion, PP, PES

and PA could all be recovered effectively and that the oily residue produced during the process did not impact identification of plastic. Although the residue may make counting and separation of fibres more complicated. Given that shorter digestions pose no risk to plastic, the optimal digestion protocol must be determined by the efficiency of the digestion. In this instance, efficiency is defined by the speed of digestion for a given cost of solution. Enzyme solution 1 costs 12 times as much as KOH for an equivalent digestion. It takes just two hours to complete the digestion with enzymes, to justify the cost KOH would need to take more than 12 times longer. If using a two-day digestion, then this is the case. It is, however, possible to reduce KOH digestions to 24 hours or overnight, which is commonly reported in the literature, e.g., Prata et al. (2021). When implementing an overnight digestion of KOH, the speed of enzyme digestions no longer justifies the difference in cost. Furthermore, the enzymes used in the present protocol development are not as widely available as KOH, which can be purchased by researchers worldwide. Whilst other enzymes are available, their cost will vary. Given the scope of this thesis and the predicted difficulty in producing suitable quantities of enzymes for marine mammal digestive tract digestions, it is recommended that future analysis be conducted using a KOH digestion. In addition, it is suggested that perhaps a shorter overnight would reduce the chance of PES degradation. Ultimately, it is necessary to harmonise protocols to allow for comparisons between regions and research groups. Consequently, KOH is a more viable option.

2.5.2 Microplastic extraction

2.5.2.1 Sediment

For sediment samples, microplastics were extracted by following the density separation protocol outlined by Coppock et al. (2017). Five 50 g (ca. 45–50 ml) sub-samples of sediment were collected from each ca. 3 L grab sample. Exceptions included sediment samples collected on 4th December 2018 and 19th December 2019. Two of the three grab samples collected in 2018 were less than 3 L and were insufficient for subsampling. Therefore, as many subsamples as possible were collected from these grab samples and the remainder of subsamples were made up from the remaining successful grab. The grab samples collected in December were all 3 L, however, due to lab access restrictions with the Covid-19 pandemic, only two subsamples were collected from one of the grabs instead of the full five. This

resulted in 12 samples instead of the 15 collected for all other sampling trips. Each 50 g sample was suspended in 700 ml of filtered 1.5 g cm^{-3} zinc chloride ($ZnCl_2$) solution. A magnetic stirrer plate agitated the sample for 5 minutes which was followed by a 5-minute settling period. After this settling period, three rapid pulses of the magnetic stirrer were used to displace and trapped air bubbles. The solution was allowed to settle until no sediment was suspended. This ranged from 15 minutes to, on one occasion, 7 hours. When all the sediment had settled, the ball valve was closed and the solution vacuum filtered through a 32 µm white nylon filter with a vacuum pump and dried at 50 °C in glass Petri dishes.

2.5.2.2 Biota

Biota were defrosted prior to dissection. Fish and crabs were defrosted overnight or on the morning of dissection. Marine mammals were left covered outside to thaw for ca. 48–72 hours. Shrimp were defrosted in one hour. The mass (Ohaus ranger 3000 balance, nearest 0.1 g), length (to nearest mm) and sex of each individual was recorded. In addition, for crabs, carapace width was measures at the widest point between the 4th pair of anterolateral teeth with a rule (to nearest mm). The standard length, total length (to tip of hind limb) and axillary girth were recorded for all seals as described by Committee on Marine Mammals (1967). Through dissection, the digestive organs (GIT and stomach/GM) and gills of the fishes and crabs were recovered, each was placed in separate 50 ml Falcon™ tubes. The GM was opened prior to digestion to allow the solution to reach the material inside. Shrimp and amphipods were cut in half dorsoventrally to expose the GIT and gills and then individually stored in 50 ml Falcon™ tubes. Polychaetes were processed whole.

Seals and porpoises were dissected at the NHM following Pugliares et al. (2007). In addition, the condition of the specimen and any scars, wounds and parasites were recorded. Parasites were collected for the NHM collections. Further samples, including tissue, stomach contents and puss, were collected for molecular analysis and for the NHM collections. The entire GIT was removed and was split into compartments (e.g., fore stomach, pyloric stomach, intestines) with cable ties used either side of each compartment to prevent contamination once cut. This also allowed for independent examination of each compartment. The stomach, and each of its sections if appropriate, was examined separately to the intestines. The latter were examined in sections a few inches long. The GIT lining was rinsed with filtered water and

examined for parasites. The GIT content was rinsed onto a 300 µm metal sieve. It is therefore likely that small microplastics will be missed in the present study. It was, however, not feasible to filter the contents through a small sieve without it clogging. The contents needed to be filtered to extract parasites for the NHM collections and to aid diet analysis. Once filtered, the GIT content was stored in multiple 50 ml Falcon™ tubes ahead of microplastic extraction.

The baleen whales could not be transported to the NHM for dissection. Instead, post-mortem examinations were conducted in the field by CSIP, collected under contract to Department for Environment, Food & Rural Affairs (Defra) in 2019. All post-mortem investigations were conducted using standard operating procedures; cetaceans were moved to and examined at a PLA managed facility at Erith. During the examination, baleen samples were collected and if there were signs of recent feeding, the gastrointestinal tract was sampled. Six and ten baleen plates were collected from *Megaptera novaeangliae* and *Balaenoptera borealis*, respectively. No stomach samples were collected.

Digestion

Each 50 ml Falcon™ tube was filled with 10% KOH solution, so that the GIT, gills, GM, shrimp, amphipod or polychaete sample was completely immersed. Digestion occurred overnight at 50 °C with the exception of crabs which were digested at 60 °C. After digestion, the solution was filtered through a 32 µm white nylon filter with a vacuum pump and the tubes were rinsed three times or until all visible material was removed. Filters were dried at 50 °C in glass Petri dishes. Once dried, these were sealed with Parafilm® until they could be visually inspected for plastic.

Baleen plates were separated with a clean scalpel and rinsed with filtered water. The filtrate was collected in a 3 L plastic container. The keratin fringe of each plate was briefly inspected under the Leica MZ 6 microscope using mounted pins and any items remaining on the plates after rinsing were recovered. The filtrate from each plate was vacuum filtered across two 32 µm nylon filters and briefly dried in a 50 °C oven. Once dried, samples were sealed in glass Petri dishes with Parafilm®.

Filters were visually examined under a Leica MZ 6 dissection microscope using mounted pins and forceps using 16–64 times magnification with a detection limit of 32 µm.

Plastic items were identified with reference to Lusher et al. (2020a). All recovered items were photographed and either stored in individual glass specimen tubes with plastic stoppers or on damp filter paper in a sealed plastic Petri dish with a diameter of 90 mm. The latter was used for a few sediment samples only. Items were described by colour and shape and were measured (length and width) using Image J version 1.52a (Rasband, 2018). Shape was classified as either a film, fibre, fragment or a tangle of fibres. In some instances, items did not fit these criteria and were listed as 'other'. This category contained three resin-like, putties that do not fit within current classification (Rochman et al., 2019). Due to time constraints as a result of the Covid-19 pandemic not all particles could be measured in ImageJ. Plastics that were analysed with FTIR were prioritised as they comprised a representative subset of samples (described below).

Tangles of fibres were weighed on a Sartorius MC5 balance (to the nearest 0.001 g) and an estimate of the number of items in the tangle calculated. Counting and photographing all items recovered from one tangle comprising over 100 items took several hours. Consequently, it was deemed impractical to precisely document all items from some tangles, instead estimates were made of their abundance.

The proportion of samples containing microlitter, the frequency of microlitter abundance and the diversity of items, as defined by the number of forms (shape and colour combinations) of plastics in a sample, was reported.

2.6 Microplastic identification with FTIR

A representative subset of 10% of these particles was analysed by FTIR and compared to commercial and in-house spectral libraries (Supplementary Material, S1). The subsample comprised 10% of each previously assigned colour and shape combinations for each species studied as well as sediment (i.e., 10% of red fibres were analysed from *P. flesus* as well as 10% from sediment samples). Two FTIR instruments were used for infrared analysis of microplastics. The libraries used to identify polymers, however, were consistent for all analysis. A PerkinElmer Spectrum One FTIR spectrometer, with an AutoIMAGE FTIR Microscope System PerkinElmer attachment was initially used alongside OMNIC Picta. Individual items from the subset of plastics were individually analysed with a background spectrum collected before analysis of every item. A total of 16 scans were collected for each

item, with the average result being used to generate an absorption spectrum between 500 and 4,000 cm^{-1}. Later samples were analysed with a Nicolet iN10 MX Infrared Microscope in OMNIC Picta. Absorption spectra were collected with an MCT-A detector over 16 scans at a resolution of 4 cm^{-1} in the range 650–4,000 cm^{-1}. Similarly, a background spectrum was collected before analysing each item. Both instruments were cooled with liquid nitrogen to collect the clearest spectra. Polymer identity was confirmed by visual comparison with library spectra rather than with a percentage match threshold. Where recorded, a percentage match is also reported in the data.

2.7 Quality assurance

2.7.1 General controls against contamination

All work was conducted in a clean laboratory with sticky contamination control mats at the entrance. Only 100% cotton clothing and 100% cotton laboratory coats were permitted in the clean laboratory. The cotton laboratory coat was dyed purple to better identify contamination and reduce the number of white fibres present in controls. All work was conducted in a laminar flow hood. All doors were kept shut to reduce airborne contamination from adjoining spaces. All equipment was washed three times with filtered distilled water (32 µm metal sieve) and examined under a dissection microscope before and between dissections and visual examinations. Glassware was triple rinsed with filtered distilled water. When samples were required to be diluted, filtered distilled water was used. For all work nitrile gloves were worn. Procedural blanks of filtered distilled water were run for all preliminary digestions. Blanks were left for the duration of the longest digestion. In addition, empty Petri dishes were used to record airborne contamination during dissections, digestions, filtration and visual examination. Potential sources of contamination highlighted by these measures were removed from the laboratory. All samples remained covered, whenever possible, including in the oven, during filtration, digestion and density separation. Filtered samples were covered by Petri dish lids and sediment-microplastic isolation units were covered with foil.

2.7.2 Controls for sediment samples

In addition to the above controls, prior to each density separation, the sediment-microplastic isolation units were primed with 700 ml $ZnCl_2$ solution following the protocol of Coppock et al. (2017). The solution was filtered and the filter discard along with any microplastics collected. This ensured the unit was free from contamination prior to collecting samples and controls.

Contamination in procedural blanks was averaged and removed from all samples. Whilst for airborne contamination, each control was averaged for the samples processed at the time of the control. This was to account for days when the laboratory was shared and contamination was expected to be higher. It is assumed that this approach was more accurate than applying average contamination to all samples.

2.7.3 Controls for biota samples

Due to limited space, marine mammal necropsies could not be conducted in the clean laboratory. Instead, for pinnipeds and porpoises, which could be transported to the NHM for dissection, everyone involved was required to wear orange or white DuPont Tychem F coveralls. Those undertaking the internal examination of the seal wore orange, whilst people only contributing to the external examination wore white. Work surfaces were wiped down with 80% ethanol and a cellulose tissue prior to any work taking place.

2.7.4 Correcting for contamination

Samples were corrected for recorded contamination based on particle form, e.g., corrected for red fibres, fragments etc. recovered from controls. Contamination in procedural blanks was averaged and removed from all samples. Whilst for airborne contamination, each control was averaged for the samples processed at the time of the control. This was to account for days when the laboratory was shared and contamination was expected to be higher. It is assumed that this approach was more accurate than applying average contamination to all samples.

2.8 Conclusion

The Thames Estuary has many potential sources of plastic pollution. Macro- and microplastics are abundant but have previously only been studied independently of each other. By applying the protocols described in the present chapter, macro- and microplastics will be quantified in the estuary and the most polluting sources will be identified.

Chapter 3

Macrodebris in the Thames Estuary

3.1 Introduction

Monitoring litter and identifying its sources is crucial to addressing how it leaks into the environment and determining the effectiveness of mitigation measures (Ryan et al., 2020). Monitoring microplastics to determine the success of measures to reduce plastic entering the environment poses technical issues due to their small size and difficulty assigning to a source. As such, combining macroplastic and microplastic monitoring would couple the identification of sources of plastic and identifying the risk from microplastics.

Macroplastic records often focus on shorelines (e.g., Storrier et al., 2007; Blettler et al., 2019; Fischer, 2019; Thames21, 2019a, b; Bernardini et al., 2020) and surface waters (e.g., Crosti et al., 2018; González-Fernández et al., 2018; Castro-Jiménez et al., 2019; Van Emmerik and Schwarz, 2019; Vriend et al., 2020) due to ease of access and observation. However, macroplastics are also present in the sub-surface water column, on the seafloor, and riverbeds accumulating near urban centres and in gyres (Barnes et al., 2009; Eriksen et al., 2014; Morritt et al., 2014). Additionally, coastal plastic can originate from land and sea-based sources as well as travelling large distances (Chassignet et al., 2021).

Whilst monitoring plastic on beaches and the foreshore is relatively easy, shorelines are complex systems with dynamic fluctuations in plastic accumulation and the foreshore may only provide evidence for plastic trapped in the riverine system and not for plastic moving out to sea. An alternative approach to address local plastic inputs is to sample riverine plastic. This sampling strategy can determine runoff from land-based sources and assess the effectiveness of mitigation on land (Ryan et al., 2020; Winston et al., 2020).

Rivers transport thousands of tons of plastic to the sea. For example, in European rivers, an average of 250 items are transported to the sea per hour (Van Calcar and Van Emmerik, 2019). Furthermore, Gasperi et al. (2014) reported that, per Parisian, up to 8.2 g of plastic was floating in the River Seine per annum. This is equivalent to every inhabitant of Paris throwing a plastic bottle in the river every year (Recycling Today, 2015; American Samoa Power Authority Material Management Office, online).

Models have determined that the main factors affecting plastic discharges from rivers are the distance of the river from waste sources, land-use and the relative length of an area's coastline. For instance, countries with a small surface area compared to the length of their coastline, such as the Philippines, are estimated to discharge more plastic waste from their rivers. Indeed, seven of the ten most polluting rivers modelled by predicted by Meijek et al. (2021) are in the Philippines. Despite their importance for plastic transport, few studies focus on freshwater environments (13%) and the majority (76%) focus on microplastics rather than macrodebris, which is often not categorised in rivers (Winton et al., 2020). Additionally, waste compositions are significantly different in marine and riverine environments, thus monitoring one without the other fails to address all sources and endpoints of plastic pollution (Winton et al., 2020). Even fewer studies address seasonal variation in plastic abundance in rivers (Van Calcar and Van Emmerik, 2019). Since rivers are not static systems and are greatly influenced by seasonal changes, such as weather patterns, ongoing monitoring through the year is key to our understanding of plastic impacts and fate.

Using product and brand audits, developed through these monitoring strategies, can further highlight the target audience to maintain impact. This could be through legislation on product's manufacture, levies on its purchase or public educational campaigns. It is important to note that an audit such as this cannot prove intent to litter but can demonstrate if particular products or waste streams are most commonly polluting a river. This is pertinent as stopping plastic at source is more achievable than removing it from the environment. Certainly, it is easier to target sources of macroplastic pollution compared to microplastic.

As a heavily urbanised river, with a large population within its catchment, the River Thames is at risk of plastic pollution and therefore, is an ideal location to perform a brand audit survey. Additionally, the Thames feeds into an OSPAR region (Region II: the greater North Sea) which is monitored for marine litter (OSPAR Commission, 2014) and comparisons could be made between datasets. Furthermore, citizen science projects have collected long-term records of litter on the Thames foreshore (e.g., Fischer, 2019; Thames21, 2019b; Bernardini et al., 2020), though records of plastic pollution in the river are limited. Perhaps the only study to investigate subsurface plastic occurrence is Morritt et al. (2014). The study reported that large numbers of items were flowing along the Thames riverbed. There is even less evidence for plastic present in the water column with a single study on micro- and

mesoplastics in surface water at Greenwich and Putney (Rowley et al., 2020). Macrodebris, however, was not reported. Surface macroplastics are collected by the PLA who employs a fleet of passive debris collectors which were constructed to retain surface water driftwood and debris but increasingly plastic is recovered. Sub-surface plastic is unmonitored, largely due to costs and restrictions on boating activity in the tidal Thames.

The present chapter aimed to address the gaps in knowledge highlighted above. Firstly, since there is no ongoing monitoring of plastic in the River Thames, only ad hoc citizen science collections along the foreshore, this chapter will quantify plastic abundance providing some baseline data building on evidence gathered by Morritt et al. (2014). Secondly, the present chapter aimed to provide the first evidence of sub-surface macroplastic pollution suspended in the water column. Comparisons were made between this midwater plastic and the concentrations on the riverbed. Lastly, seasonal variation in macroplastic abundance was recorded through repeat sampling in the estuary. Comparison of seasonal samples aimed to highlight the factors affecting plastic accumulation in the river as well as potential sources of pollution. For instance, glitter was more abundant in the Thames following celebratory events and festivals, such as LGBT+ Pride in London (Rowley et al., 2020). Hence, the hypotheses of this chapter are 1) macroplastic will be abundant in the Thames Estuary; 2) macroplastic will be more abundant on the riverbed compared to the water column and 3) macroplastic abundance will not vary seasonally, with plastic abundant in the estuary all year round.

3.2 Materials and methods

Macrodebris was collected as described in Chapter 2. Once collected, samples were cleaned and sorted into categories. Where possible, brands and other interesting details were noted. Some macrodebris items were considered of note due to, for example, their age or likely country of origin. Details about these notable items were obtained through citizen science using social media and online communities. Images were shared on social media and crowdsourcing used to assign a product or brand to the item.

Statistical analysis was completed in R version 4.0.3 (R Core Team, 2020) with “ggplot2” (Wickham, 2016). A linear model (LM) compared CPUE between seasons (December 2018 – September 2018) and depth of trawls (midwater or benthic). A chi-squared

test was used to compare the proportion of macrodebris types between benthic and midwater trawls.

Model 3.1 – LM(CPUE ~ Season + Depth)

Daily rainfall data were collected by the Met Office (2006) and accessed through the Centre for Environmental Data Analysis. The closest station to the sampling site was Erith: Manor Road P STA (51.4772, 0.19336). This station could not be used, however, as it ceased recording data in 1979. Instead, the next closest station was used: Dartford S WKS (station site number 6762). Only rainfall recordings seven days prior to sampling and the day of sampling were analysed. Dartford station is located at 51.466, 0.23488 and has been collecting data from 1927 to the present. The rain gauge number is 291628.

3.3 Results

More than 1,300 macroplastic pieces were recovered from 60 trawls on eight separate Erith visits (Table 3.1). Model 3.1 revealed that significantly more plastic was recovered per minute trawling on the riverbed than in the water column ($p < 0.05$, R^2 0.2665, *F*-statistic 6.45, degrees of freedom 14; Figure 3.1). On average CPUE for benthic samples was 2.75 ± 2.44 items per minute (mean ± standard deviation) compared to 0.57 ± 0.42 items per minute in midwater samples. The greatest number of items recovered from a single 15-minute trawl was 247 items. There was no significant difference in CPUE between sampling seasons ($p > 0.05$) and, as such, it was removed from model 3.1. Plastic ranged from 1 cm to 170 cm in the longest dimension, with an average length of 15.9 ± 19.4 cm in midwater samples and 11.3 ± 14.2 cm in benthic samples (Figure 3.2).

The mean, per Erith visit, cumulative rainfall over the recorded eight-day period was 28.13 ± 15.33 mm (Supplementary Material, S5 & S6). The greatest cumulative precipitation occurred in June 2019 (52.7 mm), but the greatest rainfall on a single day occurred on 28th August 2020 with 17.5 mm, closely followed by 18th July 2020 with 17.1 mm (Figure 3.3).

Table 3.1. Macrodebris was collected in a beam trawl from Erith in the River Thames on eight dates.

Date of sampling	Number of benthic trawls	Number of midwater trawls	Number of benthic items	Number of midwater items
4th December 2018 (Winter)	5	3	61	41
6th March 2019 (Spring)	4	5	35	23
14th June 2019 (Summer)	2	5	127	12
2nd October 2019 (Autumn)	4	5	31	17
19th December 2019 (Winter)	4	4	156	18
19th March 2020 (Spring)	3	3	68	28
21st July 2020 (Summer)	3	3	191	30
3rd September 2020 (Autumn)	4	3	449	63
Total	29	31	1,118	232

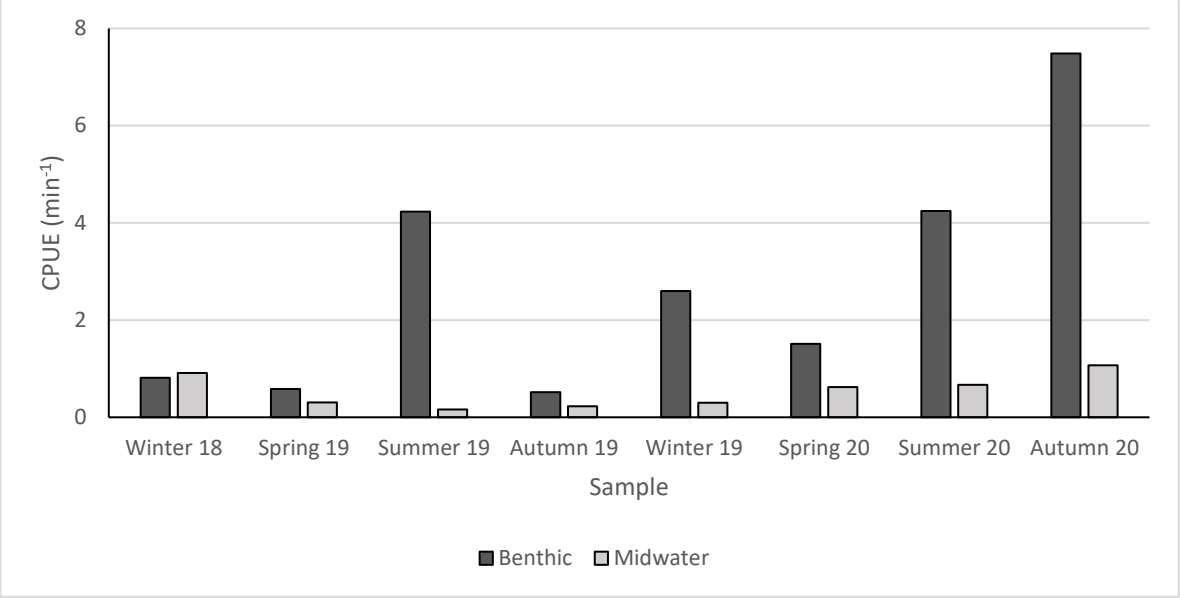


Figure 3.1. Catch per unit effort (number of plastic items per minute sampling) for benthic and midwater samples collected on eight dates between December 2018 and September 2020. Benthic samples contained significantly more plastic debris, but no significant seasonal variation was found.

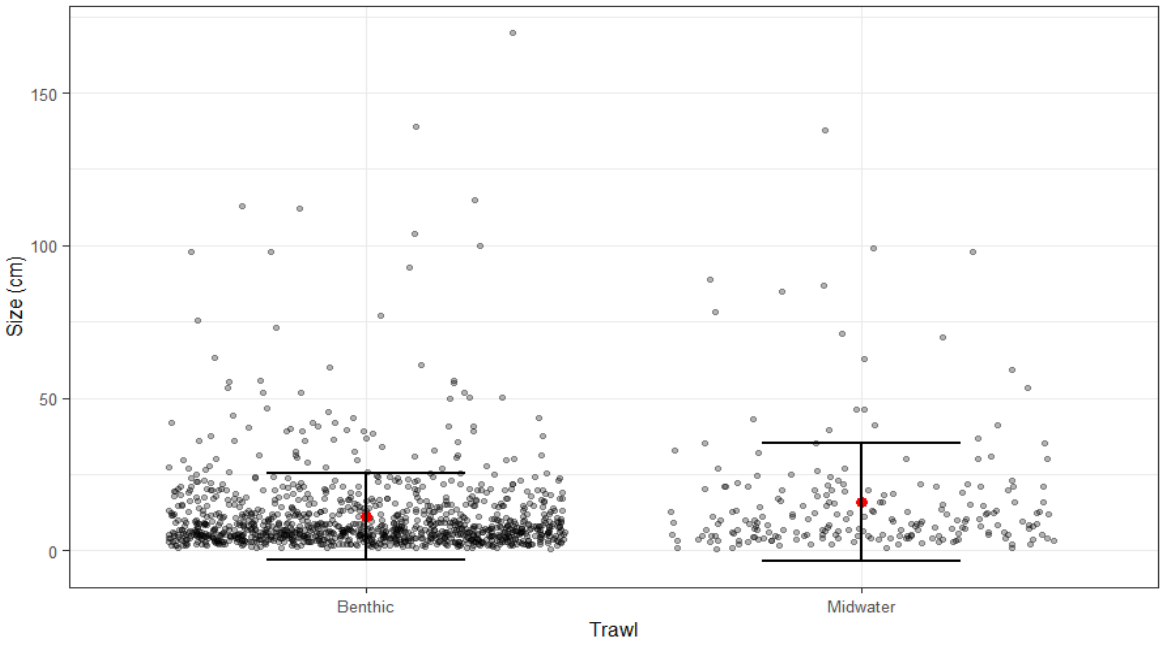


Figure 3.2. Size (cm) of macroplastic items recovered from midwater and benthic trawls at Erith, River Thames. Grey points represent individual measurements of items collected, with a red point for the mean. Error bars represent ± standard deviation.

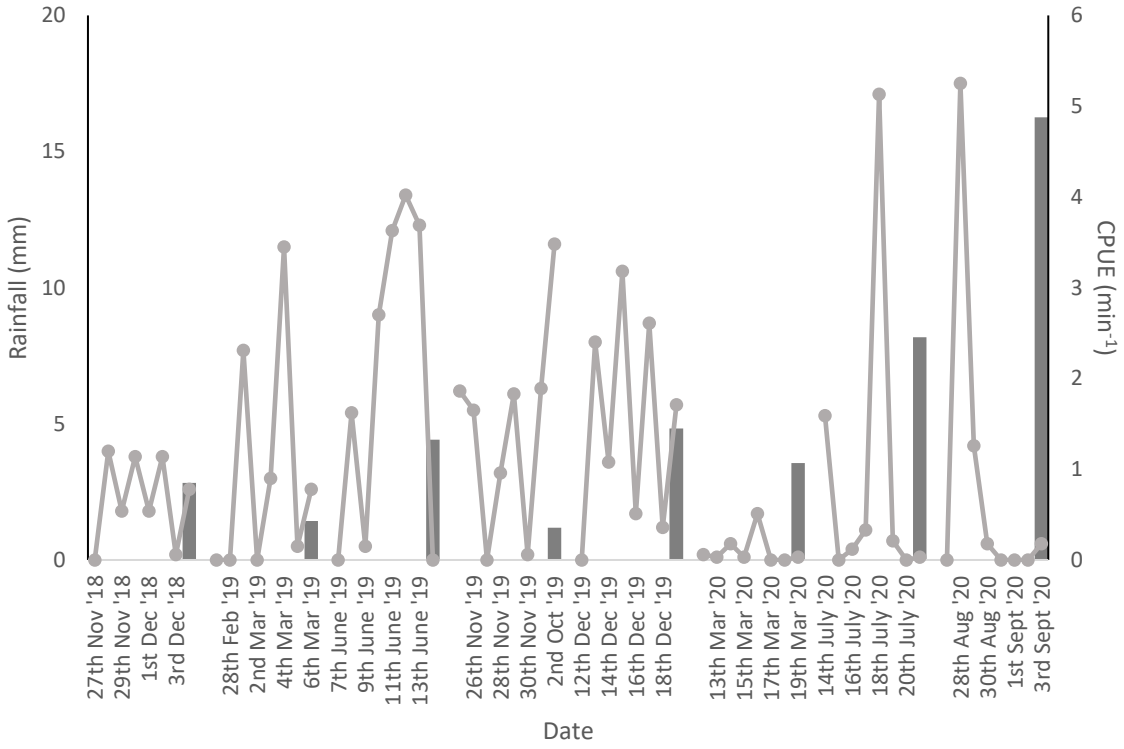


Figure 3.3. Daily rainfall (Met Office, 2006) for the week leading up to sampling and the day of sampling (primary y-axis) and CPUE (secondary y-axis).

Of the 1,335 items recovered from the River Thames, less than 1% (11 items) were non-plastic, being made of glass, metal and leather. Over a third of macrodebris items (40.3%) were packaging, mostly food packaging (207 items; Table 3.2; Figures 3.4, 3.6, 3.7). Cellophane wrappers from cigarette cartons and filters and, in one instance, a whole pack of cigarettes were also common (101 pieces). The second most abundant types of macrodebris were plastic bags (129 items; Figure 3.10), feminine hygiene products, mostly sanitary pads (105 items; Figure 3.5) and tangles of multiple plastic items (76 items), mostly consisting of degraded wet wipe or sanitary pad which captures rope, vegetation and other plastic items flowing past. An additional 20 wet wipes were recovered which had yet to form tangles of debris and vegetation. The proportion of macrodebris types did not differ between midwater and benthic trawls (χ^2 = 10.344, degrees of freedom = 6, p = 0.1109; Figure 3.4).

Table 3.2. Types and quantities of macrodebris collected from the River Thames.

Type of debris	Number of items
Packaging	538
of which food related	207 (subset of above)
of which cigarette related	101 (subset of above)
Plastic bags	129
Sanitary products	105
Tangles	76
Fishing and industry related items	28
Wet wipes	20
String	10
Textiles	5
Balloon	5
Other	22
Non-identifiable	397

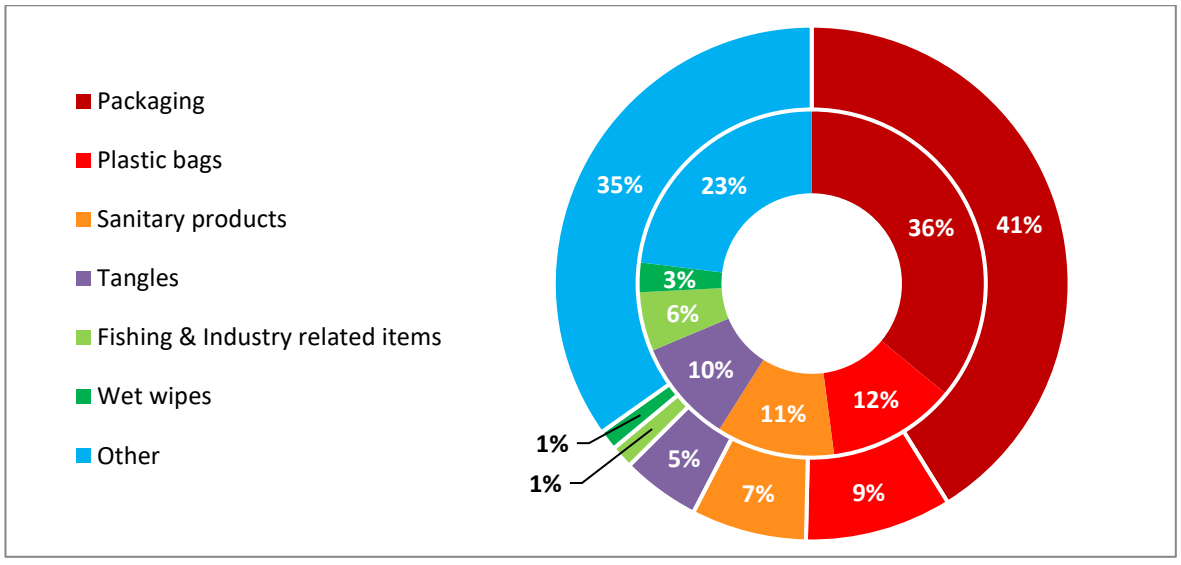


Figure 3.4. Types of macrodebris collected from the River Thames separated from midwater samples (inner ring) and benthic samples (outer ring). The proportion of plastic categories did not differ between midwater and benthic trawls (χ^2 = 10.344, degrees of freedom = 6, p = 0.1109).

Figure 3.5. A selection of sanitary pads recovered from Erith, Thames Estuary between December 2018 and September 2020. Scale bars = 2 cm.

Product branding was identifiable on 155 items, namely packaging and carrier bags. These products belonged to 61 brands (Supplementary Material, S7), most commonly Mondelēz International Inc., Mars Incorporated and PepsiCo. Samples with branding were dominated by confectionary and snack food packaging. Almost all Mondelēz packaging was from Cadbury's chocolate bars. Similarly, Mars products were all wrappers from chocolate-based products. PepsiCo was represented almost entirely by Walkers crisp packets.

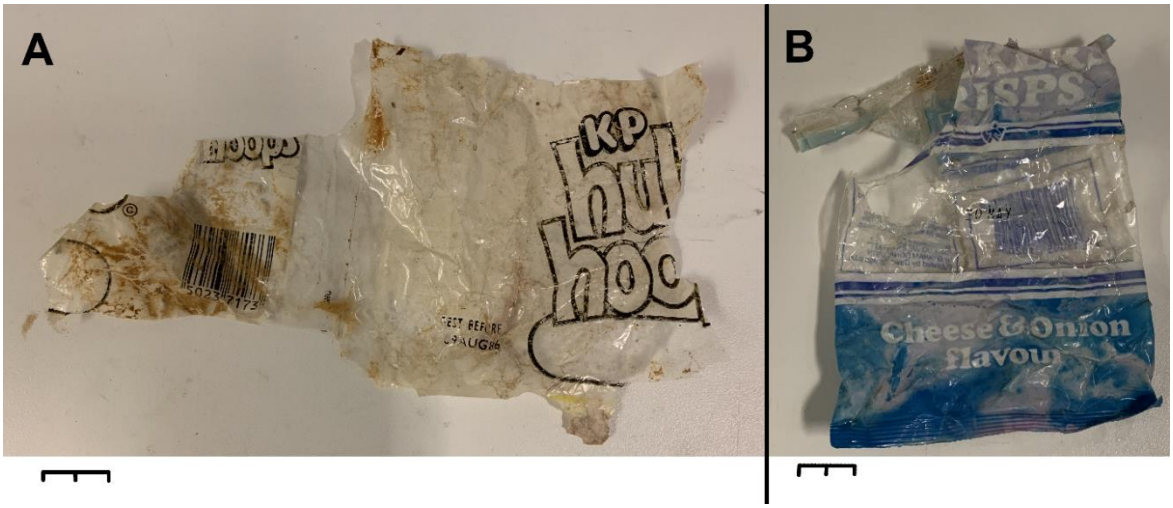


Figure 3.6. Two crisp packets, both over 30 years old. A) A KP Hula Hoops packet for which the age could be determined by a best before date printed on the front (09 Aug 86); B) A Walkers crisp packet assumed to be 30 years old based on similar reports (Tracey, 2018) and changes in the Walkers logo (Logopedia, 2020). Scale bars = 2 cm.

3.3.1 Notable items

Several notable items were recovered from the Thames during sampling. The age of some items was estimated using branding, promotional releases or best before dates. Some of this information was gathered from online communities. These communities are unverified sources (e.g., Wikipedia and fandom sites). They were included, however, as they provide insight into the approximate age of the plastic recovered from the Thames. It is assumed that, whilst the dates may not be precise, the rough age of the plastic can be identified.

<u>The impact of the Covid-19 pandemic on plastic waste in the Thames Estuary</u>

Personal protective equipment (PPE) items were rarely recovered in the Thames Estuary. The only PPE items recovered were a wrapper for shoe covers and a single-use plastic glove.

<u>Food packaging over time</u>

The rough age could be identified for 11 items recovered from the Thames Estuary. The oldest pieces of plastic were both 30-year-old crisp packets: Hula Hoops and Walkers cheese and onion packets (Figure 3.6). The Hula Hoops packet can be dated due to a best

before date printed on the front (e.g., “09 AUG 86”). Original flavour Hula Hoops have always been red (Hula Hoops, 2019) and this packet has clearly leached the dye from the packaging. Determining the age of the Walkers crisp packet was less simple. The approximate age of the packet was first identified due to its similarity to a packet recovered on the Cornish coast (Tracey, 2018). By examining the logo on the packet, it can be confirmed that the product was manufactured between 1980 and 1994 and was therefore 25–39 years old when recovered in December 2019 (Logopedia, 2020).

Figure 3.7 shows two crisp packets recovered from the Thames which contain promotional products. Figure 3.7A is a fragment of crisp packet advertising Lucas Films, the producer of the Star Wars films. Through the official Star Wars website, it is possible to identify the crisps this fragment is from (Figure 3.7B). Character Jar Jar Binx from Star Wars: Episode I – The Phantom Menace can be seen on the front of the packet (Newbold, 2014), his ear is still visible on the fragment recovered from the Thames. This fragment, recovered in December 2019, was produced in 1999 and is 20 years old. In the early 90s Walkers crisps began to give away collectable Tazos in promotional packets (Morgans, 2019; Wikipedia, 2020). Looney Tunes were the first set to be released in 1994, with sets released in 1995 and 1996 (Milkcap Mania, 2009; Morgans, 2019; Wikipedia, 2020). In Figure 3.7C a fragmented date is visible next to a copyright symbol. With reference to the citations above, it is possible to determine that this packet was produced in 1995. The Walkers logo on the back of the packet matches that from this period (Logopedia, 2020). This plastic, recovered in June 2019, was 24 years old.

Figure 3.7. Crisp packets recovered from the Thames identifiable by promotional giveaways. A) Monster Munch packet with Lucas Films logo; B) Promotional packet of Monster Munch (image from starwars.com) released in 1999 to promote Star Wars: Episode I – The Phantom Menace. The fragment in 3.5A matches this packaging; C) Walkers cheese and onion crisps with promotional Looney Tunes Tazos. Scale bars = 2 cm.

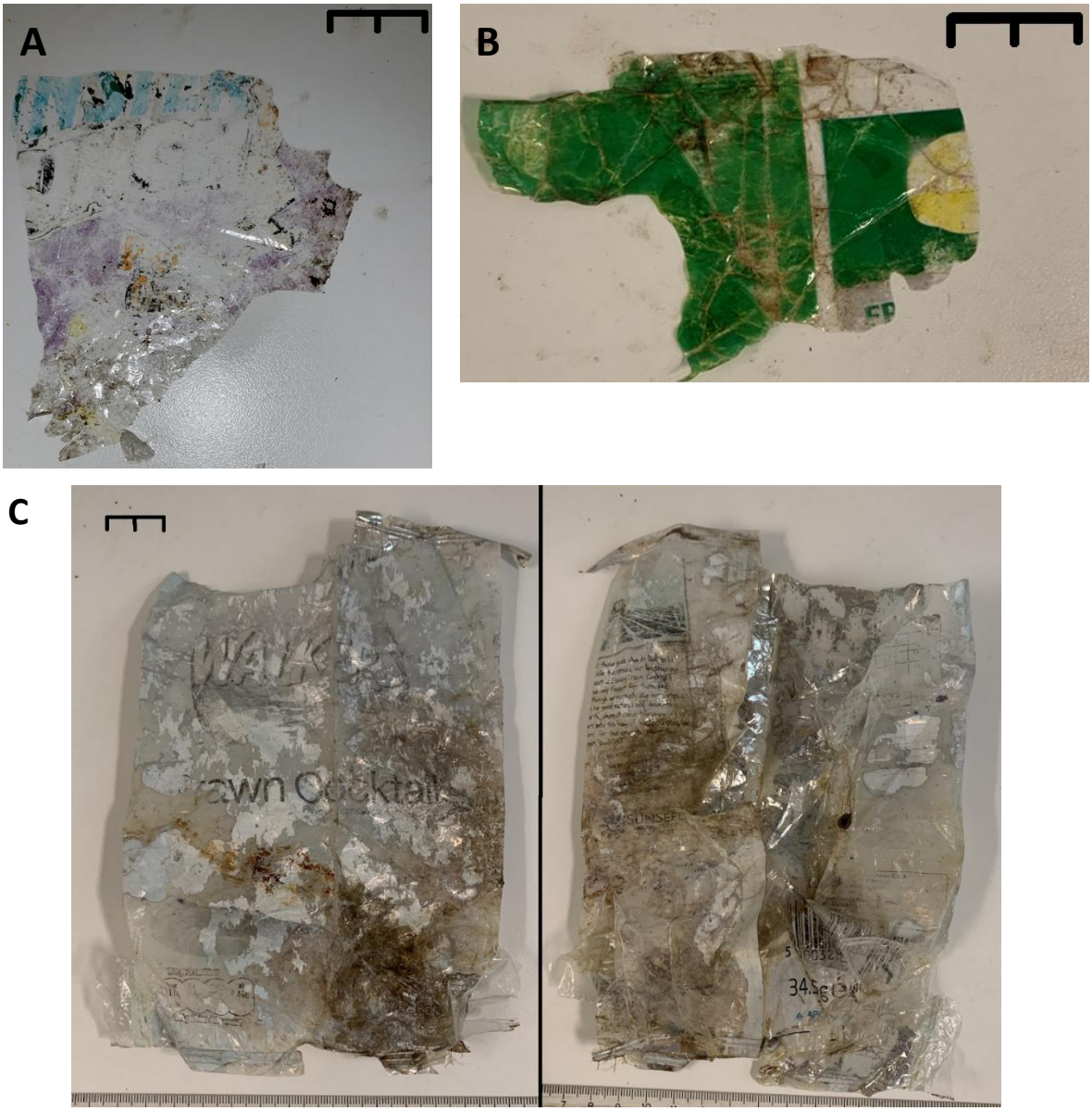


Figure 3.8. Crisp packets recovered from the Thames. The age of these items was estimated by changes in logo designs. A) Monster Munch crisp packet recovered in March 2019; B) Walkers crisp packet recovered in December 2019; C) Walkers prawn cocktail crisps with logos for SunSeed oil and the Carbon Trust, recovered in October 2019. Scale bars = 2 cm.

Figure 3.8 illustrates how changes in the design of logos can be used to estimate the age of a piece of packaging. In 1998, the orange monster was added behind the Monster Munch logo where it would remain until 2006 (Logopedia, 2019a). This Monster Munch packet (Figure 3.8A) was recovered from the Thames in March 2019 and was therefore

between 13 and 21 years old. Figure 3.6B, C are Walkers Classic crisp packets. Up until 2007, the Walkers logo contained a gold circle with rough edges, including three spikes in the circumference (Logopedia, 2020). Neither of the logos on these crisp packets have these juts out of the logo and therefore can only be a maximum of 12 years old. Figure 3.8B contains a white strip on the packaging with an F and an R still visible. Walkers packaging was updated to read "FRESH TASTE GUARANTEED" in 2015 – 2019 (Logopedia, 2019b). Therefore, this crisp packet is less than 4 years old. Figure 3.8C also contains logos for the Carbon Trust and SunSeed oil. SunSeed oil was introduced to Walkers crisps in 2006 (Logopedia, 2020), but the design of the packet resembles the product from 2007–2011. Between 2011 and 2013, the SunSeed logo was removed from the front of the packaging and is present on the back of the packet in Figure 3.8C. Furthermore, the logo of the Carbon Trust was updated to have a footprint rather than the letter C (Logopedia, 2019b). The Carbon Trust was set up in 2001 and Walkers was the first company to have their logo on its products, but the logo was the letter C as previously stated. A report from the Carbon Trust published in 2008 highlighted the change in design to a footprint, therefore the crisp packet must be produced later than 2008 (Carbon Trust, 2008). Both Walkers crisp packets were recovered in 2019 and it can be confirmed that neither were produced that year as the packaging was updated to contain the Union Flag (Dawood, 2019; Logopedia, 2019b).

The age of a Ribena carton (Figure 3.9) could also be estimated. Printed on the product is producer SmithKline Beecham which merged with Glaxo Wellcome in 2000 (GSK, 2018). This carton was recovered in July 2020 and is, therefore, a minimum of 20 years old.

Figure 3.9. Plastic film coating to a Ribena carton recovered from Erith, River Thames in July 2020.

Advertising on carrier bags

In the present study, 9.7% of items were carrier bags and many were large fragments (Figure 3.10A) and even whole bags were recovered. Many manufacturers of plastic bags can be identified due to the large logos on the product (Figure 3.10D, G). When logos are missing or damaged, colour scheme, font and wording assisted identification. Figure 3.10B, E, F are Poundland bags; they can be identified by the turquoise and white designs as well as references to “£1”, “amazing value” and “top brands” (Qureshi, 2016). Whilst Figure 3.10A does not have a logo present, the green colouration and reference to a supermarket indicates

it is an ASDA carrier bag. This was confirmed by looking up the award referenced on the bag. In 2012, ASDA won the award for the 15th year in a row (Halliwell and Zuke, 2012). The bag was recovered from the Thames in December 2018, when the award had been received for the 21st year in a row (Kupelian, 2018). Thus, the carrier bag was 10-years old when recovered from the Thames and yet remained largely intact. Figure 3.8C was more difficult to recognise due to the damage to the bag and its ink fading. It is distinctly patterned, had a simple font and also contained stars or snowflakes, suggesting a seasonal bag. The word "you" is clearly visible on the bag and is from the Marks and Spencer tagline "your M&S". A similar bag can be seen at Alamy.com from 2008. Therefore, carrier bags recovered from the Thames could also be 10 years old.

Figure 3.10. Carrier bag fragments recovered from the Thames, for which the brand has been identified. A) ASDA; B) Poundland (sponsoring Macmillan Cancer Support); C) Marks and Spencer; D) Sainsbury's; E) Poundland; F) Poundland; G) Greggs.

Macroplastics from international sources

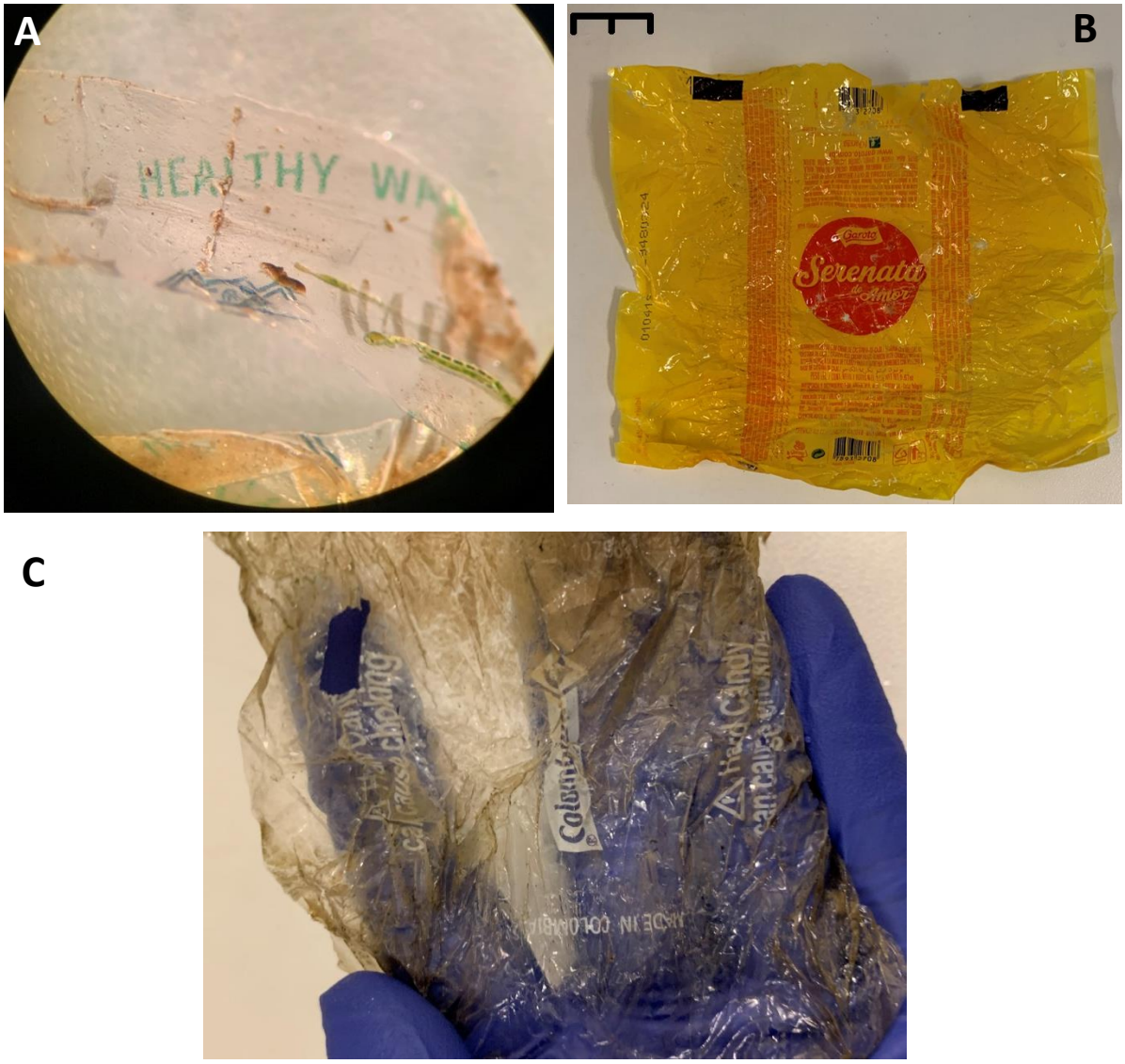


Figure 3.11. International products recovered from the Thames. A) Nabeghlavi healthy water (Georgia); B) Garoto chocolate (Brazil); C) Colombina sweets (Colombia). Scale bar for B = 2 cm.

Three items possibly originating from international sources, imports or tourism were recovered in the present study (Figure 3.11). Figure 3.11A is fragmented such that the brand name cannot be read, but a logo is visible. Similarly, before Figure 3.11C was recovered, a fragment of plastic from the same brand was found for which half the logo was missing. Both items were initially identified through Twitter (McGoran, 2019a, b) where users shared photos and possible brands that matched what was visible on the item in the post. Figure

3.11A was successfully identified as Georgian Nabeghlavi healthy water. The clear plastic fragment, which was observed in a tangle of plastic recovered in March 2019, is likely the wrapper which seals screw tap bottles. This item is unlikely to originate from UK sources, unless transported by a tourist or international citizen visiting the UK, as Nabeghlavi do not export to Britain (Nabeghlavi, online). It is possible that this plastic fragment of litter entered the river through international vessels dropping waste or was washed in from the sea and became entangled in wet wipes and fibres on the riverbed. Figure 3.11B and C, however, may have originated from UK sources as both Colombina and Garoto export to the UK (Colombina, 2017; Garoto, 2018).

3.4 Discussion

The results from the present study demonstrate that the Thames is highly contaminated with macroplastic waste. This reinforces the findings of Morritt et al. (2014), which were the first to highlight the abundance of litter on the Thames riverbed. In the present study, for every minute of trawling, an average of 0.5–2.7 items of plastic were collected. Plastic debris was recovered from the water column and the riverbed, being more abundant on the latter, with the average CPUE being five times greater. The present study reveals that litter, especially ropes, wet wipes and sanitary pads, can become entangled with vegetation and can become embedded in the sediment. This contributes to the abundance of plastic in benthic samples. Indeed, Williams and Simmons (1997b) noted that vegetation increased the retention of litter on the foreshore. Similarly, Ballas et al. (2001) determined, through modelling, that vegetation and physical barriers, such as obstructions in the watercourse, were the dominant factors controlling litter movement in rivers. It has also been reported that over 90% of floating litter washes ashore (Ryan and Perold, 2021). It is therefore likely that litter is retained for longer on the riverbed compared to the water surface. Certainly, less buoyant litter travels further in a river than buoyant waste (Ryan and Perold, 2021). It may also have more opportunities to fragment due to physical abrasion with the sediment and interactions with biota. Biofilm formation increases with residence time in the environment (Weinstein et al., 2016) and biofilms can encourage interactions with biota (Hodgson et al., 2018). Therefore, prolonged retention of macroplastics in rivers can increase microplastic abundance. This could then pose risks to benthic fauna that would be exposed to these greater microplastic concentrations.

The samples collected on 3rd September 2020 benthic trawl one (247 items), 21st July 2020 benthic trawl three (142), 19th December 2019 benthic trawl three (98) and 14th June 2019 (95) contained the most plastic. Erith is situated at a bend in the Thames Estuary. Samples collected from the south bank are closer to the outside of the bend where water flows faster, whereas samples collected from the north shore are nearer to the slower flowing waters of the inside bend. Consequently, plastic likely accumulates on the north shore at Erith. Certainly, microplastic abundance in estuaries has been linked to the energy of hydrodynamic conditions with higher concentrations in less dynamic regions (Jiwarungrueangkul et al., 2021). The trawls that crossed this inner bend – namely trawls on 19th December 2019 and 3rd September 2020 – did not, however, recover the most plastic (Supplementary Material, S3). Most trawls were performed on the straight between two bends (Supplementary Material, S3) where water is fastest flowing in the centre channel. The September and July 2020 benthic trawls were closest to the north bank and thus water current may play a role here. It has been noted that the flood tide convergence zone in the middle channel of an estuary can retain floating plastic and even transport it upstream (Vermeiren et al., 2016).

Most of the litter in the present study appeared to be from British sources, with few items possibly of foreign origin identified (6 pieces). Similarly, Williams and Simmons (1997a) found most litter on UK beaches originated from Britain. Storrier et al. (2007), however, investigated litter on the west coast of Scotland and found litter came from both local and non-local sources. Indeed, Ryan and Perold (2021) noted that plastic moved not only from riverine sources out to the sea, but from the sea into estuaries.

Storrier et al. (2007) noted that sewage-related debris was one of the main sources of plastic on the British coast as well as beach visitor-generated litters, fishing debris, shipping waste, fly tipping and street runoff. Similarly, Williams and Simmons (1999) proposed that sewage-related debris and fly tipping were the main sources of litter in the River Toff, UK. Litter recovered from the Thames Estuary in the present study could have originated from visitors to the river and foreshore, runoff from urban centres and landfill sites as well as sewage-related debris and industrial sources on or near the river. Certainly, plastic in European rivers originated from similar sources. Winton et al. (2020) reported that consumer products were the most abundant forms of plastic in European rivers (59%), arguing that

these could be targeted by changes to business practices, consumption habits and policy. Common items in European rivers include food wrappers, bottles, bags, cigarette butts and smoking-related items, sanitary items, cotton bud sticks, takeaway containers, cups and straws/stirrers/cutlery (Crosti et al., 2018; Gonzalález-Fernández et al.,2018; Winton et al., 2020). Many of these items have also been recovered in the Thames (Morritt et al., 2014; Bernardini et al., 2020), with an average of 27.7 items m^{-2} present on the Thames foreshore in Greater London (Bernardini et al., 2020). Whilst it has long been proposed that plastic in estuaries comes from urban centres, recent hydrodynamic models of a Brazilian estuarine complex have demonstrated that plastic indeed originates from land-based sources in the inner estuary (Krelling et al., 2017). The same models predict that this litter remains in the estuary for only a short period, with most having a residence time of less than five days.

For the present study, the types of plastic recovered from the Thames did not significantly differ between midwater and benthic samples. Although not significant, a slightly higher proportion of benthic samples were packaging compared to midwater trawls. Additionally, more tangles were present in the midwater, with tangles present in the water column also being larger on average (33 ± 25.5 cm compared to 23.8 ± 15.6 cm in benthic samples). This is likely the result of numerous large tangles (52% of midwater tangles) collected in trawls from December 2018. Over half the tangles in midwater samples originate in these trawls and they were on average larger than those collected in the other trawls (47.1 ± 24.9 cm compared to 18.3 ± 16.6 cm). In December 2018, for logistical reasons, benthic trawls were collected before midwater trawls. During all other sampling dates midwater trawls were collected prior to benthic samples.

Peak discharge at Erith can reach 5,000 m^3s^{-1} (Rowley et al., 2020) and thus floating debris could be quickly moved downstream and eventually out to the North Sea. Indeed, Bernardini et al. (2020) reported a distinct difference in the type and abundance of plastic that floated and sank in the Thames, with more floating plastic. Van Emmerik and Schwarz (2019) reported that rain helped to flush plastics out of the River Seine, transporting plastic from cities to the sea. This may also go some way to explaining the increase in plastic abundance with proximity to the sea (Fischer, 2019).

Beach cleans have commonly been used to monitor plastic pollution on the River Thames foreshore (e.g., Fischer, 2019; McConville et al., 2020). Seo and Park (2020) noted that more plastic washed ashore than was found floating in the surface waters, however, fouling and sinking plastic accounted for a large portion of debris. Indeed, Ryan and Perold (2021) determined that most floating litter is deposited on the foreshore. Thus, the type of plastic found on the foreshore and riverbed might differ with some plastic sinking, other items getting deposited on the shore and some floating out to sea. Certainly, Thames21 (McConville et al., 2020) recovered different types of plastic at different points along the tideway, reporting known hotspots for wipes, carrier bags and plastic bottles. The Marine Conservation Society and Thames21 reported that in the tidal Thames wet wipes were the dominant type of plastic-containing debris (Fischer, 2019), whilst the present study found packaging was most abundant.

The abundance of packaging recovered in the present study (40%) reflects plastic production, with nearly 40% of demand for plastic in Europe in 2018 coming from packaging (PlasticsEurope, 2019b). Similarly, packaging is dominant in the tributaries of the Thames (Fischer, 2019) and on the foreshore, accounting for 75% of items recovered between 2016 and 2017 (Thames21, 2017). Indeed, Rowley et al. (2020) found that identifiable fragments of packaging dominated microplastics recovered from the Thames water column. Food products such as plastic bottles are a common sight on many British shores (Williams and Simmons, 1997a; Storrier et al., 2007) and indeed worldwide (Van Emmerik and Schwarz, 2019). Food packaging made up 38% of the packaging recovered in the present study, mostly consisting of snack food wrappers. Two thirds of identifiable waste recovered by Thames foreshore beach cleans was also food packaging (Thames21, 2017) with 85,000 plastic bottles collected since 2016 (Thames21, 2019b). Nearly half of these bottles are for water, with Coca-Cola products being the second highest proportion (Thames21, 2017, 2019b). Wrappers from plastic water bottles and Coca-Cola bottles were also recovered in the present study. Indeed, a citizen science brand audit of litter in the UK found that the beverage industry was responsible for over half of the reported litter (Stanton et al., 2022). Proximity to food outlets is one factor that can influence the abundance of this waste (Storrier et al., 2007). It has been demonstrated that extensive networks of bins can reduce the number of wrappers littering the environment (Storrier et al., 2007).

Only 78% of plastic used in the UK is retrieved (British Plastics federation, 2019). Furthermore, only 40–50% of packaging is recycled in the UK (British Plastics Federation, 2019; PlasticsEurope, 2019b). Despite increases in recycling rates and energy recovery, 25% of plastic in Europe is still sent to landfill (PlasticsEurope, 2019b) where plastic can runoff into the environment. Runoff from a landfill site on the north shore of the Thames at Erith could be one source of the litter on the riverbed. Additionally, leachates from landfill runoff are considerably higher than those of aquatic environments and in sewage effluent and therefore might pose severe health risks, such as endocrine disruption, to organisms in the estuary (Teuten et al., 2009). Littering is another possible pathway, one that Bernardini et al. (2020) proposed would have a lesser effect on plastic pollution in a few years as policy and behavioural changes are implemented. The Earthwatch Institute reviewed the available data for plastic abundance in European rivers and reported that 12% of identifiable plastic litter was food packaging (Kragh et al., 2019). This report noted that 8.3 billion packets of crisps are eaten and 0.3 billion packets are littered the UK each year. Kragh et al. (2019) went on to highlight that an effective method for preventing this litter was to reduce consumption of these products, reduce the amount of packaging, improve recycling facilities and test the sustainability of this process.

In many UK rivers and on beaches, sewage-related debris is the dominant form of litter (e.g., Williams and Simmons, 1996; 1999; O'Neill, 2019; Thames21, 2019a). In the present study sewage-related debris made up nearly 8% of waste. On the south bank of the Thames at Hammersmith, 23,000 wet wipes were recovered by citizen scientists in just two hours (Thames21, 2019a). The study noted that mounds of wipes could reach 50 m across and 1 m high. It has been estimated that in the past decade wet wipe accumulations on the British shoreline have increased by 400% (Ó Brian et al., 2020). Sanitary pads whilst abundant in rivers are comparatively rare in marine systems, with the opposite trend seen with plastic straws, stirrers and cutlery (Winton et al., 2020). It is, therefore, imperative to ensure a better understanding of plastic pollution in rivers which have previously been neglected. Input of sanitary waste from WWTPs is due to them being flushed down the toilet. Williams and Simmons (1996) estimated that 72% of sanitary towels used in the UK were flushed and it has been reported that 1.5–2 billion items could be flushed in the UK (O'Neill, 2019; Wen, 2020). Typically, these items are improperly disposed of by 18–24-year-olds (Thames21, 2019a). In

addition to introducing sanitary items to rivers, flushing such items uses more water compared to toilet paper (Alda-Vidal et al., 2020). Further to the environmental implications of flushing wipes and feminine hygiene products, there are economic and hygiene implications from the "fat bergs" produced in the sewer systems (Mitchell et al., 2017; Alda-Vidal et al., 2020; Atasağun and Bhat, 2020a). In the UK, many sewer systems are antiquated and not designed to transport the abundance and diversity of sewage currently present (Alda-Vidal et al., 2020; McCoy et al., 2020). It is estimated that these blockages cost £88 million a year to remove in the UK (Alda-Vidal et al., 2020) and can increase the risk of flooding (Lebreton and Andrady, 2019).

In recent years, there has been an increase in the diversity of wipe products and a rapid increase in "flushable" varieties (Atasağun and Bhat, 2020a), with 1 million tonnes of non-woven wipes and sanitary pads produced in the UK in 2016 (Ó Brian et al., 2020). Many organisations are campaigning for clearer regulations on labelling for wet wipes to discourage flushing (O'Neill, 2019; Thames21, 2019a, b; McCoy et al., 2020) as well as increased educational campaigns (Storrier et al., 2007; Ó Brian et al., 2020) and production of plastic-free period products (O'Neill, 2019). In response, Fleur Anderson, MP for Putney, has proposed a bill to ban plastic in wet wipes (Anderson, 2021) and Defra has announced that the Government is considering action against plastic in wet wipes (Defra, 2021). Despite labelling regulations being in place, Ó Brian et al. (2020) found that all seven studied non-flushable wipes investigated failed to adhere to guidelines. Logos were too small and often hidden behind seals and folds. Alda-Vidal et al. (2020) noted that, historically, there has been a focus on raising awareness, but that lack of understanding is not the only factor influencing irresponsible flushing. Societal and cultural factors also play a role, with females required to discretely dispose of sanitary items and a stigma around "unclean" products such as wipes and pads. The study also noted that labelling on wet wipe packets proved ineffective in preventing flushing. In industry tests of wipe dispersal, it has been concluded that only biodegradable materials should be used in production (Atasağun and Bhat, 2020b).

Goldberg (1995) and Green et al. (2015) highlighted that benthic litter can prevent light and nutrients reaching fauna and result in the formation of anoxic sediment. Indeed, McCoy et al. (2020) found that layers of wet wipes on the foreshore of the Thames created an anoxic layer of sediment reducing colonisation by the invasive Asian clam, *Corbicula*

fluminea. Even biodegradable plastics, when present on the seafloor, produce anoxic conditions after a few weeks (Green et al., 2015). Anoxic sediment retains higher concentrations of phenolic compounds such as Bisphenol A, an endocrine disruptor (Teuten et al., 2009) and litter cover can reduce the foraging ability of benthic fauna (Aloy et al., 2011). Consequently, mitigating their input is vital for the health of the river.

Williams and Simmons (1996) demonstrated that sanitary pads initially show a rapid break up in the environment, with the backing strip remaining intact. This is reflected in the items recovered in the present study. The majority (90%) of sanitary products (Wen, 2020) and wipes (Ó Brian et al., 2020) contain plastic, including PET, high density polyethylene (HDPE), polyethylene vinyl acetate, low density polyethylene (LDPE), polystyrene (PS), polyurethane, PP and polyallomer (Woeller and Hochwalt, 2015; Munoz et al., 2018; Ó Brian et al., 2020). So-called flushable wipes also contain semi-synthetic components such as viscose and lyocell (Atasağun and Bhat, 2020a), with many sanitary pads also containing a cellulose core (always®). Break up of these items, either in the environment (Williams and Simmons, 1996) or in WWTPs (Alda-Vidal et al., 2020), likely adds to microfibre abundance in the river (Ó Brian et al., 2020). Indeed, both large items of litter (Storrier et al., 2007; Morritt et al., 2014) and microplastics (Horton et al., 2017; Rowley et al., 2020) are more abundant closer to sewage outfalls. Fibres shed from wet wipes and sanitary pads are known to be retained by benthic infauna in the Thames (McCoy et al., 2020) and sanitary pad fragments have been found in the GM of Thames crabs (McGoran et al., 2020; also see Chapter 5, Figure 5.4).

Studies on seasonal variation in plastic abundance are rare (Van Calcar and Van Emmerik, 2019). In the River Rhône, more plastic was recovered in May (spring) rather than in winter as predicted by the authors, with this variation left unexplained (Van Calcar and Van Emmerik, 2019). Seasonal change could be influenced by rainfall and river flow as reported by Castro-Jiménez et al. (2019). When the river discharge is greater more plastic is transported to the sea (Vriend et al., 2020). Van Emmerik and Schwarz (2019) reported that seasonal increased rainfall could result in up to 10 times more plastic being transported to the sea. Peak transport was in March after heavy rain from September onwards. Global river inputs of plastic waste to the sea are greater in May to October, with 75% of emissions occurring in this period (Lebreton et al., 2017). This was, however, driven by monsoons in

China and is not directly applicable to the Thames. The same study reported that there was a reduction in plastic abundance in January. In the Thames, microplastic abundance in the surface water peaked in July and August upstream of London and in September downstream, with a reduction in concentrations in October (Rowley et al., 2020). Similarly, mesoplastic abundance peaked in September for both sites (Katharine Rowley, pers. Comm.). No such trends were reported in the midwater samples in the present study. Similar to the downstream results of Rowley et al. (2020), Crosti et al. (2018) reported a peak in surface plastic in autumn and a decrease over spring with the lowest concentrations in summer. On the Irish west coast, microfibres in sediment peaked in December near a WWTP (Ó Brian et al., 2020). Heavy rain from storm events resulted in the increased discharge from CSOs which released sanitary products and wipes into the environment. These fragmented and resulted in a greater abundance of white fibres. Additionally, rainfall increased the mobilisation of land-based plastics and remobilisation of plastic on the foreshore and riverbed (Van Emmerik and Schwarz, 2019). Records of plastic abundance on the foreshore of the tidal Thames, suggest that floating, lightweight plastic is flushed onto reedbeds after heavy rainstorms. Peak litter abundance is recorded in the month following a storm (e.g., Storm Eleanor, January 2018; Storm Lorenzo, September 2019; McConville et al., 2020). No significant seasonal variation, however, was reported in the present study. One reason could be that plastic in the riverbed accumulates for a long period and therefore seasonal changes does not significantly impact this environment. Indeed, some plastic items recovered in this study were over 20 years old and may have been present in the estuary for some time. Additionally, as reported by McConville et al. (2020), plastic in the Thames accumulates on the foreshore and saltmarshes after heavy rain and perhaps, therefore, surface water plastic is most likely to show some sort of seasonality rather than items below the surface.

There was no seasonal variation in plastic abundance recorded in the present study. It is likely that litter, once embedded in sediment, can remain there for long periods, and thus, seasonal changes to plastic inputs minimally affect CPUE of litter. It can be noted, however, that peak rainfall events in July 2020 and September 2020, align with high abundances of debris in samples (Figure 3.3). Ryan and Perold (2021) noted that litter was more abundant on beaches during rainfall events, decreasing afterwards as litter was flushed into the surrounding wetlands. This is not a definitive pattern with the greatest cumulative

precipitation being recorded in June 2019 when a CPUE of 2.46 was recorded. This is not significantly different from the average CPUE which with the addition of standard deviation equates to ca. 3.1. Similarly, a high level of rain (11.6 mm) was reported on 2nd October 2019, which corresponds with the lowest reported CPUE. It is possible that there is a delay between heavy or prolonged rain and increased plastic runoff into the river. Certainly, deposition of plastic on riverbanks after flood events occurred sometime after the event (Williams and Simmons, 1997b). WWTPs often have an overflow capacity to cope when inputs are high, such as when rain is heavy or persistent. The capacity of these reservoirs is, however, not limitless and once capacity is reached untreated sewage is discharged through CSOs. It is possible that the rainfall events described above were not prolonged enough to result in CSO release. Perhaps if sampling had continued for a period of several days, more macroplastics would have been recovered. Over a quarter of the sewage-related debris (wet wipes and sanitary pads) recovered in the present study were found in trawls from September 2020. These trawls occurred after the greatest volume of rain was recorded in a single day during the study period. Additionally, sewage-related debris made up the highest proportion of recovered debris in samples from June 2019 after the greatest continuous rainfall. Thus, it is likely that rainfall is related to release of debris from CSOs but not necessarily total plastic abundance. Indeed, Williams and Simmons (1997b) reported that flood events resulted in increased inputs from CSOs.

Solutions to plastic pollution often emphasise the importance of consumer behavioural changes, with less responsibility placed on producers and suppliers of plastic packaging. Rhein and Schmid (2020) explored consumer awareness of plastic packaging to understand what factors would drive this behavioural change. The study reported five main consumer beliefs: 1) consumers are aware that lots of plastic packaging is produced and used, with some deeming it unnecessary; 2) consumers often feel powerless, unable to avoid plastic and sometimes feel their choices have little effect on plastic production; 3) consumers feel that some decisions influence company changes, for example, avoiding packaged fruit; 4) consumers are aware that plastic is useful and that packaging can be more hygienic than alternatives and 5) consumers are aware that large quantities of plastic are polluting the environment. In Europe, most plastic packaging is not recycled (PlasticsEurope, 2018a). This may be due to limitations in recycling facilities or could be down to consumers not

understanding which items can be recycled or, in some cases, not caring. Indeed, many German consumers believed that plastic pollution was mostly produced by developing countries and that they were responsible for little of the plastic in the ocean (Rhein and Schmid, 2020). This attitude could lead to laziness towards recycling. In the UK, however, there are added concerns that infrastructure is not in place to meet recycling demands (British Plastics Federation, 2019). With the cost of recycled material being greater than virgin plastic (Moore, 2008), recycling by consumers alone is not sufficient to curb the increasing tide of plastic into the environment. Instead, it is necessary for governments, companies, consumers and scientists to co-operate to drive positive change forward (Karasik et al., 2020).

The impact of the Covid-19 pandemic on plastic waste in the Thames Estuary

The use of disposable PPE has increased in the UK in response to the Corona-19 virus pandemic. In 2020, over 10 million single use PPE items were supplied a day, with over 420 million facemasks and 1.3 billion gloves being used over a four-month period (Shams et al., 2021). In addition to the use of PPE, the Covid-19 pandemic has driven greater consumer command for single-use plastic packaging (Shams et al., 2021). Some of this material is discarded inappropriately littering the streets and environment. It is likely that an increased amount of waste, especially plastic which is typically lightweight, is polluting the environment as a result of the pandemic. Shams et al. (2021) noted that illegal waste disposal in the UK had increased threefold in 2020 during the pandemic. It is therefore possible that PPE and other plastic litter is entering the River Thames. On 19th March 2020 a trawling trip was completed for the present study. This date representing the start of lockdown in London. Two subsequent trawling trips were also completed (21st July and 3rd September). Samples were collected during these trips after restrictions began to ease in the UK. It was proposed that the later samples might contain elements of PPE. The only items recovered that match this description was a single glove, collected June 2019, and a wrapper for shoe covers, obtained December 2019. Neither item was related to the pandemic as they were collected before PPE was in common use by the British public. There was an increase, however, in the proportion of blue plastic films in samples. Whilst the origin of these films cannot be certain, one suggested source could be fragmented single-use gloves which are typically shades of blue. The present study found no significant impact of Covid-19 on the plastic in the Thames, finding that even wet wipe abundance did not increase post-lockdown. It is suspected that over the

coming months and years, the impact of the pandemic will become clearer and that the scope of the current sampling regime was not suitable for assessing changes in plastic use over a short time frame.

3.5 Conclusion

Macroplastic is abundant and long lasting in the environment. Using different approaches to estimate the age of macroplastic items, it is evident that, if disposed of incorrectly, plastic can pollute the environment for decades. As plastic is in the environment for long periods, it has the potential to harm organisms either through entanglement, deoxygenation of sediment or ingestion. Most of the sampled plastic in this study had accumulated on the riverbed where the temperature is colder and UV exposure is reduced which can inhibit the degradation of plastic. It is persistence in the environment, however, which increases the opportunities for macroplastics to fragment through physical processes (sediment and biota) and raises the risk of potential exposure to biota. Thus, it is important to monitor macro- and microplastics.

Chapter 4

Microplastic accumulation in sediment from the Thames Estuary

4.1 Introduction

Plastic entering the environment ultimately fragments, generating increasingly smaller fractions (Weinstein et al., 2016; Andrady, 2017; Andrady, 2022; Sipe et al., 2022). These small particles (micro- and nanoplastics) are widely bioavailable and pose a risk to biota (Wright et al., 2013; Galloway et al., 2017). Monitoring environmental microplastic concentrations is key to quantifying risk and evaluating the success of mitigation strategies.

Whilst past literature has focussed on marine habitats, there is growing evidence of contamination in riverine and estuarine environments. A review by Vermeiren et al. (2016) found that evidence for contamination of estuaries is increasing but there is a gap in understanding the sinks and sources of plastic pollution in these systems. Further still, the understanding of temporal distributions is hindered by a lack of long-term monitoring of microplastic pollution. Indeed, Windsor et al. (2019a) noted that there is a lack of research into the residence time of riverine plastics. The River Thames is no exception, with just a handful of ad hoc studies into contamination in the river, the estuary and its tributaries (Horton et al., 2017; Rowley et al., 2020; Devereux et al., 2022). The present chapter provides evidence of microplastic abundance in the Thames Estuary over a two-year period (December 2018 – September 2020).

The present study aimed to address seasonal variation in microplastic abundance and ingestion. As with macroplastics, inputs of microplastics can vary over time. Additional factors could influence microplastic abundance when ingestion is considered. Species abundance in the estuary and animal life history will determine when a species is most at risk of microplastic ingestion. Sediment samples provide data on microplastic accumulation and retention in the environment, whilst fish and shrimp species will provide an insight into differing ingestion rates over time. Insight into microplastic exposure to estuarine species is vital given that they have regularly been overlooked in favour of marine species. It is even more key when considering that some evidence suggests that freshwater fish species more commonly ingest microlitter than marine species (Wootton et al., 2021). Rainfall data were collected from the

Met Office database and used to inform any seasonal variation recorded in the present study. An additional aim of the present chapter was to compare ingestion between fish species common in the estuary and determine whether some species are more at risk than others. The hypotheses are 1) microplastic will be abundant in the estuary in all samples as plastic accumulates in fine sediments such as those found in the Thames Estuary and 2) microplastic abundance will vary seasonally due to environmental factors, such as rainfall, affecting inputs.

4.2 Materials and methods

Sample collection and microplastic extraction is described in Chapter 2.

4.2.1 Daily Rainfall

Daily rainfall data were collected by the Met Office (2006) and accessed through the Centre for Environmental Data Analysis. Rainfall was recorded for the seven days prior to sampling and the day of sampling.

4.2.2 Statistical analysis

Statistical analysis was conducted in R version 4.0.3 (R Core Team, 2020) with the following packages: "ggplot2" (Wickham, 2016), "MASS" (Venables and Ripley, 2002) and "multcomp" (Hothorn et al., 2008). Generalised linear models (GLMs) were used to compare the amount of plastic per gram of sediment across sampling dates. The best fit model was determined by comparison of AIC scores. Non-significant variables were systematically removed until only significant factors remained. Significant variables were then analysed with a pair-wise Tukey post-hoc test.

Model 4.1 – GLM(Microlitter per gram ~ Date, family = gamma (link = log))

4.3 Results

4.3.1 Contamination

Contamination recorded in controls for sediment samples included clear fibres, clear films, blue fibres, black fibres, red fibres, purple fibres, green fibres, grey fibres, yellow fibres, white fibres, white films. On average 3.8 microplastics from each 50 g sediment sample were likely contamination (Figure 4.1). The limit of detection (LOD), calculated as mean

contamination + 3 SD, was 10.1 particles per sample or 0.2 per gram. The limit of quantification (LOQ), mean + 10 SD, was 25.0 particles per sample or 0.5 per gram. In total, 78% of samples were above the LOD and 50% were above the LOQ.

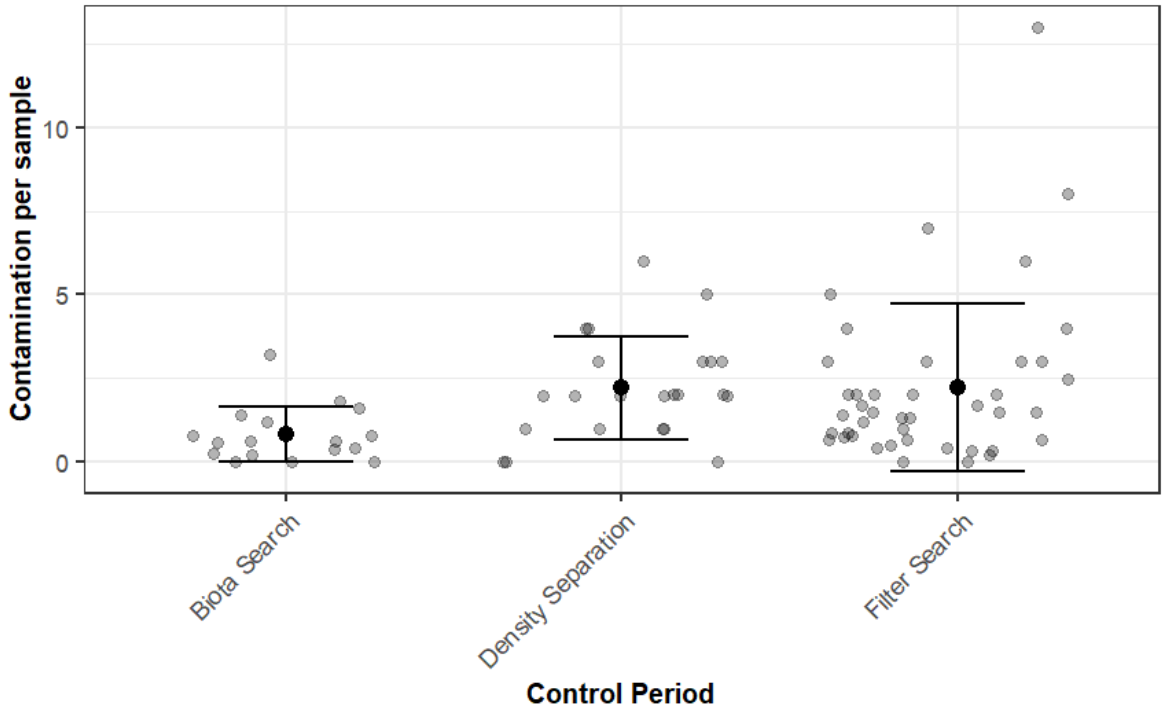


Figure 4.1. Microlitter concentrations in laboratory controls at each stage of processing (removal of biota in sediment, density separation, visual microplastic ID on filters). Grey points are individual values, black points are the means and bars are ± standard deviation.

Results were corrected for recorded contamination not LOD/LOQ. All results below are after contamination has been accounted for in the data.

4.3.2 Microlitter content in sediment

Sediment from all sampling trips contained litter, with a total of 5,557 pieces of microplastic and microlitter recovered. Mean (± SD) contamination per gram was 1.0 ± 1.1 (1,000 ± 1,100 kg^{-1}, Figure 4.2). Average microplastic and microlitter load ranged from 0.17 ± 0.13 and 2.56 ± 1.25 pieces per gram (Table 4.1, Figure 4.3).

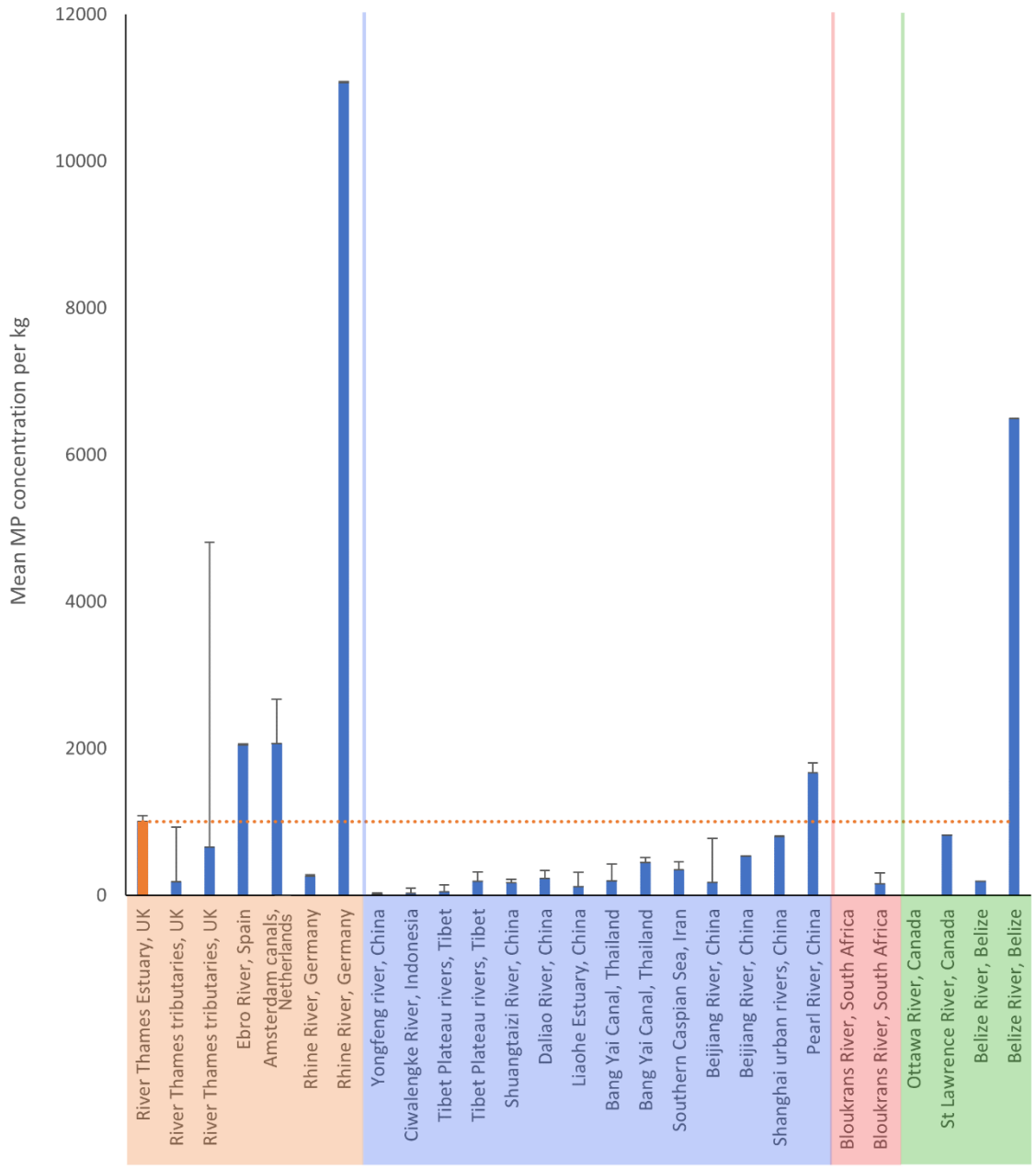


Figure 4.2. Global mean microplastic/litter concentrations in riverine and estuarine sediments (per kg). Locations are highlighted by region, orange are concentrations from Europe, blue from Asia, red from Africa and green from the Americas. Whiskers are ± standard deviation. More details in Supplementary Material (S8).

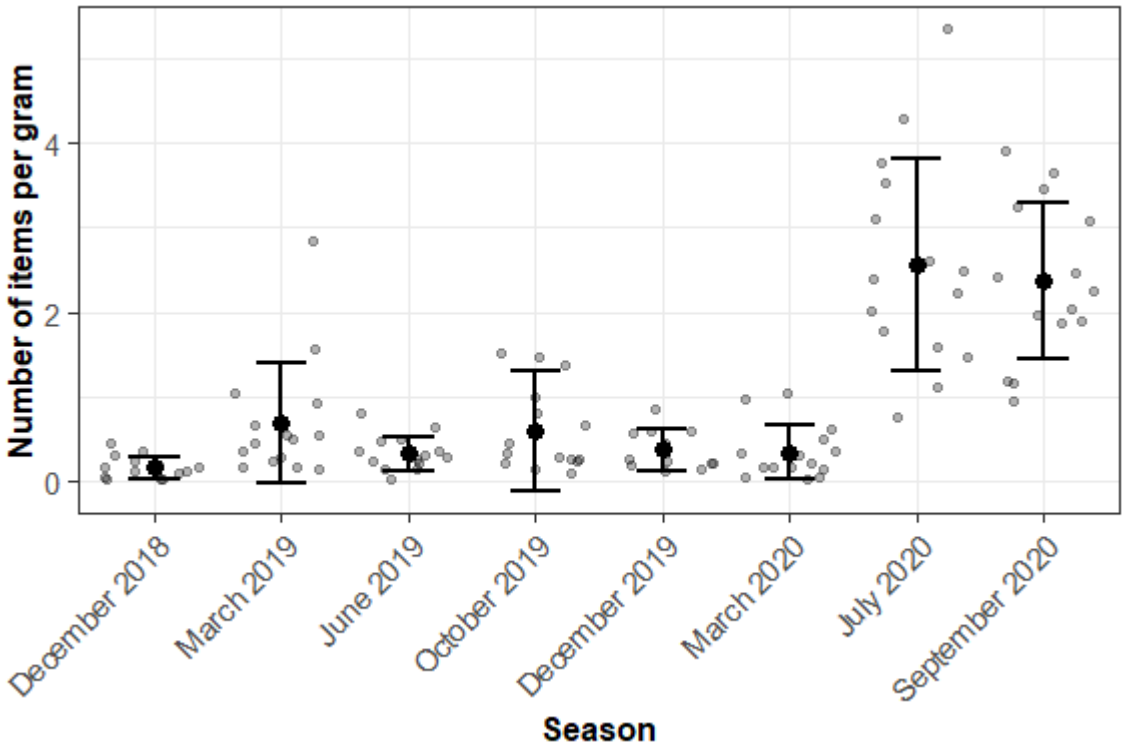


Figure 4.3. Microlitter concentrations in sediment varied significantly between sampling dates. Grey points are individual values, black points are the means and bars are ± standard deviation. More detailed results are available in Supplementary Material (S10).

AIC scores for error distributions for Model 4.1 are as follows: Poisson infinite, Negative Binomial 236.0038, Gamma (link = identity) 109.9593, Gamma (link = log) 108.2189 and Gamma (link = inverse) 107.9230. Gamma (link = inverse) had the best fit and was therefore chosen for Model 4.1. The model revealed that sampling date was significant ($p < 0.05$). July 2020 and September 2020 contained significantly more plastic per gram on average (2.6 ± 1.3 and 2.4 ± 0.9 respectively) compared to March and October 2019 (0.7 ± 0.7 and 0.6 ± 0.5 respectively) which contained significantly more plastic than the other sediment samples per gram (Figure 4.3).

Table 4.1. Microlitter abundance in sediment from Erith. Diversity of litter refers to the number of colour and form combinations recovered (i.e., red fibres, red beads). Means are reported with standard deviation. More detailed results are available in the Supplementary Material (S10).

	Dec 2018	**Mar 2019**	**June 2019**	**Oct 2019**	**Dec 2019**	**Mar 2020**	**July 2020**	**Sept 2020**
Sediment								
Sample size	15	15	15	15	12	15	15	15
Average (per gram)	0.17 ± 0.13	0.70 ± 0.71	0.34 ± 0.20	0.61 ± 0.71	0.38 ± 0.24	0.35 ± 0.31	2.56 ± 1.25	2.37 ± 0.92
Average diversity	4.64 ± 2.44	8.8 ± 4.66	6.4 ± 2.90	7.67 ± 3.39	5.5 ± 2.54	6.83 ± 3.92	18.4 ± 5.52	20.4 ± 3.72
Average size (µm)	2,758 ± 4,536	1,754 ± 1,422	1,899 ± 2,745	1,948 ± 1,294	1,423 ± 1,030	3,169 ± 5,941	2,052 ± 1,580	1,701 ± 1,508

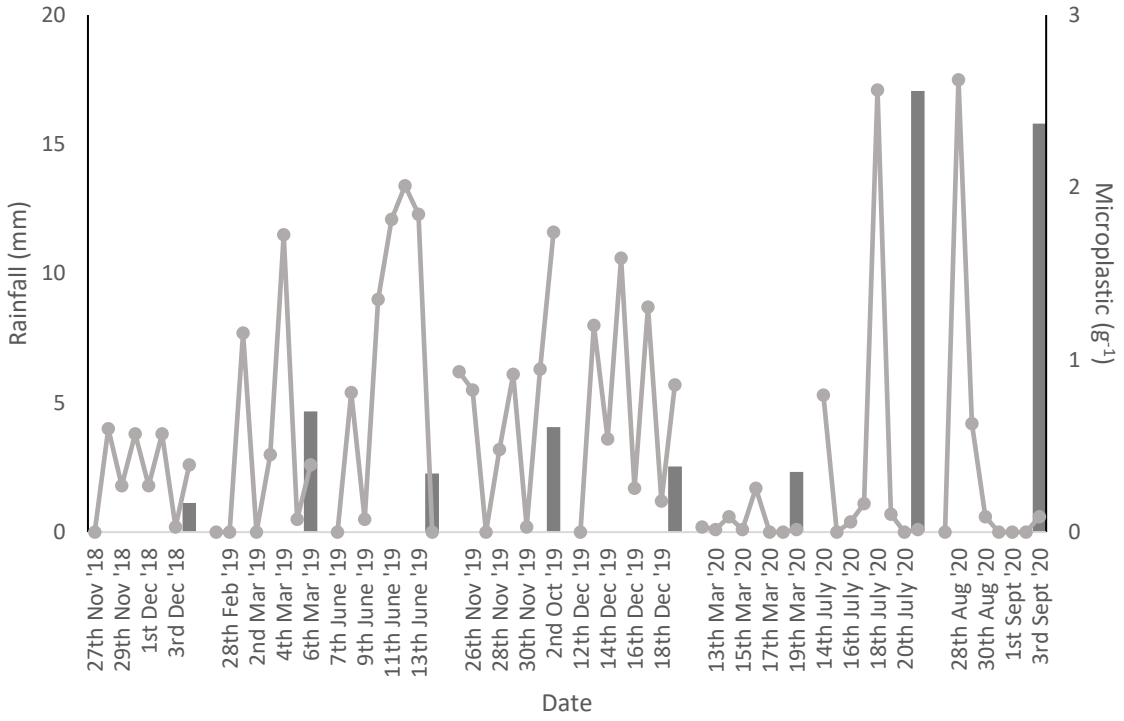
4.3.4 Rainfall

On average, rain fall leading up to sampling was 28.13 ± 15.33 mm, with the greatest rainfall over the recorded eight-day period occurring in June 2019 (Supplementary Material, S5 & S6). July and August 2020 experienced over 17 mm of rain in a single day prior to sampling. This was the greatest amount of rain recorded in a single day (Figure 4.4A).

4.3.5 Types of microlitter present

The most abundant form of microlitter per sample per date is available in the Supplementary Material (S10). Clear and white fibres are the most abundant form of microlitter in sediment across all sampling sessions (Figure 4.5). Black and red fibres are also relatively common on all sampling dates, with blue fibres being more common in March 2019, July 2020 and September 2020.

A



B

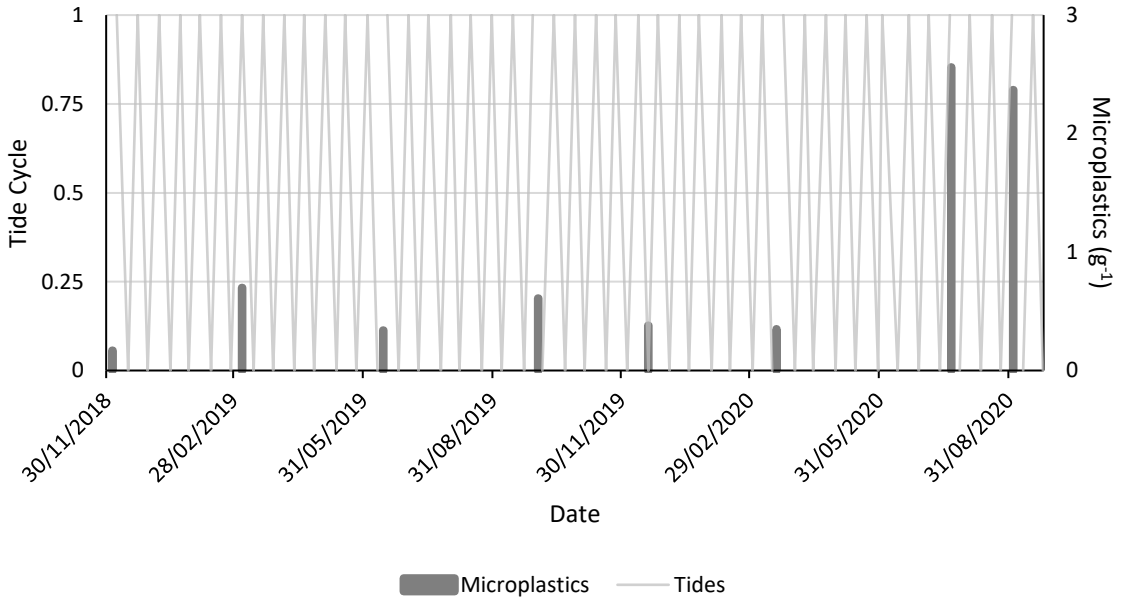


Figure 4.4. Mean microlitter abundance in sediment per gram (secondary y-axis) plotted against A) Daily rainfall (Met Office, 2006) for the week leading up to sampling (primary y-axis); B) Spring-neap tide cycle where 1 is a spring tide and 0 represents a neap tide. Tide data was collected using the PLA tide prediction tool (http://tidepredictions.pla.co.uk/).

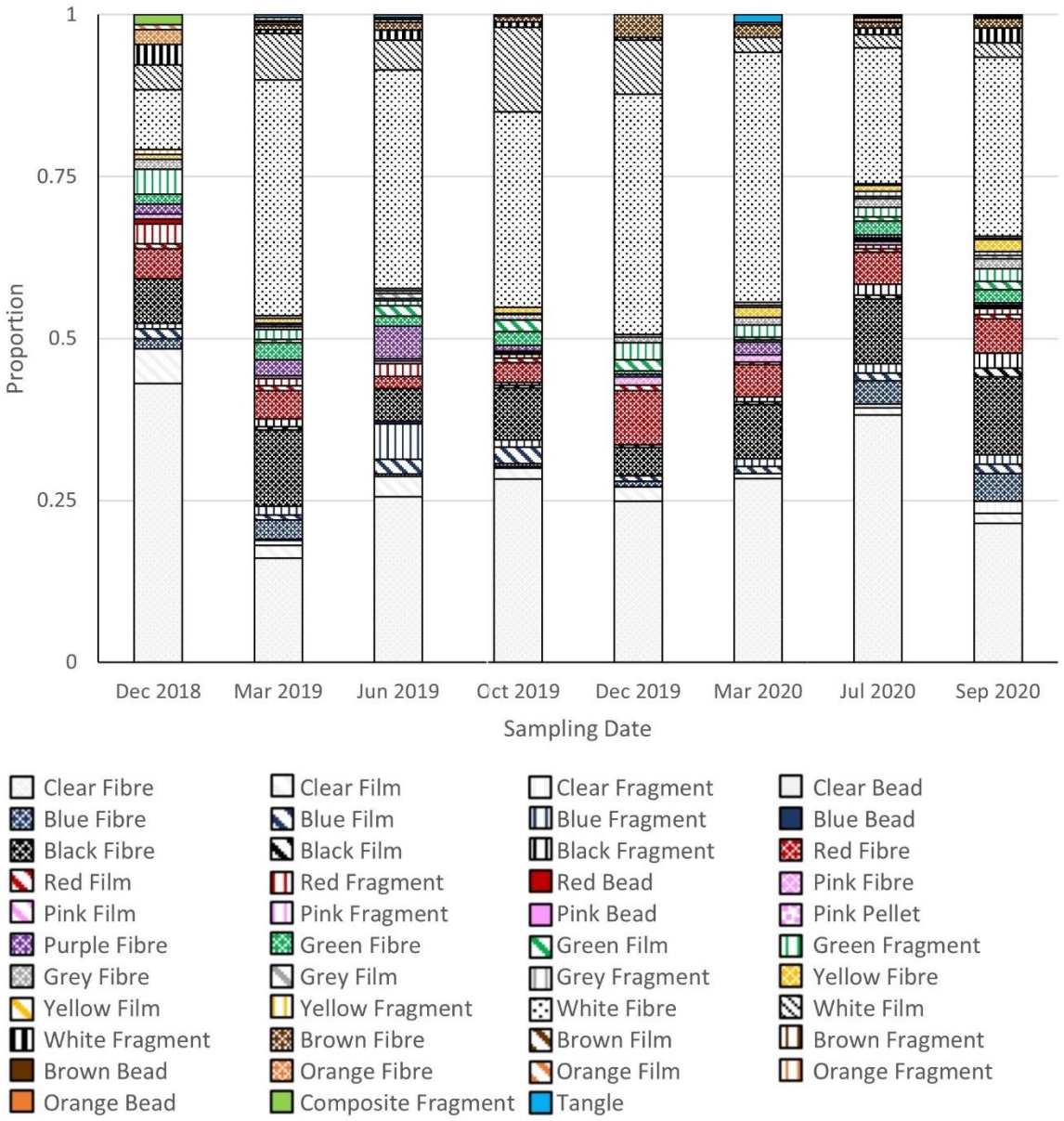


Figure 4.5. Variation in microplastic forms (colour and shape) in sediment samples compared between sampling dates.

The average length of microplastics in sediment ranged from 1,423 ± 1,031 µm in December 2019 and 3,169 ± 5,941 µm in March 2020 (Supplementary Material, S10). Of the items measured, taking the longest length of each item, 344 were small microplastics, 781 were large microplastics (mostly between 1 mm and 2 mm long, 428 items), 47 were

mesoplastics and 2 were macroplastics. Beads measured an average 411.5 ± 263.6 µm in the longest dimension, whilst fibres measured 2,253.2 ± 4,512.4 µm, films measured 2,833.5 ± 9,533.7 µm, fragments measured 1,104.7 ± 1,034.7 µm and tangles measured 1,185.4 ± 879.6 µm in the longest dimension (Figure 4.6).

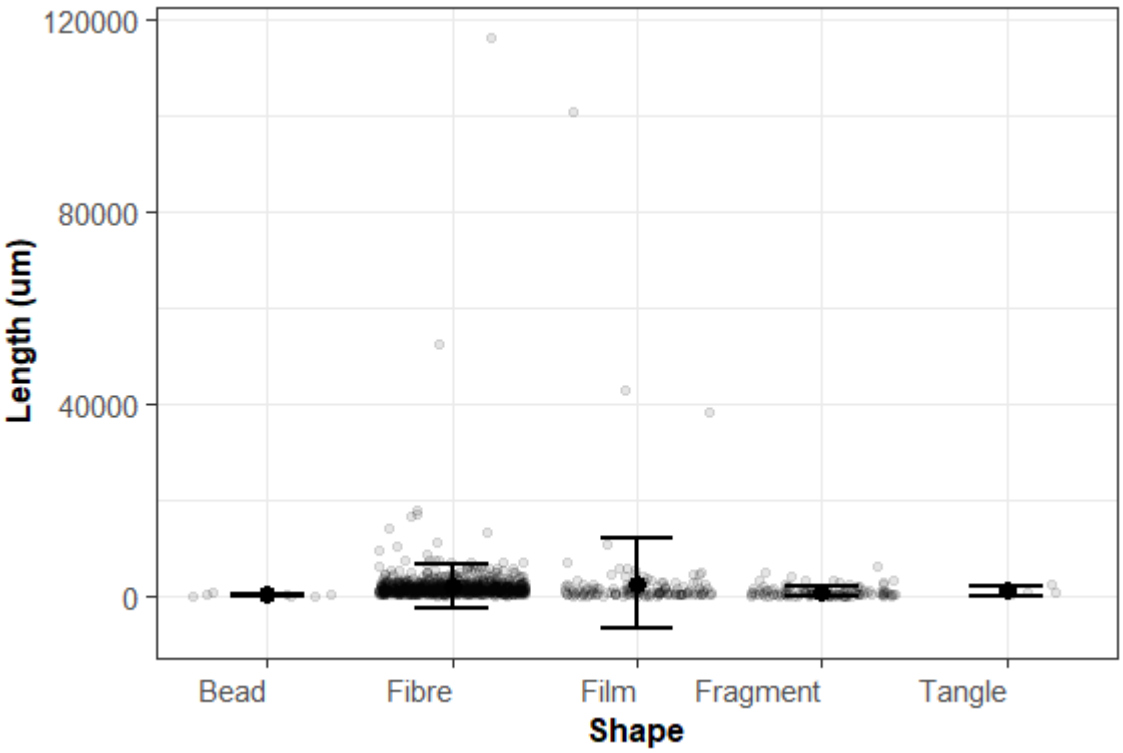


Figure 4.6. Length of microlitter by their form (bead, fibre, film, fragment or tangle). Grey points are individual values, black points are the means and bars are ± standard deviation.

A total of 696 items recovered from sediment were analysed by FTIR. Nearly all (99%) items matched library spectra, most of which were plastic (87%) rather than cellulosic and semi-synthetic compounds (11%). Whilst a library match threshold was not used to confirm polymer identities, 98% of matches did produce a match greater than 70%. The most common polymers in sediment were PP (36%), PES (17%), cellulosic polymers (11%), PE (high density and low density, 9%), acrylic (9%) and PA (7%). Polymer abundance did not vary greatly between sampling dates with PP being most abundant in six of eight samples and PES being the next most abundant in almost all samples.

4.4 Discussion

The concentration of microplastic in sediment was, on average, 1,000 ± 1,100 microplastics per kg (dry weight) peaking in June 2020 and September 2020 (2,560 ± 1,250 microplastics per kg and 2,370 ± 920 microplastics per kg respectively). The results of the present study are higher than previous accounts (Horton et al., 2017), although previous estimates have focused on large microplastics (≥ 1mm). Aside from the recorded concentrations, the results of Horton et al. (2017) were similar to the present study, with PP and PES being most dominant and fibres being most abundant at most sites. The majority of plastic was between 1 mm and 2 mm, as found by Horton et al. (2017), despite a finer mesh size being used in the present study. Whilst using a finer mesh size provides a more accurate representation of the plastic contamination in an ecosystem, lower cost methods estimating the abundance of only large microplastics are a valuable monitoring tool that are likely easier to implement on a large scale. It is important to note, however, that the size of microplastics ingested varies between organisms and that smaller microplastics can be eaten by a wider range of species.

Whilst not the highest recorded concentration in riverine sediment, the average contamination in the present study was far higher than many estimates from estuaries and rivers worldwide. Overall, European rivers contain a higher concentration of microlitter than those from Asia, Africa and the Americas. Greater microlitter concentrations were recorded in sediment with the urban canals in Amsterdam (Leslie et al., 2017) and the Ebro River, Spain (Simon-Sánchez et al., 2019) which had mean concentrations twice as high as the present study. Higher still were certain sites in the Rhine, Germany (Mani et al., 2019). Microlitter in the Rhine, however, were dominated by particles between 11–500 µm, which far smaller than reported in the present study. Outside of Europe, the concentrations reported in the Thames are greater than most in Africa (e.g., Nel et al., 2018) and Asia (e.g., Jiang et al., 2019; Xu et al., 2020; Ghayebzadeh et al., 2021) but far less than those in Belize (Silburn et al., 2022). The relatively high concentrations found in the present study are in line with other estimates of microplastic pollution in the Thames. Indeed, Rowley et al. (2020) found that surface water contamination was higher than estimates for many rivers around the world. The peaks in microplastic concentration in June 2020 and September from the present study corresponded to the highest CPUE of macroplastic (Chapter 3), with the greatest amount of macroplastic

collected in a single trawl occurring in a benthic sample on 3rd September 2020. Additionally, the greatest diversity of microplastics in sediment was also reported in June 2020 and September 2020. A high diversity of plastic is often associated with urban runoff and it is likely that London and other urban centres on the River are major sources of microplastic pollution. Jang et al. (2020) analysed surface water, sediment and biota from the southern coast of South Korea. They found that samples from urban areas contained highly diverse assemblages of microplastics, whilst samples collected next to aquafarms contained more polystyrene from buoys and rural samples contained PP from ropes and fishing gear. The same study (Jang et al., 2020) found a greater abundance of microplastics in urban and aquafarm sites compared to rural areas. It is surprising then, that given the high population density of Chinese cities, higher concentrations of microplastics were recorded in the present study compared to the Beijiang River (Wang et al., 2017). The Beijiang River is a tributary of the Pearl River and passes through Qingyuan City which has a population of over 4 million with 18 WWTPs discharging into the river. Given that this is just part of the 52,068 km^2 Beijiang catchment and compared with the Thames watershed of 16,000 km^2 encompassing 15 million residents (Environment Agency, 2016), it raises concerns for the health of this iconic river. Wang et al. (2017) noted that the WWTPs in Qingyuan City treated 425 thousand tons of sewage a day with a 90% efficiency. This was still likely a high source of pollution in the river. It is possible that flow rate and other environmental factors are preventing microplastics accumulating in the sediment and they instead stay suspended in the water column. London is operating an antiquated sewage system and it is possible that, with CSOs and the potential for illegal discharging of raw sewage, that the Thames is exposed to large volumes of untreated municipal waste which contribute to the high volumes of microplastics recorded in the sediment. Certainly, Leslie et al. (2017) concluded that the concentrations of microplastics in the Amsterdam canals was similar to that of the wastewater effluents being discharged in the area.

Similar to the results discussed in Chapter 3, following the heavy rain in July and August 2020 an increased abundance of microplastics was reported in the sediment. Additionally, as seen with the macroplastic, there was no spike in microplastic abundance following the longest rainy period in June 2019. As previously discussed, sewage-related debris is suspected to be most impacted by heavy or prolonged rainfall. The sediment in the present study was

dominated by clear and white microfibres and microplastics identified by FTIR to be mostly PP and PES. Both these polymers are used in the production of wet wipes and sanitary pads and these products have been recovered from the River Thames (McCoy et al., 2020). Sanitary pads and wet wipes are known to create artificial “reefs” in the Thames and, as noted in Chapter 3, can accumulate on the riverbed after becoming entangled in vegetation or clumping together. This close association with the riverbed likely allows for the accumulation of fragmented fibres in the sediment.

Despite this evidence of increased inputs of microplastics following rainfall events, Cheung et al. (2019) noted that estuarine systems are highly dynamic with concentrations decreasing tenfold just two hours after the event. Indeed, high flow rates can flush pollutants away from sites that are otherwise hotspots for pollution, such as sewage outfalls (Windsor et al., 2019b). Additionally, evidence from an estuary in Thailand, suggests that strong hydrodynamic forces during heavy rain events can limit microplastic deposition in sediment (Jiwarungrueangkul et al., 2021). Certainly, in the Thames Estuary, which is naturally a turbid, strongly tidal estuary with strong vertical mixing (Uncles and Mitchell, 2011), heavy rainfall increases ebb velocities resulting in localised flushing of sediment. As a result of increased seaward currents, freshwater residence time in the estuary is lower in winter compared to summer (0.5 months in winter and 3 months during summer droughts, Uncles and Mitchell, 2011). The same is likely true of plastic in the estuary.

Some estuaries are known as salt wedge estuaries, which occurs when fast flowing freshwater runs over the top of saltwater. This then results in high sedimentation, and therefore potential microplastic accumulation, at the salinity front (Vermeiren et al., 2016). This effect is reported in the Rio de la Plata Estuary in South America for example. The strong vertical mixing in the Thames Estuary, however, prevents the formation of a salt wedge. Instead, the Thames is a slightly stratified estuary with intense mixing (Preddy, 1954). The literature suggests that partially mixed estuaries, such as the Thames, are less likely to accumulate contaminants. It is proposed instead that they are greater sources of macro- and microplastics to the sea, but evidence is lacking and warrants further investigation (Vermeiren et al., 2016). Whilst the salinity front might not aid microplastic deposition at Erith, it is naturally a region of high sedimentation as part of the mud reaches in the estuary. In this region, there is a flow of water up the estuary near the riverbed which tends to lead to the

accumulation of litter (Uncles and Mitchell, 2011). Additionally, partially mixed estuaries may receive microplastic heavy sediment from marine sources (Vermeiren et al., 2016). Indeed, Ryan and Perold (2021) recovered marine litter over 1 km into the Zandvlei Estuary, South Africa.

Uncles and Mitchell (2011) reported that fortnightly spring-neap tides occurred in the Thames Estuary which periodically increase vertical mixing. Samples collected in December 2018, June 2019, October 2019 and March 2020 were collected mid cycle, with the October 2019 and March 2020 samples occurring closer to a neap tide than a spring. December 2019 samples were collected on a neap tide and March 2019, July 2020 and September 2020 were collected on spring tides. The latter three sampling dates had the highest mean concentration of microplastics in sediment. Partially mixed estuaries, such as the Thames, have greater vertical mixing during neap tides with more transverse distribution with spring tides (Greyer et al., 2008). It is possible that the combination of heavy rain and a spring tide in July 2020 and September 2020 resulted in more microplastics being flushed down to Erith from upstream. Whereas, despite prolonged rain, neap tides and tides mid cycle may hinder the deposition of plastic in the sediment, resulting in lower recorded concentrations.

Sediment samples in the present study were collected at slack water as this yielded the most success with the Ekman grab. Slack water is additionally when suspended sediment settles to the riverbed (Uncles and Mitchell, 2011) and thus recorded microplastic concentrations might be slightly elevated at this time.

In addition to the above large scale factors affecting plastic distribution, local factors can influence particle movement. For instance, wind direction has a significant effect. More plastic accumulates windward than leeward, and strong winds over the estuary's surface can cause a downward flux of plastic in the surface water. The strength of the effect, however, decreases with increased depth (Vermeiren et al., 2016). Ultimately, the combination of all these factors makes estuaries a very dynamic system for which microplastic distribution, movement and accumulation are poorly understood. The movement of plastics through the estuary by hydrodynamic forces can then influence the ingestion of microplastics. Ferreira et al. (2018) found that fish in the upper estuary ingested longer fibres than those in the lower estuary, presumably due to the rapid movement of smaller particles downstream.

4.5 Conclusion

Microlitter abundance in the environment, specifically the sediment, varies seasonally. Due to their small size and abundance, microplastics are widely bioavailable and estimating their abundance in the environment is key to evaluating potential risk. Estuaries are complex systems with many factors affecting the distribution of microplastics. To better understand how these factors contribute to microplastic accumulation in sediment and estuarine inputs to the sea more data are needed. Sampling on a finer scale and collecting more environmental data during sampling would address the knowledge gaps highlighted in the present study. Additionally, longer term studies could better determine causation of fluxes in microlitter abundance and likely cover greater variation in weather conditions, such as the drought observed in the summer of 2022 in Southeast England.

Chapter 5

High prevalence of plastic ingestion by *Eriocheir sinensis* and *Carcinus maenas* in the River Thames

This chapter has been published in *Environmental Pollution* and is available at https://doi.org/10.1016/j.envpol.2020.114972. The co-authors of this paper are Prof David Morritt, Dr Paul Clark and Mr Brian Smith. All co-authors assisted with sample collection, resource management and reviewing and editing the manuscript. Prof Morritt and Dr Clark additionally assisted with project conceptualisation, funding acquisition, method development and supervision.

5.1 Introduction

Plastics are produced in vast quantities with global production showing year on year increases. In 2018, 359 million tonnes of plastic were produced globally (PlasticsEurope, 2019b). It is now well documented that plastic waste is polluting the environment. Estuaries are particularly affected, acting as hotspots for plastic accumulation (Browne et al., 2010; Wright et al., 2013). Litter accumulates near estuarine inputs to marine habitats (Galgani et al., 2000) and, indeed, thousands of pieces of plastic litter have been found flowing along the Thames riverbed, including carrier bags, sanitary pads and food packaging (Morritt et al., 2014). These large plastic items can fragment (Barnes et al., 2009; Andrady, 2011), eventually becoming microplastics; items <5 mm. Additionally, some microplastics are produced to this size range, such as microbeads and nurdles. Microplastics are well established as a significant threat to aquatic organisms (Lusher et al., 2017; Foley et al., 2018) and are ingested by many organisms (Lusher et al., 2017). Ingestion can have severe negative impacts by reducing feeding rates, resulting in the dilution of nutrients, reducing growth, hormone levels and reproductive output and affecting enzyme action and the gut microbiome (Galgani et al., 2010; Liu et al., 2019). After ingestion, microplastics may be translocated to other tissues, including the brain, gills and gonads (Crooks et al., 2019). Additionally, microplastics contain and adsorb chemicals, such as POPs, which can also have negative effects once ingested (Moore, 2008; Wright et al., 2013; Lusher et al., 2017).

The Thames has a large catchment of 16,000 km^2 encompassing 15 million residents (Environment Agency, 2016) and passes through large urban centres e.g., Oxford, Reading and London. This river is in constant use by pleasure craft, ferries, passenger transport, cargo vessels and trans-oceanic shipping. Currently, the tidal Thames, eastward of Teddington Weir, Middlesex, is the recipient of huge quantities of untreated sewage overflow discharged from an antiquated Victorian system. Consequently, the Thames is vulnerable to domestic and industrial wastewater pollution. Microplastics have been reported at various sites in the Thames watershed (Horton et al., 2017; Greenpeace, 2019; Rowley et al., 2020) and it is one of the most microplastic-contaminated rivers in the UK (Greenpeace, 2019). Indeed, concentrations of microplastics, not including fibres which are abundant in the Thames (Horton et al., 2017, 2018; McGoran et al., 2017, 2018), recovered in the Thames are comparable to some of the highest recordings worldwide (Rowley et al., 2020). This high contamination is likely to have a detrimental effect on the river wildlife. Microplastic particles can be ingested by a range of aquatic species, but there is presently a bias towards fish and a lack of studies understanding plastic ingestion by invertebrates, particularly those in freshwater systems (De Sá et al., 2018). To date, one fish species in the freshwater reaches of the river (Horton et al., 2018) and nine estuarine fish species, as well as the brown shrimp, *Crangon crangon* (McGoran et al., 2017, 2018) have been found to ingest plastic. A recent study also reported plastic ingestion in the invasive Asian clam, *Corbicula fluminea* (McCoy et al., 2020).

Brachyuran crabs make up an important part of the Thames megafaunal community. *Carcinus maenas*, the native shore crab, and *Eriocheir sinensis*, the invasive Chinese mitten crab, are common in the estuary (Clark and Rainbow, 1997). The latter is now well established in the Thames (Morritt et al., 2013; Clark et al., 2017). It was first reported in the Thames in 1935 and thought to have made its way from Germany where the species was introduced as larvae in the ballast water of international ships in 1912 (Clark and Rainbow, 1997; Weber, 2008). Due to the high numbers present in the estuary, its large size, feeding activity (Webster et al., 2015; Mills et al., 2016) and general aggressive behaviour, *E. sinensis* is a threat to native biodiversity, competing both for food and habitats (Clark and Rainbow, 1997). Indeed, the species is considered one of the world's worst invasive species (Lowe et al., 2000; DAISIE, 2009) and is a threat to fishing industries, in terms of damage to fish stocks and equipment

(Wójcik-Fudalewska et al., 2016). Studies have demonstrated that *C. maenas* are negatively impacted by the presence of *E. sinensis*, with the invasive species displacing native crabs from refugia, making them vulnerable to predation (Gilbey et al., 2008).

Decapod crustaceans contain a GM in the foregut (McGaw and Curtis, 2013) which is used to masticate food. Evidence suggests that this structure can accumulate high quantities of plastic, with 67–83% of *Nephrops norvegicus* (Norway lobster) on the west coast of Scotland containing microplastics (Murray and Cowie, 2011; Welden and Cowie, 2016a). Furthermore, microplastics can be retained in the gut, liver, gills and gonads of crabs (Yu et al., 2018). Despite this evidence, their abundance and the potential threat they pose to the Thames Estuary, plastic ingestion by *E. sinensis* has scarcely been investigated (aside from passing mentions by Robbins, 1996 and Clark and Rainbow, 1997). Similarly, Thames *C. maenas* has not been studied in this regard.

The aim of the present study was to investigate plastic ingestion by *C. maenas* and *E. sinensis* in the Thames Estuary and expand the understanding of ingestion beyond fish species in the area. The hypotheses were 1) plastic ingestion would not differ between the species as both occupy a similar niche and are both generalist feeders and 2) plastic would be more prevalent in the GM, based on previous studies (Murray and Cowie, 2011; Welden and Cowie, 2016a).

5.2 Materials and methods

5.2.1 Sampling

A beam trawl with a standard mesh size of 80 mm and an additional 16 mm fine mesh insert was deployed from fishing vessel *Boy Daniel* SD4 at Erith, Thames Estuary. Samples were collected every three months (December 2018 – October 2019). Between two and five benthic trawls were conducted per sampling session at a depth of 9.2–13 m (Figure 5.1). Brachyuran crabs were collected, identified and stored on ice overnight until they could be frozen the following morning at −20 °C. A total of 135 crabs (Table 5.1) were collected over a year, comprising *C. maenas* and *E. sinensis*.

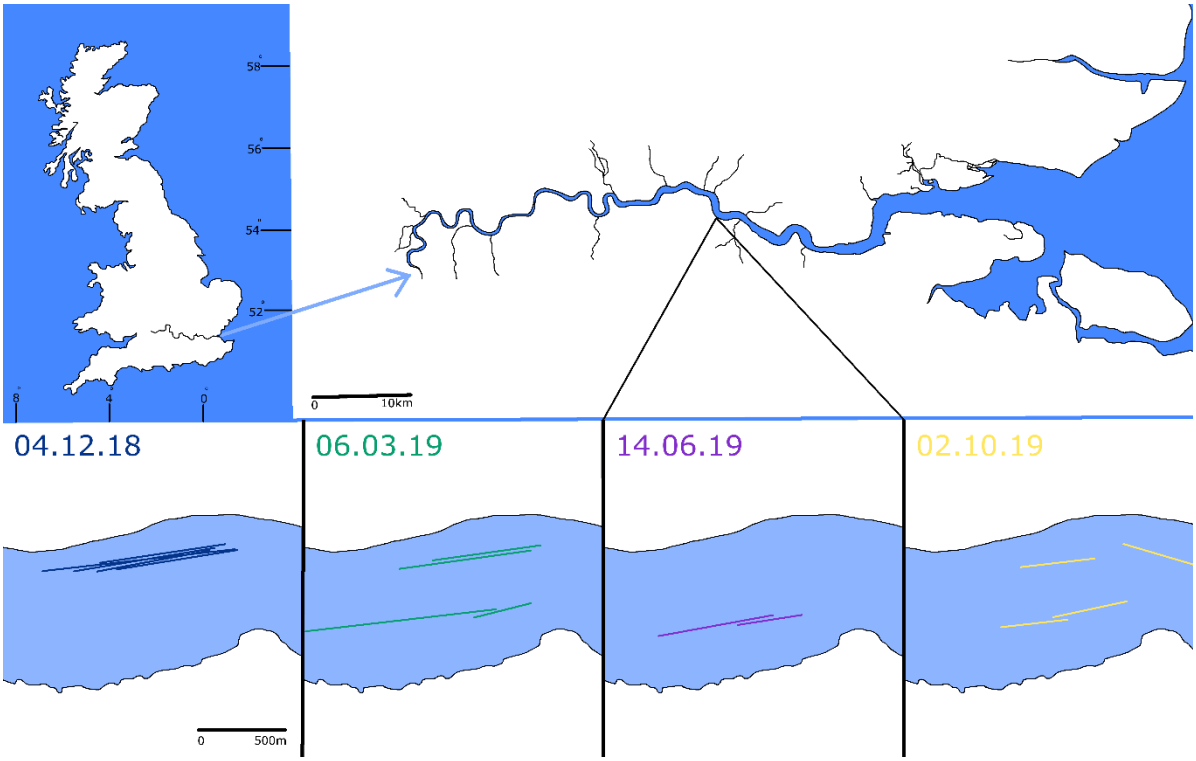


Figure 5.1. Benthic trawls were undertaken in the Thames Estuary at Erith Rands, UK on 4th December 2018 (blue), 6th March 2019 (green), 14th June 2019 (purple) and 2nd October 2019 (yellow). Scale bars = 10 km and 500 m. Map drawn by A. McGoran.

Table 5.1. *Carcinus maenas* and *Eriocheir sinensis* were sampled from Erith in the River Thames on four dates.

Date of sampling	**Number of Trawls**	**Number of *C. maenas* caught**	**Number of *E. sinensis* caught**
4th December 2018 (Winter)	5	2	37
6th March 2019 (Spring)	4	0	3
14th June 2019 (Summer)	2	70	1
2nd October 2019 (Autumn)	4	22	0
Total	15	94	41

5.2.2 Sample preparation

The mass (Ohaus ranger 3000 balance, nearest 0.1 g), length (to nearest mm) and sex of each individual was recorded. In addition, the carapace width was measured at the widest point, between the 4th pair of anterolateral teeth with a rule (to nearest mm).

The carapace was removed from defrosted crabs and the GM, GIT and gills were extracted and stored separately in 15 ml Falcon^{TM} tubes. Tubes were refrozen prior to digestion. A 10% KOH solution was used to remove organic material from the sample. The volume of solution added was at least twice the volume of the sample. The solution was heated to 60 °C and left to digest overnight in an oven. GMs were resistant to digestion and effectively protected the contents inside. Therefore, they had to be dissected to allow the KOH to enter the cavity. The liquid from digestions was removed using vacuum filtration through a 32 µm nylon mesh. The tubes were rinsed with filtered distilled water a minimum of three times or until all visible material had been removed. Filters were stored in glass Petri dishes and dried at 60 °C. Once dried, samples were sealed with Parafilm® until further processing.

Filters were examined under a Leica MZ 6 microscope using mounted pins and forceps under 16–64 times magnification with a detection limit of 32 µm. All recovered items were photographed and stored in individual glass specimen tubes. Items were described by colour and shape and were measured (length and width) using Image J. Shape was classified as either a film, fibre, fragment or a tangle of fibres. In some instances, items did not fit these criteria and were listed as 'other'. This category contained three resin-like, putties that do not fit within current classification (Rochman et al., 2019). Tangles of fibres were weighed on a Sartorius MC5 balance (to the nearest 0.001 g) and an estimate of the number of items in the tangle calculated. Counting and photographing all items recovered from one tangle comprising over 100 items took several hours. Consequently, it was deemed impractical to precisely document all items from some tangles, instead estimates were made of their abundance.

5.2.3 FTIR spectroscopy

Analysis of plastic pieces was undertaken using a PerkinElmer Spectrum One FTIR spectrometer, with an AutoIMAGE FTIR Microscope System PerkinElmer attachment. A representative subsample of ca. 10% (105 items overall) of each colour and shape combination (i.e., clear fibres, clear films and clear fragments) was analysed as per the recommendation of Lusher et al. (2017). A background spectrum was made before analysis and updated between samples. For each individual item, 16 scans were collected with the average result being used to generate an absorption spectrum between 500 and 4,000 cm^{-1}. This spectrum was compared to the 'NHM Plastic Collection'. This library was a collection of spectra collated from micro-FTIR and ATR-FTIR analyses of virgin plastics provided by plastic manufacturers.

5.2.4 Controls for contamination

A clean, white cotton laboratory coat and non-sterile, single-use gloves were worn for all laboratory work, including dissection, digestion and filter searches. The laboratory coat in the present chapter was used prior to the use of purple dye. Samples were covered as much as possible to reduce exposure to airborne contamination. Equipment and laboratory bench space were cleaned with filtered (32 µm nylon mesh) 80% industrial methylated spirit and white paper roll prior to work and between samples. Equipment, such as scalpels, forceps, scissors, mounted pins and dissection boards, were inspected for plastics prior to use and were rinsed with filtered distilled water (32 µm metal mesh) until all contamination was removed. KOH was diluted with filtered distilled water and kept in glass bottles that had been rinsed three times with filtered distilled water.

Controls to measure airborne contamination were implemented. Empty Petri dishes were placed in the laboratory to monitor environmental contamination at all stages of processing. During digestions, procedural blanks were run. Falcon™ tubes were filled with filtered distilled water and underwent the same process as the specimens for digestion, filtration and searching. Plastics recovered in the controls were analysed using the methods described for plastics recovered from samples. Contamination for each shape and colour plastic was calculated for each specimen (see below). Average contamination was only removed for digestions. For dissections and filter searching, however, contamination was

calculated independently for each crab. Thus, if contamination was higher on a particular day, only the crabs processed on that day would have a higher level of contamination. Plastics were removed from analysis if they did not exceed the calculated contamination. Where the volume of plastic matching the description of a contaminant plastic exceeded that of the calculated contamination, the count was reduced to compensate. For example, if the contamination for black fibres in crab 1 was one and it had ingested three black fibres, only two were reported.

$$\text{Contamination} = (S_1/N_1) + (A) + (S_2/N_2)$$

S_1 = Sum of items of a particular shape and colour (i.e., clear fibres, black films) collected in airborne controls during dissection, N_1 = Number of crabs dissected during the control (S_1), A = Average number of plastics of a particular shape and colour (i.e., clear fibres, black films) collected from procedural blanks, S_2 = Sum of items of a particular shape and colour (i.e., clear fibres, black films) collected in airborne controls during filter searches, and N_2 = Number of filters covered during the control (S_2).

Fibres from the nylon filters used in the present study were considered a potential source of contamination. But upon inspection under the microscope, fibres were found to be easily identifiable, retaining a uniform wave along their length. Thus, fibres matching this description were removed from samples. To further prevent these fibres contaminating samples, filters were rinsed with filtered distilled water and wiped with lab roll on each side. This was repeated until all visible fibres had been removed from the filter. Filters were stored in glass Petri dishes that had been rinsed with filtered distilled water. FTIR analysis was not conducted on controls.

5.2.5 Statistical analysis

Statistical analysis was conducted using R version 3.4.2 (R Core Team, 2017) with the following packages: “ggplot2” (Wickham, 2016), “MASS” (Venables and Ripley, 2002) and “multcomp” (Hothorn et al., 2008). GLMs were developed to understand the variables that influenced the number of crabs affected by plastic and the number of plastic pieces in individuals. The season of sampling (winter – December, spring – March, summer – June, autumn – October), carapace width, sex, species and organs were investigated. GLMs were

compared with AIC and BIC scores so that only reduced models with the significant factors were used for analysis. Non-significant variables were removed until three GLMs were generated. To analyse the number of individuals affected by microplastics, binomial models were used. To compare the amount of plastic present per individual (crabs that ingested no plastic were removed from analysis, except when tangles were being compared due to the low number of tangles remaining in *C. maenas*), Poisson or Gamma error distributions were used. When categorical variables were significant, the results were interpreted using Tukey pairwise comparisons. Similarly, when interaction terms were significant, the results were interpreted by model reductions and ANOVA comparisons.

Model 5.1 – GLM(Proportion with litter ~ Season + Sex, family = binomial)

Model 5.2 – GLM(Plastic in Organs ~ Species, family = Gamma (link = inverse))

Model 5.3 – GLM(Number of Tangles in Organs ~ Species, family = Poisson)

5.3. Results

5.3.1 Contamination

Airborne contamination was reported at all stages of processing (see Table 5.2 for average contamination per individual). Contamination included clear, blue, black, brown, red, grey, white, green and yellow fibres, clear film, blue and green fragments and rarely tangled fibres. Results were corrected for contamination as described in the methods.

5.3.2 Plastic content in crabs

Carcinus maenas and *E. sinensis* were affected across all sampling seasons were affected by plastic in either the GIT, GM or gills (see Table 5.2). Overall, 71.3% and 100% of *C. maenas* and *E. sinensis*, respectively, contained at least one item (fibre, film, fragment or tangle of fibres). A total of 874 items were collected from the 135 individuals after contamination was removed.

Model 5.1 compared the proportion of individuals affected by plastic. It revealed that there was no statistical difference in the number of *C. maenas* and *E. sinensis* affected by microplastics (either in the GIT, GM and/or gills). Although, significantly more female crabs

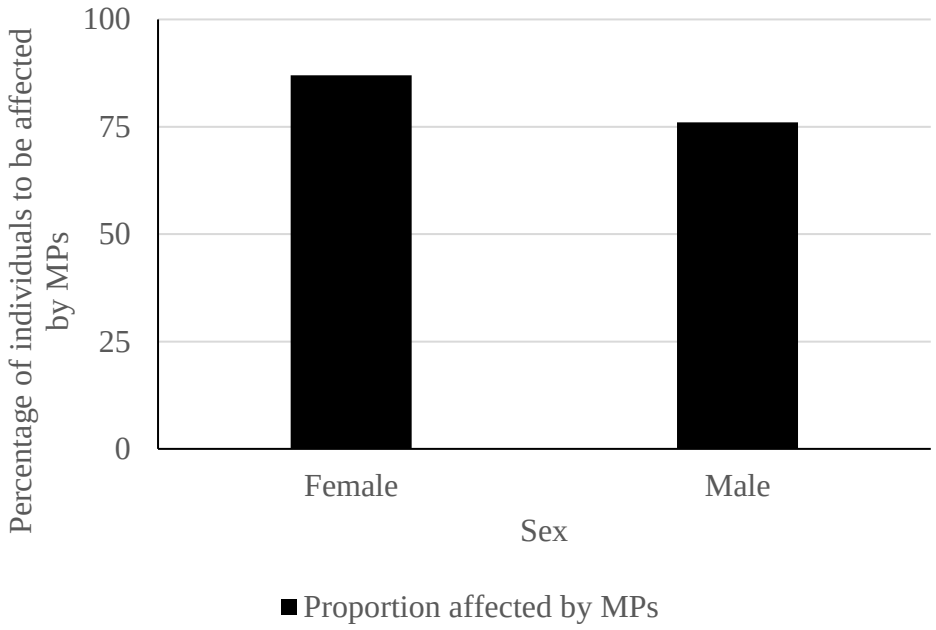
were affected by plastic than males ($p < 0.05$, 76% of males compared to 87% of females, Figure 5.5), with less plastic-affected individuals in the summer (June 2019; 66% of individuals were affected in summer, 91% in autumn, 97% in winter and 100% in spring; $p < 0.001$).

Plastic concentrations did not differ between individuals in any GLM. Model 5.2 demonstrated that the organs of *E. sinensis* contained more plastic than those of *C. maenas* ($p < 0.001$, an average 7.4 items per organ of *E. sinensis* (5.5 with zeros included) and 3.1 per organ of *C. maenas* (1.1 when zeros included)) and that there was no statistical difference in the amount of plastic per organ.

All *E. sinensis* had plastic in the GM, 68.3% in the GIT and 14.6% in the gills. Whereas only 45.7% of *C. maenas* had plastic in the GM, 46.8% in the GIT and 16.0% in the gills. The number of items recovered from each organ also differed (Figure 5.3), with mean number of items in the GM, GIT and on the gills, respectively for *E. sinensis* 10.3, 3.1, 0.2 and *C. maenas* 1.9, 1.2, 0.3.

In June 2019, 70 *C. maenas* were caught and thought to have recently moulted, with exoskeletons yet to fully harden. There was no indication that *E. sinensis* had recently moulted in any samples. Moulting may reduce plastic load as 51.9% of *C. maenas* which showed no signs of moulting recently had plastic in the GM compared to only 17.6% of *C. maenas* which had recently undergone ecdysis.

A



B

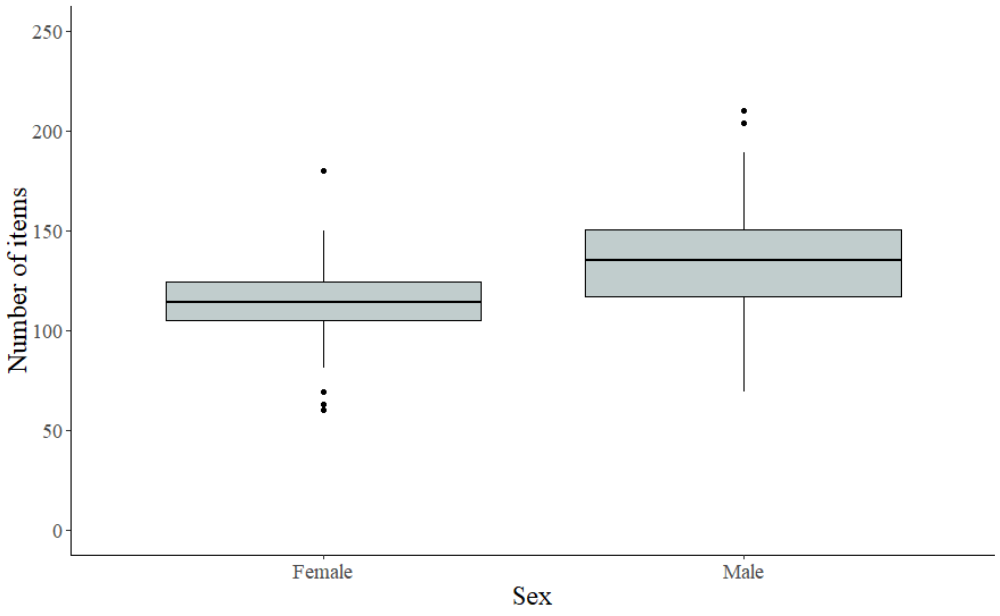


Figure 5.2. A) Proportion of male and female *Carcinus maenas* and *Eriocheir sinensis* affected by plastic; B) The number of fibres affecting each individual from *Carcinus maenas* and *Eriocheir sinensis* combined as estimated during filter searching. Whiskers represent largest and smallest values within 1.5 × interquartile range above 75th percentile and below 25th percentile. Dots represent values outside this range.

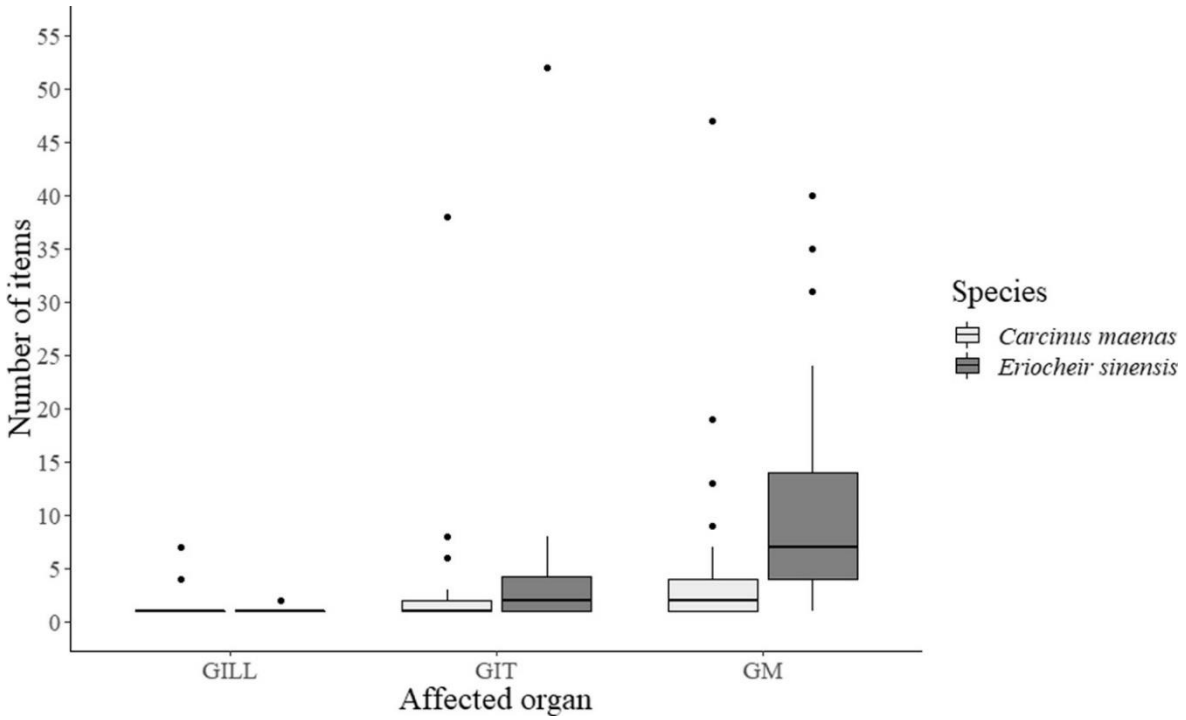


Figure 5.3. The mean number of items present in the GM, GIT and on the gills of *Carcinus maenas* (light grey) and *Eriocheir sinensis* (dark grey) when tangles are treated as a single item. Bars represent the interquartile range (Q3–Q1) and median. Whiskers represent the range of results (Q1 + 1.5 × IQR and Q3 + 1.5 × IQR) with outliers plotted as dots.

Table 5.2. *Carcinus maenas* and *Eriocheir sinensis* were sampled from the Thames Estuary on four dates. Crabs were measured, weighed and dissected. The data is presented in this table along with records of contamination.

Species	Date	Minimum carapace width (mm)	Maximum carapace width (mm)	Average contamination per individual ± SD	% of individuals with items in gills, GM and/or GIT	Average number of plastics per individual ± SD (Fig. 3.2)
Common shore crab, *Carcinus maenas* (Linnaeus, 1758)	Dec 2018	23	48	8.99 ± 0	50	1 ± 0.82
	Mar 2019	NA	NA	NA	NA	NA
	June 2019	21	57	7.76 ± 0.31	65.7	2.59 ± 3.50
	Oct 2019	26	55	8.78 ± 0.83	90.9	6.14 ± 5.33
Chinese mitten crab, *Eriocheir sinensis* (H. Milne Edwards, 1853)	Dec 2018	34	70	8.49 ± 0.32	100	11.35 ± 7.91
	Mar 2019	46	68	8.84 ± 0	100	5.67 ± 2.26
	June 2019	37	37	7.88 ± 0	100	10 ± 0
	Oct 2019	NA	NA	NA	NA	NA

5.3.3 Types of microplastic present

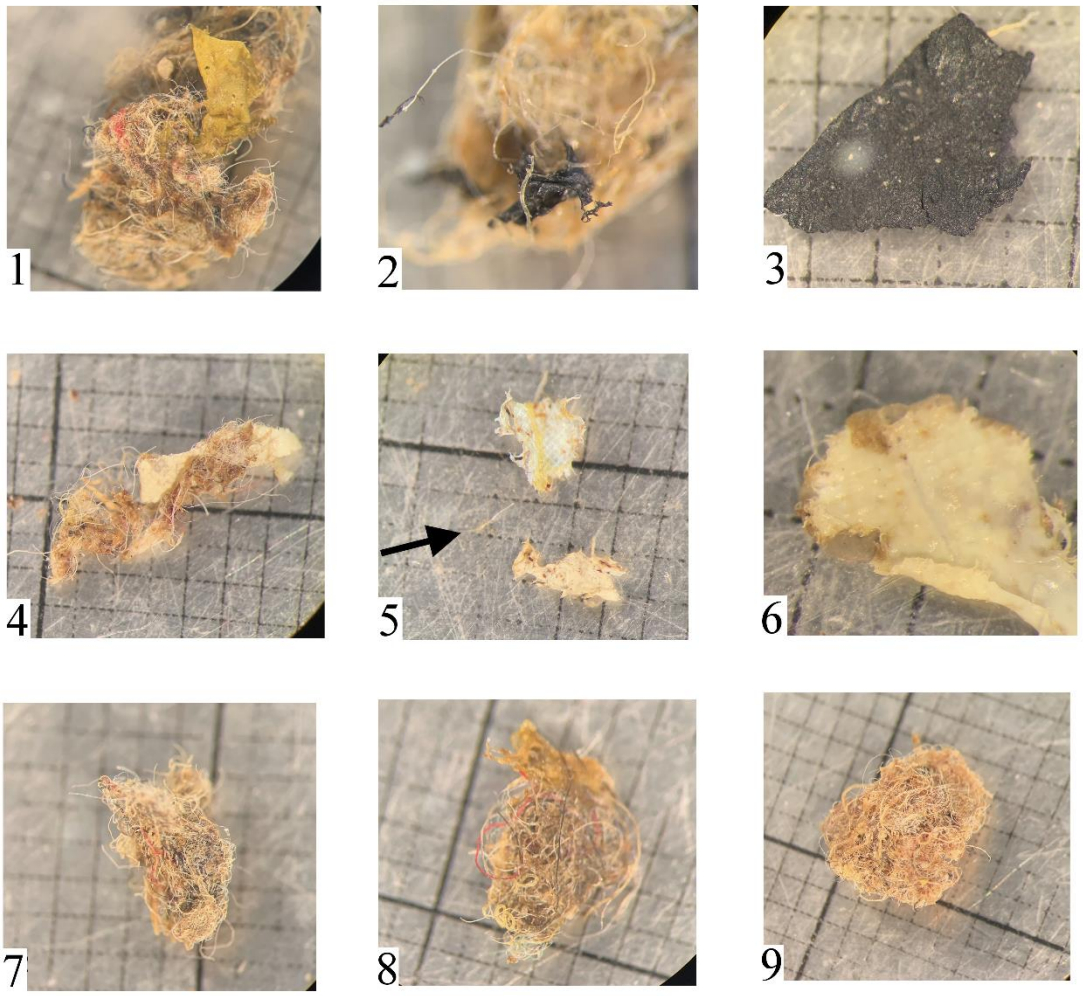


Figure 5.4. Examples of plastic recovered from the gastric mills of *Eriocheir sinensis* specimens caught in December 2018 and displayed on a 1 mm grid. 1) Tangle of fibres and yellow balloon fragment (100+ items); 2) A black film (possibly a plastic bag); 3) A rubber-like fragment; 4) Tangle of fibres and sanitary pad fragment (ca. 40 items); 5) Two fragments of sanitary pad and a clear fibre; 6) Close up of a sanitary pad fragment – note the checked pattern common to sanitary pad fragments; 7) Tangle of fibres (100+ items); 8) Tangle of fibres (ca. 70 items); 9) Tangle of fibres (100+ items).

Fibres (78%) were by far the most abundant form of plastic recovered, with an additional 7.8% of items were tangles of fibres. Tangles also sometimes contained films and other shapes of plastic (Figure 5.4). Films (9.6%) and fragments (4.0%) were also commonly

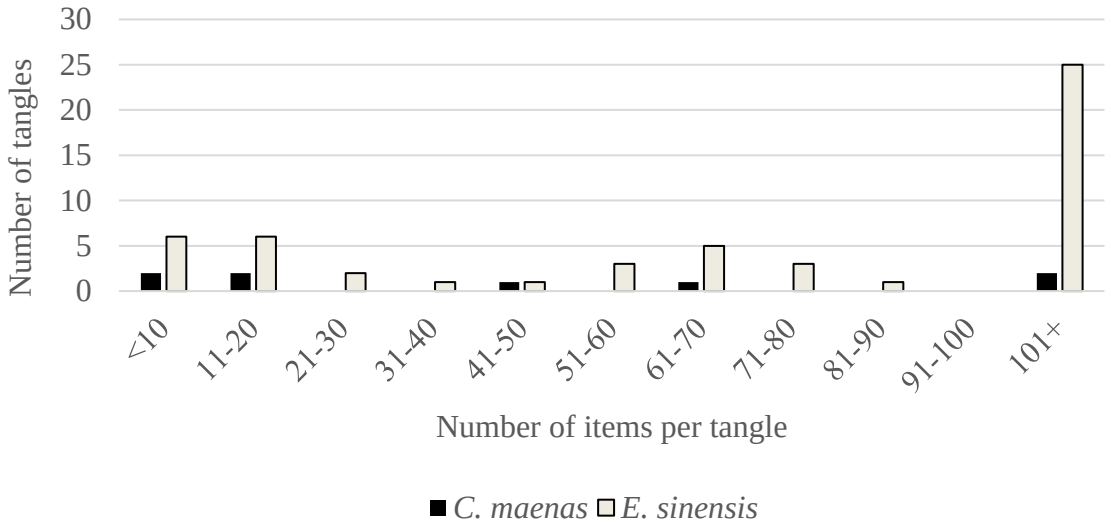
recovered from the crabs. Tangled fibre knots were recovered from 49 crabs (36.3%) and ranged from less than 10 items to over 100 (Figure 5.5A, average 28.4 items per tangle from *C. maenas* and 66.1 per tangle from *E. sinensis*). Tangles weighed between < 0.001 mg and 15.449 mg (Figure 5.5B, average of 0.312 mg in *C. maenas* and 1.900 mg in *E. sinensis*).

Model 5.3 compared the number of tangles per organ between *E. sinensis* and *C. maenas*, highlighting a difference between the species ($p < 0.001$). On average, each organ of *E. sinensis* contained 0.45 tangles of fibres (zeros included) whereas the organs of *C. maenas* only contained an average 0.05 tangled knot (zeros included).

Plastic was recovered in a variety of colours, including clear (25.1% of items), white (21.9%), black (15.7%), blue (11.8%), red (5.7%), brown (3.4%), grey (2.9%), yellow (2.2%), green (1.5%), orange (0.6%), pink (0.6%), silver/gold/glitter-like (0.6%), purple (0.5%). The remaining 7.8% of items were tangles of fibres that contained multiple colours. Items ingested included numerous blue films, fragments of balloon, fragments of sanitary pads (Figure 5.4) and elastic bands.

Items recovered from *C. maenas* and *E. sinensis* ranged from 52 µm to 34 mm in length (average: 2.1 mm) and 34 µm and 32 mm (average: 3 mm) respectively. Fibres were between 47 µm and 34 mm long (average: 2.5 mm) and 2 µm and 183 µm wide (average: 17.2 µm). Tangles of fibres were on average larger (5 mm) ranging from 153 µm to 14 mm. The shortest item analysed by FTIR was 146 µm. Items between 34 µm and 145 µm were not analysed by FTIR. It must be noted that there is some uncertainty in visual identification of microplastics in this size range. Only 20 items recovered were in this size range, mostly from the GIT of *C. maenas*.

A



B

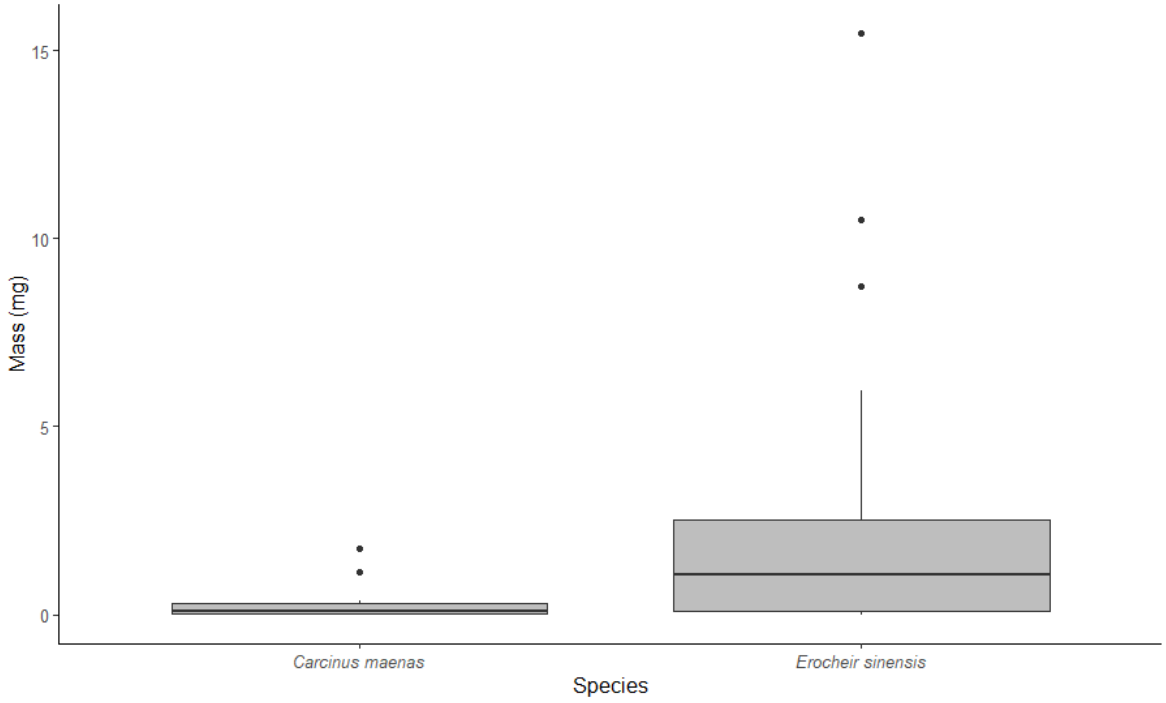


Figure 5.5. A) Estimated number of items (mostly fibres) in tangled knots from *Carcinus maenas* and *Eriocheir sinensis*; B) Mass (mg) of tangled knots from *Carcinus maenas* and *Eriocheir sinensis*.

Of the analysed subset, 72% of items were confirmed to be synthetic and 10% were semi-synthetic (viscose and cellophane). Among these samples, nine synthetic and semi-synthetic polymers and polymer mixes were identified (Figure 5.6). The most common polymers were PP and PES.

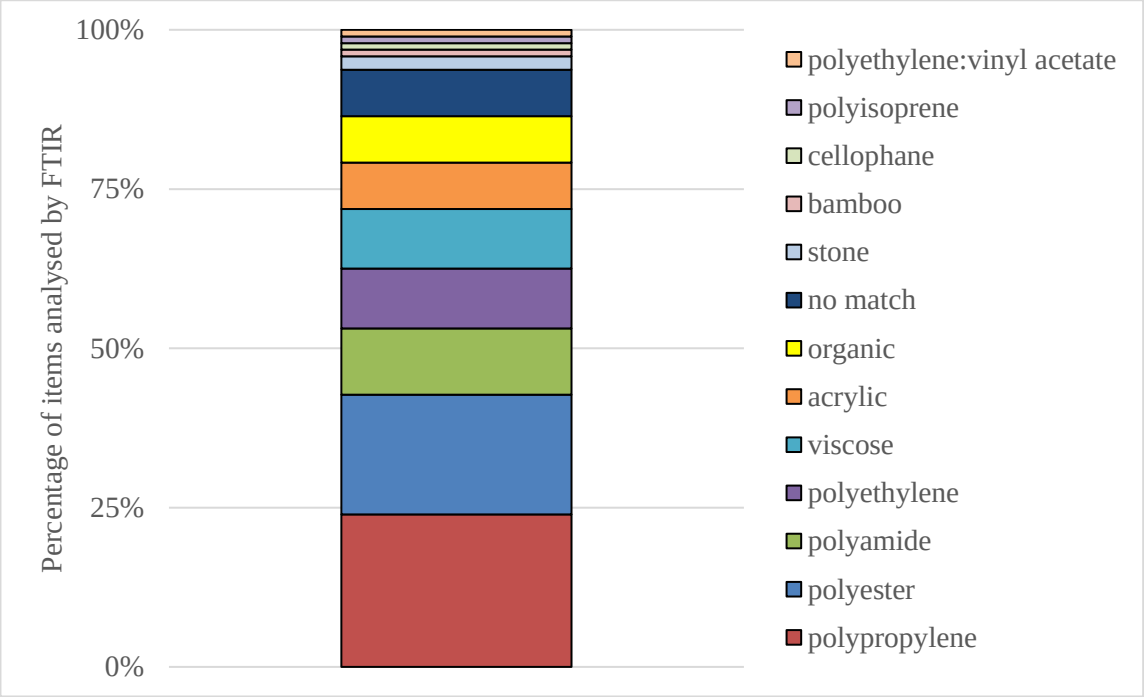


Figure 5.6. Analysis by FTIR on 10% of recovered items (n = 105) revealed the most common polymers were polypropylene and polyester. Organic and semi-synthetic polymer were also identified.

5.4. Discussion

Prior to the present study, plastic ingestion and retention in the Thames had been recorded solely in fish, one shrimp species, *Crangon crangon*, and a bivalve, *Corbicula fluminea*. Although, plastic ingestion had been reported in *Carcinus maenas* (Farrell and Nelson, 2013; Watts et al., 2014, 2015) and *Eriocheir sinensis* (Yu et al., 2018; Liu et al., 2019) under laboratory conditions, little is known about exposure in the wild, with no evidence from the Thames. Estimates of plastic ingestion by Thames fish varies, with some species reportedly ingesting no plastic (e.g., *Chelidonichthys cuculus*, red gurnard, and *Gadus morhua*, Atlantic cod; McGoran et al., 2018), although these species were represented by only a small sample (*n* = 5, *n* = 1 respectively). In comparison, up to 75% of European flounder, *Platichthys*

flesus, ingested plastic (McGoran et al., 2017). Horton et al. (2018) reported that 33% of *Rutilus rutilus* (common roach) ingested plastic which is similar to the overall plastic ingestion (28%) reported by McGoran et al. (2018). The presence of plastic in the digestive tract has been shown to fluctuate in fish (McGoran et al., 2017, 2018), suggesting a short retention time. In all studies in the Thames thus far, as in many similar investigations (Pinheiro et al., 2017), fibres are the most abundant form of plastic pollution, with an average of three fibres in the GIT (McGoran et al., 2018). Whilst brown shrimp in the Thames were reported to only ever ingest one fibre and that this was only 6% of sampled individuals (McGoran et al., 2018). This is considerably lower than estimates in the North Sea population (Devriese et al., 2015). Indeed, not enough is known about plastic concentrations in the Thames or the factors that affect plastic ingestion to determine why these studies differ so greatly. Watts et al. (2015) proposed that *Carcinus maenas* was vulnerable to microplastic ingestion due to its abundance, wide distribution and broad diet; factors that also apply to *E. sinensis* (Clark and Rainbow, 1997). In the present study *C. maenas* and *E. sinensis* are highly contaminated by plastic pollution, with most individuals containing items in at least one of the sampled organs. Several crabs contained tangles of fibres where some appeared to have accumulated over 100 fibres, as was seen in *E. sinensis*. This is considerably more than has been reported in fish (McGoran et al., 2018). The proportion of individuals containing microlitter was not statistically different between the two crab species which supports our first hypothesis.

The proportion of affected animals did not differ between the species; however, sampling season was significant. Due to unbalanced seasonal samples between species, it is difficult to determine seasonal and species level differences. Fewer crabs contained plastic in summer (June 2019), which is when most *C. maenas* were collected, however, only one *E. sinensis* was obtained in this sample. It is thought that this seasonal difference is more related to species than temporal changes as seasonal samples tended to have a biased split between species.

The microplastics recovered from the crabs could have originated from their environment and prey. Microplastics were recovered from the gills and some items could have been retained during irrigation of the gills with contaminated water. Watts et al. (2014) noted that microbeads could accumulate on the gills for up to 21 days. In the present study, fewer gills had plastic on them compared to in the GIT and GM (ca. 15% of gills and over 45%

of GITs/GMs). Additionally, mean plastic contamination on the gills was less than one microplastic per individual whilst the concentration in the GIT and GM ranged from 1.2 to 10.3 items. Thus, the main route of exposure to plastics is through ingestion rather than plastic suspended in the water column. This is also the case in Hong Kong mangroves where crabs repeatedly ingest more plastic than is retained on the gills (Not et al., 2020). Feeding mode further affects the rates of plastic ingestion, with detritivorous crabs ingesting the greatest amount of plastic. Omnivorous crabs were the next most contaminated, ingesting more plastic than predatory crabs, which ingested more than filter-feeding crabs (Not et al., 2020). *Carcinus maenas* and *E. sinensis* are omnivores and are therefore likely to ingest high quantities of plastic. *Eriocheir sinensis* is also known to ingest detritus which could explain its higher plastic load (Wójcik-Fudalewska et al., 2019).

Over half (69.1%) of *C. maenas* and 100% of *E. sinensis* sampled in the present study ingested plastic (plastic in the GIT and/or GM). Plastic prevalence was considerably higher in the present study compared to previous records of ingestion, where on average 13% of Polish and Portuguese *E. sinensis* ingested plastic (Wójcik-Fudalewska et al., 2016). Despite the difference in the proportion of individuals affected, in the Thames, Portuguese and Polish populations, all had fibres and tangles as the most common items. Indeed, almost all crabs that ingested plastic had ingested tangles (70%), ranging from 0.5 mm to 5 mm (Wójcik-Fudalewska et al., 2016). In the present study most *E. sinensis* also contained tangled fibres (98% of crabs to ingest plastic had ingested tangles), which were on average 5 mm across, but were in some instances larger (up to 14 mm). Similar accumulation was reported in laboratory studies (Watts et al., 2014, 2015).

The organs of *E. Sinensis* contained twice as much plastic on average as the organs of *C. maenas*. This contradicts hypothesis one, however, the number of individuals affected did not differ between species. It is likely that factors other than species differences also have a role in the accumulation and retention of microplastics. *Eriocheir sinensis* exhibits an annual migration in the River Thames during the reproductive period. In the autumn, adults migrate downstream to reproduce and juveniles upstream to occupy freshwater habitats (Morritt et al., 2013). Consequently, *E. sinensis* could be exposed to a variety of plastic concentrations. Migrating long distances, in comparison to the movement of *C. maenas*, might increase the number of opportunities to ingest plastic. For instance, *C. maenas* in the estuary will only be

exposed to local sewage outfalls whilst *E. sinensis* may pass several outfalls on its downstream migration. Maturing in the freshwater reaches of the River Thames, *E. sinensis* may be exposed to more plastic than crabs in the brackish estuary due to their close proximity to London and other urban centres. Similarly, Carreras-Colom et al. (2018) reported the highest rates of ingestion in the deep-water shrimp *Aristeus antennatus* nearest to major cities. Additionally, *E. sinensis* is a burrowing crab (Clark and Rainbow, 1997), a lifestyle which has been shown to increase exposure to plastic. Iribarne et al. (2000) reported that the burrowing activity of the crab *Chasmagnathus granulate* (now *Neohelice granulate*) resulted in the retention of more plastic on the sediment surface as well as in the sediment. Clark and Rainbow (1997) noted that many crabs contained mud in their stomach. In the present study, 4.9% of *E. sinensis* and 16.0% of *C. maenas* had ingested sediment.

Tangled fibres were treated as one item since precise counts of constituent items could not be established. Nearly all *E. sinensis* (95.1%) contained these tangles, many contained over 100 fibres. In comparison, only 10.6% of *C. maenas* contained such tangles. If items from tangles were treated as separate fibres, there would be a clear difference in plastic ingestion between the two species. Comparatively, only one in four of the 39.2% of *A. antennatus* which ingested plastic had tangles in the GM (Carreras-Colom et al., 2018). Microplastics ingested by crabs can be retained for several weeks (Watts et al., 2014, 2015). Many of the fibres recovered from these crabs were tangled into knots similar to those found in *N. norvegicus* (Murray and Cowie, 2011; Welden and Cowie, 2016a) and found in the present study. Watts et al. (2015) proposed that the action of the GM in *C. maenas* differed to that of *N. norvegicus* and that the species was therefore better equipped to egest fibres before they became tangled by the GM, by shredding microfibres into smaller fractions. It is not known if the mechanical function of the GM differs between *E. sinensis* and *C. maenas*. The anatomy of the digestive system in *C. maenas* and *E. sinensis* is similar, which makes it difficult to determine why one species is more affected than the other. Carreras-Colom et al. (2018) suggested that tangles of fibres in the environment were a similar size to the copepod prey of *A. antennatus* and that tangles were misidentified and ingested. Although this seems highly unlikely as the presence of tangled fibres is isolated to Crustacea, differences in prey could also impact plastic ingestion between *C. maenas* and *E. sinensis*.

The proportion of fish populations ingesting plastic can fluctuate between studies (McGoran et al., 2017, 2018) and it is possible that fibres do not have a long retention time in these species. The tangled fibres recovered from the GM of crabs, however, could potentially block the opening to the GIT and prevent microplastics from being egested, increasing their retention time. It was proposed that Thames fish could egest microplastics without complication (McGoran et al., 2018) and *C. maenas* faecal pellets can contain fibres (Watts et al., 2015), demonstrating that these do not remain in the digestive tract indefinitely. In the present study, however, some shore crabs were noted to contain plastic even when there was no evidence of recent feeding, indicating that plastic has a longer retention time than food. Similarly, *N. norvegicus* females, which undergo periods of starvation whilst ovigerous, still retained plastic in the stomach (Murray and Cowie, 2011). It has been demonstrated that fibres are retained in the GIT of *C. maenas* longer than microbeads (Watts et al., 2014, 2015).

In the present study, more females more frequently contained plastic than males. Yet, Wójcik-Fudalewska et al., (2016) reported no sex difference in ingestion by most Portuguese and Polish *E. sinensis* populations, except in the Gulf of Gdansk where more males ingested plastic. Murray and Cowie (2011) found no difference between male and female *N. norvegicus* sampled from the Firth of Clyde, whilst Welden and Cowie (2016a) reported that males ingested less plastic. Both *Nephrops* studies concluded that larger individuals had larger GMs and were therefore better able to excrete plastics, requiring larger items to block the GIT than smaller individuals (Murray and Cowie, 2011; Welden and Cowie, 2016a). Similarly, Wójcik-Fudalewska et al., (2016) concluded that females mitten crabs, which are typically smaller than males, ingested less plastic due to their size. They did not propose that stomach size was important, but that larger animals consumed more food. Females may require more energy when breeding to support egg production; during the present study 92% of female *E. sinensis* were carrying eggs, while no ovigerous *C. maenas* were collected. Female *E. sinensis* overwinter in deep water before spawning their eggs (Clark et al., 1998). To survive this period, females may increase their feeding rate and thus their exposure to plastics.

Panning (1939) noted that smaller individuals undergo ecdysis more frequently and this could be a mechanism for fibre release. Indeed, Welden and Cowie (2016a) noted that female *N. norvegicus* grow slower than males, therefore moulting less. It is possible that in *C. maenas* and *E. sinensis* in the Thames females also grow at a different rate or that their

smaller digestive system requires smaller plastic items to be blocked. In the present study, moulting certainly affected the number of items recovered from the GM. *Carcinus maenas* and *E. sinensis* lose all or part of their GM during ecdysis (Panning, 1939; Sheridan et al., 2016; Sheridan and O'Connor, 2018) and there is the potential for fibres to be trapped in the exuvia and not retained post-moult (Welden and Cowie, 2016a).

In laboratory studies, the swimming crab *Necora puber* (Crooks et al., 2019) and *C. maenas* (Farrell and Nelson, 2013) were both been found to accumulate microplastics through trophic transfer. Like the knots recovered in the present study, microbeads were reported to accumulate in the stomach (Watts et al., 2014; Crooks et al., 2019), if only briefly (up to 4 hours, Farrell and Nelson, 2012). Since plastic can be retained by *C. maenas* and *E. sinensis* for long periods in a variety of tissues (Farrell and Nelson, 2013; Watts et al., 2014; Yu et al., 2018), there is the potential for microplastics to be transferred up trophic levels with predation. McGoran et al. (2018) suggested biomagnification as one of the potential reasons plastic ingestion was greater in fish than in shrimp from the Thames. In laboratory conditions, it has been demonstrated that *C. maenas* can accumulate plastic from contaminated prey (Farrell and Nelson, 2013). This, however, may not be environmentally realistic. Farrell and Nelson (2013) provided 24 mussels (*Mytilus edulis*) with ca. 411 million microbeads in 9.6 L water. Of which nearly 58 million particles were retained after a 1-hour exposure. *Carcinus maenas* were then fed a single mussel and left for 21 days. Only an estimated 16.2 million microbeads (0.28%) from the mussels were retained by crabs. Lorenz et al. (2019) recovered an average 234.5 ± 254.3 particles per kg of sediment in the North Sea which was significantly greater than the concentration in the surface water (27.2 ± 52.5 particles per m^3). This is far less than the concentration *M. edulis* was exposed to by Farrell and Nelson (2013). Indeed, Li et al. (2018) also reported that *M. edulis* sampled from coastal waters around the United Kingdom ingested less plastic than Farrell and Nelson (2013) used in their laboratory experiments. Between 1.1 and 6.4 items of debris per individual were recovered and seawater sampled at each site ranged from 1.5 to 6.7 items per litre, an average of 3.5 items per litre. Microplastic abundance in the environment is not only lower that that tested but retention by mussels is also lower. If 0.28% of the plastic recovered by Li et al. (2018) were ingested by a crab, between 0.3 and 1.8 items would be retained. This is considerably lower than what was found in the present study. There are few investigations into fibre ingestion and retention

(De Sá et al., 2018) and as such it is not yet possible to determine whether the fibres present in the stomach of *C. maenas* and *E. sinensis* was through ingestion of contaminated prey. But certainly, if these crabs were predated upon, for instance by otters (Weber, 2008), a large number of plastic items, and potentially a high dose of associated chemical pollutants, could be transferred.

It has been suggested that most plastic ingested by decapods is derived from fishing gear (Murray and Cowie, 2011; Wójcik-Fudalewska et al., 2016); an unlikely source in the Thames as there are no commercial fisheries in the immediate vicinity of Erith. There is, however, a large amount of industry, shipping and recreational boating on the river, with potential contamination from ropes and other equipment from these sectors. Additionally, washing machine outputs and WWTPs are well-documented sources of plastic waste (Browne et al., 2011; Dubaish and Liebezeit, 2013; Free et al., 2014; Ziajahromi et al., 2017b) and it was noted as a potential route of exposure for fish in the Thames Estuary (McGoran et al., 2018). PES was the second most abundant polymer in the present study, which reflects global yarn production trends. Globally, PES accounts for 68% of fibre demand and half of the PES yarn produced is used in the clothing industry (Klein et al., 2020). Thus, shedding clothes is likely the main route of plastics entering the Thames. Sewage-related debris is also common in the Thames, especially wet wipes and sanitary products (Morritt et al., 2014; Thames21, 2018; McCoy et al., 2020), both of which can break up into fibres and fragments of plastic. Due to the high load of plastic in the GM of *E. sinensis*, especially in tangles, it was possible to identify specific items that were ingested. A black film, possibly from a plastic bag was recovered from one individual. The same individual had also ingested fragments of a yellow balloon, black rubber and a white film identified as a fragment from a sanitary pad. Including this individual, 7.3% of *E. sinensis* were thought to have ingested fragments of sanitary pads. Large items of plastic collected in the nets during sampling also included sanitary products, rope, food packaging and carrier bags. Evidence for ingestion of balloons has also been reported in seabirds and is linked to high risk of mortality. An animal which has ingested a balloon is 32 times more likely die than an individual that ingested hard plastic (Roman et al., 2019). Given the abundance of balloons in European freshwater systems (13th most abundant item; Winton et al., 2020) it is necessary to consider policy changes to balloon releases. Elastic bands were also identified in the GM of *E. sinensis*. Similar items have been reported in the GIT of seabirds

(Parslow and Jefferies, 1972; National Trust, 2019) and birds of prey (Carlin et al., 2020), sometimes remaining tangled in the crop. One explanation for their ingestion is mistaken identity as prey (e.g., worms).

In the present study plastic tangles filled the GM. This is thought to have severe negative effects on the health and fitness of the animal. *Nephrops norvegicus* showed significant reductions in mass following microplastic ingestion (Welden and Cowie, 2016b). Following ingestion of 0.3%, 0.6% and 1% of plastic in feed (homogenised mussels), *C. maenas* also demonstrated reduced feeding and a decline in their growth potential (Watts et al., 2015). Wójcik-Fudalewska et al. (2016) suggested that *E. sinensis* were likely to be affected by false satiation which would result in malnutrition and may lead to starvation. Indeed, Yu et al. (2018) reported that mass gain and growth rate decreased with increased plastic ingestion in *E. sinensis*. In addition, crabs demonstrated increased oxidative stress which was correlated with plastic load. Microplastic ingestion reduced enzyme activity and expression in *E. sinensis* and affected the gut microbiome (Liu et al., 2019). Yu et al. (2018), however, used microbeads to demonstrate the negative impacts of plastic ingestion, whilst fibres are most commonly ingested microplastics in environmental conditions. A review by Wright et al. (2013) noted that fibres were the most harmful form of microplastics, and evidence shows that fibre could have greater negative effects when ingested compared to microbeads (Ziajahromi et al., 2017a). For example, the water flea *Ceriodaphnia dubia* exposed to microfibres suffered greater detrimental effects compared to microbead exposure. Additionally, carapace deformities only developed in the presence of microfibres (Ziajahromi et al., 2017a). A review by Foley et al. (2018), however, found more evidence of negative effects in studies which used spherical microplastics compared to fibres and, therefore, more research is required into the effects of ingestion at environmentally realistic levels.

5.5 Conclusion

The present study provides the first evidence for microplastic ingestion by brachyuran crab species in the Thames. *Eriocheir sinensis* and *Carcinus maenas* are heavily affected by plastic pollution in the River Thames, typically fibres from wastewater and industry. It is possible that this plastic load could be transferred via predation to other animals in the Thames. For example, fish could ingest juvenile crabs and top predators, such as seals, might

predate on both. Many questions remain about the differences in plastic exposure between the species, the effect of ecdysis on fibre release and the impacts of plastic ingestion in these species. What is clear is that fish alone are not an adequate proxy for plastic pollution in an environment and a selection of species must be studied to attain a true representation.

5.6 Addendum

Subsequent to the publication of the present chapter the following information, from an unpublished undergraduate thesis, was brought to my attention. Plastic ingestion is not a new threat to wildlife, with evidence of plastics in the digestive tracts of *Eriocheir sinensis* collected in 1995 and 1996. Between 16% and 53% of examined crabs contained nylon fibres, 5% to 30% containing plastic and up to 9% containing rubber (Robbins, 1996). A high proportion of *E. sinensis* had ingested mud and it is possible that plastic accumulated in the sediment could have been ingested accidentally. Other prey items included eggs or seeds, fish, shells and algae.

Additionally, a presentation at microplastic conference Micro2020: Fate and impact of microplastics: knowledge and responsibilities (Carreras-Colom et al., 2020) highlighted experimental evidence that the morphology of GM of *C. maenas* does not promote tangle formation. The study also demonstrated that fibres regardless of length (0.5 mm, 2mm or 4 mm) were mostly released in faecal pellets within 24 hours. Small fibres were found densely packed together in faecal pellets. Photographs in the presentation appeared to show possible fragmentation of large fibres, although this was not discussed by Carreras-Colom et al. (2020). In the present chapter an individual *C. maenas* caught in June 2019 (140619CM18) was found with ten blue fragments in the GM and a further seven in the GIT (Figure 5.7). The blue fragments in the GIT, however, were found close together. Consequently, given the evidence presented by Carreras-Colom et al. (2020), it is now proposed that a larger fragment of plastic was possibly broken up in the GM and the resulting smaller particles aggregated together and would be egested in a faecal pellet.

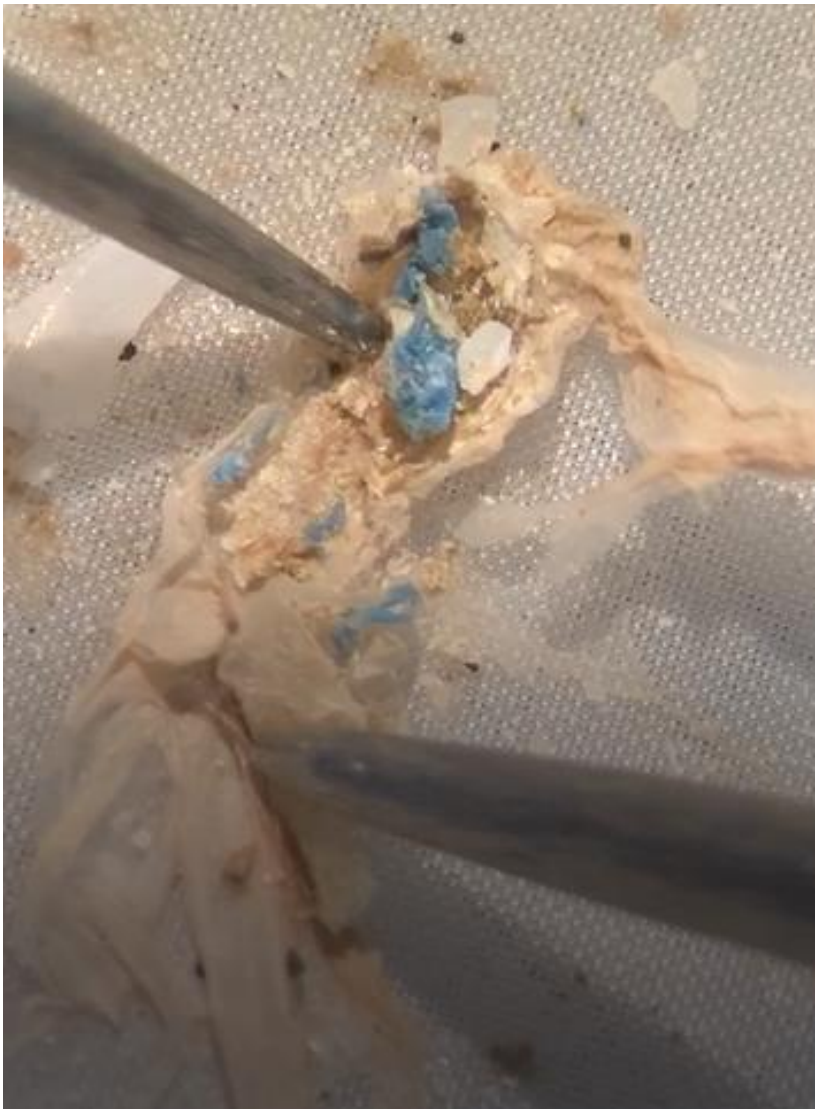


Figure 5.7. Blue fragments in the GIT of *Carcinus maenas* recovered from the Thames Estuary in June 2019. GIT is photographed on a 32 µm mesh nylon filter.

Chapter 6

Plastic in the Thames Estuary food web

6.1 Introduction

As demonstrated in the previous chapters, microlitter is abundant in the environment and is readily ingested by biota. Yet, entire food webs are understudied with a focus on a few select species. Trophic transfer of microplastics is poorly understood, with limited environmental studies to evidence plastic moving through the food chain (Lwanga et al., 2017; D'Souza et al., 2020). Despite this, trophic transfer has long been proposed as a route of exposure (Eriksson and Burton, 2003; Boerger et al., 2010; Ramos et al., 2012; Bravo Rebolledo et al., 2013; Wright et al., 2013; Desforges et al., 2015). Plastic ingestion has been reported in hundreds of species across several trophic levels (Wright et al., 2013; Gall and Thompson, 2015; Lusher et al., 2017). Consumed plastic may be retained in the digestive tract for a variable amount of time, up to weeks (Foley et al., 2018) and may therefore be passed to predators if the host is consumed. Ingestion of microplastics can also occur if plastic adheres to prey items (e.g., fish eggs and seaweed; Au et al., 2017; Foley et al., 2018). Certainly, in rivers, low trophic level invertebrates (Windsor et al., 2019b) and fish (Horton et al., 2018; McGoran et al., 2017, 2018) ingest microplastics. Consequently, it has been suggested that plastics or associated chemicals could bioaccumulate or biomagnify (Wright et al., 2013; Foley et al., 2018).

Several studies have reported that predators ingest significantly more plastic than other feeding strategists (Courtene-Jones et al., 2017; Fang et al., 2018) with Fang et al. (2018) reporting that trophic transfer was the main factor influencing exposure. Bour et al. (2018), however, found trophic level to be insignificant, with prey species impacting plastic exposure of predators. Similarly, for the deep-water shrimp, *Aristeus antennatus*, microplastic ingestion was affected by the ecological niche of its prey species (Carreras-Colom et al., 2018). The organisms which contributed the most to plastic exposure were polychaetes, isopods, a decapod crustacean and gammarid amphipods (Carreras-Colom et al., 2018). Additionally, Carlin et al. (2020) reported that piscivorous birds of prey, such as osprey (*Pandion haliaetus*), ingested less microplastic than raptors, such as the red-shouldered hawk (*Buteo linneatus*), which fed on small mammals. Alternative feeding strategies also result in microplastic

ingestion. For example, deposit feeders can ingest a diversity of plastic forms (Courtene-Jones et al., 2017). Fang et al. (2018) noted that omnivores were also exposed to higher quantities of plastic, but in contrast filter feeders were more selective with regards to particle size or were better able to egest particles and as such may be less likely to ingest microplastics (Bour et al., 2018). Feeding strategy does not always predict microplastic exposure, with some filter feeders able to eject larger microplastics in pseudofaeces (Zhao et al., 2018) and insect larvae in riverine sediments ingesting plastic across all sampled taxa regardless of feeding strategy (Windsor et al., 2019b).

In laboratory studies, microspheres can translocate to the haemolymph of bivalve suspension feeders and accumulate for up to 48 days (Browne et al., 2008). The longer microplastics remain within an organism the greater the likelihood that those particles will be passed to predators through predation of the host. *Carcinus maenas* fed contaminated *Mytilus edulis* also consumed the plastic that the bivalves had ingested. Plastics were recovered from crab haemolymph up to 21 days after initial exposure (Farrell and Nelson, 2013). Additionally, Murray and Cowie (2011) demonstrated that plastics from prey can accumulate in the gut of langoustine. Similarly, mysid shrimp ingest plastic through consumption of contaminated zooplankton (Setälä et al., 2014) and fish have also been found to accumulate plastic through trophic transfer in the lab (Cedervall et al., 2012).

Whilst laboratory studies into the transfer of plastics through the food chain are relatively common, data from the field are rare. In many cases, movement of plastic from prey to predator is assumed to occur. It has been proposed that the presence of plastic in fish could have been due to ingestion of sediment or contaminated prey, such as plankton (Boerger et al., 2010; Ramos et al., 2012). Indeed, Ferreira et al. (2019) found a link between prey species and microplastic contamination. Contamination controls were, however, minimal and no chemical analysis of the particles was conducted. Desforges et al. (2015) concluded that salmon would be exposed to over 90 pieces of plastic a day through predation on plankton after reporting that zooplankton in the Northeast Pacific Ocean had ingested plastic. In turn, fishes could then be passing plastic to top predators (Bravo Rebolledo et al., 2013; Wright et al., 2013). Plastic pieces have been recovered from the scats of fur seals (*Arctocephalus* sp.) and were suspected to have originated from their prey, such as lanternfish

(*Electrona subaspera*) (Eriksson and Burton, 2003), which have previously been found to ingest plastic (Boerger et al., 2010; Davidson and Asch, 2011).

Quantifying plastic transfer through the food web *in natura* is difficult. Gut evacuation times vary (Lusher et al., 2017) and as such microplastic retention time may differ between species. Therefore, plastic ingestion and its movement between organisms is a complex system. This pathway requires further clarification and the need for more research into the potential trophic transfer of plastics has been highlighted (Wright et al., 2013).

To overcome the limitations of environmental and laboratory studies, Nelms et al. (2018) fed wild fish to captive seals in a plastic-free enclosure and compared plastic abundance in the fish with the seal scats. Both the scats (48%) and the fish (32%) contained plastic. Nelms et al. (2018) concluded that plastic moved from the fish to the seals. It was assumed that the fish mistook the plastic for prey rather than ingesting contaminated plankton as the fragments were too large to be consumed by plankton. Using previous estimates of plastic load in prey invertebrates, D'Souva et al. (2020) examined trophic transfer using environmental data. The authors proposed that the concentration of plastic excreted by Eurasian dippers (*Cinclus cinclus*) was equivalent to that obtained through contaminated prey and, as such, trophic transfer was the main route of exposure. The study also highlighted transfer of plastic from parent to nestling through provisioning as well as accumulation up trophic levels.

Research into microplastic ingestion in the Thames has thus far focussed on fishes (McGoran et al., 2017; Horton et al., 2018). Estuaries are often dominated by a few numerous species and research into microplastics has focussed on such species (Selleslagh and Amara, 2008). Goby (*Pomatoschistus* sp.), European flounder (*Platichthys flesus*), sea bass (*Dicentrarchus labrax*) and plaice (*Pleuronectes platessa*) are highly abundant in European estuaries (Selleslagh and Amara, 2008; Selleslagh et al., 2009). Indeed, in the Thames, flounder, common goby, sand goby and sea bass are abundant (Clark et al., 2017). Fishes are an important part of the Thames food chain with numerous species found in the estuary dominating the diet of local marine mammals (ABP Marine Environmental Research Ltd, 2013). Only one microplastic study in the Thames to date has included a prey species in addition to fish (McGoran et al., 2018), with another study solely examining microplastic

retention in a bivalve (McCoy et al., 2020) and a single harbour porpoise from the mouth of the estuary included in Nelms et al. (2019). Analysing the diet of fishes and their predators exposes potential sources of plastic as well as documenting ingestion in neglected groups.

Estuarine fish are commonly used to monitor water quality and human impacts on the aquatic environment (Selleslagh and Amara, 2008; Selleslagh et al., 2009). Indeed, fish are used as an indicator of anthropogenic influences on transitional waters as part of the European Water Framework Directive (WDF – Directive 2000/60/EC; Amara et al., 2009). *Platichthys flesus* are a major component of demersal biota in European estuaries, widespread and abundant, and are commonly species used for environmental monitoring (Kirby et al., 2004; Amara et al., 2009; Marchand et al., 2010). European flounder have been used to monitor microplastic pollution (McGoran et al., 2017, 2018; Kazour et al., 2018). Basing food webs analysis around species used in water quality monitoring as well as microplastic investigations allows for assessment of multiple stressors in an environment. Therefore, it is important to determine the key prey species of *P. flesus* that could act as a route of exposure to microplastics.

Fish in turn can pass microplastics to their predators. *Phocoena phocoena* and *H. grypus* have been reported in the Thames Estuary (ABP Marine Environmental Research Ltd., 2013; Castello y Tickell and Barker, 2015). Nearly half (45%) of the world's population of *H. grypus* are native to the UK (Thompson and Duck, 2010; SCOS, 2016). Most of the UK's seal populations are in Scotland and therefore have been the focus of many surveys (Thompson and Duck, 2010; Castello y Tickell and Barker, 2015). Over the past decade, UK populations of harbour seals (*Phoca vitulina*) have been in decline. Nevertheless, populations on the East coast of England, such as the Thames, have remained stable or fluctuating (SCOS, 2016; Olsen et al., 2017). *Halichoerus grypus* populations, however, are on the rise in the UK (Thompson and Duck, 2010; SCOS, 2016). Indeed, a recent Zoological Society of London (2021) report noted that marine mammal populations in the tidal Thames were increasing. Despite this, *Phocoena phocoena* is considered a threatened species (ABP Marine Environmental Research Ltd., 2013). Globally, populations have been stable since 1994 (OSPAR Commission, 2016) but the species is still threatened due to by-catch (OSPAR Commission, 2008). All three of the above marine mammals are large and long lived, with *H. grypus* growing up to 300 kg, *Phoca vitulina* reaching 100 kg and *Phocoena phocoena* weighing between 45 kg and 70 kg, with the

pinnipeds living for 20–30 years. Furthermore, in the Thames, there were over 2,000 seal sightings between 2004 and 2014 (Castello y Tickell and Barker, 2015), with 938 seals counted in the estuary during a comprehensive survey (Zoological Society of London, 2014). The ten-year report by Castello y Tickell and Barker (2015) noted that seals have been reported as far upstream as Hampton Court Palace and have been observed in large groups at Southend-on-Sea, Greenwich and Crossness. *Phocoena phocoena* was less commonly sighted in the estuary with only 398 sightings in the same period but this species has also been reported in the inner Thames Estuary (Castello y Tickell and Barker, 2015).

Due to a competition for fish stock between fishermen and seals, the diet of seals has been monitored for many decades (Thompson and Duck, 2010; Brown et al., 2012; Méheust et al., 2015; Hammond and Wilson, 2016; Vincent et al., 2016). By understanding the diet of seals and other marine mammals, it is possible to identify which species might be exposing top predators in the Thames to microplastic contamination. In the UK, seals feed mainly on small, demersal fish, such as flatfish, sand eels, cod, whiting and ling (Thompson and Duck, 2010; Brown et al., 2012). On average, an adult *H. grypus* will consume 7 kg of cod, or 4 kg of sand eels, each day (Thompson and Duck, 2010; SCOS, 2016) and *Phoca vitulina* being smaller only require 3–5 kg of food a day (SCOS, 2016). Similarly, *Phocoena phocoena* needs to consume ca. 4 kg of food per day to meet its energy requirements (Ross et al., 2016). Due to their fast metabolism, *P. phocoena* need to feed multiple times a day. It is estimated that food passes through the digestive system in as little as 3.5 hours (Ross et al., 2016), whilst *Phoca vitulina* take between 2 hours 35 minutes and 6 hours 15 minutes to pass food through the digestive system (Markussen, 1993). Hard remains, such as otoliths, can remain in the digestive tract of *H. grypus* for up to 88 hours (Hammond and Grellier, 2006), but most prey is likely to be in the digestive system for fewer than 24 hours (Bowen and Harrison, 1994).

In addition to trophic level, seasonal variation in both fish abundance and microlitter concentrations, as discussed in Chapter 4, may affect rate of ingestion and it is vital to understand the impact of these factors. Indeed, a review of research into the trophic transfer of microplastics concluded that studies of temporal variation in microplastic ingestion and emissions were a necessary future step (Gouin, 2020). This knowledge gap is addressed in the present thesis in addition to quantifying microlitter accumulation in the Thames Estuary food web from wild-caught fauna and stranded marine mammals. The hypotheses tested were 1)

fibres will be the most abundant form of microlitter, 2) ingestion will vary seasonally, 3) high trophic level organisms and predators will ingest more microlitter than lower trophic levels and prey species, 4) larger pieces of litter will be ingested by bigger species, and 5) for marine mammals, litter will be more abundant in the stomach compared to the intestines as reported by Bravo Rebolledo et al. (2013) and Nelms et al. (2018).

6.2 Materials and methods

Full methods can be found in Chapter 2. The taxa included in this chapter are amphipods, polychaetes, shrimp, fishes, pinnipeds and cetaceans.

6.2.1 Statistical analysis

Statistical analysis was conducted in R version 4.0.3 (R Core Team, 2020) with the following packages: "ggplot2" (Wickham, 2016), "MASS" (Venables and Ripley, 2002) and "multcomp" (Hothorn et al., 2008).

For analysis of temporal variation in microlitter ingestion, species were only included in analysis if they were recovered on most of the eight sampling dates. If fewer than ten individuals of a species were recovered on a sampling trip, it was deemed insufficient for seasonal comparisons and individuals collected on that date were removed from analysis. GLMs were used to compare the proportion of individuals containing microlitter and microlitter frequency (zeros were removed to account for skewed data) in the GIT by sampling date, species, length and mass.

GLMs were used to compare the proportion and amount of plastic contamination (zeros were removed to account for skewed data) between trophic levels. A linear model (LM) compared amount of plastic contamination (zeros were removed to account for skewed data) when size variation was controlled for (i.e., microplastic per mm standard length). Size was controlled for by length rather than mass as it was not possible to weigh all amphipods, polychaetes and marine mammals. The best fit model was determined by comparison of AIC scores. Non-significant variables were systematically removed until only significant factors remained. Significant variables were then analysed with a pair-wise Tukey post-hoc test.

Model 6.1 – GLM(Ingestion ~ Date + Species, family = binomial)

Model 6.2 – GLM(Ingested Microplastics ~ Date + Species + Length + Mass, family = gamma (link = inverse))

Model 6.3 – GLM(Proportion with litter ~ Trophic level, family = binomial)

Model 6.4 – GLM(Amount of microplastics ~ Trophic level, family = gamma (link = identity))

Model 6.5 – LM(Amount of microplastics per mm ~ Trophic level)

6.3 Results

6.3.1 Material collected

Sample size for biota, namely amphipods, polychaetes, shrimp, fishes, pinnipeds and cetaceans, collected is reported in Table 6.1. Further information for the stranded baleen whales is available in the Supplementary Material (S15).

6.3.2. Contamination

Controls contained clear fibres, clear films, blue fibres, blue films, blue fragments, black fibres, black films, black fragments, brown fibres, red fibres, red films, white fibres, white films, grey fibres, green fibres, green films, green fragments, yellow fibres, pink fibres, purple fibres, purple fragments and tangled fibres. For one individual of *H. grypus* all green fragments were removed from analysis as they were suspected to be contamination from a lab sponge. On average 1.6 microplastics per sample were suspected contamination. The LOD and LOQ for *Corophium volutator* were 1.6 and 1.9, with 36% above these values before correction. For *H. diversicolor,* LOD and LOQ were 1.6 and 1.9, with 17% above these values. For *Crangon,* LOD and LOQ were 2.1 and 3.3, with 3% and 2% above these values. For fishes, LOD and LOQ were 2.0 and 3.1, with 13% and 8% of GITs and 11% and 6% of gills above these values. For marine mammals, the LOD and LOQ were 93.3 and 246.3, with 40% and 20% above these values.

Results below were corrected by recorded contamination and not LOD/LOQ.

6.3.3 Microlitter in biota

Microlitter abundance is summarised in Table 6.1.

Table 6.1. Microlitter abundance (GIT and gills, where applicable) for River Thames fauna sampled between December 2018 and September 2020. More details are available in the Supplementary Material (S15).

Species	**Sample size**	**% with litter**	**Mean abundance**	**Standard deviation**
Invertebrates				
Corophium volutator	384	53%	0.90	1.14
Crangon crangon	367	27%	0.39	0.95
Hediste diversicolor	76	26%	0.43	0.85
Fishes				
Agonus cataphractus	6	100%	3.33	1.86
Chelidonichthys lucerna	13	69%	1.69	1.89
Dicentrarchus labrax	16	44%	0.94	1.29
Merlangius merlangus	30	67%	1.47	1.46
Osmerus eperlanus	202	45%	0.96	1.43
Platichthys flesus	258	65%	2.39	3.43
Pomatoschistus minutus	1	100%	2.00	NA
Solea solea	187	44%	1.35	2.78
Sprattus sprattus	1	0%	0.00	NA
Trisopterus luscus	102	52%	1.26	1.73
Marine mammals				
Halichoerus grypus	4	100%	214.75	70.73
Phocoena phocoena	1	100%	102	NA
Megaptera novaeangliae	1	100%	74	NA
Balaenoptera borealis	1	100%	90	NA

Microlitter ingestion by invertebrates

Over half (53%) of the *Corophium volutator* collected contained microlitter. Collectively, 349 pieces of litter were present which averaged 0.91 ± 1.14 (mean ± SD) items per amphipod. Blue fibres were most abundant making up 43% of microlitter recovered. The average variety in litter forms was 0.77 ± 0.89. A quarter (26%) of the 76 *H. diversicolor* contained microlitter with 33 pieces of litter recovered. On average, 0.43 ± 0.85 items were present per polychaete with an average diversity of forms of 0.34 ± 0.62. The most common form of litter was purple fibres (48%). In total, 367 *Crangon crangon* were sampled, of which 26% contained microlitter. Overall, 142 items were recovered, averaging 0.39 ± 0.95 per shrimp. As with *Corophium*, the most abundant form was blue fibres (42% of recovered plastic). On average 0.33 ± 0.62 forms of litter were present per individual.

Microlitter ingestion by fishes

In total 816 fish were dissected and 54% were found to contain litter, with a combined 1,295 items recovered. Of this, 725 were recovered from the GIT and 570 from the gills. When all fish are considered together, on average, 1.59 ± 2.62 pieces of microlitter were present per individual. Clear fibres were most commonly ingested and blue fibres were the most common item on the gills. Fish ingested an average of 1.63 ± 1.79 forms of litter.

Of the prey items recovered from the GIT of fishes during the present study, ten individuals of *Crangon* and five amphipods were intact enough to examine for microplastics. Any plastic ingested by these individuals was included in the count of plastic ingested by the fish they were recovered from and did not go towards the average ingestion by shrimp or amphipods. Of these individuals two shrimp and one amphipod ingested plastics (Figure 6.1).

Microlitter ingestion by marine mammals

Halichoerus grypus and *Phocoena phocoena* ingested an average of 214.75 ± 70.73 microlitter and 102 ± 0 microlitter, respectively (Table 6.1). Clear fibres made up 66% and 71% of litter in *H. grypus* and *P. phocoena*, respectively. The abundance of clear fibres in *H. grypus* was driven by one individual (SS2021_147) which had ingested over 600 debris items. Excluding this individual resulted in blue fibres becoming the most abundant form of litter (24% of ingested items). The combined average diversity of plastic forms ingested by *H.*

grypus and *P. phocoena* was 9.80 ± 2.59 per individuals. Of the four seals and one porpoise investigated, only one individual had more microlitter in the stomach than the intestines (25 items compared to three items).

No samples from the GIT could be collected for the humpback whale or sei whale as there was no evidence of recent feeding. A total of 96 and 92 suspected microplastic items were recovered from the baleen plates of *M. novaeangliae* and *B. borealis*, respectively. FTIR revealed that all black fragments and films were organic and, as such they were removed from analysis. This left 74 microlitter items from *M. novaeangliae* and 90 pieces from *B. borealis*. Average microlitter concentrations per baleen plate are recorded in Table 6.2. Most recovered microplastics were black fibres (Figure 6.2)

Fibres, particularly clear and blue fibres, were the most abundant form of microlitter ingested.

Table 6.2. Plastic contamination on baleen from two Mysticeti species stranded in the River Thames, UK. Averages are reported with standard deviation. Exposure per animal is estimated from average microplastic abundance per plate and published estimates of the number of baleen plates in Mysticeti. The lower estimate is derived from the average minus the standard deviation multiplied by the lowest estimates of baleen number, whilst the upper estimate is the mean plus standard deviation multiplied by the highest recorded baleen count.

Species	**Average per plate**	**Exposure per animal**
M. novaeangliae	12.33 ± 6.38	1,607–7,484
B. borealis	9 ± 6.57	532–6,384

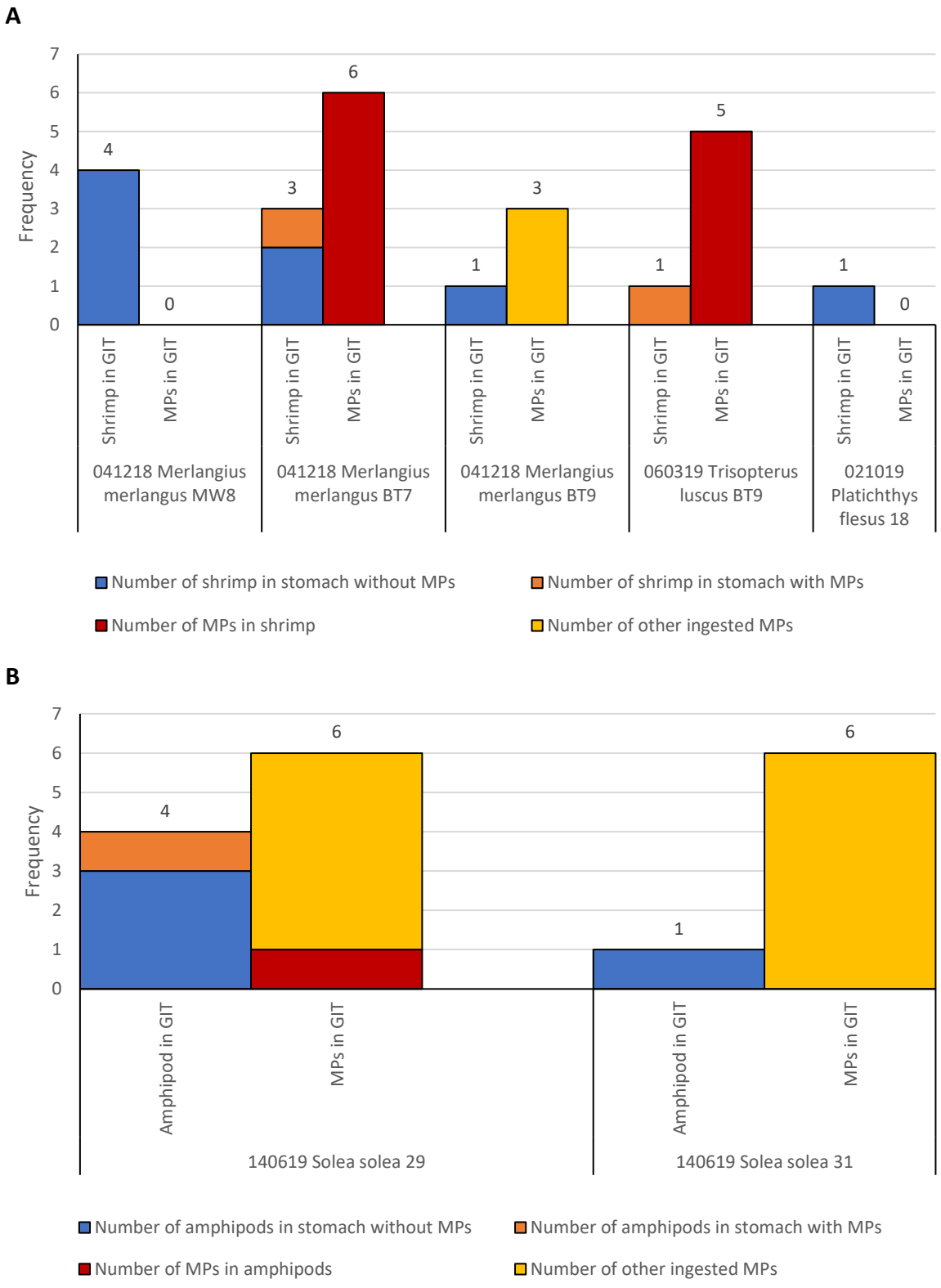
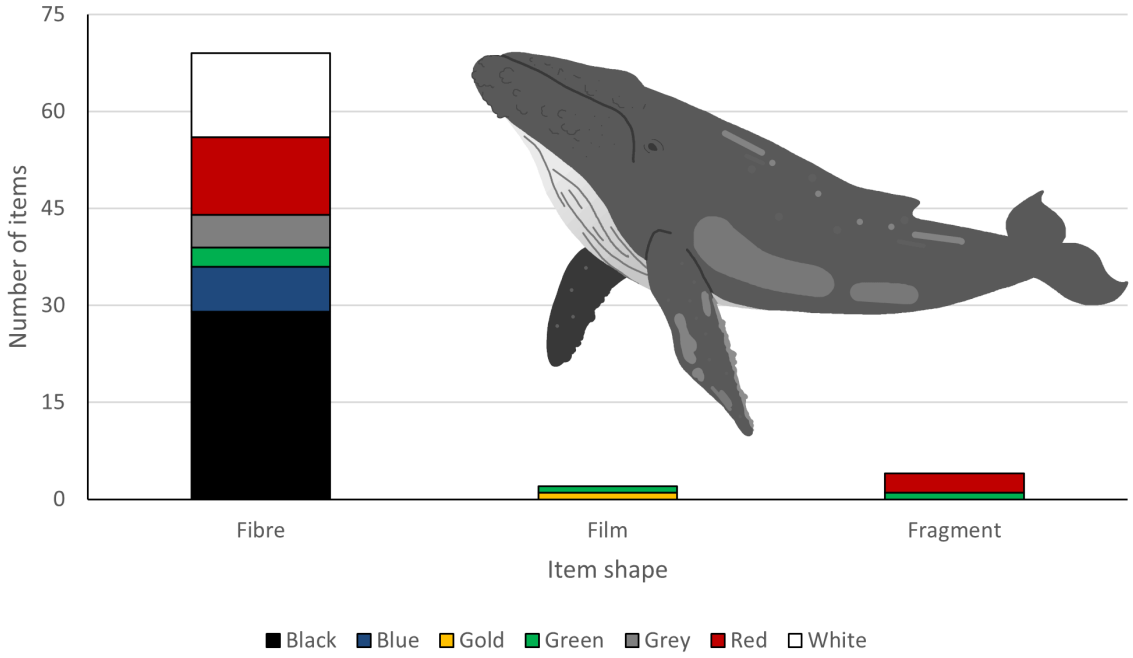


Figure 6.1. Plastic ingestion by prey recovered from the GIT of fishes from the River Thames. A) The number of shrimps, recovered from fishes, to ingest microplastics (MPs) and the amount of MPs from shrimp in the diet of fishes; B) The number of amphipods, recovered from fishes, to ingest MPs and the amount of MPs from amphipods in the diet of fishes.

A



B

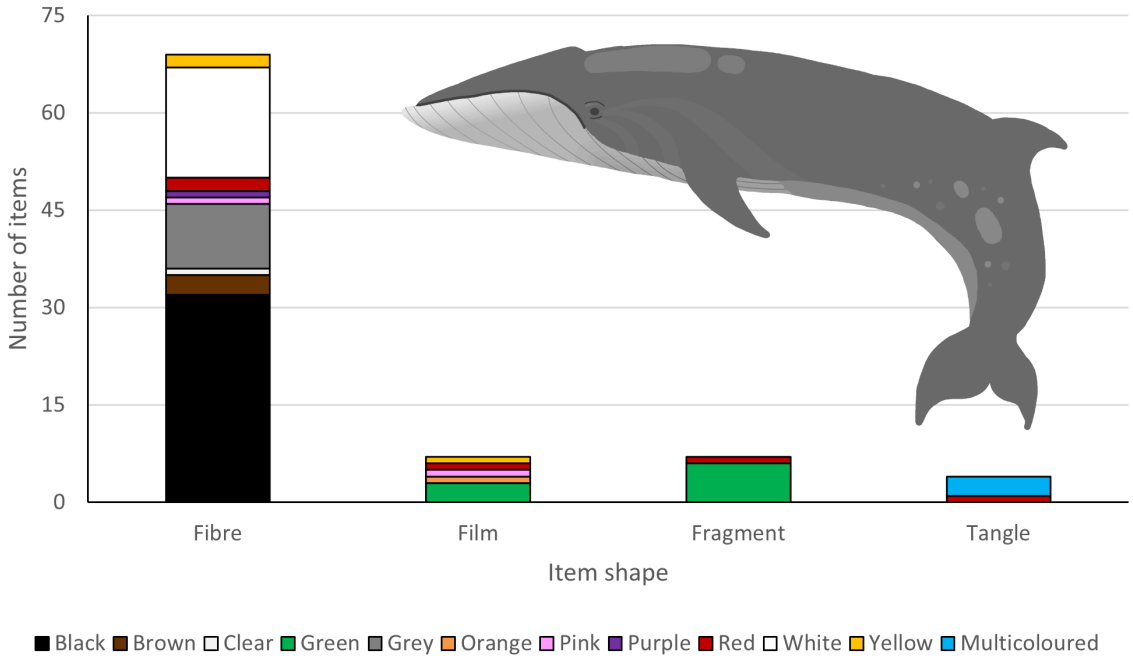


Figure 6.2. Microplastic form (shape and colour) on baleen recovered from A) *Megaptera novaeangliae* and B) *Balaenoptera borealis.*

FTIR analysis of microlitter in biota

A total of 61 items were analysed by FTIR for *Corophium volutator*, 100% matched library spectra with all matches over 70%. Only 5% of items were identified as plastic and the remaining 95% were cellulosic. The most common plastic was PE. All analysed items recovered from *H. diversicolor* (39 items) matched library spectra, 97% producing a match of at least 70%. No items were identified as plastic and 97% were cellulosic. FTIR analysis was not conducted on items from *Crangon crangon*.

Only seven items were analysed by FTIR for *M. merlangus* all of which produced matches with library spectra. Only 71% produced matches greater than 70%. Over a quarter of items were plastic (29%) and the remaining 71% were cellulosic. A total of 82 items were analysed from *O. eperlanus*, with 99% matching library spectra. Most produced a match greater than 70% (94%). Most commonly items were identified as cellulosic fibres (77%) with only 20% matching plastics. Of the items that matched plastic library spectra, most were PES and PP. A total of 131 items were analysed from *P. flesus* with 95% of items matching library spectra and 96% producing a match greater than 70%. Of the identifiable items, 63% were plastic and 37% were cellulosic. The most common polymers were PES and PP. Sixty-eight items were analysed for *S. solea*, all of which matched library spectra and 93% producing a match greater than 70%. Over half of these items were cellulosic (54%) and 43% were plastic, the most common of which were PES (13% of all items), PP (10%) and acrylic (9%). Several fish species were not included in the food web due to low sample sizes or because they were not identified as key prey species. Seventeen items were analysed by FTIR for the remaining fish species (*Agonus cataphractus*, *Chelidonichthys lucerna*, *Dicentrarchus labrax*, *Pomatoschistus minutus* and *Sprattus sprattus*). Of these 94% were identifiable, with 38% being plastic and 63% being cellulosic. PES was the most abundant plastic (31% of all items).

In total 113 items recovered from *H. grypus* were analysed by FTIR. Library spectra matches were found for 90% of analysed items, with 84% having matches greater than 70%. Only 14% of items were plastic, most commonly acrylic, whilst 80% were cellulosic. Of the 43 items analysed from *P. phocoena* 95% matched library spectra, 95% of which produced a match of at least 70%. A quarter of items were plastic (24%), most common of which was polyacrylate, and 76% was cellulosic.

For the two mysticete whales, fourteen microplastics were analysed with FTIR for both species. In both cases semi-synthetic fibres were most abundant. Baleen from *M. novaeangliae* contained viscose (36%), PVC (14%), PES (7%), PS (7%) and polybutylene (7%). A gold film did not match any library spectra. Baleen from *B. borealis* contained viscose (57%), PES (14%), PS (7%) and acrylic (7%).

6.3.4 Seasonal variation in microlitter ingestion

Species considered for comparison of seasonal variation in microlitter ingestion were *P. flesus*, *Solea solea*, *O. eperlanus*, *T. luscus*, and *C. crangon*. *Trisopterus luscus* was present in almost all samples between December 2018 and September 2020, but after removing samples with fewer than ten individuals only four samples remained. As a result, *T. luscus* was removed from seasonal analysis. Model 6.1 demonstrated that the proportion of individuals ingesting microlitter between sampling dates varied ($p < 0.05$, Table 6.3). The greatest proportion of individuals to ingest plastic was in December 2018 (75%) followed by March and June 2019 (42% and 43% respectively). The proportion of animals ingesting microlitter in December 2018 was significantly greater than March and June 2019 which were significantly higher than all other sampling dates. Between 42% (September 2020) and 96% (December 2019) of *P. flesus* contained litter. Microlitter contamination in *S. solea* ranged from 9% (July 2020) to 81% (June 2019). Between 13% (March 2020) and 100% (March 2019) of *O. eperlanus* ingested microlitter. *Crangon crangon* ingested microlitter to a lesser degree across all sampling dates, with between 8% (June 2019) and 34% (September 2020) of containing microlitter. Species was also significant in Model 6.1 ($p < 0.05$, Figure 6.3, Figure 6.4). The species with the highest proportion of individuals which ingested microlitter was *P. flesus* and *S. solea* (49% and 33% respectively) which was significantly greater than *O. eperlanus* and *C. crangon* (28% and 27% respectively).

AIC scores for error distributions in Model 6.2, where the frequency of microlitter ingestion was investigated, were as follows: Poisson 1,281.064, Negative Binomial 1,229.486, Gamma (link = identity) 1,085.159, Gamma (link = log) 1,075.254 and Gamma (link = inverse) 1,065.912. The distribution with the best fit was Gamma (link = inverse). The model revealed that the length and mass of biota were not statistically significant. Both species and sampling date were significant ($p < 0.05$). The greatest mean amount of litter ingested was in June 2019

(4.1 ± 4.3) which was significantly greater than the average plastic consumption in all other seasons. *Platichthys flesus* ingested significantly more plastic on average than *S. solea* (3.2 ± 3.5, 2.3 ± 1.9 respectively), which ingested significantly more plastic that *O. eperlanus* and *C. crangon* (1.7 ± 0.9, 1.4 ± 1.4 respectively).

Blue and clear fibres were dominant in the diet of *P. flesus*, both being the most abundant form of plastic in about half of samples. Likewise, blue fibres were the most abundant form of microplastics in *S. solea*. For four of the seven samples containing the species blue fibres were predominant, the samples contained more clear, red and purple fibres. The diet of *O. eperlanus* was always dominated by blue fibres, regardless of season with between 31% and 55% of all ingested items being blue fibres. Similarly, blue fibres were the most ingested form of plastic by *C. crangon*. In all seasons, blue fibres were most abundant, with red fibres equally prominent in October 2019 and purple fibres equally common in June 2019.

The average diversity of microplastics differed between species and seasons. *Platichthys flesus* ingested the greatest diversity of microplastic forms in December 2018 (2.9 ± 1.5) and the least diversity in September 2020 (0.5 ± 0.6). Ingested items were between 60 µm and 77.9 mm long. Diversity of microplastic form was similar for *S. solea* with the greatest diversity in June 2019 (2.3 ± 1.8) and the lowest diversity in July 2020 (0.1 ± 0.3), ingesting items 67 µm to 62.3 mm long. Similarly, diversity in *O. eperlanus* ranged on average from 0.2 ± 1.5 and 2.0 ± 1.2 in March 2020 and March 2019/June 2019 respectively. *Osmerus eperlanus* contained debris between 32 µm and 37.6 mm in length. The diversity of microplastic forms was lowest in *C. crangon*. On average the diversity was 0.1 ± 0.3 in June 2019 and 0.5 ± 0.7 in September 2020. *Crangon* ingested litter between 75 µm and 7.8 mm in length.

Table 6.3. Seasonal microlitter abundance in biota samples from Erith, River Thames, UK. Means are reported with standard deviation. More detailed results are available in the Supplementary Material (S10).

	Dec 2018	**Mar 2019**	**June 2019**	**Oct 2019**	**Dec 2019**	**Mar 2020**	**July 2020**	**Sept 2020**
Platichthys flesus								
Sample size	45	18	38	34	31	31	30	31
% with litter	96	61	84	62	52	58	43	42
Average (all)	4.53 ± 3.25	1.78 ± 1.9	4.84 ± 5.52	1.06 ± 1.04	1.87 ± 2.53	2.13 ± 3.59	0.63 ± 0.89	0.58 ± 0.85
Solea solea								
Sample size	0	33	36	37	6	14	34	27
% with litter	NA	70	81	32	17	36	9	41
Average (all)	NA	1.45 ± 1.25	4.14 ± 5.11	0.57 ± 0.96	0.17 ± 0.41	0.57 ± 0.85	0.09 ± 0.29	0.85 ± 1.35
Osmerus eperlanus								
Sample size	1	21	10	14	33	32	50	40
% with litter	0	100	80	71	39	13	36	38
Average (all)	0	2.64 ± 1.76	2.6 ± 1.9	1.29 ± 1.07	0.85 ± 1.44	0.16 ± 0.45	0.58 ± 0.93	0.73 ± 1.26
Crangon crangon								
Sample size	0	13	50	50	50	104	50	50
% with litter	NA	23	8	22	34	26	34	38
Average (all)	NA	0.23 ± 0.44	0.08 ± 0.27	0.28 ± 0.57	0.44 ± 0.76	0.38 ± 0.75	0.5 ± 0.79	0.7 ± 1.91

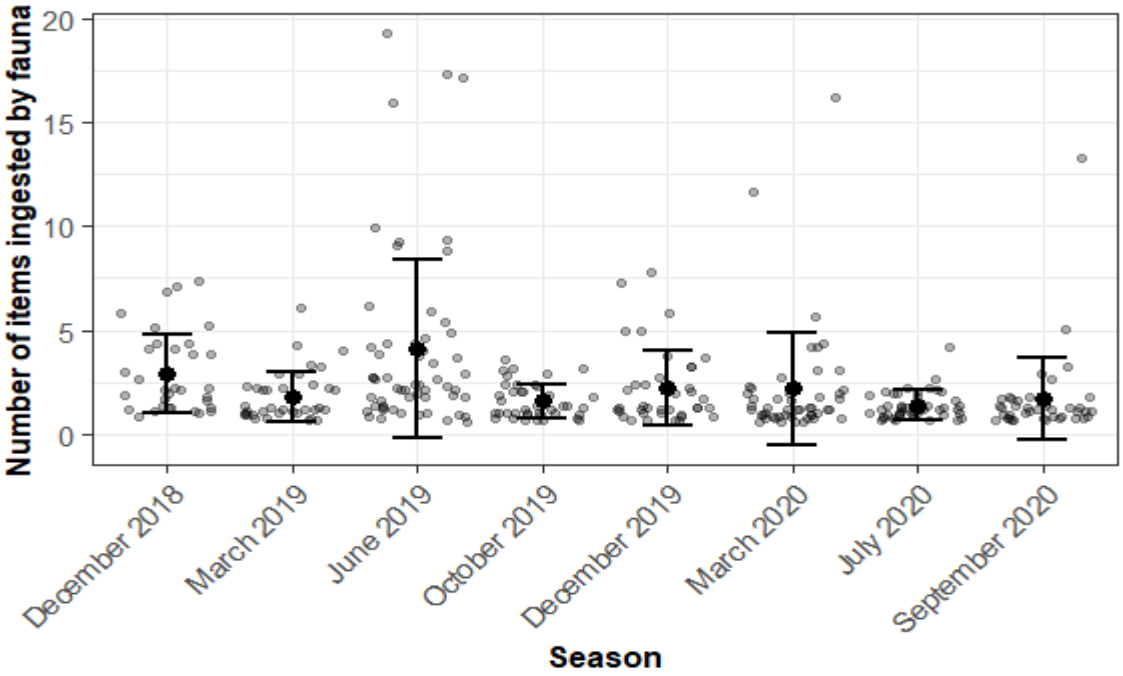


Figure 6.3. Microlitter ingestion by River Thames fauna varied significantly between sampling dates. Grey points are individual values, black points are the means and bars are ± standard deviation.

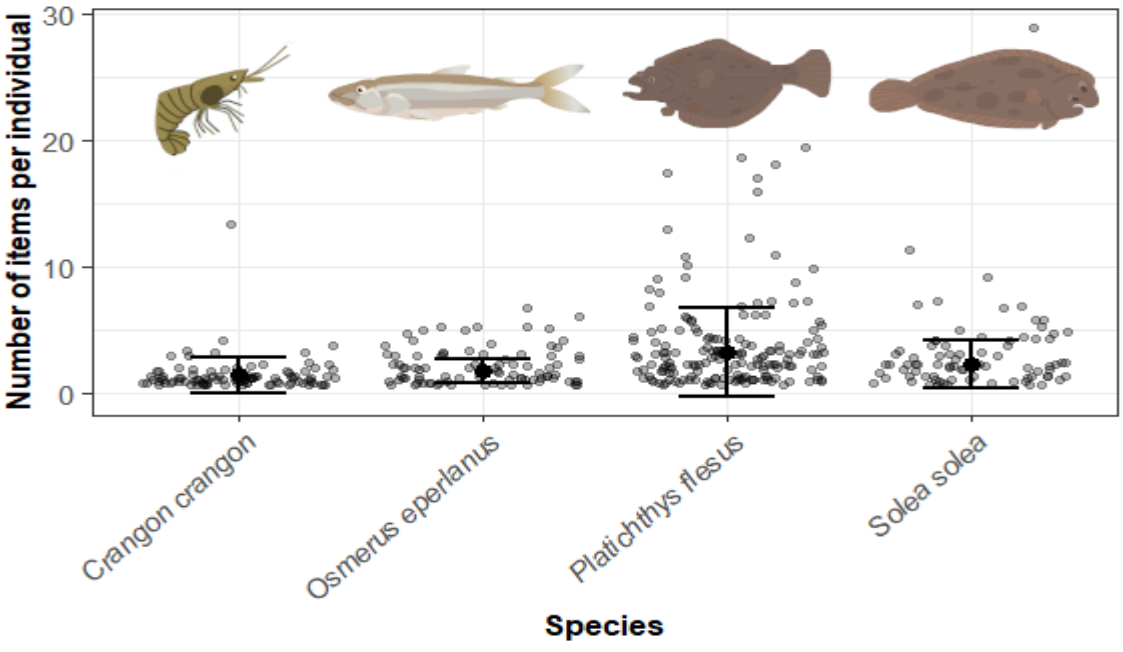


Figure 6.4. Microlitter ingestion by River Thames fauna varied significantly between species. Grey points are individual values, black points are the means and bars are ± standard deviation.

6.3.5 Dietary analysis

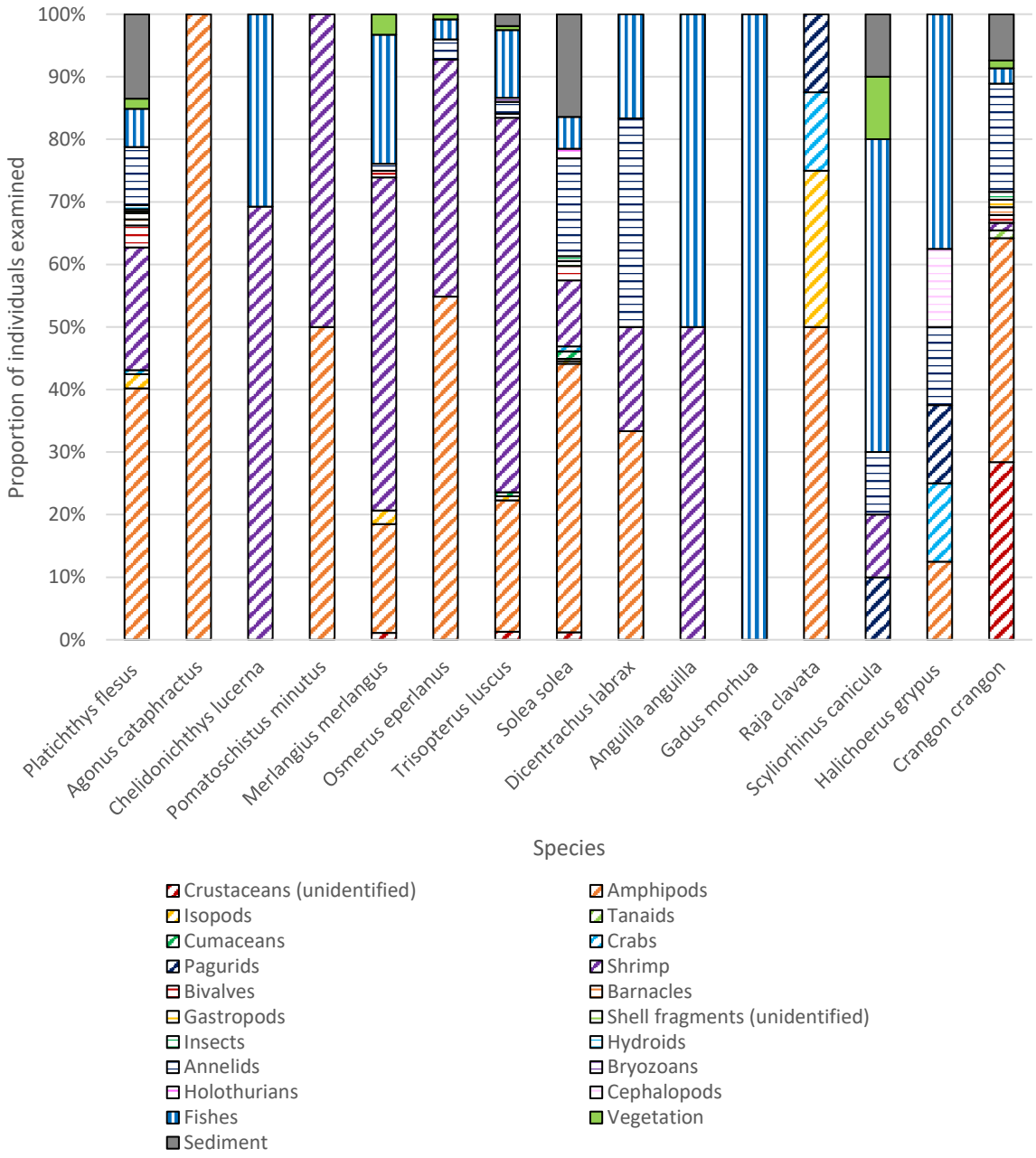


Figure 6.5. The most common dietary items of fishes, shrimp and marine mammals in the Thames Estuary. Prey items were identified to the lowest taxonomic level and grouped, unless identified to species level. Full results available in the Supplementary Material (S13).

All prey items recovered from Thames fishes are reported in the Supplementary Material (S13) with the most common prey items included in Figure 6.5. From the fish specimens collected prior to the present study, four species common in the Thames (*P. flesus*,

M. merlangus, *S. solea*, *T. luscus*) were selected for analysis. The only prey items consistent across species were ray-finned fish and caridean shrimp. The diet of *P. flesus* consisted of amphipods (68% of individuals ingested *Corophium volutator* and 2% ingested gammarids), polychaetes (24%, mostly Nereididae), fish (19%), bivalves (9%) and *Crangon crangon* (7%). Other items in the diet included hydroids, vegetation, sediment, insect larvae and mysid shrimp. *Merlangius merlangus* ingested shrimp (48% *C. crangon*, 3% other caridean shrimp and 31% mysids), amphipods (45%, including *Corophium volutator* and gammarids), fish (10%), isopods (7%), bivalves (3%) and vegetation (10%). Decapods (53% *Crangon crangon*, 6% other caridean shrimp and 6% brachyuran crab), bivalves (35%) and polychaetes (29%) were common in the diet of *S. solea*. Other prey items included fish, annelids, cumaceans, insect larvae and potential holothurians. All *T. luscus* ingested *C. crangon*. Other items recovered from the diet were cumaceans, bryozoans, vegetation, polychaetes and fish (all 16%). It was not possible to identify which species of fish were ingested from these samples, which mostly consisted of tissue.

During the present study, prey items were recovered from samples during dissection and after digestion. The results were comparable to those of the previously examined specimens. Of the 258 examined *P. flesus*, 157 contained identifiable prey items. The most common prey items were amphipods sp. (45% of individuals with prey), *Corophium* (34%), shrimp (unidentified, 28%) and stones/sediment (20%). Other prey items included polychaetes (5%), *Crangon* (5%), isopods (4%), fish (4%), bivalves (3%), barnacles (2%), gastropod shells (2%), vegetation (2%), *Carcinus maenas* exuviae (1%), damselfly nymph (<1%) and shell fragments (unidentified, <1%). Shrimp dominated the diet of both *T. luscus* (100 of 102 studied individuals contained identifiable prey) and *M. merlangus* (29 of 30) with 88% and 86% of fish ingesting shrimp, respectively. Fish remains were also commonly recovered from both species (16% and 55%, respectively) with amphipods making up a large portion of the diet for *T. luscus* (33%). Over 180 *S. solea* were collected in the present study, of which 145 had identifiable items in the GIT, most commonly amphipods (76%), sediment (29%) and polychaetes (23%). Other items included shrimp and fish. In the present study, 202 individuals of *O. eperlanus* were collected and 102 of these contained identifiable prey. The diet of *O. eperlanus* was almost exclusively shrimp and amphipods, with a slightly higher proportion of individuals predating up amphipods (67%) than shrimp (46%). For all studied

species, it is unclear whether the barnacles, bivalves, gastropods and shell fragments are truly prey items or whether it is accidental consumption along with sediment and vegetation.

Of the 116 *C. crangon* collected in McGoran et al. (2018), 53 contained material in the GIT (Figure 6.5). The most commonly ingested items were crustacean remains (unidentified, 43%), polychaetes (23%) and amphipods (19%) including *Corophium volutator* (8%). Only 24 of the 367 *Crangon crangon* collected in the present study contained prey, most commonly amphipods (58%), including at least one species identifiable as *Corophium volutator*. Polychaetes and sediment were also identified. The remaining prey species (*C. volutator* and *H. diversicolor*) contained no identifiable prey and as a result no dietary analysis could be conducted.

Of the two top predator species studied (*H. grypus* and *P. phocoena*, *n* = 5), only two individuals, both *H. grypus*, contained identifiable prey. Fish dominated the diet and whole fish present in the GIT were identified as garfish (*Belone belone*; Supplementary Material, S12). Otolith identification is reported in Figure 6.6. Otoliths were also recovered from 24 fish (11 *M. merlangus,* 10 *T. luscus*, 1 *O. eperlanus*, 1 *P. flesus* and 1 *Chelidonichthys lucerna*). *Solea solea* was the most commonly ingested species (83% of *M. merlangus*, 100% *C. lucerna*). Other species present in the diet of *M. merlangus* included European smelt (*Osmerus eperlanus*), goby, herring or sprat, *M. merlangus* and a possible eel *(Anguilla anguilla*).

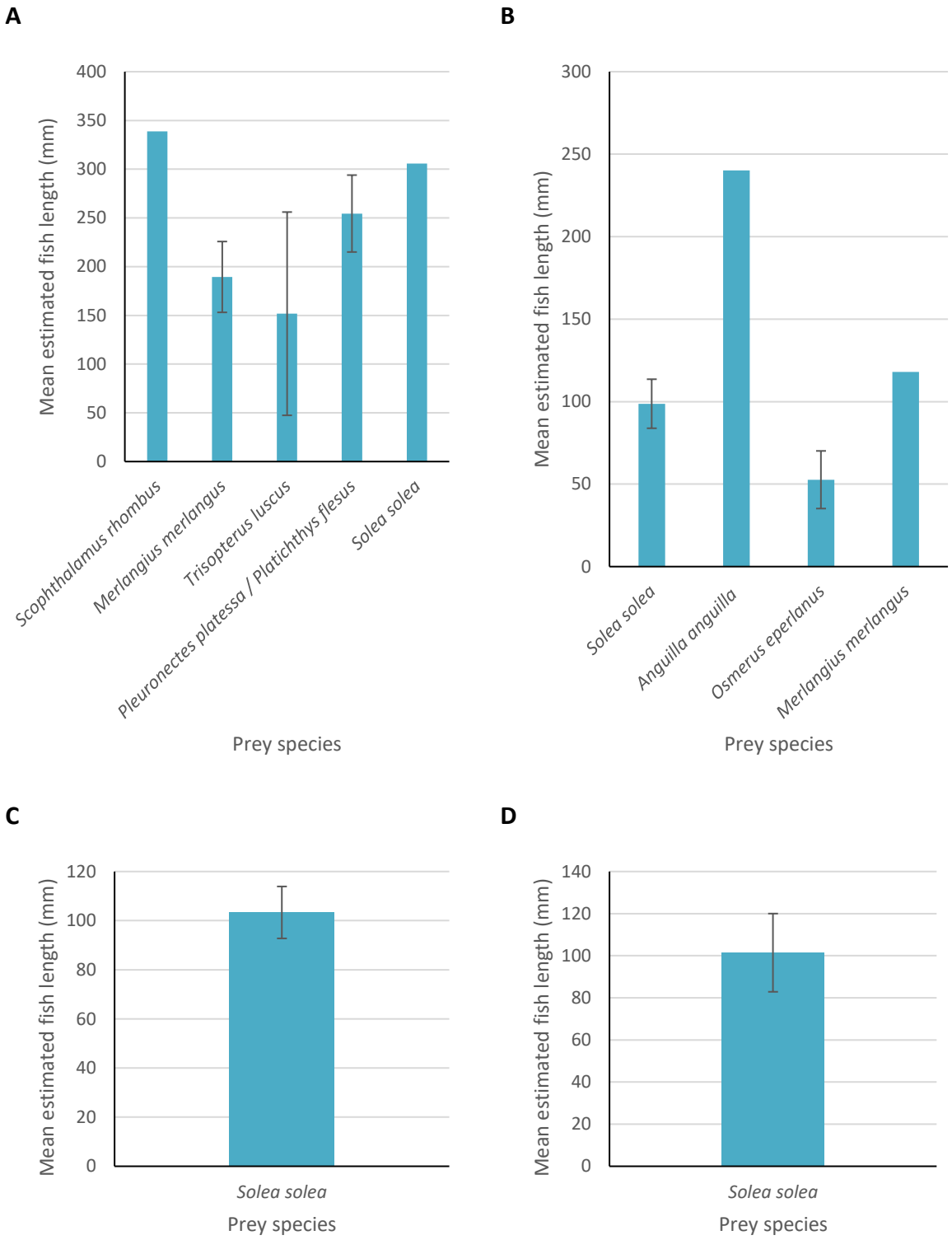


Figure 6.6. Fish remains recovered during dietary analysis, including otoliths and skeletal remains. Where possible, fish size was estimated using Härkönen (1986) and Camphuysen and Henderson (2017). A) *Halichoerus grypus* (grey seal), B) *Merlangius merlangus* (whiting), C) *Chelidonichthys lucerna* (tub gurnard), D) *Trisopterus luscus* (pouting).

6.3.6 Food web structure

The results of dietary analysis informed the structure of the food web developed for analysis (Figure 6.7). Of the ten fish species sampled, only four were included in the Thames food web: *M. merlangus*, *Platichthys flesus*, *S. solea* and *T. luscus*. The average amount of plastic ingested by these species is summarised in Table 6.1 and the averages for trophic levels three and four are reported in Figure 6.8. At the base of the food web are *Corophium volutator* and *Hediste diversicolor*. These species are present in the diet of most fish and *Crangon crangon*, which forms level 2. *Crangon crangon* is fed on by many fishes, mostly commonly midwater species such as *M. merlangus*, but is not directly predated upon by top predators. Levels 3 and 4 consist of the most common fish species in the Thames that are also present in the diet of top predators. Level 3 contains *P. flesus and S. solea*. Whilst there was no evidence of flounder in the diet of *Halichoerus grypus* or *Phocoena phocoena*, otoliths of *Pleuronectes platessa* were recovered and the species occupy a similar niche and are comparable in terms of plastic exposure. *Merlangius merlangus* and *T. luscus* make up level 4. These species have a higher proportion of fish in the diet, notably *S. solea*, and are thus more likely to ingest plastic if trophic transfer and bioaccumulation occur in this system. Finally, level 5 is the top predators in the estuary, *H. grypus* and *Phocoena phocoena*.

As a quantitative comparison to the qualitative dietary assessment, the EcoSim model with EcoPath (ICES, 2017; Mackinson et al., 2018) was used. The model provided quantifiable trophic levels for North Sea biota from 1991. The model is not directly comparable to the Thames as feeding strategies, and therefore potentially trophic level, might differ between the open sea and an estuarine environment. The model produced the following trophic levels: infauna 2.87–2.95, shrimp 3.05, large crabs 3.76, small gadoids 3.80, sole 4.00, juvenile whiting 4.25, whiting 4.36, flounder 4.38, large gadoids 4.51, baleen whales 4.44, and seals 4.97. The main difference between the observed and modelled trophic levels, is that *P. flesus* moves from level 3, with *S. solea*, to level 4, with *T. luscus* and *M. merlangius*.

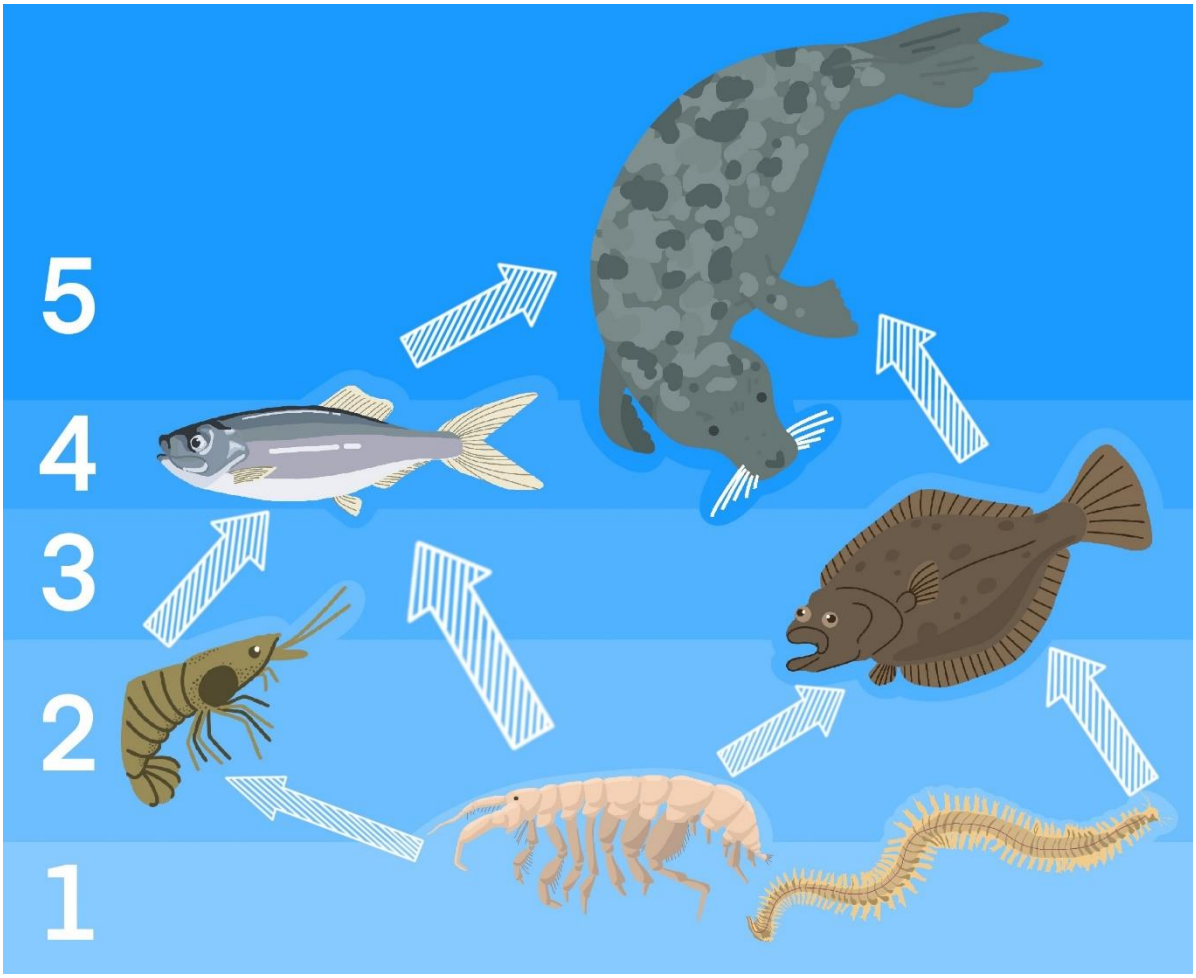


Figure 6.7. Simplified Thames Estuary food web developed from dietary analysis in the present study. Based on their position in the food web, species have been assigned a level of 1-5. *Corophium volutator* and *Hediste diversicolor* are level 1, *Crangon crangon* are level 2, *Platichthys flesus* and *Solea solea* are level 3, *Trisopterus luscus* and *Merlangius merlangus* are level 4 and the top predators *Halichoerus grypus* and *Phocoena phocoena* are level 5.

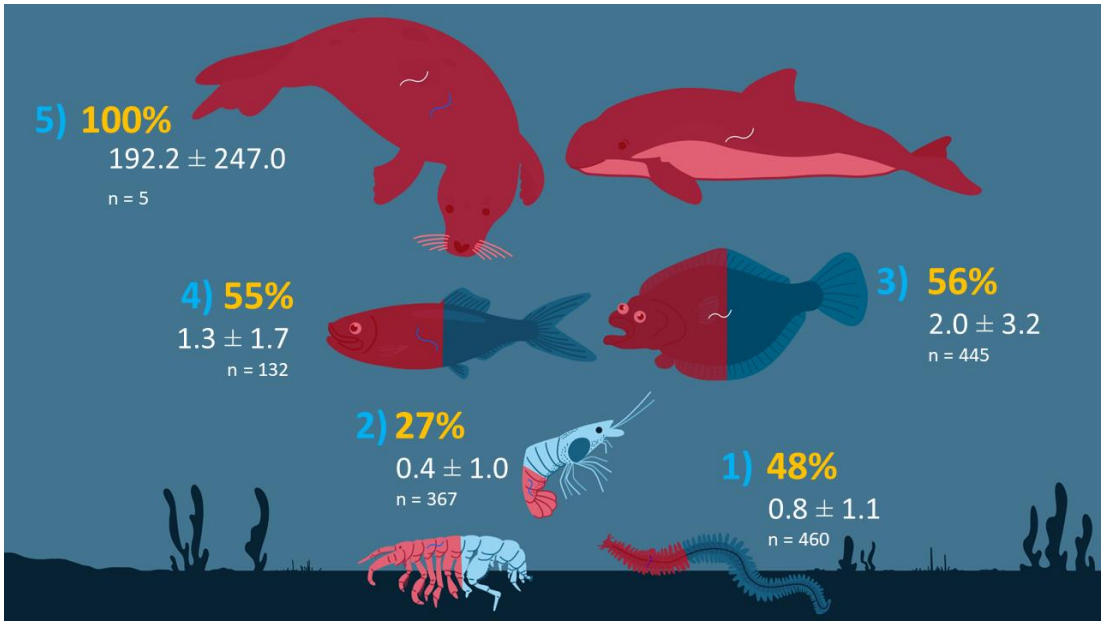


Figure 6.8. Microlitter ingestion per trophic level. Level 1: *Corophium volutator*, *Hediste diversicolor*; level 2: *Crangon crangon*; level 3: *Solea solea*, *Platichthys flesus*; level 4: *Merlangius merlangus*, *Trisopterus luscus;* level 5: *Halichoerus grypus, Phocoena phocoena*.

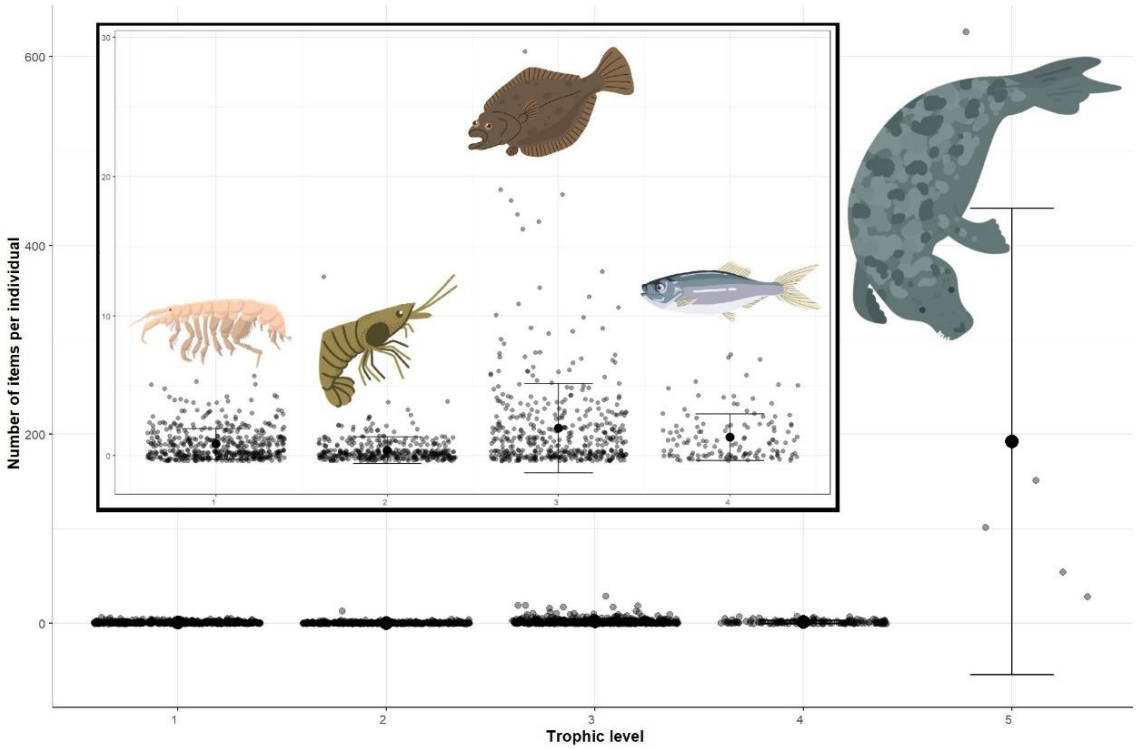


Figure 6.9. Microlitter ingestion per trophic level. Grey points are individual values, black points are the means and bars are ± standard deviation.

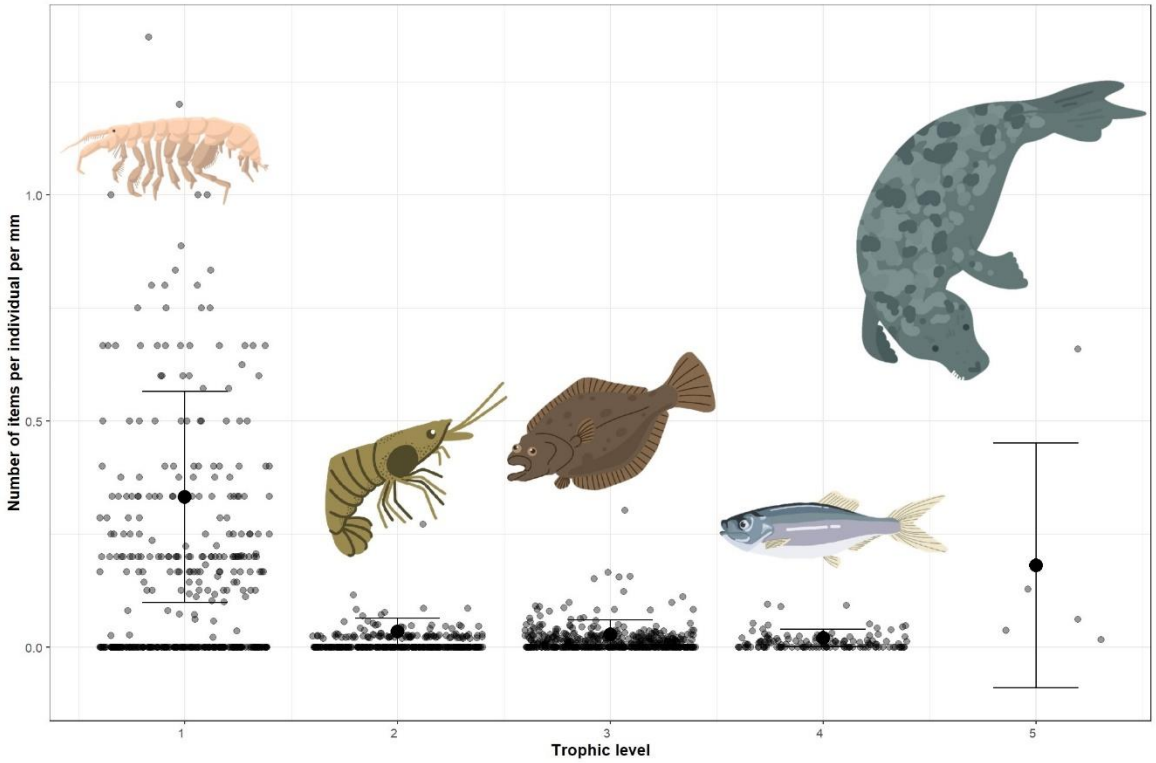


Figure 6.10. Microplastic ingestion per trophic level when the size of organisms is controlled for. Zeros were removed from the dataset to account for skew. Grey points are individual values, black points are the means and bars are ± standard deviation.

Table 6.4. Predicted microlitter exposure of top predators in the Thames Estuary based on the number of fish which would need to be consumed to reach their required daily intake (7 kg for grey seals and 4 kg for harbour porpoise).

	Halichoerus grypus			*Phocoena phocoena*		
Species	**Consumption**	**Mean**	**SD**	**Consumption**	**Mean**	**SD**
Merlangius merlangus	22	33.0	33.0	12	18.0	18.0
Platichthys flesus	19	45.6	64.6	11	26.4	37.4
Solea solea	17	23.8	47.6	10	14.0	28.0
Trisopterus luscus	46	59.8	78.2	26	33.8	44.2

Microplastic per trophic level are summarised in Figure 6.8 and 6.9. Using the trophic levels determined through dietary analysis, Model 6.3 determined that a significantly higher proportion of individuals ($p < 0.01$) from trophic levels 3–5 contained microlitter compared to level 1, which was in turn statistically greater than level 2. The EcoSIM modelled trophic levels find a similar significant difference ($p < 0.01$) with the highest proportion of biota to ingest microlitter in levels 4 and 5, followed by levels 1 and 3 and level 2 with the lowest proportion. The amount of plastic contaminating trophic levels was also significantly different ($p < 0.05$) as determined by Model 6.4 (AIC scores: Poisson 3,588.717, Negative Binomial 2,466.566, Gamma (link = identity) 2,206.123, Gamma (link = log) 2,206.123, Gamma (link = inverse) 2,206.123). Level 2 and level 1 were significantly less contaminated than level 4, which is below level 3, which in turn is less that level 5 (Figure 6.9). As with Model 6.3, when run with the EcoSIM modelled trophic levels, Model 6.4 produces similar results ($p < 0.05$; AIC scores: Poisson 3,609.843, Negative Binomial 2,478.799, Gamma (link = identity) 2,222.910, Gamma (link = log) 2,222.910, Gamma (link = inverse) 2,222.910). In this version of the model, level 1 and 2 contain the fewest microlitter items, followed by levels 3 and 4, with level 5 biota ingesting the greatest amount of marine litter. When size is accounted for in Model 6.5, trophic level is still statistically significant ($p < 0.01$, $R^2 = 0.3964$, F-statistic = 480.9, degrees of freedom = 620). On average, 0.06 ± 0.1 microplastics were present per mm, with a variance of 0.02. When zeros were removed to account for the skewed data, the mean was 0.1 ± 0.2 with a variance of 0.04. Trophic level 1 had significantly more microplastics present for its size (0.3 ± 0.2 compared to 0.03 ± 0.03, 0.03 ± 0.03, 0.02 ± 0.02 and 0.06 ± 0.05 for trophic levels 2–5 respectively; Figure 6.10). The proportion of individuals to ingest microlitter and the amount ingested is significantly higher in higher trophic level organisms, supporting hypothesis three. Predicted ingestion from fish size and mean contamination in these species can be used to predict exposure from Thames marine mammals (Table 6.4). When size is considered, however, lower trophic level organisms ingest more litter for their size. This indicates that trophic level and feeding strategy alone are not indicators for microlitter ingestion.

6.3.7 Types of microlitter present

Diversity of microlitter forms was on average 0.77 ± 1.47 in *Corophium volutator*, 0.34 ± 1.3 in *Hediste diversicolor*, 0.33 ± 1.25 in *Crangon crangon*, 1.09 ± 2.05 in fishes and 9.75 ± 2.99 in *P. phocoena* and *Halichoerus grypus* (Supplementary Material, S15).

Blue fibres were the dominant form of microlitter in fauna across almost all trophic levels (level 1 41% of microlitter, level 2 42%, level 3 23%, level 4 38%, level 5 20%, Figure 6.11). Level 3 fauna had a similar proportion of clear fibres to blue fibres (24%) and level 5 fauna were contaminated with more clear fibres than blue fibres (32%). After blue fibres, the most common litter forms in level 1 fauna were purple fibres (24%) and black fibres (11%). In level 2 it was red fibres (14%) and purple fibres (12%), whilst black fibres (10%) were most abundant after blue and clear fibres in level 3 fauna. In level 4 fauna clear fibres were the second most abundant form of litter (14%) followed by red fibres (12%) and in level 5 fauna black fibres were the most abundant (14%) after clear and blue fibres. Following a different trend, white fibres (29%) and clear fibres (28%) were most dominant in sediment with black fibres third most abundant (8%).

The average length of litter in sediment was 1,961 ± 2,315 µm (Chapter 4). Whilst litter measured between 14 µm and 5.5 mm in trophic level 1, between 75 µm and 7.8 mm in trophic level 2, between 60 µm and 77.9 mm in trophic level 3, between 39 µm and 34.6 mm in trophic level 4 and between 62 µm and 29.8 mm in trophic level 5 (Figure 6.12; Supplementary Material, S15). Small microlitter (as defined by Hanke et al., 2013) make up the largest portion of particles in trophic levels 1 and 2, whilst large microlitter are more common in sediment and trophic levels 3–5. Mesoplastics and macroplastics are rare (Figure 6.13). Most plastic in the sediment and ingested by fauna is less than 100 µm in width (sediment: 78%, trophic level 1: 99%, trophic level 2: 95%, trophic level 3: 92%, trophic level 4: 93%, trophic level 5: 89%). Plastic items wider than 1 mm were only recorded in sediment and trophic levels 3–5. This only accounted for between 1% and 2% of plastic. In general, higher trophic level organisms, which are typically larger, ingest larger plastic items. This supports hypothesis four.

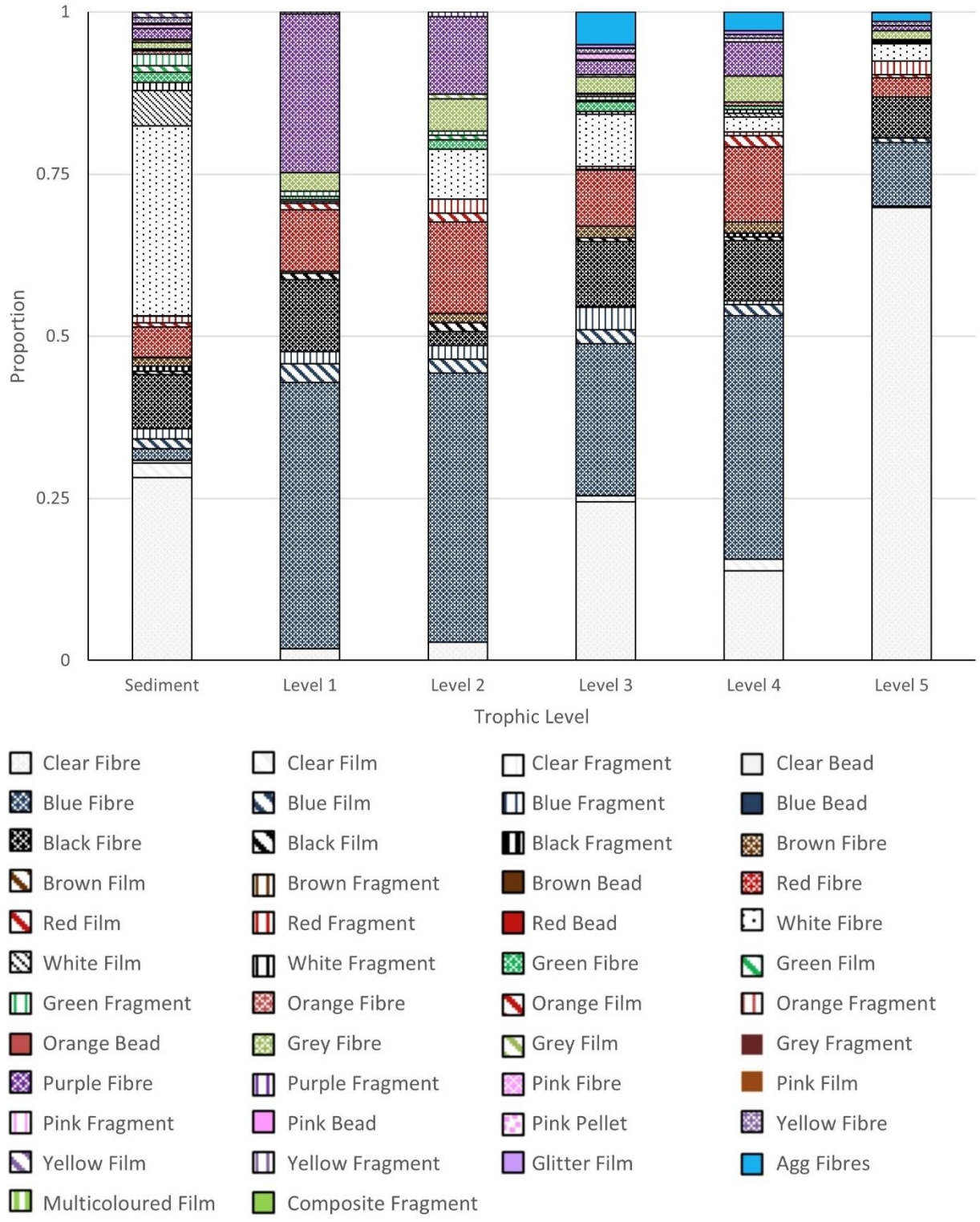


Figure 6.11. Microplastic form (shape and colour) diversity between trophic levels, where trophic level 1 is *Corophium volutator* and *Hediste diversicolor*, level 2 is *Crangon crangon*, level 3 is *Solea solea* and *Platichthys flesus*, level 4 is *Merlangius merlangus* and *Trisopterus luscus* and level 5 is *Halichoerus grypus* and *Phocoena phocoena*, and sediment (Chapter 4).

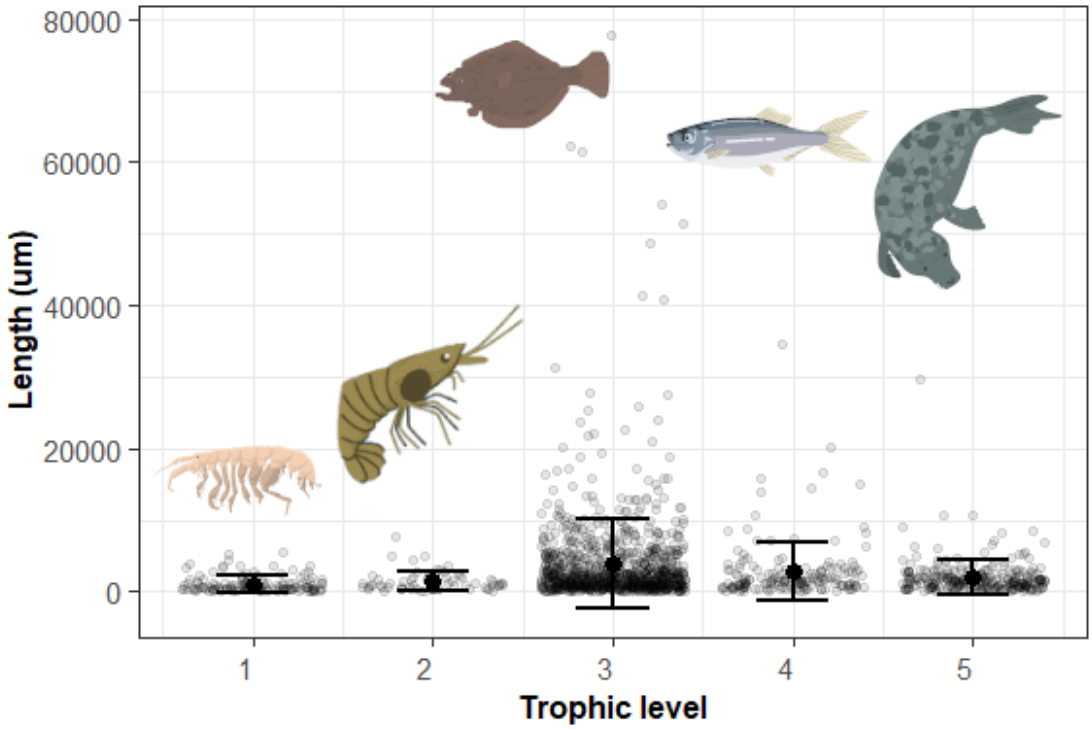


Figure 6.12. Length of plastics between trophic levels, where trophic level 1 is *Corophium volutator* and *Hediste diversicolor*, level 2 is *Crangon crangon*, level 3 is *Solea solea* and *Platichthys flesus*, level 4 is *Merlangius merlangus* and *Trisopterus luscus* and level 5 is *Halichoerus grypus* and *Phocoena phocoena*. Grey points are individual values, black points are the means and bars are ± standard deviation.

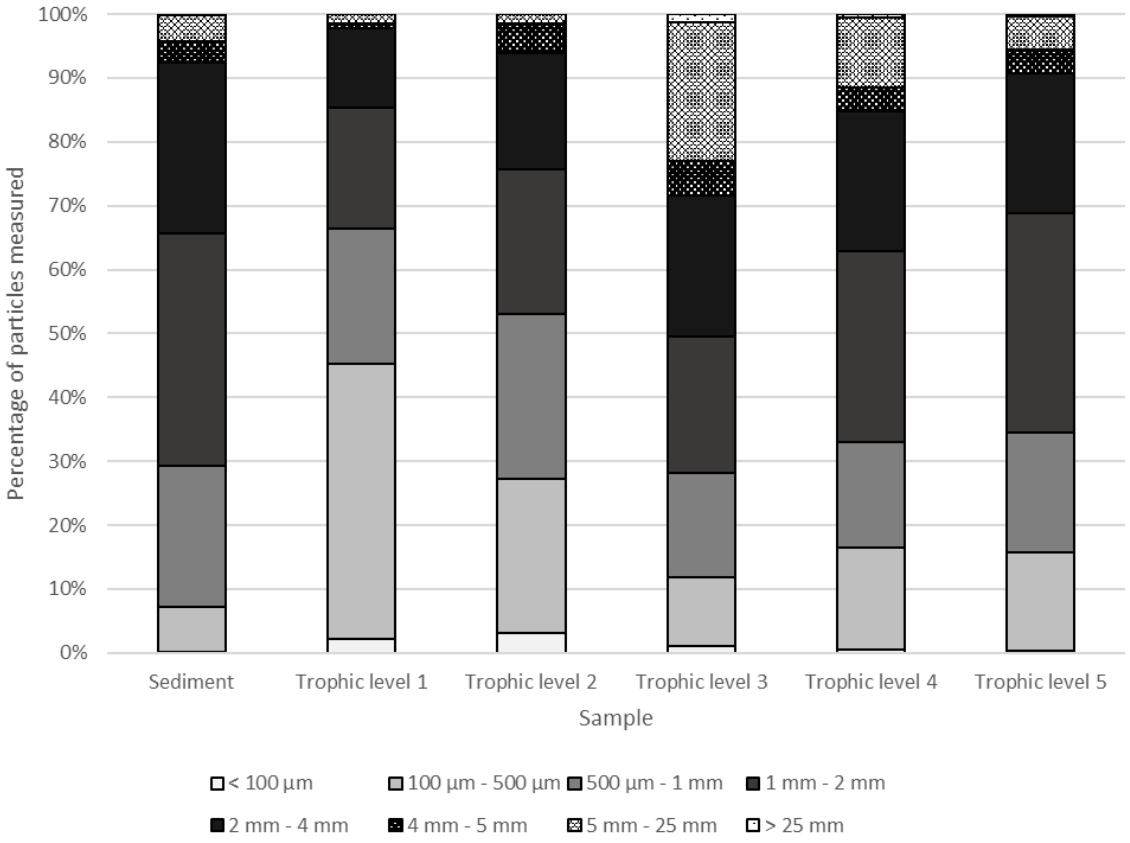


Figure 6.13. Length of particles divided by size fractions between trophic levels, where trophic level 1 is *Corophium volutator* and *Hediste diversicolor*, level 2 is *Crangon crangon*, level 3 is *Solea solea* and *Platichthys flesus*, level 4 is *Merlangius merlangus* and *Trisopterus luscus* and level 5 is *Halichoerus grypus* and *Phocoena phocoena*.

6.4 Discussion

Biota in the Thames Estuary were shown to ingest microlitter throughout the sampling period, regardless of species and feeding strategy. Ingestion ranged on average from 0.39 ± 0.95 items per individual of *C. crangon* to 214.75 ± 70.73 items per individual of *H. grypus*. Fibres were most commonly ingested, as predicted by hypothesis 1. Temporal variation was reported as well as significant differences between trophic level, supporting hypotheses 2 and 3. The greatest proportion of individuals contaminated with microlitter in the GIT occurred in December 2018 and predators ingest the most litter when organism size is not considered.

With regards to seasonal variability, the highest proportion of individuals ingesting litter was recorded in December 2018, March 2019 and June 2019. Sediment samples collected on these dates (Chapter 4), conversely, generally had low concentrations of

microlitter. Both a greater proportion of individuals were contaminated by microlitter and a higher average number of items were recovered from fauna in June 2019, which corresponds with the greatest cumulative rainfall over the eight-day period leading up to sampling. As microlitter ingestion varied significantly, in terms of both the proportion of contaminated individuals and the amount of litter ingested, hypothesis 2 is supported. Similarly, Daniel et al. (2020) found that shrimp (*Fenneropenaeus indicus*) were most contaminated by microplastics during the monsoon season in India and Devriese et al. (2015) noted that *C. crangon* ingested more microplastics in October compared to March. It was proposed that this was due to increased riverine inputs from higher volumes of rainfall. The same was true of subadult grunt (*Pomadasys ramosus*) which ingested the most plastic in the rainy season in Brazil. Roughneck grunt (*Haemulidae corvinaeformis*), however, ingested the most plastic in the dry season when its diet was predominantly a bivalve (*Anomalocardia flexuosa*). In the rainy season, *P. ramosus* fed on benthic inverts and therefore diet, especially feeding on benthic organisms (Silva et al., 2018), might be a more significant factor than rainfall. Indeed, Ferreira et al. (2018; 2019) reported that microplastic ingestion was greatest in the rainy season but concluded that individuals which ingested more fish also ingested more microplastics. During the lifecycle of estuarine fish, there are ecological shifts which prevent conflict between species and conspecifics of a different age. This can also affect seasonal microplastic ingestion (Ferreira et al., 2019). Seasonal ingestion can also vary with changes in use of the estuary. For instance, increased fishing in the rainy season resulted in more blue microplastics being ingested by fish (Ferreira et al., 2018). It should be noted, however, that fishing intensity is not always correlated with plastic ingestion (Welden et al., 2018). Additionally, no commercial fishing occurs in the Thames Estuary upstream on Lower Hope Point (near the mouth of the estuary), but variations in other activities, such as recreational use, might play a role.

Crangon crangon and *O. eperlanus* ingested fewer microlitter items on average than *P. flesus* and *S. solea*. *Crangon* ingested an average of 1.4 ± 1.4 pieces per individual. Ingestion of microplastics by shrimp vary in their estimates. A similar quantity of plastic was recovered from the following carideans, *C. crangon* in Belgium (1.23 ± 0.99 per individual, Devriese et al., 2015) and *Aristeus antennatus* from the Mediterranean Sea (1.66 ± 0.11 per individual, Cau et al., 2019), which is higher than estimates from India (*Fenneropenaeus indicus* 0.39 ±

0.6 per individual, Daniel et al., 2020) and Australia (*Paratya australiensis* 0.52 ± 0.55, Nan et al., 2020). Higher quantities have, however, been reported in caridean species from Bangladesh (*Penaeus monodon* 6.6 ± 2 per individual, *Metapenaeus monocerous* 7.8 ± 2 per individual, Hossain et al., 2020), the Persian Gulf (*Penaeus semisulcatus* 7.8 per individual, Abbasi et al., 2018; *P. semisulcatus* 0.36 per gram with an average weight of 12.4 g, estimated at 4.5 items per individual, Akhbarizadeh et al., 2019) and China (*C. affinis* 29.4 per individual, Wang et al., 2019). Certainly, microplastic ingestion in *C. crangon* in the River Thames (present study) is far lower than that of brachyuran crabs (Chapter 5). Cau et al. (2019) reported that *Nephrops norvegicus* ingested more plastic on average than *A. antennatus* from the same area (5.5 ± 0.8 per individual) and proposed that *N. norvegicus* had a less selective feeding strategy which exposed it to a greater number of microplastics. Certainly, both *Carcinus maenas* and *E. sinensis* are non-selective, opportunistic scavengers and therefore may be more likely to ingest microlitter. Similarly, *Platichthys flesus* and *Solea solea* are opportunistic ambush predators which may explain the difference in microlitter ingestion between them and *C. crangon* and *O. eperlanus* in the present study. It can also be noted that both *P. flesus* and *S. solea* are closely associated with the riverbed where litter is known to accumulate (Chapter 3 & 4). Previous studies have highlighted that diversity of prey does not increase exposure to plastic but predating on endobenthic fauna increases microplastic ingestion (Carrera-Colom et al., 2018). *Platichthys flesus* and *S. solea* mostly feed on polychaetes and benthic amphipods, such as *Corophium volutator*.

Wootton et al. (2021) reviewed microplastic ingestion in fish and concluded that approximately half of all sampled fish ingested microplastics with average of 3.5 items per individual. A similar amount of plastic was recorded in a previous study of fishes in the Thames Estuary (McGoran et al., 2018) as well as in the present study. Whilst spatial variation was not investigated in the present study, Wootton et al. (2021) found that location was a significant factor in microplastic ingestion by fish. Species ecology was also significant, with detritivores ingesting more plastic particles. This could explain why *P. flesus* and *S. solea* ingested more microplastics than *O. eperlanus* and *Crangon crangon*, due to their close association with the riverbed. As ecology changes through a fish's lifecycle, plastic ingestion may also vary over time (Silva et al., 2018).

With impact of feeding strategy on microlitter ingestion, comes discussion of trophic transfer. The possibility of microlitter moving through food webs has long been a question of debate. Often studies assumed that trophic transfer occurred without studying the issue further. Researchers that have investigated the topic, in the field and in the laboratory, have provided evidence both for and against transfer, bioaccumulation and biomagnification of microplastics. Ultimately, it is a topic which requires clarification (McIlwraith et al., 2021). Au et al. (2017) noted that trophic transfer occurred regularly and concurrently with direct ingestion. A recent review (Miller et al., 2020) found that microplastics bioaccumulate but that biomagnification is not supported by the available data. Akhbarizadeh et al. (2019) and Walkinshaw et al. (2020) also concluded that biomagnification was not probable. Other reviews have agreed that biomagnification is not supported but also find that bioaccumulation is not possible (Gouin, 2020). The disagreement appears to be the result of varied definitions of bioaccumulation. Miller et al. (2020) defined bioaccumulation as the rate of egestion being less than the rate of intake, whilst Gouin (2020) defined it as an organism containing more microplastics than the surrounding environment. Gouin (2020) concluded that trophic transfer would be more influenced by translocated particles that had moved out of the GIT rather than microplastics being retained. Whilst translocation may increase the retention time in the environment, it is not the only pathway for the transfer of plastics. Many predators can ingest whole prey if it is smaller than themselves, unlike scavengers who may consume only parts of prey. The assumption by Gouin (2020) that plastic in the GIT is not likely transferred to predators is flawed. Few studies have combined the environmental relevance of field observations with the controlled approach of experimental studies. Nelms et al. (2018) used captive seals and wild caught fish to demonstrate that microplastics can be transferred to top predators via their prey.

In addition to evaluating the likelihood of biomagnification and bioaccumulation, Miller et al. (2020) addressed gaps in the literature. Their review concluded that more research is needed to understand the retention time of plastics once ingested and the fate of chemicals associated with plastics. Diepens and Koelmans (2018) modelled an Arctic food web, which included Atlantic cod and polar bears, and found that plastic-associated chemicals can bioaccumulate. It depended, however, on the specific chemical. Polychlorinated biphenyls accumulation decreased with increased plastic ingestion, whilst polycyclic aromatic

hydrocarbon concentrations increased with plastic ingestion. Batel et al. (2016) also explored the trophic transfer of plastic and chemical pollutants in combination, reporting that both accumulated in *Danio rerio* (zebrafish). Virgin microplastics caused no physical harm to the intestinal lining, but benzo[α]pyrene was desorbed in the intestine and translocated to the liver. Experiments which have exposed *Platorchestria smithi* (beach hoppers) to environmentally relevant concentration of microplastics which sorbed chemical pollutants from the natural environment found that whilst *Bathygobius krefftii* (Krefft's frillgobies) ingested contaminated beach hoppers, chemical uptake was not increased with microplastic exposure (Tosetto et al., 2017). Whilst evidence might suggest microplastics do not influence chemical uptake in fish, in fat-rich mammals, such as cetaceans, it may be more likely to pose a threat.

The present study demonstrated that the transfer of litter from prey to predators is possible in the River Thames. Three prey items recovered whole from the GIT of Thames fishes were found to contain microlitter. For two of these prey animals, the contamination they contained accounted for all items ingested by the fish predator. In addition, two individuals of *Platichthys flesus* were found with remains of *Carcinus maenas* in the GIT. Whilst no evidence of microlitter was found in the recovered remains, it highlights the possibility of the large quantities of plastic present in brachyuran crabs (see Chapter 5) being transferred to their predators. Certainly, all studied top predators in the Thames Estuary ingested microplastics and had significantly higher quantities in their GITs than lower trophic level organisms. Over 190 pieces of microlitter were ingested on average by trophic level 5 organisms whilst fauna from levels 2–4 ingested on average 1.3–2.0 microlitter items and level 1 species ingested fewer than one particle each on average. This supports the outcomes of theoretical models which have predicted that predators are more at risk of ingesting microplastics (Huang et al., 2020).

Retention of microplastics in the GIT is a key component for the potential for trophic transfer and bioaccumulation, but it is poorly understood. Bravo Rebolledo et al. (2013) reported that 12.2% of harbour seals had ingested plastic, with a greater abundance of plastic in the stomach compared to the intestine. Nelms et al. (2018) also reported that marine mammals might retain plastics in the stomach. In the present study only one of the four studied individuals of *H. grypus* had more microplastics in the stomach compared to the

intestine. This contradicts hypothesis 5 which predicted that debris would be retained in the stomach. Between eight and 25 particles were present in the stomach compared to three and 605 in the intestines. Similarly, *P. phocoena* had more microplastics in the intestines (61) compared to the stomach (41). There was, however, more plastic in the fore stomach (36) compared to the fundic stomach (4) and pyloric stomach (1). Sample size in the present study was unfortunately small, making it hard to draw conclusions. It is possible that when the stomach contains multiple compartments, such as in porpoises, the plastic is retained for a short period in the fore stomach. This might be aided by the highly folded stomach lining. The same was suggested in *Neophocaena asiaeorientalis sunameri* (East Asian finless porpoise) from China which contained more plastic at the beginning of the intestines (Xiong et al., 2018). Future studies should use a systematic examination of the intestines for marine mammals to allow for movement of microplastics through the digestive tract to be monitored.

It has been demonstrated that brine shrimp can egest microfibres more effectively compared to microbeads with a maximum retention time of 96 hours for beads. Additionally, brine shrimp have a shorter retention time if food was also consumed (Bour et al., 2020). Sea anemones readily ingest microplastics but were reported to never retain plastic for more than a few hours. Furthermore, retention time was shorter if the microplastic was previously egested then re-consumed (Diana et al., 2020). Insect larvae, which were recorded in some of the fish specimens in the present study, can evacuate microplastics from the gut naturally (Windsor et al., 2019b), suggesting they may have a low retention time. Some fish have been found to retain plastic for up to seven weeks, but on average tend to egest it after just one week (Ory et al., 2018). Whilst Bour et al. (2020) found that fish never retained plastic in the digestive tract for more than 48 hours, retention time was longer if the plastic was on the gills instead of in the GIT. Dos Santos and Jobling (1991) studied gastric emptying in *Gadus morhua* (Atlantic cod) and found that particles smaller than 2 mm easily passed through the pylorus, whilst particles 5 mm or larger were retained in the stomach for prolonged periods. Therefore, fishes might be more likely to transfer larger plastic particles to their predators. Due to the logistical limitations, a 300 µm mesh was used during the processing of top predator samples whilst a 32 µm mesh was used for filtering all digestions and density separations. As a result, smaller items ingested by *H. grypus* and *P. phocoena* may have been missed in the present study. This means that the recorded microplastic ingestion might be an

underestimation and it also hinders the comparison of the size of plastic ingested between trophic levels. Despite this, the best effort has been made to discuss variations in microplastic ingestion by size.

Plastics in the sediment samples are primarily 1 mm to 4 mm long (Chapter 4), whilst trophic level 1 organisms primarily ingest microplastics smaller than 1 mm. This suggests some selective feeding by particle size and due to the greater abundance of small microplastics in *C. volutator* and *H. diversicolor* it is likely that some bioaccumulation may occur. The proportion of small microplastics in the diet decreases up the food chain as organisms are not only receiving plastic through trophic transfer but direct consumption as well. Larger animals can ingest larger plastics, which means a wider range of plastic is bioavailable to them. The size distribution of microplastics is similar between trophic levels 3–5, which means it is possible for these particles to be transferred up the food chain. Few meso- and macroplastics were consumed, mostly by higher trophic level organisms. Fibres are the main type of large plastic ingested. Their width is far narrower that their length and whilst they may measure several millimetres in one dimension, they are only a few micrometres in another. Some large films were also ingested.

Translocation of microplastics from the GIT or gills to other tissues likely increases the chance for trophic transfer as the plastic will remain in the organism for longer. Farrell and Nelson (2013) reported the trophic transfer of microplastics from *Mytilus edulis* to *Carcinus maenas* as well as the translocation of plastic to the haemolymph of the crab. The concentrations used were, however, extremely high. Similarly, Stienbarger et al. (2021) reported that virgin and chemically-treated microplastics biomagnified up the food chain, impacting immune response and oxygen consumption in juvenile fish, but the concentrations used were unrealistically high. This is a common problem of exposure studies and investigations into biomagnification (Miller et al., 2020). Whilst translocation to several tissues has been documented (Crooks et al., 2019), the plastics involved are usually smaller than can be detected in the present study. Our understanding of the topic is still developing, but it appears that the properties of the ingested micro- or nanoplastic affect its likelihood of being translocated (McIlwraith et al., 2018).

A higher proportion of blue fibres were recovered across all trophic levels compared to sediment samples. This could indicate selective feeding on blue fibres in at least one level of the food web. Xiong et al. (2018) and Zantis et al. (2022) also noted that marine mammals frequently ingested a high number of blue fibres, highlighting that blue fibres may be particularly abundant in the environment or readily ingested by small prey species. It has been demonstrated, for instance, that planktivorous fish show a preference towards certain colours of plastic (Ory et al., 2017; 2018). In the case of Ory et al. (2017) fish ingested blue plastic of a similar size to its blue copepod prey. Fishes and marine mammals in the Thames Estuary (levels 3–5) also ingested a high number of clear fibres which are dominant in environmental samples, suggesting that level 1 and/or level 2 fauna might be biased towards ingestion of blue fibres in the environment. These fibres may then be transferred to the higher-level predators. Additionally, more red fibres and purple fibres were present in fauna compared to sediment, especially in polychaetes, amphipods and shrimp (levels 1–2). Blue fibres were, however, also common on the gills of fishes and it may be the case that blue fibres are common in the water column but not in the sediment sampled in the present study. No water samples were collected in the present study and previous studies into microplastic pollution in the surface water of the Thames excluded fibres from analysis because of contamination concerns (Rowley et al., 2020).

Whilst the evidence in the present study suggests that *Crangon*, *Corophium* and *Hediste* might be preferentially feeding on some forms of litter, the literature suggests that shrimp typically consume plastics passively. Nan et al. (2020) reported that blue fibres were the most abundant form of microplastics in both shrimp and surface waters and that therefore direct ingestion from the water occurred. The presence of clear fibres in the digestive tracts of fishes and top predators in the Thames certainly suggests that these species are directly consuming plastic in addition to the transfer of blue fibres from prey. Although, more litter was present in the digestive tract of fish than on the gills, as was also the case in estuarine brachyuran crabs (Chapter 5), suggesting that ingestion and perhaps trophic transfer is the main route of exposure rather than passive intake of contaminated water.

The present study also highlights that whilst white fibres are highly abundant in the environment, they are rarely ingested. A similar observation can be made for tangles of microplastics which are most common in trophic levels 3 and above. Perhaps the presence of

tangles in the predators in the Thames Estuary is due to the consumption of brachyuran crabs which were found to contain large tangles of plastic in the GM (Chapter 5). Certainly, a small number of crabs were recovered from the digestive tract of a few fish specimens during dietary analysis. Another discrepancy between plastic present in fauna and sediment is the abundance of white films in sediment. It has been shown that sewage-related debris, such as sanitary pads, is abundant on the riverbed and that it can be ingested by some organisms (Chapter 5). It seems that the species in the present chapter, however, are less likely to consume white films potentially from sanitary pads.

Cellulosic fibres were more abundant in fauna whilst PP was dominant in sediment samples. Similar to the abundance of blue fibres in the diet over the white fibres abundant in sediment, the presence of cellulosic fibres up the food chain suggests that microplastics are indeed transferred from prey to predators. Though it is not clear why cellulosic fibres are so abundant in fauna given they make up a relatively small portion of environmental plastics. It is not only the present study that finds PP to be the dominant polymer in the Thames Estuary. Rowley et al. (2020) found that the surface water of the Thames contained mostly PP and PE. They did not, however, record fibre abundance and therefore may not have detected the cellulosic polymers, such as viscose.

Corophium volutator ingested, on average, twice as much plastic as *Hediste diversicolor* and *Crangon crangon*. *Corophium* is a deposit feeder and this may expose it to a greater number of microplastics. *Hediste* can adopt a range of feeding strategies from grazing to carnivory and the species is also known to produce a mucus sheet to trap organic material prior to consuming it. These feeding strategies may allow the polychaete to be more selective about the food it consumes. The use of different feeding strategies therefore affects the shape of microplastics *H. diversicolor* can ingest (Porter et al., 2023). The study by Porter et al. (2023) found that filter feeding individuals ingested more fibres than deposit feeding worms; concluding that unless fibres were present in an orientation parallel to the worm, they were not bioavailable. Brine shrimp have been shown to preferentially ingest microbeads over fibres (Bour et al., 2020). How a predator detects its prey seems to be key in determining whether the animal is likely to ingest or avoid litter. Visual foragers consume debris that resembles their prey whilst chemosensory species can avoid plastics (Roch et al., 2020). Yardy and Callaghan (2020) demonstrated that the freshwater amphipod *Gammarus*

pulex could detect microplastic in food and chose food sources that did not contain plastic. Certainly, there is evidence that *Crangon crangon* might preferentially ingest fibres and may avoid microspheres (Devriese et al., 2015). By comparison, *Crangon* prey on juvenile fishes in addition to being prey for adult fishes (Selleslagh and Amara, 2008). As such, the species may be exposed to some of the plastic ingested by higher trophic organisms. The evidence from the present study, however, demonstrates that they are ingesting a significantly lower quantity of microplastics compared to the studied fishes. It is possible that juvenile fish consume less than adults. The low level of plastic present in *Crangon* suggests that the species might be better able to egest microplastics than other crustaceans in the estuary (Chapter 5). This could explain why species which ate more shrimp, such as *M. merlangus* and *T. luscus*, ingested fewer plastic than fish with an amphipod diet, including *P. flesus*. *Solea solea*, however, also consumed mostly amphipods but had a similar average ingestion to *M. merlangus* and *T. luscus*. This could be the reason why trophic levels 3 and 4 were not significantly different. *Merlangius merlangus* and *T. luscus* were placed in a higher trophic level than other fishes as they had a piscivorous diet. It is likely that the fish in the diet was in fact opportunistic foraging on juvenile sole, which as discussed may ingest fewer microplastics. Adult *S. solea* are typically 30–40 cm (Wheeler, 1978). Whereas, in the present study, estimates of fish size from otoliths recovered from the GIT of *M. merlangus* are less than 15 cm and are therefore likely to be juveniles. Indeed, *S. solea* breed in coastal waters in the spring and early summer (Wheeler, 1978) and juveniles are likely abundant in the estuary in the summer. Certainly, *S. solea* are one of the most abundant small fishes (standard length 1–10 cm) in the Thames Estuary and over 680 were recovered between September and December 2012 (Clark et al., 2017). Both *M. merlangus* and *T. luscus* may actually share the same trophic level as the flatfish in the estuary with species such as gobies occupying a lower trophic level. *Solea* and *P. flesus* occupy similar niches in the estuary, which could infer that the species would be exposed to similar plastic loads. The sample size of *S. solea* varied by season, with the largest samples occurring in the summer and autumn (see Chapter 4, Table 4.2). Whilst *P. flesus* was present in the estuary year-round. Evidence suggests that *S. solea* is present in some parts of the Thames Estuary all year but spawns in the estuary and juveniles develop there between January and June (Zoological Society of London, 2016). During this period numbers in the Thames may be higher. Migration down the Thames was proposed as one of the possible factors that allowed *Eriocheir sinensis* to ingest more plastic that *Carcinus*

maenas (McGoran et al., 2020; Chapter 5). As *S. solea* migrate to and from nursery grounds, they may be exposed to different concentrations of microplastics. Further research is required to explore spatial variation of pollution in the Thames and variation in ingestion at different life stages of *S. solea* in the estuary.

Whilst *Halichoerus grypus* ingested fish present in the estuary, *Belone belone* (garfish) was the most abundant prey item. The species is primarily an offshore fish but can be found inshore during summer and autumn (Wheeler, 1978). Whilst not likely to enter the Thames, *B. belone* start to move inshore during the late spring. The two *H. grypus* individuals that ingested garfish were reported stranded in May and October. Therefore, the seals may have fed outside of the Thames but were in the near vicinity as the remains still contained flesh and the meal was likely only a few hours old. Soft tissue would likely be fully digested within 2–6 hours based on estimates of food passage time in *Phoca vitulina* (Markussen, 1993). Many of the *B. belone* recovered were found without heads. The lack of heads in the digestive tract could be an indication that seals opportunistically forage on fish used as bait in long line fishing. The recorded diet of seals in the UK shows that the prey of *H. grypus* is dominated by sand eels whilst *Phoca vitulina* feed mainly on flatfish (Thompson and Duck, 2010; Brown et al., 2012). It is estimated that *H. grypus* would need to consume between 4 kg and 7 kg of food every day (Thompson and Duck, 2010; SCOS, 2016). *Phocoena phocoena* diet can vary seasonally and regionally but is typically dominated by sand eels and *M. merlangus* (Santos et al., 2004). *Phocoena* would need to ingest 4 kg of prey a day (Ross et al., 2016). Certainly, sand eels have been found to be a major source of microplastics for trophic transfer to higher predators (Welden et al., 2018).

It is possible to estimate marine mammal microlitter exposure using the predicted daily food intake above. Certainly, estimates of marine mammal exposure from extrapolation of prey microlitter ingestion is not a novel concept. Moore et al. (2022) estimated, based on inspection of five fish species, that *Delphinapterus leucas* (beluga whale) could be ingesting between 3,800 and 145,000 microplastics annually through trophic transfer alone. The standard length and weight of the fishes in the present study were used to estimate microlitter ingestion in Thames marine mammals. An average ratio between length (mm) and weight (g) was created for *M. merlangus*, *Platichthys flesus*, *S. solea* and *T. luscus* which were as follows: 1:0.59, 1:0.56, 1:0.38 and 1:0.40 respectively. These ratios were then used to

determine the average weight of fish ingested by *H. grypus* using the estimated length of fish from the recovered otoliths (Table 6. 4). Six otoliths of *M. merlangus* were recovered and the estimated length of the fish was between 127.6 mm and 238.6 mm (mean 189.4 ± 36.6 mm). The average weight of the fish is therefore 111.7 g. Six otoliths of *Pleuronectes platessa* and *Platichthys flesus* were recovered and the estimated fish length was between 186.7 mm and 300.2 mm (mean 254.5 ± 39.5 mm). As such, the average weight of the fish was 142.5 g. Only one *S. solea* otolith was recovered and the estimated fish length was 305.9 mm with a weight of 116.2 g. Four otoliths of *T. luscus* were recovered and the fish lengths were estimated as between 7.4 mm and 238.1 mm (mean 151.7 ± 104.3 mm). Therefore, the average weight of the fish is estimated to be 60.7 g. To consume 7 kg of prey *H. grypus* would need to consume 63 *M. merlangus*, 49 *P. flesus*, 60 *S. solea* or 115 *T. luscus*. *Phocoena phocoena* would need to ingest 36 *M. merlangus*, 28 *P. flesus*, 34 *S. solea* or 66 *T. luscus* to total 4 kg. From these calculations, it is possible to estimate the amount of microplastics Thames marine mammals are exposed to daily. But by underestimating the weight of fish, these estimates of microplastic transfer may be elevated. *Halichoerus grypus* could ingest 94.5 ± 94.5 microlitter items, 117.6 ± 166.6 microlitter, 84 ± 168 microlitter and 149.5 ± 195.5 microlitter per day from *M. merlangus*, *P. flesus*, *S. solea* and *T. luscus* respectively. *Phocoena phocoena* could ingest 54 ± 54 pieces of debris, 67.2 ± 95.2 microlitter, 47.6 ± 95.2 microlitter and 85.8 ± 112.2 microlitter per day from *M. merlangus*, *P. flesus*, *S. solea* and *T. luscus* respectively.

It should be noted that otoliths slowly decrease in size as they are digested, with larger otoliths therefore remaining in the diet longer than smaller ones. This creates a bias towards identifying larger prey species (Ross et al., 2016). As a result of this decreased otolith size, it is acknowledged that the estimates of fish size in the diet of *H. grypus* is likely an underestimate. For instance, the largest *P. flesus* collected in trawls in the present study weighed 363.4 g. The otoliths collected from the seal GIT, however, was only estimated to weigh 142.5 g. It is possible that the otoliths were degraded and thus estimates of fish size are lower than the true values. Certainly, a fish of this size would be easily ingested by both *H. grypus* and *Phocoena.* If the largest fish collected in trawls from the present study were used to estimate microlitter exposure, to consume 7kg of prey *H. grypus* would need to ingest Between 17 and 46 fish depending on species. Similarly, *P*. *phocoena* would need to consume between 10 and 26 fish to consume 4 kg. This would result in a daily exposure of ca. 24 to 46

microlitter items a day for *H. grypus* and 14 to 34 for *P*. *phocoena.* Even using the weight of fish collected from the estuary over estimated size from otoliths in the diet, microlitter load in marine mammals is underestimated compared to recorded values.

Size has been shown to be a significant factor affecting microplastic ingestion in some cases. Both Hossain et al. (2020) and Hara et al. (2020) found that larger crustaceans ingested more microplastics. The same is true for fish, with McIlwraith et al. (2018) reporting that larger fish contained more plastic. That study also concluded that translocation from the GIT to other tissues occurred more frequently in smaller individuals. In the present thesis, however, length and mass of *P. flesus*, *S. solea*, *O. eperlanus* and *C. crangon* did not significantly affect microlitter ingestion. Despite trophic transfer likely occurring in the Thames Estuary food web, the results of the present study demonstrate that when organism size is controlled for, top predators do not ingest the most microlitter. In fact, organisms from trophic level one had the highest size-specific ingestion rate for microlitter. This is likely due to the high level of microlitter in *Corophium.* It is likely that feeding strategy exposes certain species to more microlitter than through trophic transfer. Indeed, a review by Walkinshaw et al. (2020) concluded that lower trophic levels were more at risk of ingesting microplastics.

Even though microplastic biomagnification through the food chain might be negligible, the impacts of plastic consumption at the lower trophic levels could still be having considerable negative impacts on top predators. Foley et al. (2018) concluded that a reduced consumption of food by zooplankton, perhaps the most susceptible group to microplastic ingestion, and invertebrates exposed to microplastics would have knock-on effects for predators. Reduced reproductive outputs and smaller prey would mean a reduction in the available food for predators, increasing competition. Modelling predictions, however, find a negligible effect of microplastic exposure on population dynamics of both prey and predators, even when reduced feeding rate is included in the model (Huang et al., 2020).

Megaptera novaeangliae and *Balaenoptera borealis* were found to have microplastics on their baleen. Marine mammals, such as the whales in the present study, are often considered sentinels for the health of the ocean (Bossart, 2011). Indeed, anthropogenic debris is well established as a significant threat to marine megafauna, through both ingestion and entanglement. Over 100 marine mammal species and all seven marine turtle species are

known to be affected by anthropogenic debris (Kühn et al., 2015; Poeta et al., 2017; Kühn and Van Franeker, 2020). Few studies, however, have examined microplastic ingestion in these large animals (Poeta et al., 2017; Nelms et al., 2019), with only one report, prior to the present thesis, of microplastic ingestion by *Megaptera novaeangliae* (Besseling et al., 2015) and no evidence of ingestion by *Balaenoptera borealis*. Estimates of exposure have been calculated for *B. borealis* based on microplastic ingestion by prey species (Burkhardt-Holm and N'Guyen, 2019). Other cetacean species have been studied but not as extensively as it is easier to sample groups such as fish. Certainly, there is a bias in documenting macroplastic ingestion and entanglement over microplastic exposure, likely due to the difficulty of recording the latter (Poeta et al., 2017).

In the present study, average microlitter abundance per baleen plate was extrapolated to estimate the abundance of microplastics in the baleen of sampled mysticete species. There are between 270 and 400 baleen plates in *M. novaeangliae* (Bradford, 2017) and between 219 and 410 baleen plates in *B. borealis* (NOAA Fisheries, online). In the present study it is estimated that up to nearly 7,500 microplastics could be present on the baleen. Comparatively, recorded concentrations in the digestive tract of these species are lower (Besseling et al., 2015) which indicates that the baleen may retain plastic for longer, whilst they are likely to be transitory in the digestive tract (Nelms et al., 2019). The increased retention time of microplastics could expose baleen whales to associated chemicals (Rochman et al., 2014) for longer than odontocete, toothed whales. Indeed, cetaceans have a reduced potential for population growth when exposed to polychlorinated biphenyls (Hall et al., 2018), which are often associated with plastics (De Frond et al., 2019; Yeo et al., 2020). It is, however, a common chemical contaminant in the environment and plastic is not the only exposure pathway. Locality of foraging, however, determines the threat of exposure to harmful chemicals (Fossi et al., 2016) and for the present sample it is unlikely that the whales were feeding immediately prior to stranding.

It has long been suggested that filter-feeding as a feeding strategy can result in organisms being more susceptible to microplastic ingestion (Moore, 2008). Mussels have been used as a model organism to record this exposure (Farrell and Nelson, 2013; Van Cauwenberghe and Janssen, 2014; Van Cauwenberghe et al., 2015; Li et al., 2018; Phuong et al., 2018). Since 2012, it has been proposed that filter-feeding megafauna are also highly

impacted by microplastics (Fossi et al., 2012; Fossi et al., 2014; Germanov et al., 2018). Indeed, Poeta et al. (2017) and Kühn and Van Franeker (2020) noted that mysticete whales most frequently ingested microplastics compared to other marine mammals. In the present study, pinnipeds and porpoises ingest far less plastic than recorded in the Mysticeti. This could be due to the size of the animals in addition to feeding strategy. Certainly, models predict that filter feeding exposes whales to microplastics that are present in sea water. Zantis et al. (2022) collected scats from sei and Bryde's whales recording plastic occurrence and identifying the main prey items to predict exposure to microplastics through trophic transfer. The study reported that over 24,000 particles could be ingested by filter feeding whales per mouthful of prey. Comparatively, water samples produced estimates of exposure four orders of magnitude lower. Zantis et al. (2022) concluded that trophic transfer was the main route of exposure and that whales could ingest three million particles a day. Like the present study, blue and black fibres were dominant in the scats, mostly cellulosic and suspected to originate from textiles, cigarette filters and tampons.

6.5 Conclusion

In conclusion, microplastics are present at all levels of the Thames food web. The present chapter provides evidence to support the likely transfer of microplastics from prey to predators, but whilst higher quantities of plastic and semi-synthetic microparticles were detected in top predators, when the size of the animal is considered, biomagnification does not seem likely. The present chapter also demonstrated that selective feeding at one level of the food web may affect the diversity of microplastics present higher up the food chain. It is clear that, whilst some species might be more likely to ingest certain forms of plastic, others are less discriminatory. Certainly, evidence suggests that filter feeding mysticete species are prone to ingesting large quantities and diversities of microplastics. Further investigation is needed into the retention time of microplastics at each level of the food web and the potential impacts of plastic ingestion. Additionally, as highlighted by Au et al. (2017), microplastic exposure studies typically focus on primary microplastics, typically microbeads. Whilst, in reality, a greater diversity of plastics are present in the environment and ingested by wild-caught biota. Future studies should explore the consequences of ingesting a diverse array of particles and explore plastics that are harder to obtain than microbeads. This chapter demonstrates that sampling multiple species in a sampling program provides a truer

representation of environmental contamination in an ecosystem than monitoring a single species alone.

Chapter 7

Conclusions, implications and future directions

In summary, this thesis has tested a range of microplastic extraction techniques and applied them to the quantification of subsurface macroplastics and of microplastics in sediment and biota. Evidently, both macro- and microlitter are abundant in the River Thames estuary and a combined monitoring strategy for both can provide evidence for both sources of plastic and the potential risk it poses. Yet, it must be noted that ecotoxicological assessments are needed to truly determine risk. Furthermore, there is temporal variation in microlitter accumulation in sediment as well as in ingestion by fishes and shrimp. As such, monitoring should be carried out seasonally rather than annually to truly capture the pattern of contamination in the study system. Indeed, monitoring ingestion by biota is indicative of contamination in the surrounding environment (Gouin, 2020). Monitoring and research should also consider species selection carefully. As demonstrated in the present thesis, microlitter ingestion can vary greatly between species. For instance, brachyuran crabs, like other crustaceans, can accumulate large tangles of plastic in the GM but this varies by species and is less frequently reported in other groups, such as fishes. This highlights the need to understand the functional morphology of selected bioindicator species and how this might impact microplastic ingestion and retention. Feeding strategy and trophic level might also play a role. Certainly, data collected for the present thesis suggest that trophic transfer likely occurs in the food web, however, biomagnification is unlikely. Filter feeders, such as baleen whales, might be at greater risk due to their non-selective feeding strategy. Indeed, studies have demonstrated that even within one species, differing feeding modes can greatly impact the amount and type of plastic that is bioavailable (Porter et al., 2023). The present thesis has highlighted the knowledge gaps in our understanding of trophic transfer and the microplastic pollution in ecosystems as a whole, using the Thames Estuary as a case study. For the Thames, further research into small fish species, such as gobies which occupy intermediate trophic levels; spatial data and modelling are needed to better address sources of pollution and the movement of microlitter within the estuary. In addition, subsurface water samples are required to better understand the distribution of microlitter in the water column which can then be used to further our understanding of microlitter exposure for fauna in the estuary.

7.1 Methodology

The protocols used in the present thesis (e.g., 10% KOH digestion compared to enzymatic and other chemical digestions) were deemed the most suitable. They were efficient, combining effective recovery of microplastics during extraction and low cost. It was concluded that both recovery and cost need to be considered when moving towards method standardisation. Protocols need to be suitable for large scale monitoring as well as ad hoc studies and budget is usually a limiting factor. KOH was found to be an affordable and effective solution for the digestion of organic matter. During digestion of oily fish (e.g., *Merlangius merlangus*) it was noted that a residue formed over the supernatant. For the sake of comparability and standardisation, KOH digestions were used for all species with no modifications to account for this residue. It was observed that the oily residue could be punctured, and the supernatant filtered. The residue then coated the filter which impeded the recovery of fibres. Whilst this is a limitation of the current protocol, it is not thought to have significantly reduced microplastic counts as the results are similar to those in the literature. This highlights, however, that standardisation might not be the best approach moving forward. Instead, aligning methods so that they are harmonised, providing comparable results without the need for strict standards, would allow for more flexibility and ensure that protocols can be optimised for the samples being analysed.

No quality assurance was conducted for sediment extraction methods as were undertaken with digestion protocols. Whilst not discussed in Chapter 2, $ZnCl_2$ and the use of a sediment-microplastic isolation unit are an effective and easy to use protocol for the recovery of microplastics from sediment. Due to its high density, $ZnCl_2$ can recover high density polymers which might be missed by cheaper options, such as NaCl. It should be noted, however, that in the present thesis microplastic recovery was not verified with spiked samples. A $ZnCl_2$ density separation was used by another member of the lab group who endorsed the methodology (Daniella Hodgson, pers. comm.). As such the protocol was implemented in the present thesis, with the added benefit that it would allow for comparisons to be made between the studies. The results presented in the thesis are comparable to concentrations in other estuaries, thus providing evidence that $ZnCl_2$ is a robust method for the extraction of microlitter from environmental samples. It is of note that the fine muddy sediments in the Thames Estuary resulted in fine sediment being suspended even after the

solution was allowed to settle in the SMI unit. This visibly masked some microplastics on filters, which meant that the time to search filters increased. This could be resolved by splitting the solution across several filters. Alternatively, the use of a fluorescent dye, such as Nile red, could increase the visibility of plastics in samples and reduce the screening time (Maes et al., 2017a; Nalbone et al., 2021; Prata et al., 2021).

With regards to FTIR analysis, a 10% subset is typically advised (Lusher et al., 2017). The MSFD suggested that all particles less than 100 µm be analysed by FTIR, or other suitable means. In addition, 10% (up to 50 items) of larger plastic pieces should be analysed per year or sampling occasion, whichever is least frequent (Hanke et al., 2013). In the present thesis it was not possible to portion FTIR analysis by particle size. Due to logistical constraints, such as laboratory access, microplastics were analysed by FTIR prior to being measured in ImageJ. The size of the subset analysed by FTIR in the present study was deemed sufficient since 1,150 items were analysed. If 50 items were analysed per sampling season only 400 items would have needed analysis. Certainly, more than 50 items were analysed for almost all studied species except for *Hediste diversicolor* (39 items), *M. merlangus* (7 items) and *Crangon crangon* (0 items). Due to the time constraints with the FTIR analysis posed by the Covid-19 pandemic, no FTIR analysis could be conducted for *C. crangon*. Only 142 items were recovered from the species and thus only 14 items would have been analysed. After contamination was controlled for, *H. diversicolor* only ingested 33 items. As such all items recovered from the species were analysed. *Merlangius merlangus* was not common in samples, with only 30 individuals collected. As such, whilst a 10% subset was analysed other species were prioritised if time allowed for additional analysis.

The detection limit of the present thesis was limited by the filter pore size. This was primarily 32 µm, apart from marine mammal GITs which were filtered with a 300 µm mesh. Most items recovered in the present thesis were larger than 32 µm, but some litter that was smaller was also recovered. For instance, a 14 µm object was recovered from *Corophium volutator*. A smaller mesh size would increase the number of microplastics recovered and give a more accurate representation of microplastic ingestion, but this would be impractical for marine mammals where prey items and parasites need to be recovered prior to digestion. This is in line with recommendations from Lusher and Hernandez-Milian (2018) who suggest maximising sample collection whilst minimising effort. One possible compromise would be to

collect a subsample for this prey/parasite analysis and to digest the remaining majority of material to filter with a smaller mesh. Alternatively, stacked sieves, as suggested by Lusher and Hernandez-Milian (2018), could also be used. Although this requires additional time to filter all size fractions. With regards to processing mammal digestive tracts for microplastic extraction, the findings from the present thesis indicate that systematic sampling the length of the GIT could provide insight into the retention time of litter. Indeed, this approach has been implemented for parasitology (Dr Natalia Fraija, pers. comm.).

Contamination was strictly monitored for the present thesis. The literature is vague in its accounts of how contamination controls are collected and accounted for in the data. Whilst there is a movement towards transparency in reporting, as yet there is no standardised way to remove contamination from results. Typically, an average of reported contamination from procedural blanks is removed from all samples. This method is the simplest to apply. In the present thesis, every effort was made to thoroughly report contamination control measures as this is deemed an essential step for harmonisation of protocols and is necessary to have truly comparable data. In the present thesis, controls during dissection and filter searching were averages just for the animals processed under that specific control. This allowed for more accurate contamination estimates. For instance, if the lab space was shared on a certain day only samples analysed on that day would be affected by elevated contamination. Recent studies have recommended using LOD and LOQ (e.g., Uhl et al., 2019; Carlsson et al., 2021). These are calculated as 3× standard deviation and 10× standard deviation respectively. This approach is usually taken when all microplastics have been analysed by FTIR. LOD and LOQ are applied to each polymer type separately, e.g., PES or PP. It is possible to apply the technique in the same way that FTIR subsets were identified in the present study, i.e., LOD and LOQ calculated for red fibres, films, fragments and bead separately. Given the diversity of plastic recovered and the way controls were calculated in the present thesis this was deemed unfeasible. Instead, LOD and LOQ values for count data of all microlitter, not subdivided by polymer of form, are reported. The data are, however, still corrected for the items found in controls rather than LOD.

7.2 Macrodebris: future monitoring of plastic pollution, mitigation strategies and solutions

There is no statutory river monitoring in the UK (McConville et al., 2020). The present study certainly highlights that ad hoc monitoring of plastic pollution is inadequate to identify and address trends and temporal variation in plastic accumulation and transport. It is my view that regular and long-term plastic monitoring of riverine plastic debris should be funded in addition to implementing policies addressing the production and use of plastic products. Indeed, since the Government claims that implemented mitigation measures result in delayed reductions in the field, then they should be invested in monitoring the pollution to ensure effective strategies are enforced. To verify the success of implemented measures, such as the construction of the Thames Tideway Tunnel to reduce the input of sewage-related debris to the Thames, frequent monitoring before and after construction is required. Baseline data need to be collected before any conclusion on the success of measures can be made, and with a system as dynamic as the Thames Estuary, monitoring should occur frequently. The conclusion below is based on the results of the present study which just presents a snapshot of Thames pollution. An ongoing monitoring programme could provide even greater insight into plastic accumulation and movement and, therefore, provide even more useful in combating the issue.

Riverine plastic pollution is often cited as a route for plastic from terrestrial sources to enter the sea. Floating litter is well document, even in the Thames Estuary. Yet, the plastic in the sub-surface water column and on the riverbed has rarely been studied due to the difficulty of sampling. Chapter 3 is the first study to compare plastic abundance on the Thames Estuary riverbed to subsurface floating plastic debris, providing evidence for how varied types of litter can be transported in different compartments in the water column. Indeed, the data can be used to inform transport models, guide mitigation measures at source and aid clean up initiatives within the estuary or on the coastline once litter has drifted downstream. The results of the present thesis indicate that plastic accumulates on the riverbed to a greater degree than it is transported in the water column. On the riverbed it may be retained for long periods, with several 30-year-old plastic items recovered. Further study is needed to determine the retention time of this benthic debris and the rate at which it fragments into microplastics which can then be ingested (as was documented in Chapters 5 and 6). It was also noted that the colour of recognisable products was not always as expected (i.e., a Hula

Hoops packet was white not red and a Milka chocolate bar was blue rather than purple), indicating that dyes, and likely other chemicals, leach from the products. These chemicals could have negative impacts for the wildlife in the estuary and the effects of this plastic debris needs to be explored further. Quantifying macroplastic pollution is key to identifying the major sources in the estuary, especially given that it can be difficult to determine what products microplastics may have originated from. By identifying common sources of pollution and dispersing the information to the public, press and policy makers it is hoped that the present thesis can have a positive effect on the health of the Thames and other rivers. The present study highlighted that one of the main sources of plastic in the Thames Estuary was CSOs, which are responsible for the abundance of wet wipes and sanitary products on the riverbed and foreshore. Moore (2008) noted that catchment systems can be installed in storm drains and pumping stations to remove macroplastic. This action could reduce plastic in the estuary if applied in the many major urban catchments along the River Thames.

The quantity of plastic packaging on the Thames foreshore has increased over 20 years and volunteering efforts have not successfully reduced its abundance (McConville et al., 2020). The findings in the present thesis align with those in the literature, noting that packaging and single-use plastics are common pollutants. Single-use plastic has received considerable attention from the press and policy makers. As a result of the abundance of single-use items in the environment, many activists and organisations have called for extended producer responsibility. At present, much of the responsibility lies on the public with advertising and policy driven towards the consumer not recycling enough, improperly disposing of waste and choosing to shop responsibly. Whilst consumer demand can direct the course of a company to an extent, manufacture of a product is not solely driven by consumer demand. Indeed, a survey by Greenpeace UK (2017) demonstrated that six of the world's largest soft drink producers were taking no steps towards reducing single-use plastic but instead opted to create thinner products that used less material. Certainly, in the UK, soft drinks companies are some of the biggest plastic polluters (Stanton et al., 2022). In 2020, Coca-Cola defended its production of so many single-use plastic bottles. An executive of the company announced that Coca-Cola would continue to produce plastic bottles as it believed customers preferred them to alternatives, such as cans and glass bottles, as they could be resealed (Bandoim, 2020).

Whilst studies into the amount and type of macrodebris in the environment are common, brand audits are not usually conducted alongside these studies. Brand audits are powerful tools that can be used to inform policy (Stanton et al., 2022), especially those targeted at producers, such as extended producer responsibility. Without such tools, it is harder to determine which industries to target and it is not possible to identify top polluting companies. Extended producer responsibility would dictate that companies should implement effective practices to eliminate plastic entering landfills. A proposed scheme under extended producer responsibility is buyback initiatives where companies are required to collect or buy back their single-use products. This would promote recycling and reuse of single-use plastics which would otherwise end up in landfill. This in turn would impact the manufacture of products that organisations were now required to dispose of themselves. It is noted, however, that such schemes must be profitable to be successful and that a lack of enforcement currently sees such schemes failing (Vimal et al., 2022). A deposit return scheme has been proposed in the UK to tackle single-use plastic bottles (Smith et al., 2018, Defra, 2019). A similar scheme has been used in Germany, with bottles returned to manufacturers to recycle or reuse (Rhein and Sträter, 2021). Additionally, reducing the number of bottles that are incinerated and sent to landfill could greatly decrease CO_2 emissions (House of Commons Environmental Audit Committee, 2017). Plastic bottles were rarely recovered in the present study highlighting the importance of monitoring more than one environmental compartment. Bottles are likely quickly deposited on the foreshore or remain afloat, especially if the cap remains on the bottle trapping air inside. Indeed, they are common at so-called 'floating' sites on the Thames foreshore. Extended producer responsibility and a deposit return scheme could see this plastic waste reduced in the Thames. Certainly, when disposal of products is the responsibility of the manufacturer, they are more likely to consider disposal in the initial product design. For instance, in an attempt to reduce the company's impact on the environment, Coca-Cola recently announced that it had produced a prototype for a 100% paper bottle that was completely recyclable (Coca-Cola, 2020). It must be noted, however, that alternatives to plastic are not always better for the environment, with glass bottles having a greater negative impact compared to plastic bottles in all tests (Brock and Williams, 2020). For many, reduced consumption of products is considered the only way to mitigate waste and negative environmental impacts. Continued consumption with alternative materials may still come at a cost.

"Bioplastics" are one such alternative to traditional plastics. The term can be used to refer to several products including biodegradable and compostable plastics as well as plastics produced from plant biomass rather than utilising fossil fuels (UNEP, 2015). It has been demonstrated that labelling of such products can result in increased littering as items are misinterpreted as safe for the environment (Keep Los Angeles Beautiful, 2009; British Plastics Federation, 2019). Certainly, a biodegradable plastic dog waste bag was recovered in the present study. It cannot be known how long the bag was in the Estuary, but it was intact when recovered and may have been deliberately littered. For many bioplastics to biodegrade, however, the plastic needs to be treated in an industrial composter which typically requires temperatures of 70 °C. Certainly higher than the temperatures in the Thames Estuary. In Europe, to be labelled as a biodegradable or compostable product, at least 90% of the material must be converted to CO_2 withing 6 months (UNEP, 2015). Without an industrial composter, it is not known how long these products may take to degrade. Additional concerns arise when considering products that promote fragmentation rather than degradation. Many bioplastics contain pro-oxidants that result is plastic fragmenting. Products containing pro-oxidants contribute to microplastic concentrations, may not degrade and cannot be recycled in traditional schemes (UNEP, 2015). I believe that further evaluation of environmental decomposition times is required and that this information should be made clear to the consumer through product labelling. Consumer surveys can be used to identify gaps in consumer knowledge, and this can be addressed with clear product labelling. Indeed, better product labelling of wet wipes, a common item in the present study, would reduce the amount found in the Thames. Furthermore, there is now a campaign from Fleur Anderson MP to ban plastic in wet wipes.

It has been suggested that producer compliance fees be increased to better cover the costs of collecting and sorting waste; this is being considered by the Government (House of Commons Environmental Audit Committee, 2017). Indeed, the British Plastics Federation (2019) claims to support extended producer responsibility and agreed that the cost of collection and recycling of waste be moved away from local councils. The group also proposed, however, that educational campaigns are implemented to make consumers more active in recycling, effectively shifting responsibility away from producers. Certainly, including brand audits in ongoing monitoring of macroplastic pollution would determine whether

certain producers are responsible for more waste than others and could inform targeted strategies. Additionally, brand audits utilising citizen science could incorporate some of the education outreach suggested by the British Plastics Federation (2019).

Certainly, research into environmental contamination alone cannot solve all the issues associated with plastic pollution. Outreach and social science are essential tools for understanding public opinion and behaviour. Completing this PhD at the NHM provided numerous opportunities to engage in public communication activities. From my personal experience, I discovered that many members of the public still consider recycling as their first and best action to reduce their plastic waste rather than reducing consumption of plastic products. Recycling has long been heralded as a solution to plastic waste. It was in fact the plastics industry itself that first suggested the solution in the 1980s as public concerns over plastic waste were on the rise. The recycling system, however, is flawed. At present, the proportion of plastic which is recycled is low: 31% of post-consumer waste and 41% of plastic packaging were recycled in the EU in 2016 (PlasticsEurope, 2018a). One reason plastic might not be recycled in the UK is the insufficient infrastructure in place to deal with reprocessing. The country is therefore dependent on exporting waste to be recycled (British Plastics Federation, 2019). Additionally, recycling plastic is often costlier than using virgin resin and recycling does not easily remove contaminants in the product (Moore, 2008). Furthermore, no two plastics are identical with different products containing different combinations of plasticisers, dyes and flame retardants even if they are made from the same polymer. This further hinders the promotion of recycling. To overcome these difficulties in recycling plastic, virgin plastic is included in many recycled products (British Plastics Federation, 2021). As a result of these limitations, leading researchers in marine litter have expressed concerns over circular economies which only promote recycling of products. It has been argued that a true circular economy requires a product to be recycled and reused in the same form it was created in (i.e., a plastic bottle remains a plastic bottle) and not down-cycled, with product designers and end-of-life management communicating to achieve the best product life cycle (Thompson, 2018). Ultimately, whilst recycling plastic can reduce the production of virgin plastics, it is not a solution in and of itself. Certainly, without improved technologies, the world remains to an extent dependant on virgin material. Dissemination of research to the public as well as policy makers is essential to ensuring they can take informed actions.

Citizen science monitoring, often in the form of beach cleans, can help to remove plastic from the environment and raises awareness of local pollution within a community. But, without additional regulations further up the chain, plastic pollution cannot truly be stopped. It is often described in the analogy of an overflowing bath. You go into your bathroom to find the bath is overflowing. The tap is running. There is water covering the floor. What is your first action? Do you grab a mop and bucket to remove the water building up or do you first turn off the tap? Cleaning plastic from beaches is simply removing the water from the bathroom floor whilst more continues to flood in from the tap. Ultimately, whilst monitoring and clean-ups are essential, they are not in themselves a solution and must be transformed into actionable change. Indeed, despite 97,000 plastic bottles having been removed from the Thames to date (McConville et al., 2020), litter quickly returns to the foreshore. The evidence collected by citizen science and monitoring, however, such as the data collected in the present thesis, is invaluable for decision making.

A reduction in the use and production of single-use items would prevent over 60% of the litter recovered from the Thames during beach cleans (Fischer, 2019), which also accounted for nearly 60% of the waste in the present study. Whilst removing plastic from the environment is an essential tool for data collection and improving environmental health, clean-ups are costly. In Spain, the average amount of litter removed during beach cleans was 27.6 kg per hour per person, and the average cost of removing the waste was €1 per kg (Cruz et al., 2020). As such, it would cost nearly €30 per person to conduct a one-hour beach clean. This cost would quickly mount if implemented regularly and on a large scale. This could incur considerable cost to local councils, especially given some municipalities paid far higher than the average cost for beach cleans (Cruz et al., 2020). These costs are especially great on a national scale. For instance, the NOAA in the United States spent $2 million per annum on removing lost fishing equipment as part of a project to protect the endangered Hawaiian monk seal, *Neomonachus schauinslandi* (Moore, 2008). Fishing gear is not the main type of litter recovered in the present study as there is no commercial fishing permitted on the Thames Estuary. It is, however, a major source of pollution in the ocean and some ocean plastic can move into the estuary with the tides. Cruz et al. (2020) suggested improving waste management practices and reducing the cost by the continued monitoring of how efficient beach cleans are. The House of Commons Environmental Audit Committee (2017) suggested

that beach clean-ups should receive additional funding in an effort to better reach the targets for the UN sustainability goal 14 “Life under water”, which addressed marine pollution among other issues. They also requested that a single-use plastic tax be implemented, targeting plastic production. Additionally, the cost of beach cleans could be minimised by choosing efficient labour and having targeted beach cleans. One such example of how to improve litter removing efficiency is SCRAPbook (www.scrapbook.org.uk). The organisation has invited the public to count and categorise litter from aerial photos of Scotland’s coastline and plan to use the data collected to help create targeted beach cleaning efforts. Certainly, citizen science efforts in the Thames Estuary mean that hotspots for plastic waste have already been identified. Monitoring of the riverbed could provide similar information, but this information can only be attained through trawling or setting up fyke nets in the estuary rather than with aerial photography.

Commonly policy changes have been used to target specific types of plastic that frequently leak into the environment (Karasik et al., 2020). Data, such as that presented in this thesis, are needed to address the gaps in legislation and highlight the key polluting products and waste management systems. On such example, which has quantifiable evidence of its success, was the carrier bag levy implemented by the UK Government in October 2015 (HM Government, 2018). The policy required large stores to charge 5p for plastic bags (Defra, 2018). In many stores this price has since increased to 10p per bag. Restrictions on the manufacture and sale of plastic bags have also been implemented worldwide, with 43 countries having legislation on the topic (Karasik et al., 2020). In England, the carrier bag charge successfully reduced plastic bag consumption by over 80% (Defra, 2018; HM Government, 2018). Whilst the present thesis reported carrier bags as one of the major forms of pollution in the estuary, many of the bags were several years old. It is likely that the plastic bags on the riverbed either represent historic pollution or runoff from a nearby landfill site. It demonstrates that whilst legislation on the use of certain products can reduce waste, further action is needed to tackle the pre-existing waste in the environment. This, however, would be a costly and time-intensive procedure and will not always be a practical solution.

Plastic packaging, especially food and cigarette packaging, was the most common plastic item recovered in the present study. By combining product type with the brand audit it is possible to determine that Pepsi Co.’s contribution to pollution in the Thames comes from

food rather than the beverages it is most famous for producing. An estimated 20 million crisp packets are produced in the UK each day, half of which by Walkers (Quinn, 2018). Walkers and Doritos crisp packets accounted for all the PepsiCo products recovered in the present study. The other most polluting brands were manufacturers of chocolate, such as Mondelez and Mars. This highlights the importance of targeting the food packaging industry and waste management streams. So far 111 organisations have signed the UK Plastic Pledge that by 2025 all plastic packaging will be recyclable and compostable, 70% of packaging will be recycled or composted, single-use plastic will be avoided through product redesign and an average of 30% of material in plastic packaging will originate from a recycled source (Wrap, 2018). Given that some of the packaging recovered in the present study was 30 years old, truly biodegradable alternatives to plastic would reduce the lifespan of these items and may reduce their impact on the environment. Of the organisations to sign the pledge, 68 are business members, representing various sectors of the plastic manufacture, sale and management process. These businesses are estimated to be responsible for 80% of plastic packaging in supermarkets and half of the plastic packaging in the UK (Wrap, 2018). Support from these partners is key to mitigating the impacts of plastics. Wrap provided partners with a 5-year plan to reduce plastic waste across the food and drink, clothing and textiles and electronics and technology sectors, these industries correspond with the main sources of macroplastic and microplastic pollution identified in the present study. The plan, however, is not enforced to ensure that companies remain on track (Smith et al., 2018). The plastics industry has also pledged support for mitigating plastic pollution. PlasticsEurope has published its support of the European Commission's pledge to create a more circular economy for plastic and has implemented a plan to reduce plastic waste entering the environment by 2030 (PlasticsEurope, 2019a). An example of how evidence from environmental studies can impact product design comes from beverage companies. Mounting evidence of marine life getting entangled in six-pack rings resulted in manufactures changing the formula such that the material became brittle in the environment. The resulting fragments, however, were still long-lived (Moore, 2008) and, instead, could be ingested by marine organisms. Whilst no six-pack rings were recovered in the present study, mesoplastic and microplastic fragments and films from larger products were found in the GIT of organisms in the present study. This highlights that plastic fragmentation is not a solution to plastic

pollution and that the production of microplastics needs to be considered when planning for product end of life.

7.3 Microlitter: environmental contamination, ingestion and actions to target sources of pollution

This thesis is the first to explore the transfer of microplastics from prey to predators in the Thames Estuary, including species for which there was limited evidence of exposure. Whilst no evidence of biomagnification was found in the present study, the retention time and impacts of ingesting microplastics both directly and through trophic transfer are not fully understood. Certainly, abundance of microplastics in the environment and land use relate to microplastic ingestion. The present thesis concluded that seasonal variation occurs both in microplastic abundance in the environment and in the amount ingested by estuarine fauna. To understand the implications of plastic exposure, it is also necessary to address this variation in plastic abundance. By determining the mechanism(s) that affect the variability in microplastic exposure and accumulation, solutions can be designed to mitigate the negative effects. By determining that fibres are most abundant in the environment, as was found in the present study, WWTPs and washing machine outlets have been identified as key sources of pollution. As a result, filters for washing machines have been developed.

Campaigns to reduce plastic pollution often focus on macrodebris, specifically single-use packaging, often asking consumers to reduce their use of these polluting products. Despite pressure on the consumer, governments rarely implement policies which support outreach and education (Karasik et al., 2020). Microplastics are, however, more abundant, as described in this study, and harder, if not impossible, to recover once in the environment. One of the first major policy changes addressing plastic pollution in the UK was the introduction of a ban on microbeads (HM Government, 2018). Microbeads, however, are still present in the environment. Microbeads were present in samples collected in this study, albeit in relatively small numbers. Higher quantities of microbeads were recovered from surface water samples in the Thames (Rowley et al., 2020). The study by Rowley et al. (2020) reported that microbeads were present throughout the year at Putney and Greenwich and estimated that over 5,000 microbeads could be flowing past Greenwich at any given time. This again demonstrates that monitoring one environmental compartment does not provide

a complete understanding of contamination in the system. Furthermore, without robust monitoring programmes, it is difficult to determine which sources of pollution are greatest. While microfibre contamination is far greater than that of microbeads, resolving this problem is a more complex challenge.

Microfibres, often originating from textiles, dominate microplastic compositions worldwide and the same is true in the Thames Estuary, as demonstrated in the present thesis for both sediment and biota. An estimated 22 million tonnes of textile fibres are expected to enter the marine environment between 2015 and 2050 (Ellen MacArthur Foundation, 2017). Whilst textile fibre release is recognised in the scientific literature (Napper and Thompson, 2016), to the public, clothing is a lesser-known source of plastic pollution. Additionally, few policies address microplastic pollution (Karasik et al., 2020), likely as it is harder to monitor and control. Whilst the field is not yet able to produce comprehensive risk assessments for microplastics in the environment, with crustaceans in the Thames having tangles of fibres filling the GM and plastic ingested at all trophic levels, it is evident that the potential for harm caused by microfibres is high. Policy and technology advances are required to address this source of pollution.

Fast fashion, arguably one of the causes of the rapid release of microfibres in the environment, was developed in the 1980s as apparel production accelerated and quick turnarounds of fashion trends became the norm (House of Commons Environmental Audit Committee, 2019b). Clothing production has doubled in the last 15 years (Ellen MacArthur Foundation, 2017) and the average lifetime of a garment is estimated to be only 3 years (Wrap, 2017). Despite this, over half of items do not even last a year before being disposed of (Ellen MacArthur Foundation, 2017). Current production and disposal practices are unsustainable because these produce chemical and plastic pollution (Ellen MacArthur Foundation, 2017; House of Commons Environmental Audit Committee, 2019b). Additional environmental concerns come from the water use and carbon footprint from the manufacture and cleaning of these products (Ellen MacArthur Foundation, 2017; Wrap, 2017; House of Commons Environmental Audit Committee, 2019b). Indeed, the textiles industry contributes more to climate change than international aviation and shipping combined (House of Commons Environmental Audit Committee, 2019b) and in Europe clothing sales and manufacture are the fourth highest household contributor to climate change (Wrap,

2017). Synthetic clothing can produce twice the CO_2 of an organic alternative (House of Commons Environmental Audit Committee, 2019b). Whilst switching to natural materials (e.g., cotton) can reduce the carbon footprint, it comes with its own costs for water and land use (Wrap, 2017).

The issue, however, is not simply one of environmental costs. Low-cost clothing is often produced at the expense of those manufacturing it, with many factories using child labour, forced labour and prison labour. Labour exploitation is even reported in the UK, with some factories paying below the minimum wage (Ellen MacArthur Foundation, 2017; House of Commons Environmental Audit Committee, 2019b). Stringer et al. (2020) claimed that consumers are likely to change shopping behaviours over ethical and environmental concerns but are more likely to respond to environmental and animal welfare concerns than worker welfare issues. The authors claimed that this was driven by the marketing of fast fashion companies, which promote organic materials and often neglect to discuss worker conditions. The results were, however, collected in a survey where respondents may feel pressured to appear more active and concerned. Respondents may be optimistic in their idealised responses. If companies were to be transparent and publish their supply network, it would likely see a reduction in the support for fast fashion from the consumer.

Manufacture and sale of clothing has increased considerably (Wrap, 2017), especially in the UK, where more garments are purchased per person than any other European country (ECAP, 2018). Yet, many of the products produced cannot be recycled, or can only be recycled once. Less than 1% of the materials used to manufacture clothes are recycled to make new clothes (Ellen MacArthur Foundation, 2017; House of Commons Environmental Audit Committee, 2019b). Kwon et al. (2019) concluded that clothes were likely to be discarded if they were old (worn or faded), no longer fashionable, no longer suited the wearer's age or social status or if new clothes have been purchased. Social and physical attributes, such as fashion and aging of clothing, had more influence than impacts on resources (i.e., time and money). Indeed, Degenstein et al. (2020) found that consumers were more likely to dispose of garments that showed minimal damage rather than repair them. The higher quality and more expensive items required more damage before they were likely to be thrown away. It is estimated that 300,000 tonnes of textile waste enter household waste per annum (Wrap, 2017), with 73% of garments being sent to landfill or incinerated (Ellen MacArthur

Foundation, 2017). Only a small portion of textile waste is recycled, and it is often downcycled to products that are hard to recapture, such as insulation and mattress padding. This ultimately results in the recycled product being the materials final use (Ellen MacArthur Foundation, 2017). Despite being aware of the environmental impacts of sending items to landfill, consumers were less likely to sell or donate moderately damaged garments, either due to the effort required to sell the item or a stigma around sharing damaged articles (Degenstein et al., 2020). Runoff from landfill means that these items, and fibres released from them, can still enter the environment. Certainly, in the present study, runoff from landfill was highlighted as a potential source of macroplastic in the Thames Estuary.

Removal of microfibres from the environment is difficult, thus improved waste management (Lebreton and Andrady, 2019) and textile production are key to reducing microfibre pollution (House of Commons Environmental Audit Committee, 2019b). Monitoring of riverine as well as marine habitats is then essential for determining the success of these mitigations. Yet, first background contamination, such as that in the present study, needs to be reported. The House of Commons Environmental Audit Committee (2019b) called for the Government to change taxation to reward environmentally conscious practices in the textiles industry, enforce checks by retailers across supply chains and an extension of the tax on virgin plastics to be implemented in 2022. It was suggested that the tax be applied to textile products which failed to contain 50% recycled PET. PES is the most abundant polymer in clothing and, as such, its production makes up the majority of fibre demand. PES was also found to be one of the most abundant polymers in the present study. Moreover, nearly 70% of tested garments in Brazil had a high ecological footprint (Klein et al., 2020) and a switch to recycled polymers could ensure these garments are more sustainable in the future. As a result, it was suggested that garments contain eco-labelling which informs consumers of the ecological costs of manufacturing clothes to help them make sustainable purchases.

Whilst fibres cannot feasibly be recovered from the environment, intercepting plastics in the home and in WWTPs is possible. Recently, various products have been designed to collect fibres before they are released into wastewater (Cora Ball; Guppy Friend Bag). Talvitie et al. (2017a) noted that WWTPs are extremely effective at recovering fibres from wastewater. It was demonstrated that pre-treatment removes most of the microplastics and that additional filtration improve recovery. Membrane bioreactors could remove 99.9% of

microplastics. Additionally, there has been a call to produce textiles resistant to shedding (House of Commons Environmental Audit Committee, 2019b) and such materials are being developed (Hinchcliffe, 2017). Using fabric softener is one technique which is known to help reduce microfibre output (De Falco et al., 2018b). Additionally, coatings have been proposed as a technique for reducing the shedding of fibres from washed clothes. De Falco et al. (2018a) noted that a pectin coating reduced fibre release by up to 90%. Whilst developments are forthcoming, there have been calls that Government intervention has been slow and greater input is needed (House of Commons Environmental Audit Committee, 2019b). It is not solely the responsibility of the Government to push for change, with concerns that some retailers are failing to consider environmental impacts (House of Commons Environmental Audit Committee, 2019a). Within industry, there are two main approaches to generate more sustainable fashion: pragmatic changes within the existing mainstream system and radical change that challenges pre-existing designs and results in innovative business models. Pragmatic changes include making supply chains less wasteful and more transparent and changing branding, communication and marketing at the retail level. Whilst radical changes could embrace a switch to "slow fashion", educating communities to make a difference and developing new sustainable business models (Mukendi et al., 2020). The Ellen MacArthur Foundation (2017) highlighted that the industry targets were to phase out substances of concern and microfibre release, reduce the disposability of clothes by changing design and marketing, improve recycling and collection, and move to renewable resources. A few consumer products are available to remove microplastics from washing machines. These products, however, put the onus on the consumer rather than placing responsibility with the designer and manufacturer. Whilst solutions such as these are available they can pose a financial barrier which highlights the privilege of consumers who can chose environmental alternatives whilst it might not be a possibility in every household. Additionally, whilst in tests the products reduce microfibre emissions from washing machines, the success varies between products (McIlwraith et al., 2018).

In addition to plastic fibre contamination, the results of Chapter 6 demonstrate the need to document cellulosic fibres. Cellulosic fibres have been demonstrated to be readily ingested but the present study records a lower abundance of these fibres in the sediment than biota. Historically, cellulosic fibres have been discounted and not reported, yet our

understanding of their impact on wildlife is even less than our limited understanding of risk from plastic fibre ingestion. Whilst it is important to document their presence, it is also key to distinguish them from traditional plastics as the impacts of exposure to these polymers may vary. Increased infrared analysis capabilities will allow greater proportions of plastics in samples to be analysed, providing information needed to better understand the distribution and ingestion of these polymers. It is proposed that further environmental monitoring is needed to understand the fluctuations in ingestion of both forms of microfibres coupled with studies into the effects of ingestion. Risk maps could then be produced, highlighting hotspots for plastic in the environment compared to areas of high ingestion. Investigating multiple compartments for microplastics can then be used to unpick the complex movement through the environment and food webs. It is essential to determine which organisms are most at risk, and why, to then be able to apply appropriate mitigation measures. Furthermore, monitoring needs to be inexpensive with cost-effective protocols available. Certainly, a recent study found that presence of tangles in the GM of *Nephrops norvegicus* correlated to microfibre ingestion (Carreras-Colom et al., 2022). Crustaceans which typically form tangles of plastic, could therefore be investigated for monitoring without the need of chemical digestions. This could also easily be implemented as a citizen science project since only training in dissection is required as tangles are easy to identify.

7.4 Summary

Action to tackle plastic pollution is necessary to address such a large environmental issue and has gained support from policy makers, business and the public. Successful mitigation strategies rely on robust, comparable datasets, often collected through ongoing monitoring. This thesis has provided evidence that future monitoring must include multiple environmental compartments, occur both seasonally and annually, collect macro- and microplastic, and consider entire food webs to create a complete understanding of the ecosystem in question. This novel approach, if used widely would greatly improve the value of collected data and provide a powerful tool to drive policy.

Papers published during the PhD

McGoran A.R., Cowie P.R., Clark P.F., McEvoy J.P., Morritt D., 2018. Ingestion of plastic by fish: a comparison of Thames Estuary and Firth of Clyde populations. *Marine Pollution Bulletin* 137, 12–23. https://doi.org/10.1016/j.marpolbul.2018.09.054. (Paper from MSc research). 42 citations.

McGoran A.R., Clark P.F., Smith B.D., Morritt D., 2020. High prevalence of plastic ingestion by *Eriocheir sinensis* and *Carcinus maenas* (Crustacea: Decapoda: Brachyura) in the Thames Estuary. *Environmental Pollution* 265, 114972. https://doi.org/10.1016/j.envpol.2020.114972. 13 citations.

McGoran A.R., Maclaine J.S., Clark P.F., Smith B., Morritt D., 2021. Synthetic and semi-synthetic microplastic ingestion by mesopelagic fishes from Tristan da Cunha and St Helena, South Atlantic. *Frontiers in Marine Science* 8, 78. https://doi.org/10.3389/fmars.2021.633478. 5 citations.

During the PhD there were plans to publish a manuscript quantifying microplastic contamination in a spectacled porpoise stranded in the Falkland Island and a southern right whale dolphin from the same area. Both are incredibly rare animals for which little is known. Indeed, for the NHM this would be only the third specimen of a spectacled porpoise and the first whole animal. The previous two specimens are only skeletal remains and were collected over 100 years previous. Unfortunately, the southern right whale dolphin did not arrive at the Museum before the end of this PhD. As a result, no publication prepared. The preliminary results from the spectacled porpoise are available in the Supplementary Material (S11).

References

Abbasi S., Soltani N., Keshavarzi B., Moore F., Turner A., Hassanaghaei M., 2018. Microplastics in different tissues of fish and prawns from the Musa Estuary, Persian Gulf. *Chemosphere* 205, 80–87. https://doi.org/10.1016/j.chemosphere.2018.04.076.

ABP Marine Environmental Research Ltd., 2013. Hub for London Ecology desk study part B: marine and coastal baseline [online]. Available at: http://content.tfl.gov.uk/f-abpmer-marine-baseline.pdf. [Accessed 22nd November 2021].

Ainali N.M., Bikiaris D.N., Lambropoulou D.A., 2021. Aging effects on low- and high-density polyethylene, polypropylene and polystyrene under UV irradiation: an insight into decomposition mechanism by Py-GC/MS for microplastic analysis. *Journal of Analytical and Applied Pyrolysis* 158, 105207. https://doi.org/10.1016/j.jaap.2021.105207.

Akhbarizadeh R., Moore F., Keshavarzi B., 2019. Investigating microplastics bioaccumulation and biomagnification in seafood from the Persian Gulf: a threat to human health? *Food Additives and Contaminants: Part A* 36, 1696–1708. https://doi.org/10.1080/19440049.2019.1649473.

Alamy.com [online]. Available at: https://www.alamy.com/stock-photo-ms-to-charge-on-plastic-bags-109815796.html?pv=1&stamp=2&imageid=029615B6-C4F2-45BA-89EB-6C787759BE4A&p=309357&n=0&orientation=0&pn=1&searchtype=0&IsFromSearch=1&srch=foo%3dbar%26st%3d0%26pn%3d1%26ps%3d100%26sortby%3d2%26resultview%3dsortbyPopular%26npgs%3d0%26qt%3dmarks%2520and%2520spencer%2520shopping%2520bag%26qt_raw%3dmarks%2520and%2520spencer%2520shopping%2520bag%26lic%3d3%26mr%3d0%26pr%3d0%26ot%3d0%26creative%3d%26ag%3d0%26hc%3d0%26pc%3d%26blackwhite%3d%26cutout%3d%26tbar%3d1%26et%3d0x00000000000000000000%26vp%3d0%26loc%3d0%26imgt%3d0%26dtfr%3d%26dtto%3d%26size%3d0xFF%26archive%3d1%26groupid%3d%26pseudoid%3d%26a%3d%26cdid%3d%26cdsrt%3d%26name%3d%26qn%3d%26apalib%3d%26apalic%3d%26lightbox%3d%26gname%3d%26gtype%3d%26xstx%3d0%26simid%3d%26saveQry%3d%26editorial%3d1%26nu%3d%26t%3d%26edoptin%3d%26customgeoip%3d%26c

ap%3d1%26cbstore%3d1%26vd%3d0%26lb%3d%26fi%3d2%26edrf%3d%26ispremium%3d1%26flip%3d0%26pl%3d. [Accessed 15th June 2020].

Alda-Vidal C., Browne A.L., Hoolohan C., 2020. "Unflushables": establishing a global agenda for action on everyday practices associated with sewer blockages, water quality, and plastic pollution. *Wiley Interdisciplinary Reviews – Water* 7, e1452. https://doi.org/10.1002/wat2.1452.

Alexiadou P., Foskolos I., Frantzis A., 2019. Ingestion of macroplastics by odontocetes of the Greek Seas, Eastern Mediterranean: often deadly! *Marine Pollution Bulletin* 146, 67–75. https://doi.org/10.1016/j.marpolbul.2019.05.055.

Almroth B.C., Eggert H., 2019. Marine plastic pollution: sources, impacts, and policy issues. *Reviews of Environmental Economics and Policy* 13, 317–326. https://doi.org/reep/rez012.

Aloy A.B., Vallejo Jr. B.M., Juinio-Meñez M.A., 2011. Increased plastic litter cover affects foraging activity of the sandy intertidal gastropod *Nassarius pullus. Marine Pollution Bulletin* 62, 1772–1779. https://doi.org/10.1016/j.marpolbul.2011.05.021.

Always. What are ALWAYS sanitary pads made of? [online]. Available at: https://www.always.co.uk/en-gb/tips-and-advice-for-women/always-ingredients-and-safety/what-are-always-sanitary-pads-made-of. [Accessed 8th June 2020].

Amara R., Selleslagh J., Billon G., Minier C., 2009. Growth and condition of 0-group European flounder, *Platichthys flesus* as indicator of estuarine habitat quality. *Hydrobiologia* 627, 87–98. https://doi.org/10.1007/s10750-009-9717-9.

American Samoa Power Authority Materials Management Office. Appendix A [online]. Available at: https://www.aspower.com/aspaweb/bids/RFP%20NO.%20ASPA14.1216%20ASPA%20AND%20PUBLIC%20JOINT%20VENTURE%20RECYCLING-Appendix%20A.pdf. [Accessed 24th June 2020].

Amon D.J., Kennedy B.R.C., Cantwell K., Suhre K., Glickson D., Shank T.M., Rotjan R.D., 2020. Deep-sea debris in the central and western Pacific Ocean. *Frontiers in Marine Science* 7, 369. https://doi.org/10.3389/fmars.2020.00369.

Anderson F., 2021. Ban plastic in wet wipes [online]. Available at: https://www.fleuranderson.co.uk/ban-plastic-in-wet-wipes/. [Accessed 22nd November 2021].

Andrady A.L., 2011. Microplastics in the marine environment. *Marine Pollution Bulletin* 62, 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030.

Andrady A.L., 2017. The plastic in microplastics: a review. *Marine Pollution Bulletin* 119, 12–22. https://doi.org/10.1016/j.marpolbul.2017.01.082.

Andrady A.L., 2022. Weathering and fragmentation of plastic debris in the ocean environment. *Marine Pollution Bulletin* 180, 113761. https://doi.org/10.1016/j.marpolbul.2022.113761.

Arthur C., Baker J., Bamford H., (eds), 2009. Proceedings of the international research workshop on the occurrence, effects, and fate of microplastic marine debris. National Oceanic and Atmospheric Administration, Technical Memorandum NOS-ORandR-30. [Available online at: https://repository.library.noaa.gov/view/noaa/2509/noaa_2509_DS1.pdf?].

Arturo I.A., Corcoran P.L., 2022. Categorization of plastic debris on sixty-six beaches of the Laurentian Great Lakes, North America. *Environmental Research Letters* 17, 045008. https://doi.org/10.1088/1748-9326/ac5714.

Atasağun H.G., Bhat G.S., 2020a. Advancement in flushable wipes: modern technologies and characterization. *Journal of Industrial Textiles* 49, 722–747. https://doi.org/10.1177/1528083718795910.

Atasağun H.G., Bhat G.S., 2020b. Assessing the structural, mechanical and dispersible characteristics of flushable nonwovens. *Textile Research Journal* 90, 581–592. https://doi.org/10.1177/0040517519873055.

Atici A.A., Sepil A., Sen F., 2021. High levels of microplastic ingestion by commercial, planktivorous *Alburnus tarichi* in Lake Van, Turkey. *Food Additives and Contaminants: Part A* 38. 1767–1777. https://doi.org/10.1080/19440049.2021.1941304.

Attrill M., 1992. Thames estuary benthic programme: a site by site report on the results of macroinvertebrate surveys undertaken during 1990–91. National Rivers Authority Thames Region, Reading. http://www.environmentdata.org/archive/ealit:3668.

Au S.Y, Lee C.M., Weinstein J.E., ven den Hurk P., Klaine S.J., 2017. Trophic transfer of microplastics in aquatic ecosystems: identifying critical research needs. *Integrated Environmental Assessment and Management* 13, 505–509. https://doi.org/10.1002/ieam.1907.

Aytan U., Esensoy F.B., Senturk Y., 2022. Microplastic ingestion by copepods in the Black Sea. *Science of the Total Environment* 806, 150921. https://doi.org/10.1016/j.scitotenv.2021.150921.

Ballas C.E., Williams A.T., Simmons S.L., Ergin A., 2001. A statistical riverine litter propagation model. *Marine Pollution Bulletin* 42, 1169–1176. https://doi.org/10.1016/S0025-326X(01)00133-3.

Bandoim L., 2020. Why Coca-Cola refuses to ban plastic bottles [online]. Available at: https://www.forbes.com/sites/lanabandoim/2020/01/23/why-coca-cola-refuses-to-ban-plastic-bottles/. [Accessed 1st December 2020].

Barnes D.K.A., Galgani F., Thompson R.C., Barlaz M., 2009. Accumulation and fragmentation of plastic debris in global environments. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364, 1985–1998. https://doi.org/10.1098/rstb.2008.0205.

Batel A., Linti F., Scherer M., Erdinger L., Braunbeck T., 2016. Transfer of benzo[α]pyrene from microplastics to *Artemia nauplii* and further to zebrafish via a trophic food web experiment: CYPIA induction and visual tracking or persistent organic pollutants. *Environmental Technology and Ecology* 35, 1656–1666. https://doi.org/10.1002/etc.3361.

Beck M.W., Heck K.L. Jr., Able K.W., Childers D.L., Eggleston D.B., Gillanders B.M., Halpern B., Hays C.G., Hoshino K., Minello T.L., Orth R.J., Sheridan P.F., Weinstein M.P., 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. *BioScience* 51, 633–641. https://doi.org/10.1641/0006-3568(2001)051[0633:TICAMO]2.0.CO;2.

Bergmann M., Mützel S., Primpke S., Tekman M.B., Trachsel J., Gerdts G., 2019. White and wonderful? Microplastics prevail in snow from the Alps to the Arctic. *Science Advances* 5, eaax1157. https://doi.org/10.1126/sciadv.aax1157.

Bernardini G., McConville A.J., Castillo Castillo A., 2020. Macro-plastic pollution in the tidal Thames: an analysis of composition and trends for the optimization of data collection. *Marine Policy* 119, 104064. https://doi.org/10.1016/j.marpol.2020.104064.

Bertoldi C., Lara L.Z., Mizushima F.A.D.L., Martins F.C.G., Battisti M.A., Hinrichs R., Fernander A.N., 2021. First evidence of microplastic contamination in the freshwater of Lake Guaíba. Porto Alegre, Brazil. *Science of the Total Environment* 759, 143503. https://doi.org/10.1016/j.scitotenv.2020.143503.

Besseling E., Foekema E.M., Van Franeker J.A., Leopold M.F., Kühn S., Bravo Rebolledo E.L., Heße E., Mielke L., Ijzer J., Kamminga P., Koelmans A.A., 2015. Microplastic in a macro filter feeder: humpback whale *Megaptera novaengliae*. *Marine Pollution Bulletin* 95, 248–252. https://doi.org/10.1016/j.marpolbul.2015.04.007.

Besseling E., Wegner A., Foekema E.M., van den Heuvel-Greve M.J., Koelmans A.A., 2013. Effects of microplastics on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). *Environmental Science and Technology* 47, 593–600. https://doi.org/10.1021/es302763x.

Bhuyan M.S., Venkatramana S., Selvam S., Szabo S., Hassain M.M., Rashed-Un-Nabi M., Parasivam C.R., Jonathon M.P., Islam M.S., 2021. Plastics in marine ecosystem: a review of their sources and pollution conduits. *Regional Studies in Marine Science* 41, 101539. Https://doi.org/10.1016/j.rsma.2020.101539.

Blettler M.C.M., Garello N., Ginon L., Abrial E., Espinola L., Wantzon K., 2019. Massive plastic pollution in a mega-river of a developing country: sediment deposition and ingestion by fish (*Prochilodus lineatus*). *Environmental Pollution* 255, 113348. https://doi.org/10,,1016/j.envpol.2019.113348.

Boerger C.M., Lattin G.L., Moore S.L., Moore C.J., 2010. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. *Marine Pollution Bulletin* 60, 2275–2278. https://doi.org/10.1016/j.marpolbul.2010.08.007.

Bond A.L., Hutton I., Lavers J.L., 2021. Plastics in regurgitated Flesh-footed Shearwater (*Ardenna carneipes*) boluses as a monitoring tool. *Marine Pollution Bulletin* 168, 112428. https://doi.org/10.1016/j.marpolbul.2021.112428.

Bossart G.D., 2011. Marine mammals as sentinel species for oceans and human health. *Veterinary Pathology* 48, 676–690. https://doi.org/10.1177/0300985810388525.

Bour A., Avio C.G., Gorbi S, Regoli F., Hylland K., 2018. Presence of microplastics in benthic and epibenthic organisms: influence of habitat, feeding mode and trophic level. *Environmental Pollution* 243, 1217–1225. https://doi.org/10.1016/j.envpol.2018.09.115.

Bour A. Hossain S., Taylor M., Summer M., Almroth B.C., 2020. Synthetic microfiber and microbead exposure and retention time in model aquatic species under different exposure scenarios. *Frontiers in Environmental Science* 8, 83. https://doi.org/10.3389/fenvs.2020.00083.

Bowen W.D., Harrison G.D., 1994. Offshore diet of grey seals *Halichoerus grypus* near Sable Island, Canada. *Marine Ecology Progress Series* 112, 1–11. https://doi.org/10.3354/meps112001.

Bowley J., Baker-Austin C., Porter A., Hartnell R., Lewis C., 2021. Oceanic hitchhikers – assessing pathogen risks from marine microplastic. *Trends in Microbiology* 29, 107–116. https://doi.org/10.1016/j.tim.2020.06.011.

Bravo Rebolledo E.L., Van Franeker J.A., Jansen O.E., Brasseur S.M.J.M., 2013. Plastic ingestion by harbour seals (*Phoca vitulina*) in the Netherlands. *Marine Pollution Bulletin* 67, 200–202. https://doi.org/10.1016/j.marpolbul.2012.11.035.

Bradford A., 2017. Facts about humpback whales [online]. Available at: https://www.livescience.com/58464-humpback-whale-facts.html. [Accessed on 30th March 2020].

Brennecke D., Duarte B., Paiva F., Caçador I., Canning-Clode J., 2016. Microplastics as vector for heavy metal contamination from the marine environment. *Estuarine Coastal and Shelf Science* 178, 189–195. https://doi.org/10.1016/j.ecss.2015.12.003.

British Plastics Federation, 2019. Understanding the debate about plastic. British Plastics Federation, London. 1–24.

British Plastics Federation, 2021. Plastic recycling [online]. Available at: https://www.bpf.co.uk/sustainability/plastics_recycling.aspx. [Accessed 22nd November 2021].

Brock A., Williams I., 2020. Life cycle assessment of beverage packaging. *Detritus Multidisciplinary Journal for Waste and Resources and Residues* 13, 47–61. https://doi.org/10.31025/2611-4135/2020.14025.

Browne M.A., Crump P., Niven S.J., Teuten E., Tonkin A., Galloway T.S., Thompson R.C., 2011. Accumulation of microplastics on shorelines worldwide: sources and sinks. *Environmental Science and Technology* 45, 9175–9179. https://doi.org/10.1021/es201811s.

Browne M.A., Dissanayake A., Galloway T.S., Lowe D.M., Thompson R.C., 2008. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). *Environmental Science and Technology* 42, 5026–5031. https://doi.org/10.1021/ea800249a.

Browne M.A., Galloway T.S., Thompson R.C., 2010. Spatial patterns of plastic debris along estuarine shorelines. *Environmental Science and Technology* 44, 3404–3409. https://doi.org/10.1021/es903784e.

Browne M.A., Niven S.J., Galloway T.S., Rowland S.J., Thompson R.C., 2013. Microplastics moves pollutants and additives to worms, reducing function linked to health and biodiversity. *Current Biology* 23, 2388–2392. https://doi.org/10.1016/j.cub.2013.10.012.

Burkhardt-Holm P., N'Guyen A., 2019. Ingestion of microplastics by fish and other prey organisms of cetaceans, exemplified for two large baleen whale species. *Marine Pollution Bulletin* 144, 224–234. https://doi.org/10.1016/j.marpolbul.2019.04.068.

CABI, 2012. Invasive species compendium: *Rutilus rutilus* (roach) [online]. Available at: https://www.cabi.org/isc/datasheet/66337. [Accessed 18th October 2018].

Camphuysen K.C.J., Henderson P.A., 2017. North Sea fish and their remains. Royal Netherlands Institute for Sea Research and Pisces Conservation Ltd.

Carbon Trust, 2008. Product carbon footprinting: the new business opportunity – experience from leading companies [online]. Available at: https://www.carbontrust.com/media/84928/ctc744-product-carbon-footprinting-the-new-business-opportunity.pdf. [Accessed 7th October 2019]. Update: link no longer active. Available through the Internet Archive: https://web.archive.org/web/20160311033932/https://www.carbontrust.com/media/84928/ctc744-product-carbon-footprinting-the-new-business-opportunity.pdf. [Accessed on 12th June 2020].

Carey M.J., 2011. Intergenerational transfer of plastic debris by Short-tailed Shearwaters (*Ardenna tenuirostris*). *EMU – Austral Ornithology* 111, 229–234. https://doi.org/10.1071/MU10085.

Carlin J., Craig C., Little S., Donnelly M., Fox D., Zhai L., Walters L., 2020. Microplastic accumulation in the gastrointestinal tracts in birds of prey in central Florida, USA. *Environmental Pollution* 264, 114633. https://doi.org/10.1016/j.envpol.2020.114633.

Carlsson P., Singdahl-Larsen C., Lusher A.L., 2021. Understanding the occurrence and fate of microplastics in coastal Arctic ecosystems the case of surface waters, sediments and

walrus (*Odobenus rosmarus*). *Science of the Total Environment* 792, 148308. https://doi.10.1016/j.sctotenv.148308.

Carpenter E.J., Smith Jr. K.L., 1972. Plastics on the Sargasso Sea. *Science* 175, 1240–1241. https://doi.org/10.1126/science.175.4027.1240.

Carreras-Colom E., Carrassón M., Welden N.A., 2020. Ingestion and retention of plastic fibres of different lengths by the green shore crab (*Carcinus maenas*), presented at Micro2020, November 2018. Abstract available online at: https://www.micro.infini.fr/IMG/pdf/micro2020proceedingsbook.pdf. [Accessed 23rd November 2021].

Carreras-Colom E., Cartes J.E., Constenla M., Welden N.A., Soler-Membrives A., Carrassón M., 2022. An affordable method for the monitoring of plastic fibre ingestion in *Nephrops norvegicus* (Linnaeus, 1758) and implications on wide temporal and geographical scale comparisons. *Science of the Total Environment* 810, 152264. https://doi.org/10.1016/j.scitotenv.2021.152264.

Carreras-Colom E., Constenla M., Soler-Membrives A., Cartes J.E., Baeza M., Padrós F., Carrassón M., 2018. Spatial occurrence and effects of microplastic ingestion on the deep-water shrimp *Aristeus antennatus*. *Marine Pollution Bulletin* 133, 44–52. https://doi.org/10.1016/j.marpolbul.2018.05.012.

Castello y Tickell S., Barker J., 2015. Thames marine mammal sightings survey ten year report (2004–2014). UK and Europe Conservation Programme, Zoological Society of London.

Castro-Jiménez J., González-Fernández D., Fornier M., Schmidt N., Sempere R., 2019. Macro-litter in surface waters from the Rhone River: plastic pollution and loading in the NW Mediterranean Sea. *Marine Pollution Bulletin* 146, 60–66. https://doi.org/10.1016/j.marpolbul.2019.05.067.

Catarino A.I., Thompson R., Sanderson W., Henry T.B., 2016. Development and optimization of a standard method for extraction of microplastics in mussels by enzyme digestion

of soft tissues. *Environmental Toxicology and Chemistry* 36, 947–951. https://doi.org/10.1002/etc.3608.

Cau A., Avio C.G., Dessi C., Follesa M.C., Moccia D., Regoli F., Pusceddu A., 2019. Microplastics in the crustaceans *Nephrops norvegicus* and *Aristeus antennatus*: flagship species for deep-sea environments? *Environmental Pollution* 255, 113107. https://doi.org/10.1016/j.envpol.2019.113107.

Cedervall T., Hansson L.-A., Lard M., Frohm B., Linse S., 2012. Food chain transport of nanoparticles affects behaviour and fat metabolism in fish. *PLoS ONE* 7, e32254. https://doi.org/10.1371/journal.pone.0032254.

Cera A., Pierdomenico M., Sodo A., Scalici M., 2022. Spatial distribution of microplastics in volcanic lake water and sediments: relationships with depth and sediment grain size. *Science of the Total Environment* 829, 154659. https://doi.org/10.1016/j.scitotenv.2022.154659.

Chambers S.J., Garwood P.R., 1992. Polychaetes from Scottish waters: a guide to identification, part 3 family Nereidae. National Museums of Scotland, Edinburgh.

Chassignet E.P., Xu X., Zavala-Romero O., 2021. Tracking marine litter with a global ocean model: where does it go? Where does it come from? *Frontiers in Marine Science* 8, 667591. https://doi.org/10.3389/fmars.2021.667591.

Cheung P.K., Hung P.L., Fok L., 2019. River microplastic contamination and dynamics upon a rainfall event in Hong Kong, China. *Environmental Processes* 6, 253–264. https://doi.org/10.1007/s40710-018-0345-0.

Chiba S., Saito H., Fletcher R., Yogi T., Kayo M., Miyagi S., Ogido M., Fujikura K., 2018. Human footprint in the abyss: 30 year records of deep-sea plastic debris. *Marine Policy* 96, 204–212. https://doi.org/10.1016/j.marpol.2018.03.022.

Claessens M., De Meester S., Van Landuyt L., De Clerck K., Janssen C.R., 2011. Occurrence and distribution of microplastics in marine sediments along the Belgian coast. *Marine Pollution Bulletin* 62, 2199–2204. https://doi.org/10.1016/j.marpolbul.2011.06.030.

Claessens M., Van Cauwenberghe L., Vandergehuchte M.B., Janssen C.R., 2013. New techniques for the detection of microplastics in sediments and field collected organisms. *Marine Pollution Bulletin* 70, 227–233. https://doi.org/10.1016/j.marpolbul.2013.03.009.

Clark P.F., Rainbow P.S., 1997. The Chinese Mitten crab in the Thames catchment. A report for the Environment Agency (Myles Thomas and Willie Yeomans) in three parts. Part 1 A report for the Environment Agency, 1–75 and Part 2 The appendices to the report for Environment Agency, 1–91, with Robbins R., Smith B., Dobson G., Byrne C., Lowe M., Morgan A.; Part 3 A report on available *Eriocheir sinensis* literature for the Environment Agency, with Robbins R., Cooper P. 1–27.

Clark P.F., Rainbow P.S., Robbins R.S., Smith B., Yeomans W.E., Thomas M., Dobson G., 1998. The alien Chinese mitten crab, *Eriocheir sinensis* (Crustacea: Decapoda: Brachyura), in the Thames catchment. *Journal of the Marine Biological Association of the UK* 78, 1215–1221. https://doi.org/10.1017/S002531540004443X.

Clark P.F., Stefanoudis P.V., Crimmen O.A., Pearce D., Clifton-Dey D., Morritt D., 2017. Catching invasive Chinese mitten crab while releasing the endangered European eel and other fish by-catch: the implications of fyke net design. *Aquatic Conservation* 27, 1101–1115. https://doi.org/10.1002/aqc.2779.

Coca-Cola, 2020. Coca-Cola unveils paper bottle prototype [online]. Available at: https://www.coca-colacompany.com/news/coca-cola-unveils-paper-bottle-prototype. [Accessed on 20th December 2021].

Coclough S.R., Gray G., Bark A., Knights B., 2002. Fish and fisheries of the tidal Thames: management of the modern resource, research aims and future pressures. *Journal of Fish Biology* 61, 64–73. https://doi.org/10.1006/jfbi.2002.2068.

Cole M., Coppock R., Lindeque P.K., Altin D., Reed S., Pond D.W., Sørensen L., Galloway T.S., Booth A.M., 2019. Effects of nylon microplastic on feeding, lipid accumulation, and moulting in a coldwater copepod. *Environmental Science and Technology* 53, 7075–7082. https://doi.org/10.1021/acs.est.9b01853.

Cole M., Lindeque P., Fileman E., Halsband C., Galloway, T.S., 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod *Calanus helgolandicus*. *Environmental Science and Technology* 49, 1130–1137. https://doi.org/10.1021/es504525u.

Cole M., Lindeque P., Halsband C., Galloway T.S., 2011. Microplastics as contaminants in the marine environment: a review. *Marine Pollution Bulletin* 62, 2588–2597. https://doi.org/10.1016/j.marpolbul.2011.09.025.

Cole M., Webb H., Lindeque P.K., Fileman E.S., Halsband C., Galloway T.S., 2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. *Scientific Reports* 4, 4528. https://doi.org/10.1038/srep04528.

Colombina, 2017. Colombina in the world [online]. Available at: https://www.colombina.com/corporativo/en/colombinaintheworld.php. [Accessed 15th June 2020].

Committee on Marine Mammals (1966-67), 1967. Standard measurements of seals. *Journal of Mammalogy* 48, 459–462. http://www.jstor.org/stable/1377778.

Connors K.A., Dyer S.D, Belanger S.E., 2017. Advancing the quality of environmental microplastic research. *Environmental Toxicology and Chemistry* 36, 1697–1703. https://doi.org/10.1002/etc.3829.

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

Cora Ball [online]. Available at: https://coraball.com/. [Accessed 15th October 2018].

Corcoran P.L., 2015. Benthic plastic debris in marine and freshwater environments. *Environmental Science: Processes and Impacts* 17, 1363–1369. https://doi.org/10.1039/c5em00188a.

Corcoran P.L., Norris T., Ceccanese T., Walzak M.J., Helm P.A., Marvin C.H., 2015. Hidden plastics of Lake Ontario, Canada and their potential preservation in the sediment

record. *Environmental Pollution* 204, 17–25. https://doi.org/10.1016/j.envpol.2015.04.009.

Courtene-Jones W., Quinn B., Gary S.F., Mogg A.O., Narayanaswamy B.E., 2017. Microplastic pollution identified in deep-sea water and ingested by benthic invertebrates in the Rockall Trough, North Atlantic Ocean. *Environmental Pollution* 231, 271–280. https://doi.org/10.1016/j.envpol.2017.08.026.

Cowger W., Gray A.B., Guilinger J.J., Fong B., Waldschläger K., 2021. Concentration depth profiles of microplastic particles in river flow and implications for surface sampling. *Environmental Science and Technology* 55, 6032–6041. https://doi.org/10.1021/acs.est.1c01768.

Cózar A., Echevarría F., González-Gordillo J.I., Irigoien X., Úbeda B., Hernández-León S., Palma Á.T., Navarro S., Garciá-de-Lomas J., Ruiz A., Fernández-de-Puelles M.L., Duarte C.M., 2014. Plastic debris in the open ocean. *Proceedings of the National Academy of Sciences of the United States of America* 111, 10239–10244. https://doi.org/10.1073/pnas.1314705111.

Critchell K., Hoogenboom M.O., 2018. Effects of microplastic exposure on the body condition and behaviour of planktivorous reef fish (*Acanthochromis polyacanthus*). *PLoS ONE* 13, e0193308. https://doi.org/10.1371/journal.pone.0193308.

Crooks N., Parker H., Pernetta A.P., 2019. Brain food? Trophic transfer and tissue retention of microplastics by the velvet swimming crab (*Necora puber*). *Ecology* 519, 151187. https://doi.org/10.1016/j.jembe.2019.151187.

Crosti R., Arcangelli A., Campana I., Paraboschi M., González-Fernández D., 2018. 'Down to the river': amount, composition, and economic sector of litter entering the marine compartment, through the Tiber river in the Western Mediterranean Sea. *Rendiconti Lincei Scienze Fisiche e Naturali* 29, 859–866. https://doi.org/10.1007/s12210-018-0747-y.

Cruz C.J., Muñoz-Perez J.J., Carrasco-Braganza M.I., Poullet P., Lopez-Garcia P., Contreras A., Silva R., 2020. Beach cleaning costs. *Ocean and Coastal Management* 188, 105118. https://doi.org/10.1016/j.ocecoaman.2020.105118.

D'Souza J.M., Windsor F.M., Santillo D., Ormerod S.J., 2020. Food web transfer of plastics to an apex riverine predator. *Global Change Biology* 23, 3846–3857. https://doi.org/10.1111/gcb.15139.

Dai Z., Haibo Z., Zhou Q., Tian Y., Chen T., Tu C., Fu C., Luo Y., 2018. Occurrence of microplastics in the water column and sediment in an inland sea affected by intensive anthropogenic activities. *Environmental Pollution* 242, 1557–1565. https://doi.org/10.1016/j.envpol.2018.07.131.

DAISIE, 2009. Handbook of alien species in Europe, in: Invading nature – Springer series in invasion ecology, vol. 3, XXVIII, 400pp, 200 illustrations in colour. Springer, Dordrecht, ISBN: 978-1-4020-8279-5.

Daniel D.B., Ashraf P.M., Thomas S.N., 2020. Abundance, characteristics and seasonal variation of microplastics in Indian white shrimps (*Fenneropendeus indicus*) from coastal waters off Cochin, Kerala, India. *Science of the Total Environment* 737, 139839. https://doi.org/10.1016/j.scitotenv.2020.139839.

Davidson P., Asch R.G., 2011. Plastic ingestion by mesopelagic fishes in the North Pacific Subtropical gyre. *Marine Ecology Progress Series* 432, 173–180. https://doi.org/10.3354/meps09142.

Dawood S., 2019. Walkers redesigns its crisp packaging to focus on Britishness [online]. Available at: https://www.designweek.co.uk/issues/28-january-3-february-2019/walkers-redesigns-its-crisp-packaging-focus-on-britishness/. [Accessed 12th June 2020].

De Falco F., Gentile G., Avolio R., Errico M.E., Di Pace E., Ambrogi V., Avella M., Cocca M., 2018a. Pectin based finishing to mitigate the impact of microplastics released by polyamide fabrics. *Carbohydrate Polymers* 198, 175–180. https://doi.org/10.1016/j.carbpol.2018.06.062.

De Falco F., Gullo M.P., Gentile G., Di Pace E., Cocca M., Gelabert L., Brouta-Agnésa M., Rovira A., Escudero R., Villalba R., Mossotti R., Montarsolo A., Gavignano S., Tonin C., Avella M., 2018b. Evaluation of microplastic release caused by textile washing processes of synthetic fabrics. *Environmental Pollution* 236, 916–925. https://doi.org/10.1016/j.envpol.2017.10.057.

De Frond H.L., van Sebille E., Parnis J.M., Diamond M.L., Mallos N., Kingsbury T., Rochman C.M., 2019. Estimating the mass of chemicals associated with ocean plastic pollution to inform mitigation efforts. *Integrated Environmental Assessment and Management* 15, 596–606. https://doi.org/10.1002/ieam.4147.

De Sá L.C., Oliveira M., Ribeiro F., Rocha T.L., Futter M.N., 2018. Studies of the effects of microplastics on aquatic organisms: what do we know and where should we focus our efforts in the future? *Science of the Total Environment* 645, 1029–1039. https://doi.org/10.1016/j.scitotenv.2018.07.207.

Defra, 2018. Carrier bags: why there's a charge. Policy paper [online]. Available at: https://www.gov.uk/government/publications/single-use-plastic-carrier-bags-why-were-introducing-the-charge/carrier-bags-why-theres-a-5p-charge#results-from-the-scheme. [Accessed 14th February 2019].

Defra, 2019. Introducing a Deposit Return Scheme on beverage containers [online]. Available at: https://consult.defra.gov.uk/environment/introducing-a-deposit-return-scheme/supporting_documents/depositreturnconsultia.pdf. [Accessed on 21st November 2022].

Defra, 2021. Government considering new action on wet wipes [online]. Available at: https://deframedia.blog.gov.uk/2021/11/15/government-considering-new-action-on-wet-wipes/. [Accessed on 22nd November 2021].

Degenstein L.M., McQueen R.H., McNeill L.S., Hamlin R.P., Wakes S.J., Dunn L.A., 2020. Impact of physical condition on disposal and end-of-life extension of clothing. *International Journal of Consumer Studies* 44, 12590. https://doi.org/10.1111/ijcs.12590.

Dehaut A., Cassone A.-L., Frère L., Hermabessiere L., Himber C., Rinnert E., Rivière G., Lambert C., Soundant P., Huvet A., Duflos G., Paul-Pont I., 2016. Microplastics in seafood: Benchmark protocol for their extraction and characterization. *Environmental Pollution* 215, 223–233. https://doi.org/10.1016/j.envpol.2016.05.018.

Derraik J.G.B., 2002. The pollution of the marine environment by plastic debris: a review. *Marine Pollution Bulletin* 44, 842–852. https://doi.org/10.1016/S0025-326X(02)00220-5.

Desforges J.-P.W., Galbraith M., Ross P.S., 2015. Ingestion of microplastics by zooplankton in the Northeast Pacific Ocean. *Archives of Environmental Contamination and Toxicology* 69, 320–330. https://doi.org/10.1007/s00244-015-0172-5.

Devereux R., Westhead E., Jayaratbe R., Newport D., 2022. Microplastic abundance in the Thames River during the New Year Period. *Marine Pollution Bulletin* 117, 113534. https://doi.org/10.1016/j.marpolbul.2022.113534.

Devriese L.I., Van de Meulen M.D., Maes T., Bekaert K., Paul-Pont I., Frère L., Robbens J., Vethaak A.D., 2015. Microplastic contamination in brown shrimp (*Crangon crangon*, Linnaeus 1758) from coastal waters of the Southern North Sea and Channel area. *Marine Pollution Bulletin* 98, 179–187. https://doi.org/10.1016/j.marpolbul.2015.06.051.

Diana Z., Sawickij N., Rivera jr. N.A., Hsu-Kim H., Rittschof D., 2020. Plastic pellets trigger feeding responses in sea anemones. *Aquatic Toxicology* 222, 105447. https://doi.org/10.1016/j.aquatox.2020.105447.

Diepens N.J., Koelmans A.A., 2018. Accumulation of plastic debris and associated chemicals in aquatic food webs. *Environmental Science and Technology* 52, 8510–8520. https://doi.org/10.1021/acs.est.8b02515.

Dos Santos J., Jobling M., 1991. Gastric emptying in cod, *Gadus morhua* L: emptying and retention of indigestible solids. *Journal of Fish Biology* 38, 187–197. https://doi.org/10.1111/j.1095-8649.1991.tb03105.x.

Dris R., Gasperi J., Mirande C., Mandin C., Guerrouache M., Langlois V. Tassin B., 2017. A first overview of textile fibres, including microplastics, in indoor and outdoor environments. *Environmental Pollution* 221, 453–458. https://doi.org/10/1016/j.envpol.2016.12.013.

Dris R., Gasperi J., Saad M., Mirande C., Tassin B., 2016. Synthetic fibres in atmospheric fallout: a source of microplastics in the environment? *Marine Pollution Bulletin* 104, 290–293. https://doi.org/10.1016/j.marpolbul.2016.01.0060025-326X.

Dris R., Imhof H.K., Löder M.G.L., Gaspen J., Laforsch C., Tassin B., 2018. Microplastic contamination in freshwater systems: methodological challenges, occurrence and sources, in Zeng E.Y. (ed.), Microplastic contamination in aquatic environments: an emerging matter of environmental urgency. Elsevier, pp. 51–93. https://doi.org/10.1016/B978-0-12-813747-5.00003-5.

Dubaish F., Liebezeit G., 2013. Suspended microplastics and black carbon particles in the Jade system, southern North Sea. *Water Air and Soil Pollution* 224, 1352. https://doi:10.1007/s11270-012-1352-9.

Duis K., Coors A., 2016. Microplastics in the aquatic and terrestrial environments: sources (with a specific focus on personal care products), fate and effects. *Environmental Sciences Europe* 28, UNSP 2. https://doi.org/10.1186/s12302-015-0069-y.

Earn A., Bucci K., Rochman C.M., 2021. A systematic review of the literature on plastic pollution in the Laurentian Great Lakes and its effects on freshwater biota. *Journal of Great Lakes Research* 47, 120–133. https://doi.org/10.1016/j.jglr.2020.11.002.

ECAP, 2018. Used textile collection in European cities [online]. Available at: http://www.ecap.eu.com/wp-content/uploads/2018/07/ECAP-Textile-collection-in-European-cities_full-report_with-summary.pdf#:~:text=ECAP%20-%20Used%20Textile%20Collection%20in%20European%20Cities,Europeans%E2%80%99%20provision%2C%20access%20to%20and%20consumption%20of%20clothing. [Accessed 30th June 2020].

Egger M., Quiros L., Leone G., Ferrari F., Boerger C.M., Tishler M., 2021. Relative abundance of floating plastic debris and neuston in the Eastern North Pacific Ocean. *Frontiers in Marine Science* 8, 626026. https://doi.org/10.3389/fmars.2021.626026.

Egger M., Sulu-Gambari F., Lebreton L., 2020. First evidence of plastic fallout from the North Pacific Garbage Patch. *Scientific Reports* 10, 7495. https://doi.org/10.1038/s41598-020-64465-8.

Ellen MacArthur Foundation, 2017. A new textiles economy: redesigning fashion's future [online]. Available at: https://www.ellenmacarthurfoundation.org/assets/downloads/publications/A-New-Textiles-Economy_Full-Report.pdf. [Accessed 30th June 2020].

Environment Agency, 2016. Water for life and livelihoods. Part 1: Thames river basin district River basin management plan. Environment Agency, Bristol. 1–114.

Environment Agency, 2022a. Historic landfill sites [online]. Available at: https://data.gov.uk/dataset/17edf94f-6de3-4034-b66b-004ebd0dd010/historic-landfill-sites#licence-info. [Accessed 1st April 2022].

Environment Agency, 2022b. Permitted waste sites – authorised landfill site boundaries [online]. Available at: https://data.gov.uk/dataset/ad695596-d71d-4cbb-8e32-99108371c0ee/permitted-waste-sites-authorised-landfill-site-boundaries. [Accessed 1st April 2022].

Eriksen A.M., Simonsen K.P., Rasmussen A.R., 2013. Conservation of mitochondrial DNA in fast enzyme-macerated skeletal material. *International Journal of Conservation Science* 4, 127–132.

Eriksen M., Lebreton L.C.M., Carson H.S., Thiel M., Moore C.J., Borerro J.C., Galgani F., Ryan P.G., Reisser J., 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. *PLoS ONE* 9, e111913. https://doi.org/10.1371/journal.pone.0111913.

Eriksen M., Mason S., Wilson S., Box C., Zellers A., Edwards W., Farley H., Amato S., 2013. Microplastic pollution in the surface waters of the Laurentian Great Lakes. *Marine Pollution Bulletin* 77, 177–182. https://doi.org/10.1016/j.marpolbul.2013.10.007.

Eriksson C., Burton H., 2003. Origins and biological accumulation of small plastic particles in fur seals from Macquarie Island. *Ambio: A Journal of the Human Environment* 32, 380–384. https://doi.org/10.1579/0044-7447-32.6.380.

Everaert G., Van Cauwenberghe L., De Rijcke M., Koelmans A.A., Mees J., Vandegehuchte M., Janssen C.R., 2018. Risk assessment of microplastics in the ocean: modelling approach and first conclusions. *Environmental Pollution* 242, 1930–1938 https://doi.org/10.1016/j.envpol.2018.07.069.

Fang C., Zheng R., Zhang Y., Hong F., Mu J., Chen M., Song P., Lin L., Lin H., Le F., 2018. Microplastic contamination in benthic organisms from the Arctic and sub-Arctic regions. *Chemosphere* 209, 298–306. https://doi.org/10.1016/j.chemosphre.2018.06.101.

FAO, 1974. The measurement of fish and shellfish, in Holden M.J., Raitt D.F.S. (eds), Manual of fisheries science part 2 – methods of resource investigation and their application. Food and agriculture organisation of the United Nations, Rome.

Farrell P., Nelson K., 2013. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). *Environmental Pollution* 177, 1–3. https://doi.org/10.1016/j.envpol.2013.01.046.

Faure F., Corbaz M., Baecher H., De Alencastro L.F., 2012. Pollution due to plastics an microplastics in Lake Geneva and in the Mediterranean Sea. *Archives Des Sciences* 65, 157–164. [Available at: https://www.unige.ch/sphn/Publications/ArchivesSciences/AdS%202004-2015/AdS%202012%20Vol%2065%20Fasc%201/157-164_16_Faure_65.pdf].

Fauvel P., 1923. Faune de France, 5. Polychètes errantes. Office Central de Faunistique, Paris.

Felismino M.E.L., Helm P.A., Rochman C.M., 2021. Microplastic and other anthropogenic microparticles in water and sediments of Lake Simcoe. *Journal of Great Lakes Research* 47, 180–189. https://doi.org/10.1016/j.jglr.2020.10.007.

Ferreira G.V.B., Barletta M., Lima A.R.A., Morley S.A., Justino A.K.S., Costa M.P., 2018. High intake rates of microplastics in a Western Atlantic predatory fish, and insights of a direct fishery effect. *Environmental Pollution* 236, 706–717. https://doi.org/10.1016/j.envpol.2018.01.095.

Ferreira G.V.B., Barletta M., Lima A.R.A., 2019. Use of estuarine resources by top predator fishes. How do ecological patterns affect rates of contamination by microplastics? *Science of the Total Environment* 655, 292–304. https://doi.org/10.1016/j.scitotenv.2018.11.229.

Fischer C., 2019. River Thames – polluted by single-use items [online]. Available at: https://mcsuk.org/news/river_clean_report. [Accessed 3rd June 2020].

Foekema E.M., Gruijter C.D., Mergia M.T., van Franeker J.A., Murk A.T.J., Koelmans A.A., 2013. Plastic in North Sea fish. *Environmental Science and Technology* 47, 8818–8824. https://doi.org/10.1021/es400931b.

Foley C.J., Feiner Z.S., Malinich T.D., Hook T.O., 2018. A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. *Science of the Total Environment* 631–632, 550–559. https://doi.org/10.1016/j.scitotenv.2018.03.046.

Forrest A., Giacovazzi L., Dunlop S., Reisser J., Tickler D., Jamieson A., Meeuwig J.J., 2019. Eliminating plastic pollution: how a voluntary contribution from industry will drive the circular plastics economy. *Frontiers in Marine Science* 6, 00627. Https://doi.org/10.3389/fmars.2019.00627.

Fossi M.C., Coppola D., Baini M., Guerranti C., Giannetti M., Marsili L., Panti C., de Sabata E., Clò S., 2014. Large filter feeding marine organisms as indicators of microplastic in the pelagic environment: the case studies of the Mediterranean basking shark (*Cetorhinus maximus*) and fin whale (*Balaenoptera physalus*). *Marine Environmental Research* 100, 17–24. https://doi.org/10.1016/j.marenvres.2014.02.002.

Fossi M.C., Marsili L., Baini M., Giannetti M., Coppola D., Guerranti C., Minutoli R., Lauriano G., Finoia M.G., Rubegni F., Panigada S., Bérubé M., Ramirez J.U., Panti C., 2016. Fin whales and microplastics: the Mediterranean Sea and the Sea of Cortez scenarios. *Environmental Pollution* 209, 68–78. https://doi.org/10.1016/j.envpol.2015.11.022.

Fossi M.C., Panti C., Guerranti C., Coppola D., Giannetti M., Marsili L., Minutoli R., 2012. Are baleen whales exposed to the threat of microplastics? A case study of the Mediterranean fin whale (*Balaenoptera physalus*). *Marine Pollution Bulletin* 64, 2374–2379. https://doi.org/10.1016/j.marpolbul.2012.08.013.

Fred-Ahmadu O.H., Tenebe I.T., Ayejuyo O.O., Benson N.U., 2022. Microplastics and associated organic pollutants in beach sediments from the Gulf of Guinea (SE Atlantic) coastal ecosystems. *Chemosphere* 298, 134193. https://doi.org/10.1016/j.chemosphere.2022.134193.

Free C.M., Jensen O.P. Mason S.A., Eriksen M., Williamson N.J., Boldgiv B., 2014. High-levels of microplastic pollution in a large, remote, mountain lake. *Marine Pollution Bulletin* 65, 156–163. https://doi.org/10.1016/j.marpolbul.2014.06.001.

Galgani F., Fleet D., Van Franeker J., Katsanevakis S., Maes T., Mouat J., Oosterbaan L., Poitou I., Hanke G., Thompson R., Amato E., Birkun A., Janssen C., 2010. Marine Strategy Framework Directive Task Group 10 Report on Marine Litter, in Zampoukas, N. (ed.). JRC Scientific and Technical Reports, EUR 24340 EN-2010.

Galgani F., Leate J.P., Monuedet P., Souplet A., Verin Y., Carpntier A., Goraguer H., Latrouite D., Andral B., Cadiou Y., Mahe J.C., Poulard J.C., Nerisson P., 2000. Litter on the sea floor along European coasts. *Marine Pollution Bulletin* 40, 516–527. https://doi.or/10.1016/S0025-326X(99)00234-9.

Gall S.C., Thompson R.C., 2015. The impact of debris on marine life. *Marine Pollution Bulletin* 92, 170–179. https://doi.org/10.1016/j.marpolbul.2014.12.041.

Galloway T.S., Cole M., Lewis C., 2017. Interactions of microplastic debris throughout the marine ecosystem. *Nature Ecology and Evolution* 1, 0116. https://doi.org/10.1038/s41559-017-0116.

Garoto, 2018. Export: products catalogue [online]. Available at: https://www.garoto.com.br/assets/pdf/product_catalogue_catalogo_productos.pdf. [Accessed 15th June 2020].

Gasperi J., Dris R., Bonin T., Rocher V., Tassin B., 2014. Assessment of floating plastic debris in surface water along the Seine River. *Environmental Pollution* 195, 163–166. https://doi.org/10.1016/j.envpol.2014.09.001.

Germanov E.S., Marshall A.D., Bejder L., Fossi M.C., Loneragan N.R., 2018. Microplastics: no small problem for filter-feeding megafauna. *Trends in Ecology and Evolution* 33, 227–232. https://doi.org/10.1016/j.tree.2018.01.005.

GESAMP, 2015. Sources, fate and effects of microplastics in the marine environment: a global assessment, in Kershaw P.J., (ed.), IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection. Reports and Studies, GESAMP no. 90, 96.

Ghayebzadeh M., Taghipour H., Aslani H., 2021. Abundance and distribution of microplastics in the sediments of the estuary of seventeen rivers: Caspian southern coasts. *Marine Pollution Bulletin* 164, 112044. https://doi.org/10.1016/j.marpolbul.2021.112044.

Gilbey V., Attrill M.J., Coleman R.A., 2008. Juvenile Chinese mitten crabs (*Eriocheir sinensis*) in the Thames estuary: distribution, movement and possible interactions with the native crab *Carcinus maenas*. *Biological Invasions* 10, 67–77. https://doi.org/10.1007/s10530-007-9110-4.

Godoy V., Blázquez G., Calero M., Quesada L., Martín-Lara M.A., 2019. The potential of microplastics as carriers of metals. *Environmental Pollution* 255, 113363. https://doi.org/10.1016/j.envpol.2019.113363.

Goldberg E.D., 1995. Emerging problems in the coastal zone for the twenty-first century. *Marine Pollution Bulletin* 31, 152–158. https://doi.org/10.1016/0025-326X(95)00102-S.

González-Fernández D., Hanke G., and the RiLON network, 2018. Floating macro litter in European rivers – top items, EUR 29383 EN. Publications office of the European Union, Luxembourg, JRC108172. https://doi.org/10.2760/316058.

Gouin T., 2020. Toward an improved understanding of the ingestion and trophic transfer of microplastic particles: critical review and implications for future research. *Environmental Science and Technology* 39, 1119–1137. https://doi.org/10.1002/etc.4718.

Gramentz D., 1988. Involvement of loggerhead turtle with plastic, metal, and hydrocarbon pollution in the central Mediterranean. *Marine Pollution Bulletin* 19, 11–13. https://doi.org/10.1016/0025-326X(88)90746-1.

Grant M.L., Lavers J.L., Hutton I., Bond A.L., 2021. Seabird breeding islands as sinks for marine plastic debris. *Environmental Pollution* 276, 116734. https://doi.org/10.1016/j.envpol.2021.116734.

Green D.S., Boots B., Blockley D.J., Rocha C., Thompson R., 2015. Impacts of discarded plastic bags on marine assemblages and ecosystem functioning. *Environmental Science and Technology* 49, 5380–5389. https://doi.org/10.1021/acs.est.5b00277.

Greenpeace UK, 2017. Written evidence by Greenpeace UK [online], for House of Commons Environmental Audit Committee, 2017. Plastic bottles: turning back the plastic tide. First report of session 2017–2019. Available at: http://data.parliament.uk/WrittenEvidence/CommitteeEvidence.svc/EvidenceDocument/Environmental%20Audit/Packaging/written/49799.html. [Accessed 7th January 2019].

Greenpeace, 2019. Upstream: microplastics in UK rivers [online]. Available at: https://www.greenpeace.org.uk/wp-content/uploads/2019/06/plastics_v08.pdf. [Accessed 15th July 2020].

Gregory M.R., 2009. Environmental implications of plastic debris in marine settings – entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions.

Philosophical Transactions of the Royal Society B: Biological Sciences 364, 2013–2025. https://doi.org/10.1098/rstb.2008.0265.

Greyer W.R., Chant R., Houghton R., 2008. Tidal and spring-neap variations in horizontal dispersion in a partially mixed estuary. *Journal of Geophysical Research: Oceans* 113, C07023. https://10.1029/2007JC004644.

GSK, 2018. 300 years of GSK: three centuries of innovation [online]. Available at: https://www.gsk.com/media/4573/300yrs-of-gsk.pdf. [Accessed 9th November 2020].

Gulizia A.M., Brodie E., Daumuller R., Bloom S.B., Corbett T., Santana M.M.F., Motti C.A., Vamvounis G., 2022. Evaluating the effect of chemical digestion treatments on polystyrene microplastics: recommended updates to chemical digestion protocols. *Macromolecular Chemistry and Physics* early view, 2100485. https://doi.org/10.1002/macp.202100485.

Guppy Friend Bag [online]. Available at: http://guppyfriend.com/en/. [Accessed 15th October 2018].

Hall A.J., McConnell B.J., Schwacke L.H., Ylitalo G.M., Williams R., Rowles T.K., 2018. Predicting the effects of polychlorinated biphenyls on cetacean population through impacts on immunity and calf survival. *Environmental Pollution* 233, 407–418. https://doi.org/10.1016/j.envpol.2017.10.074.

Halliwell J., Zuke E., 2012. Asda widens gap over rivals in price wars [online]. Available at: https://www.thegrocer.co.uk/fmcg-prices-and-promotions/asda-widens-gap-over-rivals-in-price-wars/229923.article. [Accessed 15th June 2020].

Halpern B.S., Wallbridge S., Selkoe K.A., Kappel C.V., Micheli F., D'Agrosa C., Bruno J.F., Casey K.S., Ebert C., Fox H.E., Fujita R., Heinemann D., Leniham H.S., Madin E.M.P., Perry M.T., Selig E.R., Spalding M., Steneck R., Watson R., 2008. A global map of human impact on marine ecosystems. *Science* 319, 948–952. https://doi.org/10.1126/science.1149345.

Hammond P.S., Grellier K., 2006. Robust digestion and passage rate estimates for hard parts of grey seal (*Halichoerus grypus*) prey. *Canadian Journal of Fisheries and Aquaculture Sciences* 63, 1982–1998. https://doi.org/10.1139/f06-092.

Hammond P.S., Wilson L.J., 2016. Grey seal diet composition and prey consumption. *Scottish Marine and Freshwater Science* 7 no. 20, pp. 52. Marine Science Scotland, St Andrews, Scotland.

Hanke G., Galgani F., Werner S., Oosterbaan L., Nilson P., Fleet D., Kinsey S., Thompson R., Palatinus A., Van Franeker J.A., Vlachogianni T., Scoullos M., Veiga J.M., Matiddi M., Alcaro L., Maes T., Korpinen S., Budziak A., Leslie H., Gago J., Liebezeit G., 2013. Guidance on monitoring of marine litter in European seas: a guidance document within the common implementation strategy for the marine strategy framework directive. MSFD Technical Subgroup on Marine Litter. JRC Scientific and Policy Reports, European Commission. https://doi.org/10.2788/99816.

Hara J., Frias J., Nash R., 2020. Quantification of microplastic ingestion by the decapod crustacean *Nephrops norvegicus* from Irish waters. *Marine Pollution Bulletin* 152, 110905. https://doi.org/10.1016/j.marpolbul.2020.110905.

Härkönen T., 1986. Guide to the otoliths of the bony fishes of the Northeast Atlantic. Danbui ApS. Biological Consultants, Denmark.

Hartline N.L., Bruce N.J., Kaba S.N., Ruff E.O., Sonar S.U., Holden P.A., 2016. Microfiber Masses Recovered from Conventional Machine Washing of New or Aged Garments. *Environmental Science and Technology* 50, 11532–11538. https://doi.org/10.1021/acs.est.6b03045.

Hermabessiere L., Rochman C.M., 2021. Microwave-assisted extraction for quantification of microplastics using pyrolysis-gas chromatography/mass spectrometry. *Environmental Toxicology and Chemistry* 40, 2733–2741. https://doi.org/10.1002/etc.5179.

Hidalgo-Ruz V., Gutow L., Thompson R.C., Thiel M., 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification.

Environmental Science and Technology 46, 3060–3075. https://doi.org/10.1021/es2031505.

Hinchcliffe T., 2017. First fleece to 'not shed' microplastics [online]. Available at: https://www.ecotextile.com/2017103123051/materials-production-news/first-fleece-to-not-shed-microplastics.html. [Accessed 23rd February 2018].

Hitchcock J.N., 2020. Storm events as key moments of microplastic contamination in aquatic ecosystems. *Science of the Total Environment* 734, 139436. https://doi.org/10.1016/j.scitotenv.2020.139436.

HM Government, 2018. A green future: our 25 year plan to improve the environment. Defra, London. 1–151.

Hodgson D.J., Bréchon A.L., Thompson R.C., 2018. Ingestion and fragmentation of plastic carrier bags and the amphipod *Orchestia gammarellus*: effects of plastic type and fouling load. *Marine Pollution Bulletin* 127, 154–159. https://doi.org/10.1016/j.marpolbul.2017.11.057.

Hodson M.E., Duffus-Hodson C.A., Clark A., Prendergast-Miller M.T., Thorpe K.L., 2017. Plastic bag derived-microplastics as a vector for metal exposure in terrestrial invertebrates. *Environmental Science and Technology* 51, 4714–4721. https://doi.org/10.1021/acs.est.7b00635.

Horton A.A., Jürgens, M.D., Lahive, E., van Bodegom, P.M., Vijver, M.G., 2018. The influence of exposure and physiology on microplastic ingestion by the freshwater fish *Rutilus rutilus* (roach) in the River Thames, UK. *Environmental Pollution* 236, 188–194. https://doi.org/10.1016/j.envpol.2018.01.044.

Horton A.A., Svendsen C., Williams R.J., Spurgeon D.J., Lahive E., 2017. Large microplastic particles in sediments of tributaries of the River Thames, UK - abundance, sources and methods for effective quantification. *Marine Pollution Bulletin* 114, 218–226. https://doi.org/10.1016/j.marpolbul.2016.09.004.

Hossain M.S., Rahman M.S., Uddin M.N., Shrifuzzaman S.M., Chowdhury S.R., Sarker S., Chowdhury M.S.N., 2020. Microplastic contamination in Penaeid shrimp from the

Northern Bay of Bengal. *Chemosphere* 238, 124688. https://doi.org/10.1016/j.chemosphere.2019.124688.

Hothorn T., Bretz F., Westfall P., 2008. Simultaneous inference in general parametric models. *Biometrical Journal* 50, 346–363.

House of Commons Environmental Audit Committee, 2017. Plastic bottles: turning back the plastic tide. First report of session 2017–2019 [online]. Available at: https://publications.parliament.uk/pa/cm201719/cmselect/cmenvaud/339/339.pdf. [Accessed 4th January 2019].

House of Commons Environmental Audit Committee, 2019a. Interim report on the sustainability of the fashion industry. Fifteenth report of the session 2017–19 [online]. Available at: https://publications.parliament.uk/pa/cm201719/cmselect/cmenvaud/1148/1148.pdf. [Accessed 30th June 2020].

House of Commons Environmental Audit Committee, 2019b. Fixing fashion: clothing consumption and sustainability. Sixteenth report of session 2017–19 [online]. Available at: https://publications.parliament.uk/pa/cm201719/cmselect/cmenvaud/1952/1952.pdf. [Accessed 30th June 2020].

Huang D., Li X., Ouyang Z., Zhao X., Wu R., Zhang C., Lin C., Li Y., Guo X., 2021. The occurrence and abundance of microplastics in surface water and sediment of the West River downstream, in the south of China. *Science of the total Environment* 756, 143857. https://doi.org/10.1016/j.scitotenv.2020.143857.

Huang Q., Lin Y., Zhing Q., Ma F., Zhang Y., 2020. The impact of microplastic particles on population dynamics of predator and prey: implications of the Lotka-Volterra model. *Scientific Reports* 10, 4500. https://doi.org/10.1038/s41598-020-61414-3.

Hula Hoops, 2019. Our story: A look back at Hula Hoops' history through its packaging… [online]. Available at: https://www.hulahoops.com/the-hoops-story/. [Accessed 12th June 2020].

Hurley R., Schell T., Crossman J., Rico A., Nawrocki B., Lavoy M., Vighi M., Nizzetto L., 2018. Microplastic dynamics in agricultural systems: insights from Spanish and Canadian case studies, presented in part at Micro2018, Lanzarote, Canary Islands, Spain, November 2018. Abstract available online at: https://micro2018.sciencesconf.org/data/pages/micro2018_proceedings_book_1.pdf. [Accessed 23rd November 2021].

Hutchins M.G., Abesser C., Prudhomme C., Elliot J.A., Bloomfield J.P., Mansour M.M., Hitt O.E., 2018. Combined impacts of future land-use and climate stressors on water resources and quality in groundwater and surface waterbodies of the upper Thames river basin, UK. *Science of the Total Environment* 631–632, 962–986. https://doi.org/10.1016/j.scitotenv.2018.03.052.

ICES, 2017. Report of the Working Group on Multispecies Assessment Methods (WGSAM), 10 – 14 October 2016, Reykjavik, Iceland. ICES CM 2016/SSGEPI:21. 94 pp.

Imran M., Das K.R., Naik M.M., 2019. Co-selection of multi-antibiotic resistance in bacterial pathogens in metal and microplastic contaminated environments: an emerging health threat. *Chemosphere* 215, 846–857. https://doi.org/10.1016/j.chemosphere.2018.10.114.

Iribarne O., Botto F., Martinetto P., Gutierrez J.L., 2000. The role of burrows of the SW Atlantic intertidal crab *Chasmagnathus granulata* in trapping debris. *Marine Pollution Bulletin* 40, 1057–1062. https://doi.org/10.1016/S0025-326X(00)00058-8.

Iroegbu A.O.C., Ray S.S., Mbarane V., Bordado J.C., Sardinha J.P., 2021. Plastic pollution: a perspective on matters arising: challenges and opportunities. *ACS Omega* 6, 19343–19355. https://doi.org/10.1021/acsomega.1c02760.

Jambeck J.R., Greyer R., Wilcox C., Siegler T.R., Perryman M., Andrady A., Narayan R., Law K.L., 2015. Plastic waste inputs from land into the ocean. *Science* 347, 768–771. https://doi.org/10.1126/science.1260352.

Jang M., Shim W.J., Cho Y., Han G.M., Song Y.K., Hong S.H., 2020. A close relationship between microplastic contamination and coastal area use pattern. *Water Research* 171, 115400. https://doi.org/10.1016/j.watres.2019.115400.

Jiang C., Yin L., Li Z., Wen X., Luo X., Hu S., Yang H., Long Y., Deng B., Huang L., Liu Y., 2019. Microplastic pollution in the rivers of the Tibet Plateau. *Environmental Pollution* 249, 91–98. https://doi.org/10.1016/j.envpol.2019.03.022.

Jiwarungrueangkul T., Phaksopa J., Sompongchaiyakul P., Tipmanee D., 2021. Seasonal microplastic variations in estuarine sediments from urban canal on the west coast of Thailand: a case study in Phuket province. *Marine Pollution Bulletin* 168, 112452. https://doi.org/10.1016/j.marpolbul.2021.112452.

Kallenback E.M.F., Rødland E.S., Buenaventura N.T., Hurley R., 2021. Microplastics in terrestrial and freshwater environments, in Bank M.S. (ed.), Microplastic in the environment: pattern and process. Remediation and Management. Springer, Cham 87–130. https://doi.org/10.1007/978-3-030-78627-4.

Kane I.A., Clare M.A., Miramontes E., Wogelius R., Rothwell J.J., Garreau P., Pohl F., 2020. Seafloor microplastic hotspots controlled by deep-sea circulation. *Science*, eaba5899. https://doi.org/10.1126/science.aba5899.

Karami A., Golieskardi A., Choo C.K., Romano N., Ho Y.B., Salamatinia B., 2017. A high-performance protocol for extraction of microplastics in fish. *Science of the Total Environment* 578, 485–495. https://doi.org/10.1016/j.scitotenv.2016.10.213.

Karasik R., Vegh T., Diana Z, Bering J., Caldas J., Pickle A., Rittschof D., Virdin J., 2020. 20 years of government responses to the global plastic pollution problem: the plastics policy inventory. NI X 20-05. Nicholas Institute for Environmental Policy Solutions, Duke University, Durham, pp. 311.

Kazour M., Jemaa S., El Rawke M., Duflos G., Hermabessiere L., Dehaut A., Le Bihanic F., Cachot J., Cornille V., Rabhi K., Khalaf G., Amara R., 2018. Juvenile fish caging as a tool for assessing microplastics contamination in estuarine fish nursery grounds.

Environmental Science and Pollution Research 25, 1–12. https://doi.org/10.1007/s11356-018-3345-8.

Keep Los Angeles Beautiful, 2009. Littering and the iGeneration: city-wide intercept study of youth litter behaviour in Los Angeles. S. Groner Associates in collaboration with Action Research.

Khuyen V.T.K., Le D.V., Fischr A.R., Dornack C., 2021. Comparison of microplastic pollution in beach sediment and seawater at UNESCO Can Gio Mangrove Biosphere Reserve. *Global Challenges* 5, 2100044. https://doi.org/10.1002/gch2.202100044.

Kirby M.F., Neall P., Bateman T.A., Thain J.E., 2004. Hepatic ethoxyresorufin o-deethylase (EROD) activity in flounder (*Platichthys flesus*) from contaminant impacted estuaries of the United Kingdom: continued monitoring 1999–2001. *Marine Pollution Bulletin* 49, 71–78. https://doi.org/10.1016/j.marpolbul.2004.01.009.

Kirstein I.V., Kirmizi S., Wichels A., Garin-Fernandez A., Erler R., Löder M., Gerdts G., 2016. Dangerous hitchhikers? Evidence for potentially pathogenic *Vibrio* spp. on microplastic particles. *Marine Environmental Research* 120, 1–8. https://doi.org/10.1016/j.marenvres.2016.07.004.

Klein J.A., Petrere Jr. M., Butturi-Gomes D., Barrella W., 2020. Textile sustainability: a Brazilian etiquette issue. *Environmental Science and Policy* 109, 125–130. https://doi.org/10.1016/j.envsci.2020.02.025.

Koelmans A.A., Bakir A., Burton G.A., Janssen C.R., 2016. Microplastic as a vector for chemicals in the aquatic environment: critical review and model-supported reinterpretation of empirical studies. *Environmental Science and Technology* 50, 3315–3326. https://doi.org/10.1021/acs.est.5b06069.

Kole P.J., Löhr A.J., van Berlleghem F.G.A.J., Ragas A.M.J., 2017. Wear and tear of tyres: a stealthy source of microplastics in the environment. *International Journal of Environmental Research and Public Health* 14, 1265. https://doi.org/10.3390/ijerph14101265.

Koutnik V.S., Alkidim S., Leonard J., DePrima F., Cao S., Hoek E.M.V., Mohanty S.K., 2021. Unaccounted microplastics in wastewater sludge: where do they go? *ACS EST Water* 1, 1086–1097. https://doi.org/10.1021/acsestwater.0c000267.

Kragh G., Winton D., Loiselle S., van Noordwijk T., 2019. Plastic rivers: reducing the plastic pollution on our doorstep. Earthwatch Institute in association with Plastic Oceans. Earthwatch Europe, Oxford, UK, pp. 20.

Krelling A.P., Souza M.M., Williams A.T., Turra A., 2017. Transboundary movement of marine litter in an estuarine gradient: evaluating sources and sinks using hydrodynamic modelling and ground truthing estimates. *Marine Pollution Bulletin* 119, 48–63. https://doi.org/10.1016/j.marpolbul.2017.03.034.

Kühn S., Bravo Rebolledo E.L., van Franeker J.A., 2015. Deleterious effects of litter on marine life, in Bergmann M., Gutow L., Klages M. (eds), Marine Anthropogenic Litter. Springer, Cham, 75–116. https://doi.org/10.1007/978-3-319-16510-3_4.

Kühn S., van Franeker J.A., 2020. Quantitative overview of marine debris ingested by marine megafauna. *Marine Pollution Bulletin* 151, 110858. https://doi.org/10.1016/j.marpolbul.2019.110858.

Kühn S., van Werven B., van Oyen A., Meijboom A., Bravo Rebolledo E.L., van Franeker J.A., 2017. The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms. *Marine Pollution Bulletin* 115, 86–90. https://doi.org/10.1016/j.marpolbul.2016.11.034.

Kumar M., Xiong X., He M., Tsang D.C.W., Gupta J., Khan E., Harrad S., Hou D., Ok Y.S., Bolan N.S., 2020. Microplastics as pollutants in agricultural soils. *Environmental Pollution* 265, 114980. https://doi.org/10.1016/j.envpol.2020.114980.

Kupelian M., 2018. 21 years as the UK's lowest price supermarket is a team effort [online]. Available at: https://corporate.asda.com/blog/2018/06/18/21-years-as-the-uks-lowest-price-supermarket-is-a-team-effort. [Accessed 15th June 2020].

Kwon T.A., Choo H.J., Kim Y.K., 2019. Why do we feel bored with our clothing and where does it end up? *International Journal of Consumer Studies* 44, 1–13. https://doi.org/10.1111/ijcs.12540.

Laist D.W., 1987. Overview of the biological effects of lost and discarded plastic debris in the marine environment. *Marine Pollution Bulletin* 18, 319–326. https://doi.org/10.1016/S0025-326X(87)80019-X.

Lau W.W.Y., Shiran J., Bailey R.M., Cook E., Stuchtey M.R., Koskella J., Velis C.A., Godfrey L., Boucher J., Murphy M.B., Thompson R.C., Jankowska E., Castillo Castillo A., Pilditch T.D., Dixon B., Koerselman L., Kosior E., Favoino E., Gutberlet J., Baulch S., Atreya M.E., Fischer D., He K.K., Petit M.M., Sumaila U.R., Neil E., Bernhofen M.V., Lawrence K., Palardy J.E., 2020. Evaluating scenarios toward zero plastic pollution. *Science* 369, 1455–1461. https://doi.org/10.1126/science.aba9475.

Lebreton L., Andrady A., 2019. Future scenarios of global plastic waste generation and disposal. *Palgrave Communications* 5, 6. https://doi.org/10.1057/s41599-018-0212-7.

Lebreton L.C.M., van der Zwet J., Damsteeg J.-W., Slat B., Andrady A., Reisse J., 2017. River plastic emissions to the world's oceans. *Nature Communications* 8, 15611. https://doi.org/10.1038/ncomms15611.

Lechner A., Keckeis H., Lumesberger-Loisl F., Zens B., Krusch R., Tritthart M., Glas M., Schludermann E., 2014. The Danube so colourful: a potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. *Environmental Pollution* 188, 177–181. https://doi.org/10.1016/j.envpol.2014.02.006.

Lenz R., Enders K., Nielsen T.G., 2016. Microplastic exposure studies should be environmentally realistic. *Proceedings of the National Academy of Sciences of the United States of America* 113, E4121-E4122. https://doi.org/10.1073/pnas.1606615113.

Leslie H.A., Brandsma S.H., Van Velzen M.J.M., Vethaak A.D., 2017. Microplastics en route: field measurements in the Dutch river delta and Amsterdam canals, wastewater

treatment plants, North Sea sediments and biota. *Environment International* 101, 133–142. https://doi.org/10.1016/j.envint.2017.01.018.

Li J., Green C., Reynolds A., Shi H., Rotchell J.M., 2018. Microplastics in mussels sampled from coastal waters and supermarkets in the United Kingdom. *Environmental Pollution* 241, 35–44. https://doi.org/10.1016/j.envpol.2018.05.038.

Li J., Song Y., Cai Y., 2020. Focus topics on microplastics in soil: analytical methods, occurrence, transport and ecological risks. *Environmental Pollution* 257, 113570. https://doi.org/10.1016/j.envpol.2019.113570.

Lincoln S., Andrews B., Birchenough S.N.R., Chowdhury P., Engelhard G.H., Harrod O., Pinnegar J.K., Townhill B.L., 2022. Marine litter and climate change: inextricably connected threats to the world's oceans. *Science of the Total Environment* 837, 155709. https://doi.org/ 10.1016/j.scitotenv.2022.155709.

Liu Y., You J., Tang Y., He Y., Breider F., Tao S., Liu W., 2022. Quantified effects of multiple parameters on inputs and potential sources of microplastics from a typical river flowing into the sea. *ACS EST Water* 2, 556–564. https://doi.org/10.1021/acsestwater.1c00354.

Liu Z., Yu P., Cai M., Wu D., Zhang M., Chen M., Zhao Y., 2019. Effects of microplastics on the innate immunity and intestinal microflora of juvenile *Eriocheir sinensis*. *Science of the Total Environment* 685, 836–846. https://doi.org/10.1016/j.scitotenv.2019.06.265.

Logopedia, 2019a. Monster Munch [online]. Available at: https://logos.fandom.com/wiki/Walkers_Monster_Munch. [Accessed 12th June 2020].

Logopediea, 2019b. Walkers Classic [online]. Available at: https://logos.fandom.com/wiki/Walkers_Classic. [Accessed 12th June 2020].

Logopedia, 2020. Walkers [online]. Available at: https://logos.fandom.com/wiki/Walkers. [Accessed 12th June 2020].

Lorenz C., Roscher L., Meyer M.S., Hildebrandt L., Prume J., Löder M.G.J., Primpke S., Gerdts G., 2019. Spatial distribution of microplastics in sediments and surface waters of the southern North Sea. *Environmental Pollution* 252, 1719–1729. https://doi.org/10.1016/j.envpol.2019.06.093.

Lowe S., Browne M., Boudjelas S., De Poorter M., 2000. 100 of the world's worst invasive alien species: a selection from the Global Invasive Species Database. Published by The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN), 12pp. First published as a special lift-out in Aliens 12, December 2000. Updated and reprinted version: November 2004. Electronic version available at: www.issg.org/booklet.pdf.

Lu H., Ziajahromi S., Neale P.A., Leusch F.D.L., 2021. A systematic review of freshwater microplastics in water and sediments: recommendations for harmonisation to enhance future study comparisons. *Science of the Total Environment* 781, 146693. https://doi.org/10.1016/j.scitotenv.2021.146693.

Lusher A.L., Bråte I.L.N., Munno K., Hurley R.R., Welden N.A., 2020a. Is it of isn't it: the importance of visual classification in microplastic characterization. *Applied Spectroscopy* 74, 1139–1153. https://doi.org/10.1177/0003702820930733.

Lusher A.L., Hernandez-Milian G., 2018. Microplastic extraction from marine vertebrate digestive tracts, regurgitates and scats: a protocol for researchers from all experience levels. *Bio-protocol* 8, e3087. https://doi.org/10.21769/BioProtoc.3087.

Lusher A.L., Munno K., Hermabessiere L., Carr S., 2020b. Isolation and extraction of microplastics from environmental samples: an evaluation of practical approaches and recommendations for further harmonisation. *Applied Spectroscopy* 74, 1049–1065. https://doi.org/10.1177/0003702820938993.

Lusher A.L., Welden N.A., Sobral P., Cole M., 2017. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. *Analytical Methods* 9, 1346–1360. https://doi.org/10.1039/C6AY02415G.

Lwanga E.H., Vega J.M., Quej V.K., Chi J.D., del Cid L.S., Chi C., Segura G.E., Gertsen H., Salanki T., van der Ploeg M., Koelmans A.A., Geissen V., 2017. Field evidence for transfer of plastic debris along a terrestrial food chain. *Scientific Reports* 7, 14071. https://doi.org/10.1038/s41598-017-14588-2.

Mackinson S., Platts M., Garcia C., Lynam C., 2018. Evaluating the fishery and ecological consequences of the proposed North Sea multi-annual plan. *PLoS ONE* 13, e0190015. https://doi.org/10.1371/journal.pone.019005.

MacLeod M., Arp H.P.H., Tekman M.B., Jahnke A., 2021. The global threat from plastic pollution. *Science* 373, 61–65. https://doi.org/10.1126/science.abg5433.

Maes T., Jessop R., Wellner N., Haupt K., Mayes A.G., 2017a. A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. *Scientific Reports* 7, 44501. https://doi.org/10.1038/srep44501.

Maes T., Van der Meulen M.D., Devriese L.I., Leslie H.A., Huvet A., Frère L., Robbens J., Vethaak A.D., 2017b. Microplastics baseline surveys at the water surface and in sediments of the North-East Atlantic. *Frontiers in Marine Science* 4, 135. https://doi.org/10.3389/fmars.2017.00135.

Mani T., Primpke S., Lorenz C., Gerdts G., Burkhardt-Holm P., 2019. Microplastic pollution in benthic midstream sediments of the Rhine River. *Environmental Science and Technology* 58, 6053–6062. https://doi.org/10.1021/acs.est.9b01363.

Marchand J., Evrard E., Guinand B., Cachot J., Quiniou L., Laroche J., 2010. Genetic polymorphism and its potential relation to environmental stress in five populations of the European flounder *Platichthys flesus*, along the French Atlantic coast. *Marine Environmental Research* 70, 201–209. https://doi.org/10.1016/j.marenvres.2010.05.002.

Markussen N.H., 1993. Transit-time of digesta in captive harbour seals (*Phoca vitulina*). *Canadian Journal of Zoology* 71, 1071–1073. https://doi.org/10.1139/z93-144.

Marsh T., Hannaford J. (eds), 2008. UK hydrometric register. Hydrological data UK series. Centre for Ecology and Hydrology, Wallingford. 1–210.

Masura J., Baker J., Foster G., Arthur C., 2015. Laboratory methods for the analysis of microplastics in the marine environment: recommendations for quantifying synthetic particles in waters and sediments. NOAA Technical Memorandum, NOS-ORandR-48, Silver Spring. 1–39.

Mato Y., Isobe T., Takada H., Kanehiro H., Ohtake C., Kaminuma T., 2001. Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. *Environmental Science and Technology* 35, 318–324. https://doi.org/10.1021/es0011498.

McConville A.J., Hall A., Harrington E., Downer K., 2020. Plastic pollution in the River Thames: results from citizen science surveys: January 2015 to December 2019 [online]. Available at: https://www.thames21.org.uk/wp-content/uploads/2020/06/FINAL-Plastic-pollution-in-the-tidal-Thames-v10.pdf. [Accessed 21st October 2020].

McCoy K.A., Hodgson D.J., Clark P.F., Morritt D., 2020. The effects of wet wipe pollution on the Asian clam, *Corbicula fluminea* (Mollusca: Bivalvia) in the River Thames, London. *Environmental Pollution* 264, 114577. https://doi.org/10.1016/j.envpol.2020.114577.

McGaw I.J., Curtis D.L., 2013. A review of gastric processing in decapod crustaceans. *Journal of Comparative Physiology B* 183, 443–465. https://doi.org/10.1007/s00360-012-0730-3.

McGoran A.R., 2019a. Does anyone recognise this logo? Today I'm being a #PlasticDetective and trying to work out what this #plastic fragment from the #Thames used to be. Can you help? [Twitter]. Available at: https://twitter.com/AlexMcGoran/status/1109116414697373696?s=20. [Accessed 15th June 2020].

McGoran A.R., 2019b. Does anyone recognise this packaging? It looks like it says made in Colombia and "COLO" followed by either an 'M', 'r' or 'n' [Twitter]. Available at: https://twitter.com/AlexMcGoran/status/1159402031884447744?s=20. [Accessed 15th June 2020].

McGoran A.R., Clark P.F., Morritt D., 2017. Presence of microplastic in the digestive tracts of European flounder, *Platichthys flesus*, and European smelt, *Osmerus eperlanus*, from the river Thames. *Environmental Pollution* 220, 744–751. https://doi.org/10.1016/j.envpol.2016.09.078.

McGoran A.R., Clark P.F., Smith B.D., Morritt D., 2020. High prevalence of plastic ingestion by *Eriocheir sinensis* and *Carcinus maenas* (Crustacea: Decapoda: Brachyura) in the Thames Estuary. *Environmental Pollution* 265, 114972. https://doi.org/10.1016/j.envpol.2020.114972.

McGoran A.R., Cowie P.R., Clark P.F., McEvoy J.P., Morritt D., 2018. Ingestion of plastic by fish: a comparison of Thames Estuary and Firth of Clyde populations. *Marine Pollution Bulletin* 137, 12–23. https://doi.org/10.1016/j.marpolbul.2018.09.054.

McIlwraith H.K., Kim J., Helm P., Bhausar S.P., Metzger J.S., Rochman C.M., 2021. Evidence of microplastic translocation in wild-caught fish and implications for microplastic accumulation dynamics in food webs. *Environmental Science and Technology* 55, 12372–12382. https://doi.org/10.1021/acs.est.1c02922.

McIlwraith H.K., Lin J., Erdle L.M., Mallos N., Diamond M.L., Rochman C.M., 2018. Capturing microfibers – marketed technologies reduce microfiber emissions from washing machines. *Marine Pollution Bulletin* 139, 40–45. https://doi.org/10.1016/j.marpolbul.2018.12.012.

Méheust E., Alfonsi E., Le Ménec P., Hassani S., Jung JL., 2015. DNA barcoding for the identification of soft remains of prey in the stomach contents of grey seals (*Halichoerus grypus*) and harbour porpoises (*Phocoena phocoena*). *Marine Biology Research* 11, 385–395. https://doi.org/10.1080/17451000.2014.943240.

Meijek L.J.J., Van Emmerik T., Van Derent R., Schmidt C., Lebreton L.C.M., 2021. More than 1000 rivers account for 80% of global riverine plastic emissions into the ocean. *Science Advances* 7, 2375–2548. https://doi.org/10.1126/sciadv.aaz5803.

Met Office, 2006 [online]. MIDAS: UK daily rainfall data. NCAS British Atmospheric Data Centre. Available at:

https://catalogue.ceda.ac.uk/uuid/c732716511d3442f05cdeccbe99b8f90. [Accessed 11th November 2021].

Milkcap Mania, 2009 [online]. Available at: https://www.milkcapmania.co.uk/Tazos/Walkers/296-Looney-Tunes.html. [Accessed 12th June 2020].

Miller M.E., Hamann M., Kroon F.J., 2020. Bioaccumulation and biomagnification of microplastics in marine organisms: a review and meta-analysis of current data. *PLoS ONE* 15, e0240792. https://doi.org/10.1371/journal.pone.0240792.

Miller R.Z., Watts A.J.R., Winslow B.O., Galloway T.S., Barrows A.P.W., 2017. Mountains to the sea: river study of plastic and non-plastic microfiber pollution in the northeast USA. *Marine Pollution Bulletin* 124, 245–251. https://doi.org/10.1016/j.marpolbul.2017.07.028.

Mills C.C., Clark P.F., Morritt D., 2016. Flexible prey handling, preference and a novel capture technique in invasive, sub-adult Chinese mitten crabs. *Hydrobiologia* 733, 135–147. https://doi.org/10,1007.s10750-016-2700-3.

Min K., Cuiffi J.D., Mathers R.T., 2020. Ranking environmental degradation trends of plastic marine debris based on physical properties and molecular structure. *Nature Communications* 11, 727. https://doi.org/10.1038/s41467-020-14538-z.

Mitchell R.L., Thamsen P.U., Gunkel M., Waschnewski J., 2017. Investigation into wastewater composition focussing on nonwoven wet wipes. *Technical Transactions* 1, 125–135. https://doi.org/10.4467/235373XCT.17.010.6107.

Moore C.J., 2008. Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. *Environmental Research* 108, 131–139. https://doi.org/10.1016/j.envres.2008.07.025.

Moore C.J., Moore S.L., Leecaster M.K., Weisberg S.B., 2001. A comparison of plastic and plankton in the North Pacific central gyre. *Marine Pollution Bulletin* 42, 1297–1300. https://doi.org/10.1016/S0025-326X(01)00114-X.

Moore R.C., Noel M., Etemadifar A., Loseto L., Posacka A.M., Bendell L., Ross P.S., 2022. Microplastics in beluga whale (*Delphinapterus leucas*) prey: an exploratory assessment of trophic transfer in the Beaufort Sea. *Science of the Total Environment* 806, 150201. https://doi.org/10.1016/j.scitotenv.2021.150201.

Morgado V., Gomes L., Bettencourt da Silva R.J.N., Palma C., 2022. Microplastics contamination in sediments from Portuguese inland waters: physical-chemical characterisation and distribution. *Science of the Total Environment* 832, 155053. https://doi.org/10.1016/j.scitotenv.2022.155053.

Morgans J., 2019. Meet the guy who invented Tazos and gave meaning to school lunchtimes [online]. Available at: https://www.vice.com/en_au/article/evyxgj/meet-the-guy-who-invented-tazos-and-gave-meaning-to-school-lunchtimes. [Accessed 12th June 2020].

Morritt D., Mills H., Hind K., Clifton-Dey D., Clark P.F., 2013. Monitoring downstream migrations of *Eriocheir sinensis* H. Milne Edwards, 1853 (Crustacea: Brachyura: Grapsoidea: Varunidae) in the River Thames using capture data from a water abstraction intake. *Management of Biological Invasions* 4, 139–147. https://doi.org/10.3391/mbi.2013.4.2.07.

Morritt D., Stefanoudis P.V., Pearce D., Crimmen O.A., Clark P.F., 2014. Plastic in the Thames: a river runs through it. *Marine Pollution Bulletin* 78, 196–200. https://doi.org/10.1016/j.marpolbul.2013.10.035.

Mourgkogiannis N., Kalavrouziotis I.K., Karapanagioti H.K., 2018. Questionnaire-based survey to managers of 101 wastewater treatment plants in Greece confirms their potential as plastic marine litter sources. *Marine Pollution Bulletin* 133, 822–827. https://doi.org/10.1016/j.marpolbul.2018.06.044.

Mukendi A., Davies I., McDonagh P., Glozer S., 2020. Sustainable fashion: current and future research directions. *European Journal of Marketing* 54, 2873–2909. https://doi.org/10.1108/EJM-02-2019-0132.

Munoz L.P., Baez A.G., McKinney D., Garelick H., 2018. Characterisation of “flushable” and “non-flushable” commercial wet wipes using microRaman, FTIR spectroscopy and fluorescence microscopy: to flush or not to flush. *Environmental Science and Pollution Research* 20, 20268–20279. https://doi.org/10.1007/s11356-018-2400-9.

Murray F., Cowie P.R., 2011. Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). *Marine Pollution Bulletin* 62, 1207–1217. https://doi.org/10.1016/j.marpolbul.2011.03.032.

Nabeghlavi. Export [online]. Available at: http://www.nabeghlavi.ge/eng/export. [Accessed 15th June 2020].

Nalbone L., Panebianco A., Giarratana F., Russell M., 2021. Nile red staining for detecting microplastics in biota: preliminary evidence. *Marine Pollution Bulletin* 172, 112888. https://doi.org/10.1016/j.marpolbul.2021.112888.

Nan B., Su L., Kellar C., Craig N.J., Keough M.J., Pettigrove V., 2020. Identification of microplastics in surface water and Australian freshwater shrimp *Paratya australiensis* in Victoria, Australia. *Environmental Pollution* 259, 113865. http://doi.org/10.1016/j.envpol.2019.113865.

Napper I.E., Baroth A., Barrett A.C., Bhola S., Chowdhury G.W., Davies B.F.R., Duncan E.M., Kumar S., Nelms S.E., Niloy M.N.H., Nishat B., Maddalene T., Thompson R.C., Koldewey H., 2021. The abundance and characteristics of microplastics in surface water in transboundary Ganges River. *Environmental Pollution* 274, 116348. https://doi.org/10.1016/j.envpol.2020.116348.

Napper I.E., Thompson R.C., 2016. Release of synthetic plastic fibres from domestic washing machines: effects of fabric type and washing conditions. *Marine Pollution Bulletin* 112, 39–45. https://doi.org/10/1016/j.marpolbul.2016.09.025.

National Trust, 2019. An uninhabited island that is so remote a permit is required to visit has been littered with thousands of elastic bands – by seabirds mistakenly thinking they are worms, the National Trust said [online]. Available at:

https://www.nationaltrust.org.uk/press-release/remote-bird-haven-found-strewn-with-elastic-bands. [Accessed 28th May 2020].

Nel H.A., Dalu T., Wasserman R.J., 2018. Sinks and sources: assessing microplastic abundance in river sediments and deposit feeders in an Austral temperate urban river system. *Science of the Total Environment* 612, 950–956. https://doi.org/10.1016/j.scitotenv.2017.08.298.

Nelms S.E., Barnett J., Brownlow A., Davison N.J., Deaville R., Galloway T.S., Lindeque P.K., Santillo D., Godley B.J., 2019. Microplastics in marine mammals stranded around the British coast: ubiquitous but transitory? *Scientific Reports* 9, 1075. https://doi.org/10.1038/s41598-018-37428-3.

Nelms S.E., Galloway T.S., Godley B.J., Jarvis D.S., Lindeque P.K., 2018. Investigating microplastic trophic transfer in marine top predators. *Environmental Pollution* 238, 999–1007. https://doi.org/10.1016/j.envpol.2018.02.016.

Newbold, 2014. Star Wars in the UK: lock S-foils in a snack position – Walkers crisps '97–'99 [online]. Available at: https://www.starwars.com/news/star-wars-in-the-uk-lock-s-foils-in-a-snack-position-walkers-crisps-97-99. [Accessed 12th June 2020].

Nizzetto L., Langaas S., Futter M., 2016. Pollution: do microplastics spill on to farm soils? *Nature* 537, 488. https://doi.org/10.1038/537488b.

NOAA Fisheries [online]. Available at: https://www.fisheries.noaa.gov/. [Accessed 30th March 2020].

Nor N.H.M., Obbard J.P., 2014. Microplastics in Singapore's coastal mangrove ecosystems. *Marine Pollution Bulletin* 79, 278–283. https://doi.org/10.1016/j.marpolbul.2013.11.025.

Not C., Lui C.Y.I., Cannicci S., 2020. Feeding behaviour is the main driver for microplastic intake in mangrove crabs. *Limnology and Oceanography Letters* 5, 84–91. https://doi.org/10.1002/lol2.10143.

Ó Brian O., Marques Mendes A.R., McCarron S., Healy M.G., Morrison L., 2020. The role of wet wipes and sanitary towels as a source of white microplastic fibres in the marine environment. *Water Research* 182, 116021. https://doi.org/10.1016/j.watres.2020.116021.

O'Brien S., Okoffo E.D., O'Brien J.W., Ribeiro F., Wang X., Wright S.L., Samanipour S., Rauert C., Toapanta T.Y.A., Albarracin R., Thomas K.V., 2020. Airborne emissions of microplastic fibres from domestic laundry dryers. *Science of the Total Environment* 747, 141175. https://doi.org/10.1016/j.scitotenv.2020.141175.

O'Neill E., 2019. Campaigning for plastic-free periods [online]. Available at: https://www.mcsuk.org/news/period-plastic. [Accessed 21st October 2020].

Olsen M.T., Islas V., Graves J.A., Onoufriou A., Vincent C., Brasseur S., Frie A.K., Hall A.J., 2017. Genetic population structure of harbour seals in the United Kingdom and neighbouring waters. *Aquatic Conservation* 27, 839–845. https://doi.org/10.1012/ac.c2760.

Ory N.C., Gallardo C., Lenz M., Thiel M., 2018. Capture, swallowing, and egestion of microplastics by a planktivorous juvenile fish. *Environmental Pollution* 240, 566–573. https://doi.org/10.1016/j.envpol.2018.04.093.

Ory N.C., Sobral P., Ferreira J.L., Thiel M., 2017. Amberstripe chad *Decapterus muroadsi* (Carangidae) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific gyre. *Science of the Total Environment* 586, 430–437. https://doi.org/10.1016/j.scitotenv.2017.01.175.

OSPAR Commission, 2008. Case report for the OSPAR list of threatened and/or declining species and habitats [online]. Available at: https://www.ospar.org/site/assets/files/44267/harbour_porpoise.pdf. [Accessed 23rd November 2021].

OSPAR Commission, 2014. Marine litter regional action plan [online]. Available at: https://www.ospar.org/documents?v=34422. [Accessed 17th June 2022].

OSPAR Commission, 2016. OSPAR background document for harbour porpoise *Phocoena phocoena* (OSPAR publication 420) [online]. Available at: https://www.ospar.org/site/assets/files/44267/harbour_porpoise_reporting.pdf. [Accessed 23rd November 2021].

OSPAR Commission 2022. Marine litter [online]. Available at: https://www.ospar.org/work-areas/eiha/marine-litter. [Accessed 10th June 2022].

Panning A., 1939. The Chinese mitten crab. *Annual Report Smithsonian Institution for 1938* 3508, 361–375.

Parker B., Andreou D., Green I.D., Britton J.R., 2021. Microplastics in freshwater fishes: occurrence, impacts and future perspectives. *Fish and Fisheries* 22, 467–488. https://doi.org/10.1111/faf.12528.

Parslow J.L.F., Jefferies D.J., 1972. Elastic thread pollution of puffins. *Marine Pollution Bulletin* 3, 43–45. https://doi.org/10.1016/0025-326X(72)90142-7.

Peng X., Chen M., Chen S., Dasgupta S., Xu H., Ta K., Du M., Li J., Guo Z., Bai S., 2018. Microplastics contaminate the deepest part of the world's ocean. *Geochemical Perspectives Letters* 9, 1–5. https://doi.org/10.7185/geochemlet.1829.

Periyasamy A.P., Tehrani-Bagha A., 2022. A review on microplastic emissions from textile materials and its reduction techniques. *Polymer Degradation and Stability* 199, 109901. https://doi.org/10.1016/j.polymdegradstab.2022.109901.

Petersen F., Hubbart J.A., 2021. The occurrence and transport of microplastics: the state of the science. *Science of the Total Environment* 758, 143936. https://doi.org/10.1016/j.scitotenv.2020.143936.

Phuong N.N., Zalouk-Vergnoux A., Kamari A., Mouneyrac C., Amiard F., Poirier L., Lagarde F., 2018. Quantification and characterization of microplastics in blue mussels (*Mytilus edulis*): protocol setup and preliminary data on the contamination of the French Atlantic coast. *Environmental Science and Pollution Research* 25, 6135–6144. https://doi.org/10.1007/s11356-017-8862-3.

Pinheiro C., Oliveira U., Viera M., 2017. Occurrence and impacts of microplastics in freshwater fish. *Journal of Aquaculture and Marine Biology* 5, 00138. https://doi.org/10.15406/jamp.2017.05.00138.

PlasticsEurope, 2016. Plastics – the facts 2016: an analysis of European plastics production, demand and waste data. PlasticsEurope, Brussels. 1–38.

PlasticsEurope, 2018a. Plastics – the facts 2017: an analysis of European plastics production, demand and waste data. PlasticsEurope, Brussels. 1–44.

PlasticsEurope, 2018b. PlasticsEurope Annual review 2017-2018. PlasticsEurope, Brussels. 1–44.

PlasticsEurope, 2019a. Plastics – the facts 2018: an analysis of European plastics production, demand and waste data. PlasticsEurope, Brussels. 1–60.

PlasticsEurope, 2019b. Plastics – the facts 2019: an analysis of European plastics production, demand and waste data. PlasticsEurope, Brussels. 1–42.

PlasticsEurope 2021. Plastics – the facts 2021: an analysis of European plastics production, demand and waste data. PlasticsEurope, Brussels. 1–42.

Poeta G., Staffieri E., Acosta A.T.R., Battisti C., 2017. Ecological effects of anthropogenic litter on marine mammals: a global review with a “black-list” of impacted taxa. *Hystrix* 28, 253–364. https://doi.org/10.4404/hystrix-00003-2017.

Porter A., Barber D., Hobbs C., Love J., Power A.L., Bakir A., Galloway T.S., 2023. Uptake of microplastics by marine worms depends on feeding mode and particle shape but not exposure time. *Science of the Total Environment* 857, 159287. https://doi.org/10.1016/j.scitotenv.2022.159287.

Prata J.C., Sequeira I.F., Monteiro S.S., Silva A.L.P., da Costa J.P., Dias-Pereira P., Fernandes A.J.S., Da Costa F.M., Duarte A.C., Rocha-Santos R., 2021. Preparation of biological samples for microplastic identification by Nile Red, *Science of the Total Environment* 783, 147065. https://doi.org/10.1016/j.scitotenv.2021.147065.

Preddy W.S., 1954. The mixing and movement of water in the estuary of the Thames. *Journal of the Marine Biological Association of the United Kingdom* 33, 645–662. https://doi.org/10.1017/S0025315400026928.

Provencher J.F., Bond A.L., Avery-Gomm S., Borrelle S.B., Bravo Rebolledo E.L., Hammer S., Kühn S., Lavers J.L., Mallory M.L., Trevail A., van Franeker J.A., 2017. Quantifying ingested debris in marine megafauna: a review and recommendations for standardization. *Analytical Methods* 9, 1454–1469. https://doi.org/10.1039/c6ay02419j.

Provencher J., Kögel T., Lusher A., Vorkamp K., Gomiero A., Peeken I., Granberg M., Hammer S., Baak J., Larsen J.R., Farmen E., 2022. An ecosystem-scale litter and microplastic monitoring plan under the Arctic Monitoring and Assessment Programme (AMAP). *Arctic Science* Just In. https://doi.org/10.1139/AS-2021-0059.

Pugliares K.R., Bogomolni A., Touhey K.M., Herzig S.M., Harry C.T., Moore M.J., 2007. Section three: pinniped necropsy technique and anatomy, in Marine mammal necropsy: an introductory guide for stranding responders and field biologists. Woods Hole Oceanographic Institution, WHOI-2007-06, 29–52.

Puskic P.S., Lavers J.L., Bond A.L., 2020. A critical review of harm associated with plastic ingestion on vertebrates. *Science of the Total Environment* 743, 140666. https://doi.org/10.1016/j.scitotenv.2020.140666.

Qian J., Tang S., Wang P., Lu B., Li K., Jin W., He X., 2020. From source to sink: review and prospects of microplastics in wetland ecosystems. *Science of the Total Environment* 758, 143633. https://doi.org/10.1016/j.scitotenv.2020.143633.

Qiao R., Deng Y., Zhang S., Wolosker M.B., Zhu Q., Ren H., Zhang Y., 2019. Accumulation of different shapes of microplastics initiates intestinal injury and gut microbiota dysbiosis in the gut of zebrafish. *Chemosphere* 236, 124334. https://doi.org/10.1016/j.chemosphere.2019.07.065.

Quinn P., 2018. Turning a Walkers crisp packet into a bench is a start [online]. Available at: https://friendsoftheearth.uk/plastics/walkers-crisp-packet. [Accessed 20th December 2020].

Qureshi W., 2016. Poundland donates £800k to UK charities from plastic carrier bag charge [online]. Available at: https://www.packagingnews.co.uk/news/environment/poundland-donates-800k-to-uk-charities-from-plastic-carrier-bag-charge-24-05-2016. [Accessed 15th June 2020].

R Core Team, 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

R Core Team, 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ramos J.A.A., Barletta M., Costa M.F., 2012. Ingestion of nylon threads by Gerreidae while using a tropical estuary as foraging grounds. *Aquatic Biology* 17, 29–34. https://doi.org/10.3354/ab00461.

Rasband W.S., 2018. ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij /, 1997-2018.

Rebelein A., Int-Veen I., Kammann U., Scharsack J.P., 2021. Microplastic fibers – underestimated threat to aquatic organisms? *Science of the Total Environment* 777, 146045. https://doi.org/10.1016/j.scitotenv.2021.146045.

Recycling Today, 2015. Weight of water bottles decreases, while recycled content increases [online]. Available at: https://www.recyclingtoday.com/article/water-bottle-weight-decreases-recycled-content-increases/#:~:text=New%20data%20compiled%20by%20Beverage,52%20percent%20to%209.25%20grams. [Accessed 24th June 2020].

Reed S., Clark M., Thompson R., Hughes K.A., 2018. Microplastics in marine sediments near Rothera Research Station, Antarctica. *Marine Pollution Bulletin* 133, 460–463. https://doi.org/10.1016/j.marpolbul.2018.05.068.

Reisser J., Slat B., Noble K., du Plessis K., Epp M., de Sonneville J., Becker T., Pattiaratchi C., 2015. The vertical distribution of buoyant plastics at sea: an observational study in the North Atlantic Gyre. *Biogeosciences* 12, 1249–1256. https://doi.org/10.5194/bg-12-1249-2015.

Rhein S., Schmid M., 2020. Consumers' awareness of plastic packaging: more than just environmental concerns. *Resources, Conservation and Recycling* 162, 105063. https://doi.org/10.1016/j.resconrec.2020.105063.

Rhein S., Sträter K.F., 2021. Intended and unintended effects of statutory deposit return schemes for single-use plastic bottles: lessons learned from the German experience. *GAIA – Ecological Perspectives for Science and Society* 30, 250–256. https://doi.org/10.14512/gaia.30.4.8.

Robbins S.M., 1996. The ecology of *Eriocheir sinensis*, the Chinese mitten crab, Undergraduate project thesis, Queen Mary and Westfield College, University of London.

Roch S., Friedrick C., Brinker A., 2020. Uptake of microplastics in fishes: practical and theoretical approaches to test existing theories. *Scientific Reports* 10, 3896. https://doi.org/10.1038/s41598-020-60630-1.

Rochman C.M., 2018. Microplastics research – from sink to source. *Science* 360, 28–29. https://doi.org/10.1126/science.aar7734.

Rochman C.M., Brookson C., Bikker J., Djuric N., Earn A., Bucci K., Athey S., Huntington A., McIlwraith H., Munno K., De Frond H., Kolomijeca A., Erdle L., Grbic J., Bayoumi M., Borrelle S.B., Wu T., Santoro S., Werbowski L.M., Zhu X., Giles R.K., Hamilton B.M., Thaysen C., Kaura A., Klasios N., Ead L., Kim J., Sherlock C., Ho A., Hung C., 2019. Rethinking microplastics as a diverse contaminant suite. *Environmental Toxicology and Chemistry* 38, 703–711. https://doi.org/10.1002/etc.4371.

Rochman C.M., Kurobe T., Flores I., Teh S.J., 2014. Early warning signs of endocrine disruption in adult fish from the ingestion of polyethylene with and without sorbed

chemical pollutants from the marine environment. *Science of the Total Environment* 493, 656–661. https://doi.org/10.1016/j.scitotenv.2014.06.051.

Rochman C.M., Tahir A., Williams S.L., Baxa D.V., Lam R., Miller J.T., Teh F.-C., Werorilangi S., Teh S.J., 2015. Anthropogenic debris in seafood: plastic debris and fibres from textiles in fish and bivalves sold for human consumption. *Scientific Reports* 5, 14340. https://doi.org/10.1038/srep14340.

Rodrigues J.P., Duarte A.C., Santos-Echeandía J., Rocha-Santos T., 2019. Significance of interactions between microplastics and POPs in the marine environment: a critical review. *Trends in Analytical Chemistry* 111, 252–260. https://doi.org/10.1016/j.trac.2018.011.038.

Roman L., Haresty B.D., Hindell M.A., Wilcox C., 2019. A quantitative analysis linking seabird mortality and marine debris ingestion. *Scientific Reports* 9, 3202. https://doi.org/10.1038/s41598-018-36585-9.

Ross S.D., Andreasen H., Andersen N.G., 2016. An important step towards accurate estimation of diet composition and consumption rates for the harbour porpoise (*Phocoena phocoena*). *Marine Mammal Science* 32, 1491–1500. https://doi.org/10.1111/mms.12318.

Rowley K.H., Cucknell A.C., Smith B.D., Clark P.F., Morritt D., 2020. London's river of plastic: high levels of microplastics in the Thames water column. *Science of the Total Environment* 740, 140018. https://doi.org/10.1016/j.scitotenv.2020.140018.

Rummel C.D., Janhke A., Gorokhova E., Kühnel D., Schmitt-Jansen M., 2017. Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. *Environmental Science and Technology Letters* 4, 258–267. https://doi.org/10.1021/acs.estlett.7b00164.

Russell M., Webster L., 2021. Microplastics in sea surface waters around Scotland. *Marine Pollution Bulletin* 166, 112210. https://doi.org/10.1016/j.marpolbul.2021.112210.

Ryan P.G., Cole G., Spiby K., Nel R., Osborne A., Perold V., 2016. Impacts of plastic ingestion on post-hatchling loggerhead turtles off South Africa. *Marine Pollution Bulletin* 107, 155–160. https://doi.org/10.1016/j.marpolbul.2016.04.005.

Ryan P.G., Perold V., 2021. Limited dispersal of riverine litter onto nearby beaches during rainfall events. *Estuarine, Coastal and Shelf Science* 251, 107186. https://doi.org/10.1016/j.ecss.2021.107186.

Ryan P.G., Pichegru L., Perold V., Moloney C.L., 2020. Monitoring marine plastics – will we know if we are making a difference? *South African Journal of Science* 116, 58–66. https://doi.org/10.17159/sajs.2020/7678.

Santos M.B., Pierce G.J., Learmonth J.A., Ross R.J., Patterson I.A.P., Beare D., 2004. Variability in the diet of harbour porpoise (*Phocoena phocoena*) in Scottish waters 1992–2003. *Marine Mammal Science* 20, 1–27. https://doi.org/10.1111/j.1748-7692.204.tb01138.x.

Santos R.G., Andrades R., Boldrini M.A., Martins A.S., 2015. Debris ingestion by juvenile marine turtles: an underestimated problem. *Marine Pollution Bulletin* 93, 37–43. https://doi.org/10.1016/j.marpolbul.2015.02.022.

Sarkar D.J., Das Sarkar S., Das B.K., Sahoo B.K., Das A., Nag S.K., Manna R.K., Behera B.K., Samanta S., 2021. Occurrence, fate and removal of microplastics as heavy metal vector in natural wastewater treatment wetland system. *Water Research* 192, 116853. https://doi.org/10.016/j.watres.2021.116853.

Schmidt C., Krauth T., Wagner S., 2017. Export of plastic debris by rivers into the sea. *Environmental Science and Technology* 51, 12246–12253. https://doi.org/10.1021/acs.est.7b02368.

SCOS, 2016. Scientific advice on matters related to the management of seal populations: 2016, 1–169.

Selleslagh J., Amara R., 2008. Environmental factors structuring fish composition and assemblages in a small macrotidal estuary (Eastern English Channel). *Estuarine, Coastal and Shelf Science* 79, 507–517. https://doi.org/10.1016/j.ecss.2008.05.006.

Selleslagh J, Amara R, Laffargue P, Lesourd S, Lepage M, Girardin M., 2009. Fish composition and assemblage structure in three Eastern English Channel macrotidal estuaries: A comparison with other French estuaries. *Estuarine, Coastal and Shelf Science* 81, 149–159. https://doi.org/10.1016/j.ecss.2008.10.008.

Seo S., Park Y.G., 2020. Destination of floating plastic debris released from ten rivers around the Korean Peninsula. *Environmental International* 138, 105655. https://doi.org/10.1016/j.envint.2020.105655.

Setälä O., Fleming-Lehtinen V., Lehtinemi M., 2014. Ingestion and transfer of microplastics in the planktonic food web. *Environmental Pollution* 185, 77–83. https://doi.org/10.1016/j.envpol.2013.10.013.

Setälä O., Lehtiniemi M., Coppock R., Cole M., 2018. Microplastics in marine food webs, in Zeng E.Y. (ed.), Microplastic contamination in aquatic environments: an emerging matter of environmental urgency. Elsevier, pp. 339-364. https://doi.org/10.1016/B978-0-12-813747-5.00011-4.

Shams M., Alam I., Mahbub M.S., 2021. Plastic pollution during COVID-19: plastic waste directives and its long-term impact on the environment. *Environmental Advances* 5, 100119. https://doi.org/10.1016/j.envadv.2021.100119.

Sheridan M., O'Connor I., 2018. Evidence of complete gastric mill ossicle loss at ecdysis in the European green crab *Carcinus maenas* (Linnaeus, 1758) (Decapoda: Brachyura: Carcinidae). *Journal of Crustacean Biology* 38, 435–442. https://www.doi.org/10.1093/jcbiol/ruy045.

Sheridan M., O'Connor I., Henderson A.C., 2016. Investigating the effect of moulting on gastric mill structure in Norway lobster (*Nephrops novegicus*) and its potential as a direct ageing tool. *Journal of Experimental Marine Biology and Ecology* 484, 16–22. https://www.doi.org/10.1016/j.jembe.2016.08.005.

Shim W.J., Hong S.H., Eo S., 2018. Marine microplastics: abundance, distribution, and composition, in Zeng E.Y. (ed.), Microplastic contamination in aquatic environments:

an emerging matter of environmental urgency. Elsevier, pp. 1–26. https://doi.org/10.1016/B978-0-12-813747-5.00001-1.

Silburn B., Bakir A., Binetti U., Russell J., Kohler P., Preston-Whyte F., Meakins B., van Hoytema N., Andrews G., Carrias A., Maes T., 2022. A baseline study of macro, meso and micro litter in the Belize River basin, from catchment to coast. *ICES Journal of Marine Science* fsab268. https://doi.org/10.1093/icesjms/fsab268.

Sillanpää M., Sainio P., 2017. Release of polyester and cotton fibres from textiles in machine washings. *Environmental Science and Pollution Research* 24, 19313–19321. https://doi.org/10.1007/s11356-017-9621-1.

Silva J.D.B., Barletta M., Lima A.R.A., Ferreira G.V.B., 2018. Use of resources and microplastic contamination in a tropical estuary. *Environmental Pollution* 242, 1010–1021. https://doi/org/10.1016/j.envpol.2018.07.038.

Simon-Sánchez L., Grelaud M., Garcia-Orellana J., Ziveri P., 2019. River Deltas as hotspots of microplastic accumulation: the case study of the Ebro River (NW Mediterranean). *Science of the Total Environment* 687, 1186–1196. https://doi.org/10.1016/j.scitotenv.2019.06.168.

Simonsen K.P., Rasmussen A.R., Mathisen P., Petersen H., Borup F., 2011. A fast preparation of skeletal materials using enzyme maceration. *Journal of Forensic Science* 56, 480–484. https://doi.org/10.1111/j.1556-4029.2010.01668.x.

Sipe J.M., Bossa N., Berger W., von Windheim N., Gall K., Wiesner M.R., 2022. From bottle to microplastics: can we estimate how our plastic products are breaking down? *Science of the Total Environment* 814, 152460. https://doi.org/10.1016/j.scitotenv.152460.

Smith L., Barton C., Pratt A., 2018. Government policy on reducing plastic waste in the marine environment. Debate pack number 2018-0107. House of Commons Library, London. 1–47.

Stanton T., Chico G., Carr E., Cook S., Gomes R.L., Heard E., Law A., Wilson H.L., Johnson M., 2022. Planet patrolling: a citizen science brand audit of anthropogenic litter in the

context of national legislation and international policy. *Journal of Hazardous Materials* 436, 129118. https://doi.org/10.1016/j.jhazmat.2022.129118.

Stienbarger C.D., Joseph J., Athey S.N., Monteleone B., Andrady A.A., Brander S.M., 2021. Direct ingestion, trophic transfer, and physiological effects of microplastics in the early life stages of *Centropristis striata*, a commercially and recreationally valuable fishery species. *Environmental Pollution* 285, 117653. https://doi.org/10.1016/j.envpol.2021.117653.

Storrier K.L., McGlashan D.J., Bonellie S., Velander K., 2007. Beach litter deposition at a selection of beaches in the Firth of Forth, Scotland. *Journal of Coastal Research* 23, 813–822. https://doi.org/10.2112/04-0251.1.

Sundt P., Schulze P.-E., Syversen F., 2014. Sources of microplastic pollution to the marine environment. Report no: M-321|2015. Norwegian Environmental Agency, 1–86.

Sussarellu R., Susquet M., Thomas Y., Lambert C., Fabioux C., Pernet M.E.J. Le Goïc N., Quillie V., Mingant C., Epelboin Y., Corporeau C., Guyomarch J., Robbens J., Paul-Pont I., Soudant P., Huvet A., 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. *Proceedings of the National Academy of Sciences of the United States of America* 113, 2430–2435. https://doi.org/10.1037/pnas.1519019113.

Takarina N.D., Purwiyanto A.I.S., Rasud A.A., Arifin A.A., Suteja Y., 2022. Microplastic abundance and distribution in surface water and sediment collected from the coastal area. *Global Journal of Environmental Science and Management* 8, 183–196. https://doi.org/10.22034/gjesm.2022.02.03.

Talvitie J., Mikola A., Koistinen A., Setälä O., 2017a. Solutions to microplastic pollution – removal of microplastics from wastewater effluent with advanced wastewater treatment technologies. *Water Research* 123, 401–407. https://doi.org/10.1016/j.watres.2017.07.005.

Talvitie J., Mikola A., Setälä O., Heinonen M., Koistinen A., 2017b. How well is microlitter purified from wastewater? – a detailed study on the stepwise removal of microliter

in a tertiary level wastewater treatment plant. *Water Research* 109, 164–172. https://doi.org/10.1016/j.watres.2016.11.046.

Tamis J.E., Koelmans A.A., Dröge R., Kaag N.H.B.M., Keur M.C., Tromp P.C., Jongbloed R.H., 2021. Environmental risks of car tire microplastic particles and other road runoff pollutants. *Microplastics and Nanoplastics* 1, 10. https://doi.org/1186/s43591-021-00008-w.

Tanentzap A.J., Cottingham S., Fonvielle J., Riley I., Walker L.M., Woodman S.G., Kontou D., Pichler C.M., Reisner E., Lebreton L., 2021. Microplastics and anthropogenic fibre concentrations in lakes reflect surrounding land use. *PLoS Biology* 19, e3001389. https://doi.org/10.1371/journal.pbio.3001389.

Tang K.H.D., 2021. Interactions of microplastics with persistent organic pollutants and the ecotoxicological effects: a review. *Tropical Aquatic and Soil Pollution* 1, 24–34. https://doi.org/10.53623/tasp.v1i1.11.

Tekman M.B., Wekerle C., Lorenz C., Primpke S., Hasemann C., Gerdts G., Bergmann M., 2020. Tying up loose ends of microplastic pollution in the Arctic: distribution from the sea surface through the water column to deep-sea sediments at the HAUSGARTEN Observatory. *Environmental Science and Technology* 54, 4079–4090. https://doi.org/10.1021/acs.est.9b06981.

Teuten E.L., Saquing J.M., Knappe D.R.U., Barlaz M.A., Jonsson S., Björn A., Rowland S.J., Thompson R.C., Galloway T.S., Yamashita R., Ochi D., Watanuki Y., Moore C., Hung Viet P., Seang Tana T., Prudente M., Boonyatumanond R., Zakaria M.P., Akkhavong K., Ogata Y., Hirai H., Iwasa S., Mizukawa K., Hagino Y., Imamura A., Saha M., Takada H., 2009. Transport and release of chemicals from plastics to the environment and wildlife. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364, 2027–2045. https://doi.org/10.1098/rstb.2008.0284.

Thames21, 2017. Empowering communities and inspiring change for rivers: annual review 2016–2017 [online]. Available at: https://thames21.org.uk/wp-content/uploads/2013/11/Thames21-Annual-review-2017-Final-Web.pdf. [Accessed 3rd June 2020].

Thames21, 2018. Record number of wet wipes found on Thames foreshore [online]. Available at: https://www.thames21.org.uk/2018/04/record-number-wet-wipes-found-thames-foreshore/. [Accessed 26th November 2019].

Thames21, 2019a. 23 thousand wet wipes discovered on stretch of Thames river bank [online]. Available at: https://thames21.org.uk/2019/04/23-thousand-wet-wipes-discovered-stretch-thames-river-bank/. [Accessed 3rd June 2020].

Thames21, 2019b. More than 27 000 single-use plastic bottles removed from the Thames in the past 12 months [online]. Available at: https://thames21.org.uk/2019/10/more-than-27-000-single-use-plastic-bottles-removed-from-the-thames-in-past-12-months/. [Accessed 3rd June 2020].

The Rivers Trust, 2022. Raw sewage in our rivers [online]. Available at: https://theriverstrust.org/key-issues/sewage-in-rivers. [Accessed 1st April 2022].

Thomas M., 1998. Temporal changes in the movements and abundance of Thames estuary fish populations, in Attrill, M.J. (ed.) A rehabilitated estuarine ecosystem: the environment and ecology of the Thames Estuary. Kluwer Academic publishers, London. 15–139.

Thompson D., Duck C., 2010. Berwickshire and Northumberland Coast European Marine Site: grey seal population status. Report to Natural England, 20100902-RFQ.

Thompson R.C., 2018. Marine litter: are there solutions to this global environmental problem? Presented at Micro2018, Lanzarote, Canary Islands, Spain, November 2018. Available at: https://www.youtube.com/watch?v=2v2-EEmvdqE. [Accessed 7th January 2019].

Thompson R.C., Olsen Y., Mitchell R.P., Davis A., Rowland S.J., John A.W.G., McGonigle D., Russel A.E., 2004. Lost at sea: where is all the plastic? *Science* 304, 838. https://doi.org/10.1126/science.1094559.

Tibbetts J., Krause S., Lynch I., Smith G.H.S., 2018. Abundance, distribution, and drivers of microplastic contamination in urban river environments. *Water* 10, 1597. https://doi.org/10.3390/w10111597.

Tinsley D., 1998. The Thames Estuary: a history of the impact of humans on the environment and a description of the current approach to environmental management, in Attrill, M.J. (ed.) A rehabilitated estuarine ecosystem: the environment and ecology of the Thames Estuary. Kluwer Academic publishers, London. 5–26.

Tobin C., Urban-Rich J., 2022. The fiber microparticle pipeline in the marine water column – from source to mitigation strategies. *Environmental Advances* 7, 100133. https://doi.org/10.1016/j.envadv.2021.100133.

Tosetto L., Williamson J.E., Brown C., 2017. Trophic transfer of microplastics does not affect fish personality. *Animal Behaviour* 123, 159–167. https://doi.org/j.anbehav.2016.10.035.

Tracey T., 2018. Boy finds 30-year-old packet of Walkers crisps [online]. Available at: https://www.independent.co.uk/news/uk/home-news/walkers-crisps-old-packet-found-beach-cornwall-boy-laurence-miller-cheese-onion-padstow-a8299676.html. [Accessed on 8th June 2020].

Turner S.D., Horton A.A., Rose N.L., Hall C., 2019. A temporal sediment record of microplastics in an urban lake, London, UK. *Journal of Paleolimnology* 61, 449–462. https://doi.org/10.1007/s10933-019-00071-7.

Uhl W., Eftekhardadkhah M., Lusher A., van Bravel B., 2019. Microplastic in raw water and finished drinking water: limits of detection and quantification and what can be concluded regarding removal. Presented at *17th International Conference on Chemistry and the Environment*, Thessaloniki, Greece, June 16–20, 2019, pp.3.

Uncles R.J., Mitchell S.B., 2011. Turbidity in the Thames Estuary: how turbid do we expect it to be? *Hydrobiologia* 672, 91–103. https://doi.org/10.1007/s10750-011-0757-6.

UNEP, 2015. Biodegradable plastics and marine litter: misconceptions, concerns and impacts on marine environments. United Nations Environment Programme (UNEP), Nairobi.

Uurasjärvi E., Hartikainen S., Setälä O., Lehtiniemi M., Koistinen A., 2020. Microplastic concentrations, size distribution, and polymer types in the surface waters of a

northern European lake. *Water Environment Research* 92, 149–156. https://doi.org/10.1002/wer.1229.

Van Calcar C.J., Van Emmerik T.H.M., 2019. Abundance of plastic debris across European and Asian rivers. *Environmental Research Letters* 14, 124051. https://doi.org/10.1088/1748-9326/ab5468.

Van Cauwenberghe L., Claessens M., Vandegehuchte M.B., Janssen C.R., 2015. Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. *Environmental Pollution* 199, 10–17. https://doi.org/10.1016/j.envpol.2015.01.008.

Van Cauwenberghe L., Janssen C.R., 2014. Microplastics in bivalves cultured for human consumption. *Environmental Pollution* 193, 65–70. https://doi.org/10.1016/j.envpol.2014.06.010.

Van Cauwenberghe L., Vanreusel A., Mees J., Janssen C.R., 2013. Microplastic pollution in deep-sea sediments. *Environmental Pollution* 182, 495–499. https://doi.org/10.1016/j.envpol.2013.08.013.

Van Emmerik T., Schwarz A., 2019. Plastic debris in rivers. *WIREs Water* 7, e1398. https://doi.org/10.1002/wat2.1398.

Van Sebille E., Wilcox C., Lebreton L., Maximenko N., Hardesty B.D., Van Franeker J.A., Eriksen M., Siegel D., Galgani F., Law K.L., 2015. A global inventory of small floating plastic debris. *Environmental Research Letters* 10, 124006. https://doi.org/10.1088/1748-9326/10/12/124006.

Vaughan R., Turner S.D., Rose N.L., 2017. Microplastics in the sediments of a UK urban lake. *Environment Pollution* 229, 10–18. https://doi.org/10.1016/j.envpol.2017.05.057.

Vega-Moreno D., Abaroa-Pérez B., Rein-Loring P.D., Presas-Navarro C., Fraile-Nuez E., Machín F., 2021. Distribution and transport of microplastics in the upper 1150 m of the water column at the Eastern North Atlantic Subtropical Gyre, Canary Island, Spain. *Science of the Total Environment* 788, 147802. https://doi.org/10.1016/j.scitotenv.2021.147802.

Venables W.N., Ripley B.D., 2002. Modern applied statistics with S. Fourth edition. Springer, New York.

Vermeiren P., Muñoz C.C., Ikejima K., 2016. Sources and sinks of plastic debris in estuaries: a conceptual model integrating biological, physical and chemical distribution mechanisms. *Marine Pollution Bulletin* 113, 7–16. https://doi.org/10.1016/j.marpolbul.2016.10.002.

Verschoor A.J., 2015. Towards a definition of microplastics: considerations for the specification of physio-chemical properties. RIVM Letter report 2015-0116. National Institute for Public Health and the Environment, Bilthoven. 1–38.

Vimal K.E.K., Agarwal V., Mathiyazhagan K., 2022. Barriers in the adoption of buyback schemes for used plastic packaging material – a contextual relationship analysis. *Resources, Conservation and Recycling* 178, 106084. https://doi.org/10.1016/j.resconrec.2021.106084.

Vincent C., Ridoux V., Fedak M.A., McConnell B.J., Sparling C.E., Leaute J-P., Jouma'a J., Spitz J., 2016. Foraging behaviour and prey consumption by grey seals (*Halichoerus grypus*) – spatial and trophic overlaps with fisheries in a marine protected area. *ICES Journal of Marine Science* 73, 2653–2665. https://doi.org/10.1093/icesjms/fsw102.

Vriend P., Van Calcar C., Kooi M., Landman M., Pikaar R., Van Emmerik T., 2020. Rapid assessment of floating macroplastic transport in the Rhine. *Frontiers in Marine Science* 7, 10. https://doi.org/10.3389/fmars.2020.00010.

Wagner S., Klöckner P., Stier B., Römer M., Seiwart B., Reemtsma T., Schmidt C., 2019. Relationship between discharge and river plastic concentrations in a rural and an urban catchment. *Environmental Science and Technology* 53, 10082–10091. https://doi.org/10.1021/acs.est.9b03048.

Walkinshaw C., Lindeque P.K., Thompson R., Tolhurst T., Cole M., 2020. Microplastics and seafood: lower trophic organisms at highest risk of contamination. *Ecotoxicology and Environmental Safety* 190, 110066. https://doi/org/10.1016/j.ecoenv.2019.110066.

Wang J., Peng J., Tan Z., Gao Y., Zhan Z., Chen Q., Cai L., 2017. Microplastics in the surface sediments from the Beijiang River littoral zone: composition, abundance, surface textures and interaction with heavy metals. *Chemosphere* 171, 248–258. https://doi.org/10.1016/j.chemosphere.2016.12.074.

Wang J., Wang M., Ru S., Liu X., 2019. High levels of microplastic pollution in the sediments and benthic organisms of the South Yellow Sea, China. *Science of the Total Environment* 651, 1661–1669. https://doi.org/10.1016/j.scitotenv.2018.10.007.

Watt J., Pierce G.J., Boyle P.R., 1997. Guide to the identification of North Sea fish using premaxillae and vertebrae. ICES Cooperative Research Report No. 220.

Watts A.J.R., Lewis C., Goodhead R.M., Beckett S.J., Moger J., Tyler C.R., Galloway T.S., 2014. Uptake and retention of microplastics by the shore crab *Carcinus maenas*. *Environmental Science and Technology* 48, 8823–8830. https://doi.org/10.1021/es501090e.

Watts A.J.R., Urbina M.A., Corr S., Lewis C., Galloway T.S., 2015. Ingestion of plastic microfibers by the crab *Carcinus maenas* and its effect on food consumption and energy balance. *Environmental Science and Technology* 49, 14597–14604. https://doi.org/10.1021/acs.est.5b04026.

Weber A., 2008. Predation of invasive species Chinese mitten crab (*Eriocheir sinensis*) by Eurasian otter (*Lutra lutra*) in the Drömling Nature Reserve, Saxony-Anhalt, Germany. *IUCN Otter Specialist Group Bulletin* 25, 104–107.

Webster J.M., Clark P.F., Morritt D., 2015. Laboratory based feeding behaviour of the Chinese mitten crab, *Eriocheir sinensis* (Crustacea: Decapoda: Brachyura: Varunidae): fish egg consumption. *Aquatic Invasions* 10, 313–326. https://doi.org/10.3391.ai.2015.10.3.06.

Weinstein J.E., Crocker B.K., Gray A.D., 2016. Macroplastics can fragment and biofilm increases overtime. *Environmental Toxicology and Chemistry* 35, 1632–1640. https://doi.org/10.1002/etc.3432.

Welden N.A., Abylkhani, A., Howarth, L.M., 2018. The effects of trophic transfer and environmental factors on microplastic uptake by plaice, *Pleuronectes plastessa*, and spider crab, *Maja squinado*. *Environmental Pollution* 239, 351–358. https://doi.org/10.1016/j.envpol.2018.03.110.

Welden N.A.C., Cowie P.R., 2016a. Environment and gut morphology influence microplastic retention in langoustine, *Nephrops norvegicus*. *Environmental Pollution* 214, 856–865. https://doi.org/10.1016/j.envpol.2016.03.067.

Welden N.A.C., Cowie P.R., 2016b. Long-term microplastic retention causes reduced body condition in the langoustine, *Nephrops norvegicus*. *Environmental Pollution* 218, 895–900. https://doi.org/10.1016/j.envpol.2016.08.20.

Wheeler A.C., 1978. Key to the fishes of Northern Europe. Frederick Warne & Co. Ltd., London, UK, pp. 184.

Wheeler A.C., 1979. The tidal Thames: the history of a river and its fishes. Routledge and Kegan Paul, pp. 228.

Wickham W., 2016. ggplot2: elegant graphics for data analysis. Springer-Verlag, New York.

Wikipedia, 2020. Tazos [online]. Available at: https://en.wikipedia.org/wiki/Tazos. [Accessed 12th June 2020].

Williams A.T., Simmons S.L., 1996. The degradation of plastic litter in rivers: implications for beaches. *Journal of Coastal Conservation* 2, 63–72. https://doi.org/10.1007/BF02743038.

Williams A.T., Simmons S.L., 1997a. Estuarine litter at the river/beach interface in the Bristol Channel, United Kingdom. *Journal of Coastal Research 13*, 1159–1165. https://jstor.org/stable/4298724.

Williams A.T., Simmons S.L., 1997b. Movement patterns of estuarine litter. *Water, Air and Soil Pollution* 98, 119–139. https://doi.org/10.1007/BF02128653.

Williams A.T., Simmons S.L., 1999. Sources of riverine litter: the River Toff, South Wales, UK. *Water, Air and Soil Pollution* 112, 197–216. https://doi.org/10.1023/A:1005000724803.

Windsor F.M., Durance I., Horton A.A., Thompson R.C., Tyler C.R., Ormerod S.J., 2019a. A catchment-scale perspective of plastic pollution. *Global Change Biology* 25, 1207–1221. https://doi,org/10.1111/gcb.14572.

Windsor F.M., Tiley R.M., Tyler C.R., Ormerod S.J., 2019b. Microplastic ingestion by riverine macroinvertebrates. *Science of the Total Environment* 646, 68–74. https://doi.org/10.1016/j.scitotenv.2018.07.271.

Winton D.J., Anderson L.G., Rocliffe S., Loiselle S., 2020. Macroplastic pollution in freshwater environments: focusing public and policy action. *Science of the Total Environment* 704, 135242. https://doi.org/10.1016/j.scitotenv.2019.135242.

Woeller K.E., Hochwalt A.E., 2015. Safety assessment of sanitary pads with a polymeric foam absorbent core. *Regulatory Toxicology and Pharmacology* 73, 419–424. https://doi.org/10.1016/j.yrtph.2015.07.028.

Wójcik-Fudalewska D.J., Normant-Saremba M., Anastácio P., 2016. Occurrence of plastic debris in the stomach of the invasive crab *Eriocheir sinensis*. *Marine Pollution Bulletin* 113, 306–311. https://doi.org/10.1016/j.marpolbul.2016.09.059.

Wójcik-Fudalewska D.J., Normant-Saremba M., Kolasa A., Anastacio P.M., 2019. Diet and feeding ecology of *Eriocheir sinensis* on the Polish coast of the Baltic Sea and in the Tagus Estuary, Portugal. *Oceanological and Hydrobiological Studies* 48, 236–246. https://doi.org/10.2478/ohs-2019-0021.

Woodall L.C., Sanchez-Vidal A., Canals M., Paterson G.L.J., Coppock R., Sleight V., Calafat A., Rogera A.D., Narayanaswamy B.E., Thompson R.C., 2014. The deep sea is a major sink for plastic debris. *Royal Society Open Science* 1, 140317. https://doi.org/10.1098/rsos.140317.

Wootton N., Reis-Santos P., Gillanders B.M., 2021. Microplastic in fish – a global synthesis. *Reviews in Fish Biology and Fisheries* 31, 753–771. https://doi.org/10.1007/s11160-021-09684-6.

Worek J., Badura X., Bialas A., Chwiej J., Kawoń K., Styszko K., 2022. Pollution from transport: detection of tyre particles in environmental samples. *Energies* 15, 2816. https://doi.org/10.3390/en15082816.

Wrap, 2017. Valuing our clothes: the cost of UK fashion [online]. Available at: http://www.wrap.org.uk/sites/files/wrap/valuing-our-clothes-the-cost-of-uk-fashion_WRAP.pdf. [Accessed 30th June 2020].

Wrap, 2018. A Roadmap to 2025 - The UK Plastics Pact [online]. Available at: http://www.wrap.org.uk/content/the-uk-plastics-pact-roadmap-2025. [Accessed 4th January 2019].

Wright S.L., Thompson R.C., Galloway T.S., 2013. The physical impacts of microplastics on marine organisms: a review. *Environmental Pollution* 178, 483–492. https://doi.org/10.1016/j.envpol.2013.02.031.

Wright S.L., Ulke J., Font A., Chan K.L.A., Kelly F.J., 2020. Atmospheric microplastic deposition in an urban environment and an evaluation of transport. *Environment International* 136, 105411. https://doi.org/10.1016/j.envint.2019.105411.

Wu C., Tanka K., Tani Y., Bi X., Liu J., Yu Q., 2022. Effect of particle size on the colonization of biofilms and the potential of biofilm-covered microplastics as metal carriers. *Science of the Total Environment* 821, 153265. https://doi.org/10.1016/j.scitotenv.2022.153265.

Xia F., Yao Q., Zhang J., Wang D., 2021. Effects of seasonal variation and resuspension on microplastics in river sediments. *Environmental Pollution* 286, 117403. https://doi.org/10.1016/j.envpol.2021.117403.

Xiong X., Chen Z., Zhang K., Mei Z., Hao Y., Zheng J., Wu C., Wang K., Ruan Y., Lam P.K.S., Wang D., 2018. Microplastics in the intestinal tracts of East Asian finless porpoises (*Neophocaena asiaeorientalis sunameri*) from Yellow Sea and Bohai Sea of China.

Marine Pollution Bulletin 136, 55–60. https://doi.org/10.1016/j.marpolbul.2018.09.006.

Xu Q., Xing R., Sun M., Gao Y., An L., 2020. Microplastics in sediments from an interconnected river-estuary region. *Science of the Total Environment* 729, 139025. https://doi.org/10.1016/j.scitotenv.2020.139025.

Yardy L., Callaghan A., 2020. What the fluff is this? – *Gammarus pulex* prefer food sources without plastic microfibers. *Science of the Total Environment* 715, 136815. https://doi.org/10.1016/j.scitotenv.2020.136815.

Yeo B.G., Takada H., Yamashita R., Okazaki Y., Uchida K., Tokai T., Tanaka K., Trenholm N., 2020. PCBs and PBDEs in microplastic particles and zooplankton in open water in the Pacific Ocean and around the coast of Japan. *Marine Pollution Bulletin* 151, 110806. https://doi.org/10.1016/j.marpolbul.2019.110806.

Youngblood K., Brooks A., Das N., Singh A., Sultana M., Verma G., Zakir T., Chowdhury G.W., Duncan E., Khatoon H., Maddalene T., Napper I., Nelms S., Patel S., Sturges V., Jambeck J.R., 2022. Rapid characterization of macroplastic input and leakage in the Ganges river basin. *Environmental Science and Technology* 56, 4029–4038. https://doi.org/10.1021/acs.est.1c04781.

Yu P., Liu Z., Wu D., Chen M., Lv W., Zhao Y., 2018. Accumulation of polystyrene microplastics in juvenile *Eriocheir sinensis* and oxidative stress effects in the liver. *Aquatic Toxicology* 200, 28–36. https://doi.org/10.1016/j.aquatox.2018.04.015.

Yu X., Huang W., Wang Y., Wang Y., Cao L., Yang Z., Dou S., 2022. Microplastic pollution in the environment and organisms of Xiangshan Bay, East China Sea: An area of intensive mariculture. *Water Research* 212, 118117. https://doi.org/10.1016/j.watres.2022.118117.

Zaki M.R.M., Zaid S.H.M., Zainuddin A.H., Aris A.Z., 2021. Microplastic pollution in tropical estuary gastropods: abundance, distribution and potential sources of Klang River estuary, Malaysia. *Marine Pollution Bulletin* 162, 111866. https://doi.org/10.1016/j.marpolbul.2020.111866.

Zantis L.J., Bosker T., Lawler F., Nelms S.E., O'Rorke R., Constantine R., Sewell M., Carroll E.L., 2022. Assessing microplastic exposure of large marine filter-feeders. *Science of the Total Environment* 818, 151815. https://doi.org/10.1016/j.scitotenv.2021.151815.

Zhang D., Liu X., Huang W., Li J., Wang C., Zhang D., Zhang C., 2020. Microplastic pollution in deep-sea sediments and organisms of the Western Pacific Ocean. *Environmental Pollution* 259, 113948. https://doi.org/10.1016/j.envpol.2020.113948.

Zhang L., Liu J., Xie Y., Zhong S., Gao P., 2021. Occurrence and removal of microplastics from wastewater treatment plants in a tropical tourist city in China. *Journal of Cleaner Production* 291, 125968. https://doi.org/10.1016/j.jclepro.2021.125968.

Zhang Q., Zhao Y., Du F., Cai H., Wang G., Shi H., 2020. Microplastic fallout in different indoor environments. *Environmental Science and Technology* 54, 6530–6539. https://doi.org/10.1021/acs.est.0c00087.

Zhao S., Ward J.E., Danley M., Mincer T.J., 2018. Field-based evidence for microplastic in marine aggregates and mussels: implications for trophic transfer. *Environmental Science and Technology* 52, 11038–11048. https://doi.org/10.1021/acs.est.8b03467.

Zhao S., Zhu L., Li D., 2016. Microscopic anthropogenic litter in terrestrial birds from Shanghai, China: Not only plastics but also natural fibers. *Science of the Total Environment* 550, 1110–1115. https://doi.org/10.1016/j.scitotenv.2016.01.112.

Ziajahromi S., Kumar A., Neale P.A., Leusch F.S.L., 2017a. Impact of microplastic beads and fibres on waterflea (*Ceriodaphnia dubia*) survival, growth, and reproduction: implications of single and mixed exposures. *Environment Science and Technology* 61, 13397–13406. https://doi.org/10.1021/acs.est.7b03574.

Ziajahromi S., Neale P.A., Rintoul L., Leusch F.D.L., 2017b. Wastewater treatment plants as a pathway for microplastics: development of a new approach to sample wastewater-based microplastics. *Water Research* 112, 93–99. https://doi.org/10.1016/j.watres.2017.01.042.

Zoological Society of London, 2014. ZSL's seal survey 2014 [online]. Available at: https://www.sites.zsl.org/inthethames/main/'ZSL's%20surveys. [Accessed 23rd November 2021].

Zoological Society of London, 2016. Conservation of tidal Thames fish through the planning process. Guidance document. Zoological Society of London, Regent's Park, London, UK, pp. 20.

Zoological Society of London, 2018. Juvenile fish ID guide for the tidal Thames. Thames Conservation Programme.

Zoological Society of London, 2021. The state of the Thames 2021: environmental trends of the tidal Thames, in McCormick H., Cox T., Pecorelli G., Debney A.J. (eds), Zoological Society of London, London, UK, pp. 90.