LIFTING THE VEIL ON MARINE LITTER

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LIFTING THE VEIL ON MARINE LITTER

Towards a better understanding of Marine Litter in the North Atlantic: Method Development, Occurrence and Impacts

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CONTENTS

Chapter 1

General introduction

Marine litter (ML) originates from many sources and causes a wide spectrum of environmental, economic, safety, health and cultural impacts. Although marine litter science has been in existence since the early 1960s and rapidly evolved over the last two decades, several crucial evidence gaps were not addressed. This thesis is dedicated to studying marine litter and microplastics in the North East Atlantic region, by investigating its distribution, bio-accumulative properties and ecotoxicological impacts on marine animals in coastal zones and seas of North West Europe. Specifically, the aim of this work is to drive method development for the monitoring of marine litter and microplastics, to increase our understanding of the presence and impacts of marine litter and microplastics. To pave the way for further research, decision making and solutions, the particular gaps this thesis set out to address were: to take stock of current evidence and progress in marine litter science, to review existing seafloor litter data and map spatial and temporal trends; to monitor microplastic pollution and setup baselines; to improve methods for microplastic sampling and analysis in a range of matrices; to undertake chronic exposure studies using environmental concentrations; to study plastic ingestion and bioaccumulation in a top predator. Several national and international frameworks have been created to target the marine litter issue e.g. the United Nations Sustainable Development Goals (SDG), The European Marine Strategy Framework Directive (MSFD), The Oslo Paris (OSPAR) Regional Action Plan for Marine Litter. The findings of this work can be linked to the requirements of these frameworks and thus contribute towards the reduction and elimination of plastic pollution.

1.1 INTRODUCTION

Humans are omnipresent and produce a lot of waste, since approximately 70% of Earth's surface is covered by water, large proportions of that waste are likely to end up in the marine environment. This trash is not nature`s treasure**.** Marine litter is defined as any solid material which has been deliberately discarded or unintentionally lost on beaches, on shores or at sea. The definition also covers materials transported into the marine environment from land by rivers, draining or sewage systems, via atmospheric deposition or winds. It includes any persistent, manufactured or processed solid material[1]. Originating from sources both on land and at sea, dominated by plastic[1,2,] marine litter comprises a wide range of other materials, including metal, wood, rubber, glass, ceramic and paper. Although marine litter is not new (e.g. amphoras), the quantities and polymers which are currently ending up in the marine environment are. Modern plastics are extremely cheap, workable, durable and long-lasting. All characteristics which make them very popular for use in a wide range of applications, but unfortunately also very persistent in the environment. Demands have been growing exponentially due to both an increase in consumerism and an increase in the number of polymers used to manufacture the things we use daily. Many of these items are single-use items, designed to be wasted, and more than often, pile up in the environment. A wide range of polymer types of different sizes can be found in the marine environment[2,3]. The unsustainable consumption and production of plastic in combination with inadequate waste management led to this accumulation[4]**.**
Pollutants that are resistant to degradation in the environment are called persistent[5]. Although synthetic polymers are not explicitly included, the plastic fraction can be considered to be a modern type of persistent pollutants, coming in different types and many sizes and shapes[6]. Microplastics (MP) are defined as all forms of plastics less than 5mm[2]. They can enter the oceans as: primary microplastics (e.g. beads from personal care products, preproduction pellets) or as secondary microplastics which are derived from larger plastic items which slowly get broken into smaller pieces[3]. High persistence (degradation half-lives of six months or more) has important implications for the behaviour of plastics in the environment. Persistent pollutants will be distributed widely, often globally, and ultimately reach (much) higher concentrations than short-lived substances emitted at the same rate[5]. High persistence thus indicates the potential for long-lasting environmental and human exposure to a pollutant that is difficult to control and reverse.

About a decade ago, marine litter research was still in its infancy with less than 200 papers on the topic published a year[7]. The different sources and large-scale implications make the marine litter issue and its solutions rather complex. Therefore, most studies published before 2010, simply focused on reporting marine litter presence in a range of sizes and matrices[7]. Still, only a fraction of marine litter is routinely monitored across a small selection of matrices (e.g. macro litter on beaches, microplastics in sediment). There is an urgent need for to quantitatively measure the number and mass of plastic particles across the marine environment, together with their residence time in each fraction, to guide exposure experiments and to constrain models[8,9]. Existing monitoring approaches vary widely and are dependent on the underlying scientific/political questions and available funding and techniques. Several methods for marine litter and MP monitoring are available, but only beach litter monitoring is somehow harmonised internationally[7]. Some analytical MP techniques are useful to define polymer types, others are useful to determine status and trends by rapidly screening large amounts of samples[10]. Overall, the absence of harmonised agreements, standardised protocols, reference materials and shared data repositories have led to a range of different sampling, analytical and assessment techniques, which makes comparison and further decision making difficult.

It's important to develop adequate methods, setup monitoring programmes, create baselines and investigate the broader implications of marine litter to find solutions and follow up progress of policy measures. The aim of this work is to drive method development for the monitoring of marine litter and microplastics, to increase our understanding of the presence and impacts of marine litter and microplastics, to provide transparent and conclusive evidence needed to manage plastic materials and their impacts better. By addressing current evidence needs we can develop the knowledge base to stop marine litter entering our environment and define future research better. While the scientific understanding of the marine litter issue is still evolving, different parts of science are focusing on distinct aspects of the problem e.g. distribution, temporal/spatial trends, impacts, efficiency of measures.

The impacts of marine litter are far reaching and include environmental and socio-economic effects[2]. There has been an enormous growth in public and political attention to the issue of marine litter and the unsustainability of modern society[4,11]. Various national and international instruments have been administered, most notably, dedicated legislation was introduced to deal with marine litter and its impact on the coastal and marine environment. In Europe, a legal framework was introduced in 2008, the Marine Strategy Framework Directive (MSFD). The MSFD incorporates an indicator specifically in relation to litter (Descriptor 10: 'marine litter does not cause harm to the coastal and marine environment') and requires evidence that Member States are moving towards Good Environmental Status (GES)[12]. The MSFD and other directives support the achievement of an EU-wide "quantitative reduction headline target" for marine litter, as agreed in the 7th Environment Action Programme[13]. Together they all form part of the wider European strategy for plastics and the circular economy[14,15]. The objectives of the Oslo Paris Convention (OSPAR), governing the North East Atlantic maritime area, and its Regional Action Plan regarding marine litter, as laid down in the Strategy for the protection of the Marine Environment of the North-East Atlantic for the years 2010-2020, are in line with the definition of Descriptor 10 of the MSFD. These regional objective are supportive of the global Rio+20 Commitment, "to take action, by 2025, to achieve significant reductions in marine debris and prevent harm to the coastal and marine environment, based on collected scientific data", with the United Nations Sustainable Development Goals established pursuant to General Assembly resolution 66/288 and 68/70 in which States noted concern and demanded action on marine debris[16] and the United Nations Decade of Ocean Science for Sustainable Development[17]. The findings of this thesis can be linked to the requirements of these frameworks and thus indirectly contribute towards the reduction and elimination of plastic pollution.

1.2 OUTLINE OF THE THESIS

The research described here aims to improve the scientific understanding of the marine litter issue, including microplastics, in the North-East Atlantic ecosystem. The research does so by addressing standardisation of marine litter and MP monitoring methods, analytical method development, field exposure of MP in both abiotic and biotic matrices, and laboratory exposure and chronic toxicity of MP to marine species.

In Chapter 2 of this thesis a review of marine litter literature is provided to reveal global distribution and accumulation areas, evaluate the ability of applied monitoring methods to detect temporal trends and assess the need for standardisation of monitoring approaches.

In Chapter 3 a methodology is developed and implemented for a long-term monitoring programme of quantities and types of litter on the seafloor in seas surrounding the UK to establish a baseline, spatial and time trends for key seafloor litter types.

In Chapter 4 a methodology is developed and implemented to determine the concentration of microplastics in sediment and surface seawater samples from the English Channel and North Sea with recommendations for a long-term monitoring programme.

Chapter 5 describes the development of a methodology to determine the concentration of microplastics in a top marine predator, the Porbeagle shark, of the North East Atlantic to assess the exposure routes and bioaccumulation potential.

The results of a long-term microplastic exposure study with juvenile Pacific oysters are described in Chapter 6. The chronic low-dose toxicity of prototypical microplastic on this secondary producer was assessed and a series of biomarkers and potential impact mechanisms were tested.

To contribute to the analytical methodology for microplastics, in Chapter 7 a new, rapid screening method for microplastics in sediments using a fluorescent dye was developed.

Lastly, in Chapters 8 and 9 of this thesis research gaps, guidance and recommendations are provided. Chapter 8 gives a review of completed European projects related to marine litter and microplastics to determine research gaps and guide future research funding. Much more work is required to develop a full understanding of the problem. In Chapter 9 our findings delivered several important insights, give clear recommendations to guide monitoring, assessments, measure development, future funding and next steps to tackle the marine litter issue.

Chapter 2

Global Distribution, Composition and Abundance of Marine Litter

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ABSTRACT

Marine litter is commonly observed everywhere in the oceans. Litter enters the seas from both land-based sources, from ships and other installations at sea, from point and diffuse sources, and can travel long distances before being stranded. Plastics typically constitute the most important part of marine litter sometimes accounting for up to 100 % of floating litter. On beaches, most studies have demonstrated densities in the 1 item m^{-2} range except for very high concentrations because of local conditions, after typhoons or flooding events. Floating marine debris ranges from 0 to beyond 600 items km^{-2}. On the seabed, the abundance of plastic debris is very dependent on location, with densities ranging from 0 to >7700 items km^{-2}, mainly in coastal areas. Recent studies have demonstrated that pollution of microplastics, particles <5 mm, has spread at the surface of oceans, in the water column and in sediments, even in the deep sea. Concentrations at the water surface ranged from thousands to hundred thousands of particles km^{-2}. Fluxes vary widely with factors such as proximity of urban activities, shore and coastal uses, wind and ocean currents. These enable the presence of accumulation areas in oceanic convergence zones and on the seafloor, notably in coastal canyons. Temporal trends are not clear with evidences for increases, decreases or without changes, depending on locations and environmental conditions. In terms of distribution and quantities, proper global estimations based on standardized approaches are still needed before considering efficient management and reduction measures.

2.1 INTRODUCTION

Anthropogenic litter on the sea surface, beaches and seafloor has significantly increased over recent decades. Initially described in the marine environment in the 1960s, marine litter is nowadays commonly observed across all oceans[7]. Together with its breakdown products, meso-particles (5–2.5 cm) and micro-particles (<5 mm), they have become more numerous and floating litter items can be transported over long distances by prevailing winds and currents[18]. Humans generate considerable amounts of waste and global quantities are continuously increasing, although waste production varies between countries. Plastic, the main component of litter, has become ubiquitous and forms sometimes up to 95 % of the waste that accumulates on shorelines, the sea surface and the seafloor. Plastic bags, fishing equipment, food and beverage containers are the most common items and constitute more than 80 % of litter stranded on beaches[19,20]. A large part of these materials decomposes only slowly or not at all. This phenomenon can also be observed on the seafloor where 90 % of litter caught in benthic trawls is plastic[21–24].

Even with standardized monitoring approaches, the abundance and distribution of anthropogenic litter show considerable spatial variability. Strandline surveys and cleanings as well as regular surveys at sea are now starting to be organized in many countries in order to generate information about temporal and spatial distribution of marine litter[25]. Accumulation rates vary widely and are influenced by many factors such as the presence of large cities, shore use, hydrodynamics and maritime activities. As a general pattern, accumulation rates appear to be lower in the southern than in the northern hemisphere. Enclosed seas such as the Mediterranean or Black Sea may harbor some of the highest densities of marine litter on the seafloor[23], reaching more than 100,000 items km^{-2}. In surface waters, the problem of plastic fragments has increased in the last few decades. From the first reports in 1972[26], the quantities of microparticles in European seas have grown in comparison to data from 2000[27]. Recent data suggest that quantities of microparticles appear to have stabilized in the North Atlantic Ocean over the last decade[28]. Little is known about trends in accumulation of debris in the deep sea. Debris densities on the deep seafloor decreased in some areas, such as in the Bay of Tokyo from 1996 to 2003 and in the Gulf of Lion between 1994 and 2009[29,30]. By contrast, in some areas around Greece, the abundance of debris in deep waters has substantially increased over a period of eight years[31,32] and on the deep Arctic seafloor of the HAUSGARTEN observatory over a period of ten years[33]. Interpretation of temporal trends is complicated by seasonal changes in the flow rate of rivers, currents, wave action, winds etc. Decreasing trends of macroplastics (>2.5 cm) on beaches of remote

islands suggest that regulations to reduce dumping at sea have been successful to some extent[34]. However, both the demand and the production of plastics reached 299 million tons in 2013 and are continuing to increase[35].

2.2 COMPOSITION

Analysis of the composition of marine litter is important as it provides vital information on individual litter items, which, in most cases, can be traced back to their sources. Sources of litter can be characterised in several ways[36]. One common method is to classify marine litter sources as either land-based or ocean-based, depending on where the litter entered the sea. Some items can be attributed with a high level of confidence to certain sources such as fishing gear, sewage-related debris and tourist litter. So-called use-categories provide valuable information for developing reduction measures[30].

Land-based sources include mainly recreational use of the coast, general public litter, industry, harbours and unprotected landfills and dumps located near the coast, but also sewage overflows, introduction by accidental loss and extreme events. Marine litter can be transported to the sea by rivers[37,38] and other industrial discharges and run-offs or can even be blown into the marine environment by winds. Ocean-based sources of marine litter include commercial shipping, ferries and liners, both commercial and recreational fishing vessels, military and research fleets, pleasure boats and offshore installations such as platforms, rigs and aquaculture sites. Factors such as ocean current patterns, climate and tides, the proximity to urban, industrial and recreational areas, shipping lanes and fishing grounds also influence the types and amount of litter that are found in the open ocean or along beaches.

Assessments of the composition of litter in different marine regions show that “plastics”, which include all petroleum-based synthetic materials, make up the largest proportion of overall litter pollution[39]. Packaging, fishing nets and pieces thereof, as well as small pieces of unidentifiable plastic or polystyrene account for the majority of the litter items recorded in this category[40]. Some of this can take hundreds of years to break down or may never truly degrade[18].

Whether or not visual observations from ships and airplanes, observations using underwater vehicles, manned or not, acoustics and finally trawling will provide the necessary detail to characterise litter and eventually define sources is not always clear. Previous notions that at a global scale most of the marine litter is from land-based sources rather than from ships, were confirmed[41]. Marine litter found on beaches consists primarily of plastics (bottles, bags, caps/lids, etc.), aluminium (cans, pull tabs) and glass (bottles) and mainly originates from shoreline recreational activities but is also transported by the sea by currents. In some cases, specific activities account for local litter densities well above the global average[39]. For example, marine litter densities on beaches can be increased by up to 40 % in summer because of high tourist numbers. In some tourist areas, more than 75 % of the annual waste is generated in summer, when tourists produce on average 10–15 % more waste than the inhabitants; although not all of this waste enters the marine environment[40].

In some areas such as the North Sea or the Baltic Sea, the large diversity of items and the composition of the litter recorded indicate that shipping, fisheries and offshore installations are the main sources of litter found on beaches[42]. In some cases, litter can clearly be attributed to shipping, sometimes accounting for up to 95 % of all litter items in a given region, a large proportion of which originates from fishing activities often coming in the form of derelict nets[43]. In the North Sea, this percentage has been temporally stable[40] but litter may be supplemented by coastal recreational activities and riverine input[44,45]. Studies along the US west coast, specifically off the coast of the southern California Bight[46–49] have shown that ocean-based sources are the major contributors to marine debris in the eastern North Pacific with, for example, fishing gear being the most abundant debris off Oregon[50].

Investigations in coastal waters and beaches around the northern South China Sea in 2009 and 2010 indicated that plastics (45 %) and Styrofoam (23 %) accounted for more than 90 % of floating debris and 95 % of beached debris. The sources were primarily land-based and mostly attributed to coastal recreational activities[51]. In the Mediterranean, reports from Greece classify land-based (69 % of the litter) and vessel-based (26 %) waste as the two predominant sources of litter[32].

2.3 DISTRIBUTION

2.3.1 Beaches

Marine debris is commonly found at the sea surface or washed up on shorelines, and much of the work on marine litter has focused on coastal areas because of the presence of sources, ease of access/assessment and for aesthetic reasons[52]. Marine litter stranded on beaches is found along all coasts and has become a permanent reason for concern. Beach-litter data are derived from various approaches based on measurements of quantities or fluxes, considering various litter categories, and sampling on transects of variable width and length parallel or perpendicular to the shore. This makes it difficult to draw a quantitative global picture of beach litter distribution. In general, methods that are used for estimating amounts of marine debris on beaches are considered cheap and reliable, but it is not clear how it relates to litter at sea, floating or not. Moreover, in some coastal habitats, litter may be of terrestrial origin and may never actually enter the sea. Most surveys are done with a focus on cleaning, thereby missing proper classification of litter items. When studies are not dedicated to specific items, litter is categorized by the type of material, function or both. Studies record the numbers, some the mass of litter and some do both[40]. Evaluations of beach litter reflect the long-term balance between inputs, land-based sources or stranding, and outputs from export, burial, degradation and cleanups. Measures of stocks may reflect the presence and amounts of debris. Factors influencing densities such as cleanups, storm events, rain fall, tides, hydrological changes may alter counts, evaluations of fluxes and, even if surveys can track changes in the composition of beach litter, they may not be sensitive enough to monitor changes in the abundance[53]. This problem can be circumvented by recording the rate, at which litter accumulates on beaches through regular surveys that are performed weekly, monthly or annually after an initial cleanup[53]. This is the most common approach, revealing long-term patterns and cycles in accumulation, requiring nonetheless much effort to maintain surveys. However, past studies may have vastly underestimated the quantity of available debris because sampling was too infrequent[54].

It is unfeasible to review the hundreds of papers on beach macro-debris, which often apply different approaches and lack sufficient detail[25]. Most studies range from a local[51] to a regional scale[55] and cover a broad temporal range. Information on sources, composition, amounts, usages, baseline data and environmental significance are often also gathered[56–58] as such data are easier collected. Most studies record all litter items encountered between the sea and the highest strandline on the upper shore. Sites are often chosen because of their ecological relevance, accessibility and anthropogenic activities and sources. Factors influencing the accumulation of debris in coastal areas include the shape of the beach, location and the nature of debris[59]. In addition, most sediment-surface counts do not take buried litter into account and clearly underestimate abundance, which biases composition studies. However, raking of beach sediments for litter may disturb the resident fauna. Apparently, a good correlation exists between accumulated litter and the amount arriving, indicating regular inputs and processes. Recent experiments with drift models in Japan indicate good correlation of flux with litter abundances on beaches[60,61]. It appears that glass and hard plastics are accumulating more easily on rocky shores[62]. Litter often strands on beaches that lack strong prevalent winds, which may blow them offshore[23,63]. Abundance or composition of litter often varies even among different parts of an individual beach[64] with higher amounts found frequently at high-tide or storm-level lines[65]. Because of this and beach topography, patchiness is a common distribution pattern on beaches, especially for smaller and lighter items that are more easily dispersed or buried[66]. It is very difficult to compare litter concentrations of various coastal areas (with different population densities, hydrographic and

geological conditions) obtained from various studies with different methodologies, especially when the sizes of debris items considered are also different. Nevertheless, common patterns indicate the prevalence of plastics, greater loads close to urban areas and touristic regions[18]. Data expressed as items m^{-2} or larger areas are more convenient for comparisons. Most studies have reported densities in the m^{-2} range (Table 2.1). High concentrations of up to 37,000 items per 50m beach line (78.3 items m^{-2}) were recorded in Bootless Bay, Papua New Guinea[67] because of specific local conditions, following typhoons (3,227 items m^{-2})[68] or flooding events (5,058 items m^{-2})[19]. Data expressed as quantities per linear distance are more difficult to compare because the results depend on beach size/width. Plastic accounts for a large part of litter on beaches from many areas with up to 68 % in California[58], 77 % in the south east of Taiwan[68], 86 % in Chile[20], and 91 % in the southern Black Sea[19]. However, other types of litter or specific types of plastic may also be important in some areas, in terms of type (Styrofoam, crafted wood) or use (fishing gear).

Table 2.1. Comparison of mean litter densities from recent data worldwide (non-exhaustive list). Ranges of values are given in parentheses

Region	Density (m−2)	Density (linear m−1)	Plastic (%)	References
SW Black Sea	0.88 (0.008–5.06)	24 (1.7–197)	91	Topçu et al. (2013)[19]
Costa do Dende, Brazil	n.d.	9.1	75	Santos et al. (2009)[69]
Cassina, Brazil	n.d.	5.3–10.7	48	Tourinho and Fillmann (2011)[70]
Gulf of Aqaba	2 (1–6)	n.d.	n.d.	Al-Najjar and Al-Shiyabet (2011)[71]
Monterey, USA	1 ± 2.1	n.d.	68	Rosevelt et al. (2013)[58]
North Atlantic, USA	n.d.	0.10 (0.2)	n.d.	Ribic et al. (2010)[72]
North Atlantic, USA	n.d.	0.42 (0.1)	n.d.	Ribic et al. (2010)[72]
North Atlantic, USA	n.d.	0.08 (0.2)	n.d.	Ribic et al. (2010)[72]
South Caribbean, Bonaire	1.4 (max. 115)	n.d.	n.d.	Debrot et al. (2013)[57]
Bootless Bay, Papua New Guinea	15.3 (1.2–78.3)	n.d.	89	Smith (2012)[73]
Nakdong, South Korea	0.97–1.03	n.d.	n.d.	Lee et al. (2013)[74]
Kaosiung, Taiwan	0.9 (max. 3,227)	n.d.	77	Liu et al. (2013)[75]
Tasmania	0.016–2.03	n.d.	n.d.	Slavin et al. (2012)[76]
Midway, North Pacific	n.d.	0.60–3.52	91	Ribic et al. (2012a)[77]
Chile	n.d.	0.01–0.25	n.d.	Thiel et al. (2013)[78]
Heard Island, Antarctica	n.d.	0–0.132	n.d.	Eriksson et al. (2013)[34]

For trends in the amount of litter washed ashore and/or deposited on coastlines, beach litter monitoring schemes provide the most comprehensive data on individual litter items. Large data sets have already been held by institutions[79] or NGO's such as the Ocean Conservancy through their International Coastal Cleanup scheme for 25 years, or the EU OSPAR marine litter monitoring program, which started over 10 years ago and covers 78 beaches[80]. The lack of large-scale trends in the OSPAR-regions is probably due to small-scale heterogeneity of near-shore currents, which evoke small-scale heterogeneity in deposition patterns on beaches[80].

Several nonlinear models were derived to describe the development of pollution of coastal areas with marine litter[79,81]. There were long-term changes in indicator debris on the Pacific Coast of the U.S. and Hawaii over the nine-year period of the study. Ocean-based indicator debris loads declined substantially while at the same time land-based indicator items had also declined, except for the North Pacific coast region where no change was observed. Variation in debris loads was associated with land- and ocean-based processes with higher land-based debris loads being related to larger local populations. Overall and at the local scale, drivers included fishing activities and oceanic current systems for ocean-based debris and human population density and land use status for land-based debris.

At local scales, concentrations of specific items may be largely driven by specific activities or new sources. For example, 41 % of the total debris from beaches in California was of Styrofoam origin, with no other explanation than an increased use of packaging, which degrades very easily[81]. Small-sized items may form an important fraction of debris on beaches. For example, up to 75 % of total debris from the southern Black Sea was smaller than 10 cm[19]. Small-sized particles include fragments smaller than 2.5 cm[41], the so-called meso-particles or mesodebris, which is, unlike macrodebris, often buried and not always targeted by cleanups, stranding fluxes are therefore difficult to evaluate. Little attention has been paid to sampling design and statistical power even though optimal sampling strategies have been proposed[53]. Densities of small-sized debris were found to be very high in some areas where, in addition to floating debris, they can pose a direct threat to wildlife, especially to birds that are known to ingest plastic[82,83].

2.3.2 Floating Marine Debris

Floating debris constitutes the fraction of debris in the marine environment, which is transported by wind and currents at the sea surface and is thus directly related to the pathways of litter at sea. Floating litter items can be transported by the currents until they sink to the seafloor, be deposited on the shore or degrade over time[84]. While the occurrence of anthropogenic litter items floating in the world oceans was reported already decades ago[85,86], the existence of accumulation zones of Floating Marine Debris (FMD) in oceanic gyres has only recently gained worldwide attention[87].

Synthetic polymers constitute the major part of floating marine debris, the fate of which depends on their physico-chemical properties and the environmental conditions. As high-production volume polymers such as polyethylene and polypropylene have lower densities than seawater, they float until they are washed ashore or sink because their density changes due to biofouling and leaching of additives. While being subject to biological, photic or chemical degradation processes, they can be physically degraded gradually into smaller fragments until becoming microplastics, which is often defined as the size fraction <5 mm. This fraction requires different monitoring techniques, such as surface net trawls, and is therefore treated elsewhere[83,88]. Floating macrolitter is typically monitored by visual observation from ships, though results from net trawls are also being reported. The spatial coverage and thus the representativeness of the quantification depends on the methodology applied. Also, observation conditions, such as sea state, elevation of the observation position and ship speed affect results.

Existing datasets indicate substantial spatial variability and persistent gradients in floating marine litter concentrations[89]. The variations can be attributed to differential release pathways or specific litter accumulation areas. Because of inconsistent reporting schemes used in scientific publications, data sets are often not comparable. Typically, item numbers are reported per surface area. Mass-based concentrations can then only be derived through estimates. Differences are found between studies in size ranges, concentration units and item categories used. As the number of pieces increases drastically with decreasing size of the observed litter items, the reporting of corresponding size classes is of high importance for comparing debris abundances among studies. Apart from the difficulty in reporting sizes correctly from shipboard observations, many publications use different size-range categories.

In addition to research activities, the quantification of floating litter is part of the assessment schemes of national and international monitoring frameworks. Monitoring of the quantity, composition and pathways of floating litter can contribute to an efficient management of waste streams and the protection of the marine environment. The European Marine Strategy Framework Directive, national programs, the Regional Sea Conventions and international agreements such as the United Nations Environmental Programme consider the monitoring of floating litter[90]. Visual assessment approaches include the use of research vessels, marine mammal surveys, commercial shipping carriers and dedicated litter observation surveys. Aerial surveys are often conducted for larger items[91]. However, available data for floating litter are currently difficult to compare because existing observation schemes (NOAA, UNEP, Hellenic Marine Environment Protection Association—HELMEPA, etc.) apply different approaches, observation schemes and category lists [41,92]. Some approaches involve the reporting by volunteers [93]. While the main principle of monitoring floating debris through visual observation is very simple, there are not many data sets, which allow a comparison of debris abundance. Some data sets are accessible as peer-reviewed publications or through reports from international organizations. However, the regions covered are very limited and monitoring occurs only sporadically.

Globally, the reported densities of floating marine debris pieces >2 cm ranges from 0 to beyond 600 items km^{-2}. Ship-based visual surveys in the North Sea German Bight yielded 32 items km^{-2} on average[94]. The integration over different surveys and seasons resulted in litter densities of 25 items km^{-2} at the White Bank area, 28 items km^{-2} around the island of Helgoland and 39 items km^{-2} in the East Frisian part of the German Bight. More than 70 % of the observed items were identified as plastics. From 2002 to 2006, aerial marine mammal surveys were used for the quantification of floating litter. Results were reported as sightings km^{-1}, ranging from 0 to beyond 1 item km^{-1}. Concentrations in coastal waters appeared to be lower than in offshore regions[95].

In the Corsican Channel at the northern Mediterranean Sea, in an offshore area of ca. 100 x 200 km between Marseille and Nice, floating debris was quantified during marine mammal surveys. A maximum of 55 pieces km^{-2} was recorded with strong spatial variability[96]. In the Ligurian Sea, data were collected through ship-based visual observation in 1997 and 2000. Between 15 and 25 objects and between 1.5 and 3.0 objects km^{-2} were found in 1997 and 2000, respectively, without specification of the size ranges used[97]. Voluntary surveys through HELMEPA made from commercial shipping vessels in the Mediterranean Sea revealed a concentration of 2 items km^{-2} with higher concentrations in coastal areas but also longer transects without any litter encounters. While plastic material accounted for the highest proportion (83 %) of litter, textiles, paper, metal and wood comprised 17 %[2]. No size ranges were given, but the described conditions during observation indicate that only larger items were considered. A large-scale survey in the Mediterranean Sea found 78 % of the observed objects larger than 2 cm to be of anthropogenic origin[98]. Plastic constituted 96 % of these. While highest densities (>52 items km^{-2}) were reported from the Adriatic Sea and Algerian basin,

lowest densities (<6.3 items km^{-2}) were recorded in the central Thyrrenian and Sicilian Sea. Densities in other areas ranged between 11 and 31 items km^{-2} [98].

Visual aerial surveys were conducted in the Black Sea, flying slow at low altitude above the Kerch Strait, the southern part of the Azov Sea and on the coastal Russian Black Sea. Concentrations in the Kerch Strait and the Azov Sea were comparable at 66 items km^{-2} and twice as high as those from the Black Sea[99]. In a visual observation study in the north Pacific, ca. 56 km off Japan, densities of 0.1–0.8 items km^{-2} with a size >5 cm were found[100]. A study at the east coast of Japan utilized surface trawl nets with a net opening of 50 cm and a mesh size of 333 µm to sample transects of 10 min at 2 knots. The size of plastic pieces captured ranged from 1 to 280 mm. Pieces >11 mm accounted only for 8 % and particles of 1–3 mm accounted for 62 % at total average litter mass of 3600 g km^{-2} [101]. Visual observation studies in southern Chilean fjords revealed 1–250 items km^{-2} >2 cm during seven oceanographic cruises from 2002 to 2005[20,102,103]. Typically, densities in the northern areas ranged from 10 to 50 items km^{-2}. An average of 0.5 items km^{-2} was reported in the waters northwest of Hawaii, close to the so-called Pacific garbage patch, compared with 9 pieces km^{-2} in southeast Asia[104]. Debris densities in the waters off British Columbia (Canada), comprised 0.9–23 pieces km^{-2} with a mean of 1.5 items km^{-2} [105], but no size range was given. In the Gulf of Mexico, 1.0–2.4 pieces km^{-2} were recorded during cetacean survey flights[106] (Table 2.2).

Floating marine debris (FMD) density in the northern South China Sea was quantified by net trawls at 4.9 (0.3–16.9) items km^{-2}, with Styrofoam (23 %) and other plastics (45 %) dominating[107]. More than 99 % of FMD was small- (<2.5 cm) or medium-sized (2.5–10 cm). Large items (10–100 cm) were detected by visual observation resulting in mean concentrations of 0.025 items km^{-2} [107]. In the northeast Indian Ocean, a large difference in the concentration of marine debris was reported between the Strait of Malacca (578 ± 219 items km^{-2}) and the Bengal Sea (8.8 ± 1.4 items km^{-2}) [108]. By contrast, concentrations >375 items km^{-2} were reported in Amon Bay, east Indonesia [109].

Table 2.2. Comparison of mean litter densities on the sea surface from worldwide data (non-exhaustive list)

Region	Density (item km^{-2}) (max)	Size range (cm)	Plastic (%)	References
North Sea	25–38	>2	70	Thiel et al. (2011)[94]
Belgian coast	0.7	n.d.	95	Van Cauwenberghe et al. (2013)[110]
Ligurian coast	1.5–25	n.d.	n.d.	Aliani and Molcard (2003)[97]
Mediterranean Sea	10.9 → 52 (194.6)	>2	95.6	Suaria and Aliani (2014)[98]
North Sea	2 (1–6)	n.d.	n.d.	Herr (2009)[95]
Kerch Strait/Black Sea	66	n.d.	n.d.	BSC (2007)[99]
Chile	10–50 (250)	>2	>80	Hinojosa and Thiel (2009)[102]
West of Hawaii	0.5	0.08 (0.2)	n.d.	Matsumura and Nasu (1997)[104]
British Columbia	1.48 (2.3)	n.d.	92	Williams et al. (2011)[105]
South China Sea	4.9 (0.3–16.9)	<2.5–10	68	Zhou et al. (2011)[111]
North Pacific	459	2	95	Titmus and Hyrenbach (2011)[112]
Strait of Malacca	579	>1–2	98.8	Ryan (2013)[108]
Bay of Bengal	8.8	>1–2	95.5	Ryan (2013)[108]
Southern Ocean	0.032–6	>1	96	Ryan et al. (2014)[113]

In 2009, a 4,400-km cruise from the American west coast to the North Pacific subtropical gyre and back, provided data during 74 h of observation, corresponding to a transect length of 1,343 km[112]. A single observer at 10 m above the sea level recorded a total of 3,868 pieces, of which 90 % were fragments and 96 % of these were plastic. Eighty-one percent of the items had a size of 2–10 cm, 14 % of 10–30 cm and 5 % of >30 cm. The density of debris increased towards the centre of the gyre, where smaller, probably older and weathered pieces were found. The authors note that visual observations are constrained by the inability to detect smaller fragments (<20 mm) and to retrieve the observed items for further analysis and concluded that visual observations can be easily conducted from ships of opportunity, which provide a useful and inexpensive tool for monitoring debris accumulation and distribution at sea.

A specific case of floating marine litter is abandoned or lost fishing gear, such as nets or longlines. These items cause significant harm when abandoned, as they continue to catch marine wildlife[82]. In 2003, a major effort, including the identification of possible accumulation areas by satellite imaging and ocean current modelling, was made to select appropriate areas for aerial surveys in search for abandoned fishing gear in the Gulf of Alaska[91]. Employing a wide range of methodologies including visual video, infrared video and Lidar imaging during 14 days of observation, 102 items of anthropogenic origin were sighted.

Modelling of oceanographic currents can help to identify pathways and accumulation areas, thus enabling source attribution[114,115]. A modelling approach in the North Sea identified seasonal signals in litter reaching the coasts[116]. The concentrations and distribution patterns of floating marine debris can be expected to change according to climatic changes[117]. The cycling and distribution of debris was modelled within the global oceanic currents[118]. Input scenarios were based on population density and major shipping lanes. A 30-year projection showed the accumulation of floating debris in ocean gyres

and enclosed seas. These studies have the potential to investigate pathways and to guide monitoring to enable effective implementation of management measures and the assessment of their efficiency. Modelling is also used to predict the pathways and impacts of large quantities of debris introduced through natural events such as tsunamis and related run-offs[119]. Single events may drastically increase local debris concentrations. A study combining available worldwide data with a modelling approach estimated the weight of the global plastic pollution to comprise 75 % macroplastic (>200 mm), 11 % mesoplastic (4.75–200 mm), and 11 and 3 % in two microplastic size classes, respectively[89]. The data suggest that a minimum of 233,400 tons of larger plastic items are adrift in the world's oceans compared to 35,540 tons of microplastics.

Floating marine litter can be considered as ubiquitous, occurring even in the most remote areas of the planet such as the Arctic[33]. Floating litter items are also present in the remote Antarctic Ocean, although densities are low and cannot be expressed as concentrations[120]. Some 42 % of the observed 120 objects south of 63°S consisted of plastic. Debris items were observed even as far south as 73°S. However, the small number of surveys and low total object counts do not allow for trend assessments. In the African part of the Southern Ocean, 52 items (>1 cm) were recorded during a 10,467 km transect survey, yielding densities ranging from 0.03 to 6 items km^{-2} [113].

The diversity and non-comparability of monitoring approaches used currently hinders a comparison of absolute pollution indicators and spatial or temporal assessments. The development and widespread implementation of protocols for monitoring, such as the ongoing efforts for the implementation of the MSFD[41], could improve the quality of data gathered. Established protocols should be accompanied by training schemes, quality assurance and control procedures. The implementation of standardized protocols in the monitoring of riverine litter may enable source allocation.

Unfortunately, data acquired by NGOs or authorities are often not published in peer-reviewed journals and are therefore not readily accessible. A joint international database would facilitate the collection of such data and improve standardization and comparability. The collection of data, e.g. on-site through tablet computer applications, the standardization of reporting formats and the streamlining of data flows would facilitate data treatment. More easily accessible data sets can then help to prioritize activities and to monitor the success of litter reduction measures.

While monitoring by human observers is a simple and straightforward approach, for large-scale and frequent surveys, automatized approaches are promising. Developing technologies may lead to the use of digital imaging and image recognition techniques for the autonomous large-scale monitoring of litter[121,122].

The implementation of international frameworks such as the EU MSFD, Regional Action Plans against Marine Litter and the agreements of the Rio 20 Conference require improvement of data availability and quality and can therefore be expected to provide the basis for coordinated assessments in the future.

2.3.3 Seafloor

Change in the nature, presence or abundance of anthropogenic debris on the seafloor is much less widely investigated than sea surface patterns. Studies typically focus on continental shelves, as sampling difficulties, inaccessibility and costs rarely allow for research in deeper waters, which accounts for almost half of the planet's surface. Deep-sea surveys are important because ca. 50 % of plastic litter items sink to the seafloor and even low-density polymers such as polyethylene and propylene may lose buoyancy under the weight of fouling[123]. While acoustic approaches do not enable discrimination of different types of debris on the seafloor except for metals and may not record

smaller objects, trawling was considered the most adequate method when taking into account mesh sizes and net opening width[41] (Figure 2.1). However, nets were primarily designed to collect specific biota leading to sample bias and underestimation of benthic litter quantities. Therefore, beam trawling has been suggested as the most consistent survey method for the assessment of benthic marine litter[124], although rather destructive to seafloor habitats because of the scraping of sediments and inhabiting biota. However, trawls cannot be used in rocky habitats or on hard substrates and they do not allow for a precise localization of individual items. Samples from trawls are likely to underestimate debris abundance and may miss some types of debris altogether such as monofilaments because of variability in the sampling efficiency for different debris items[47]. Pieces from the trawl nets themselves[125] may contaminate samples. Finally, it does not enable the assessment of impacts of litter on habitats when it contributes its own impacts on the seafloor, which are more severe for the benthic fauna and habitats than the litter items caught by trawl.

Figure 2.1. a. North Sea seafloor litter collected in 30min GOV trawl by Cefas using the RV Endeavour (picture by John Thain, Cefas) b. Litter collected by trawling in the Mediterranean Sea, France. 10 min experiment (picture by Barbaroux and Galgani, IFREMER)

Strategies to investigate seabed debris are similar to those for evaluating the abundance and composition of benthic species. Mass is less often determined for marine debris, because very large items may increase variability in measures. Although floating debris, such as that found in the highly publicized “gyres” and/ or convergence zones, is currently the focus of attention, debris accumulating on the seafloor has a high potential to impact benthic habitats and organisms. Forty-three seafloor litter studies were published between 2000 and 2013. Until recently, only few of them covered greater geographic areas or depths. Most of these studies utilised a bottom trawl for sampling as part of fish stock assessments. More recently, remotely operated vehicles and towed camera systems were increasingly used for deep-sea surveys[39] (see Figure 2.2). The geographic distribution of debris on the ocean floor is strongly influenced by hydrodynamics, geomorphology and human factors[39,126]. Moreover, there are notable temporal variations, particularly seasonal, with tendencies for accumulation and concentration of marine litter in geographic areas [22]. Interpretation of trends is, however, difficult because the ageing of plastics at depth is unknown and the accumulation of debris on the seafloor certainly began before scientific investigations started in the 1990s.

In estuaries, large rivers are responsible for substantial input of debris to the seabed[37,44]. Rivers can also transport waste far offshore because of their high flow rate and strong currents[22,126]. Alternatively, small rivers and estuaries can also act as a sink for litter, when weak currents facilitate deposition on shores and banks[23]. In addition, litter may accumulate upstream of salinity fronts being transported to the sea later, when river flow velocity is increasing.

Plastics were found on the seabed of all seas and oceans and the presence of large amounts has been reported[18,21,23] but remains uncommon in remote areas such as Antarctica, particularly in deep waters[18]. So far, deep-sea sampling has been limited to some trawls and sediments grabs. Microplastics were found in deep sea sediments from the southern Atlantic[127] and Kuril-Kamchatka-trench area[128]. Large-scale evaluations of seabed debris distribution and densities are more common

in other regions[23]. However, these studies mostly involve extrapolations from small-scale investigations mainly in coastal areas such as bays, estuaries and sounds. The abundance of plastic debris shows strong spatial variations, with mean densities ranging from 0 to more than 7,700 items km^{-2} (Table 2.3). Mediterranean sites show the greatest densities owing to the combination of a densely populated coastline, shipping in coastal waters and negligible tidal flow. Moreover, the Mediterranean is a closed basin with limited water exchange through the Strait of Gibraltar. Generally, litter densities are higher in coastal seas[129] because of large-scale residual ocean circulation patterns but also because of extensive riverine input[130]. However, debris that reaches the seabed may have been transported over considerable distances before sinking to the seafloor, e.g. because of heavy fouling. Indeed, some accumulation zones were identified far from coasts[33,131–133]. Accordingly, even in the shallow subtidal abundance and distribution patterns can differ substantially from the adjacent strandlines with plastics being the most important fraction at sea. In general, bottom debris tends to become trapped in areas of low circulation where sediments are accumulating[39,49,126]. The consequence is an accumulation of plastic debris in bays, including lagoons of coral reefs, rather than in the open sea. These are the locations where large amounts of derelict fishing gear accumulate and cause damage to shallow-water biota and habitats[82,134]. Continental shelves are considered as accumulation zones for marine debris[129], however, often with lower concentrations of debris than adjacent canyons because debris is not retained but washed offshore by currents associated with offshore winds and river plumes.

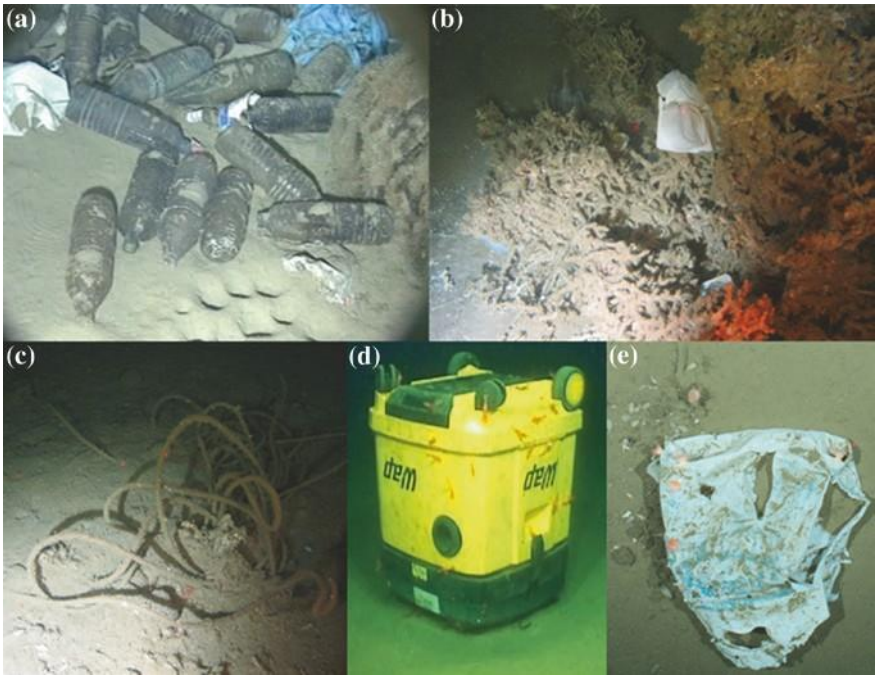

Figure 2.2. Litter on the deep seafloor. a. Plastic bags and bottles dumped 20 km off the French Mediterranean coast at 1,000 m in close vicinity to burrow holes (F. Galgani, IFREMER); b. food package entrapped at 1,058 m in deep-water coral colony; c. rope at 1,041 m depth, both from Darwin Mounds (courtesy of V. Huvenne, National Oceanography Centre Southampton (NOCS)); d. waste disposal bin or a vaccum cleaner with prawns on the seafloor off Mauritania at 1,312 m depth (courtesy of D. Jones, SERPENT Project, NOCS); e. plastic carrier bag found at ~2,500 m depth at the HAUSGARTEN observatory (Arctic) colonised by hormathiid anemones and surrounded by dead tests of irregular sea urchins (courtesy of M. Bergmann, AWI)

Table 2.3. Comparison of litter densities on the seafloor from recent data worldwide (non-exhaustive list)

Location	Habitat	Date	Sampling	Depth (m)	Density (min-max)	Plastic (%)	References
Southern China	Benthic	2009–2010	4 trawl (mesh not available)/1 dive	0–10	693 (147–5,000) items km−2	47	Zhou et al. (2011)[107]
France-Mediterranean	Slope	2009	17 canyons, 101 ROV dives	80–700	3.01 km−1 survey (0–12)	12 (0–100)	Fabri et al. (2014)[135]
Thyrenian Sea	Fishing ground	2009	6 × 1.5 ha samples, trawl, 10 mm mesh	40–80	5,960 ± 3,023 km−2	76	Sanchez et al. (2013)[136]
Spain-Mediterranean	Fishing ground	2009		40–80	4,424 ± 3,743 km−2	37	Sanchez et al. (2013)[136]
Mediterranean Sea	Bathyal/abyssal	2007–2010	292 tows, otter/ Agassiz trawl, 12 mm mesh	900–3,000	0.02– -2 3,264.6 kg km (incl. clinker)	n.d.	Ramirez-Llodra et al. (2013)[24]
Malta	Shelf	2005	Trawl (44 hauls, 20 mm mesh)	50–700	102	47	Mifsud et al. (2013)[137]
Turkey/Levantin Basin	Bottom/bathyal	2012	32 hauls (trawl, 24 mm mesh)	200–800	290 litter -2 (3,264.6 kg km)	81.1	Güven et al. (2013)[138]
Azores, Portugal	Condor seamount	2010–2011	45 dives	185–256	1,439 items km−2	No plastic/89 % fishing gear	Pham et al. (2013)[139]
Goringe Bank, NE Atlantic	Gettysburg and Ormonde seamounts	2011	4 ROV dives (124 h video, 4,832 photographs), total distance of 80.6 km	60–3,015	1–4 items·km−1	9.9/56 fishing gear	Vieira et al. (2014)[140]
US west coast	Shelf	2007–2008	1,347 sites (total, trawling, 38 mm mesh)	55–183	30 items km−2	23	Keller et al. (2010)[48]
	Slope	2007–2008		183–550	59 items km−2	n.d.	Keller et al. (2010)[48]
	Slope/bathyal	2007–2008		550–1,280	129 items km−2	n.d.	Keller et al. (2010)[48]
Mediterranean Sea, France	Shelf/canyon	1994–2009 (16 years study)	90 sites (trawls, 0.045 km^2/tow, 20 mm mesh)	0–800	76–146 km−2 (0–2,540)	29.5–74	Galgani et al. (2000) and unpub- lished data[23]

Table 2.3 (continued)

Location	Habitat	Date	Sampling	Depth (m)	Density (min-max)	Plastic (%)	References
Japan, offshore Iwate	Trench	Jamstek database	3 dives on 4,861 available,	299–400, 1,086–1,147, 1,682–1,753	15.9 items h−1	42.8	Miyake et al. (2011)[141]
Kuril-Kamchatka area (NW Pacific)	Trench/bathyal plain	2012	20 box cores (0.25 m^2) (Agassiz trawl, camera epi-benthic sledge)	4,869–5,766	60 → 2,000 micro-plastics m−2	(Trawl samples: mostly fishing gear)	Fischer et al. (2015)[128]
Fram Strait, Arctic	Slope	2002–2011 (5 surveys)	One OFOS camera tow $year^{-1}$, 5 transects (1,427–2,747 m^2)	2,500	3,635 (2002)–7,710 (2011) items km−2	59	Bergman and Klages (2012)[33]
Northern Antarctic Peninsula and Scotia Arc	Slopes/bathyal	2006	32 Agassiz trawls	200–1,500	2 pieces only	1 plastic	Barnes et al. (2009)[18]
Monterey Canyon, California	From margin to abyssal	1989–2011	ROVs, 2,429 km^2 in total	25–3,971	632 items km−2	33	Schlining et al. (2013)[49]
ABC islands, Dutch Caribbean	Sandy bottoms to rocky slopes	2000	24 video transects, submersibles	80–900	2,700 items km−2 (0–4590)	29	Debrot et al. (2014)[142]

Only few studies have assessed debris below 500 m depth[21,23,33,39,48–50,126,128,130,131,140,141,143,144]. Trends in deep-sea pollution (1992–98) were observed off the European coast with an extremely *variable* distribution and debris accumulating in submarine canyons[23]. Anthropogenic debris was recorded down to 7,216 m depth in video surveys from the Ryukyu Trench[141]. Litter was primarily composed of plastic and accumulated in deep-sea trenches and depressions. Accordingly, several authors[39,126,143] concluded that submarine canyons may act as a conduit for the transport of marine debris into the deep sea. Recent studies conducted in coastal deep-sea areas along California and the Gulf of Mexico[47,49,130] confirmed this pattern. Also, an analysis of the composition and abundance of man-made, benthic marine debris collected in bottom trawl surveys at 1,347 randomly-selected stations along the US west coast in 2007 and 2008 indicated that densities increased significantly with depth, ranging from 30 items km^{-2} in shallow (55–183 m) to 128 items km^{-2} in the deepest waters surveyed (550–1,280 m)[48]. Higher densities at the bottom were also found in particular areas such as those around rocks, wrecks as well as in depressions or channels[126]. Deep submarine extensions of coastal rivers influence the distribution of seabed debris. In some areas, local water movements transport debris away from the coast to accumulate in zones of high sedimentation. In the case of the Mississippi river, for example, the front canyon was a focal point for litter, probably due to bottom topography and currents [130]. Under these conditions, the distal deltas of rivers can fan out in deeper waters, creating areas of high accumulation. Many authors[46,126,130] show that circulation may be influenced by strong currents occurring in the upper part of canyons, which decrease rapidly in deeper areas resulting in an increased confinement with a litter distribution that seems to be temporally more stable as a consequence.

A great variety of human activities such as fishing, urban development and tourism contribute to the distribution pattern of debris on the seabed. Debris from the fishing industry is prevalent in fishing areas[47,49,140]. This type of material may account for a high proportion of debris. In the eastern China Sea[129], 72% of debris is made of plastic, mainly pots, nets, octopus jars, and fishing lines. Investigations using submersibles at depths beyond the continental shelf and canyons have revealed substantial quantities of debris in remote areas. Between 0.2–0.9 pieces of plastic per linear kilometer were observed at the HAUSGARTEN observatory (2500 m) in the Fram Strait (Arctic)[131]. Fifteen items, of which 13 were plastic, were observed during one dive between 5,330 and 5,552 m ('Molloy Hole'), which reflects the local funnel-like topography and downwards directed eddies acting as particle trap. Litter quantities doubled between 2002 and 2011 in the HAUSGARTEN area[33]. The accumulation trends reported in that study raise concern as degradation rates of most polymers in deep-sea environments are assumed to be even slower due to the absence of light, low temperature and oxygen concentrations.

2.3.4 Microplastics

Similar to large debris, there is growing concern about the implications of the diverse microparticles in the marine environment, which are particles between 5mm – 1 µm[27,41]. Most microparticles are tiny plastic fragments known as microplastics, although other types of microparticles exist, such as fine fly ash particles emitted with flue gases from combustion, rubber from tyre wear and tear as well as glass and metal particles, all of which constantly enter the marine environment. The abundance and global distribution of microplastics in the oceans appeared to have steadily increased over past decades[145–147], while a decrease in the average size of plastic litter has been observed over this time period [18]. In recent years, the existence of microplastics and their potential impact on wildlife and human health has received increased public and scientific attention[83,148,149].

Microplastics comprise a very heterogeneous assemblage of particles that vary in size, shape, color, chemical composition, density, and other characteristics. They can be subdivided by usage and source as (i) 'primary' microplastics, produced either for indirect use as precursors (nurdles or virgin resin pellets) for the production of polymer consumer products, or for direct use, such as in cosmetics,

scrubs and abrasives and (ii) 'secondary' microplastics, resulting from the break- down of larger plastic material into smaller fragments. Fragmentation is caused by a combination of mechanical forces, e.g. waves and/or photochemical processes triggered by sunlight. Some 'degradable' plastics are even designed to fragment quickly into small particles, however, the resulting material does not necessarily biodegrade [150]. There are various sources of microplastics and pathways into the oceans[36].

In order to understand the environmental impacts of microplastics, many studies have quantified their abundance in the marine environment. One of the major difficulties in making large-scale spatial and temporal comparisons between existing studies is the wide variety of methods that have been applied to isolate, identify and quantify marine microplastics[151]. For meaningful comparisons to be made and robust monitoring studies to be conducted, it is therefore important to define common methodological criteria for estimating abundance, distribution and composition of microplastics[88].

Microplastics normally float at the sea surface because they are less dense than sea- water. However, the buoyancy and specific gravity of plastics may change during their time at sea due to weathering and biofouling, which results in their distribution across the sea surface, the deeper water column, the seabed, beaches and sea ice[18,28,146,152–155]. Until now, only a limited number of global surveys have been conducted on the quantity and distribution of microplastics in the oceans[83]. Most surveys focused on specific oceanic regions and habitats, such as coastal areas, regional seas, gyres or the poles[27,154,156]. Concentrations of microplastics at sea vary from thousands to hundreds of thousands of particles km^{-2} and latest reports suggest that microplastic pollution has spread throughout the world's oceans from the water column[145] to sediments even of the deep sea[28,87,127,128,132,145,146,154,157,15828,87,89,127,128,132,145,146,154,158]. Recently, microplastics were also recorded from Arctic sea ice in densities two orders of magnitude higher than those previously reported from highly contaminated surface waters, such as those of the Pacific gyre[155]. This has important implications considering the projected acceleration in sea ice melting due to global climate change and concomitant release of microplastics to the Arctic marine ecosystem.

Time-series data on the composition and abundance of microplastics are sparse. However, available evidence on long-term trends suggests various patterns in microplastic concentrations. A decade ago, the broad spatial extent and accumulation of this type of contamination was already demonstrated[27]. They found plastic particles in sediments from U.K. beaches and archived among the plankton in samples dating back to the 1960s with a significant increase in abundance over time. More recent evidence indicated that microplastic concentrations in the North Pacific subtropical gyre have increased by two orders of magnitude in the past four decades[159]. However, no change in microplastic concentration was observed at the surface of the North Atlantic gyre for a period of 30 years[28].

Less is known about the composition of microplastics in the oceans. Evidence suggests a temporal decrease in the average size of plastic litter[18,89]. Studies based on the stomach contents of shearwaters (*Puffinus tenuirostris*) in the Bering Sea also indicated a decrease in 'industrial' primary pellets and an increase in 'user' plastic between the 1970s and the late 1990s[160] but constant levels over the last decade[43]. Similarly, long-term data from The Netherlands since the 1980s show a decrease of industrial plastics and an increase in user plastics, with shipping and fisheries being the main sources[43].

2.4 SUMMARY & CONCLUSIONS

Marine debris is now commonly observed everywhere in the oceans and available information suggests that marine debris is highly dynamic in space and time. However, we need standardized methodologies for quantification and characterisation of marine litter to be able to achieve global estimates. Litter enters the sea from land-based sources, from ships and other installations at sea, from point and diffuse sources, and can travel long distances before being deposited. While plastic typically constitutes a lower proportion of the discarded waste, it represents the most important part of marine litter with sometimes up to 95 % of the waste and has become ubiquitous even in remote polar regions. However, trends are not clear with quantities having slightly decreased over the last 20 years in some locations, notably in the western Mediterranean. At the same time no change in litter quantities are evident in the convergence zones from oceanic basins or beaches. In other locations, however, including the deep seafloor, densities have increased.

Accumulation rates vary widely with factors such as proximity of urban activities, shore and coastal uses, wind and ocean currents. These enable the accumulation of litter in specific areas at the sea surface, on beaches or on the seafloor. Before an accurate estimate of global debris quantities can be made, basic information is still needed on sources, inputs, degradation processes and fluxes. For this and because there is considerable variation in methodology between regions and investigators, more valuable and comparable data must be obtained from standardized sampling programs. In terms of distribution and quantities, important questions concerning the balance between the increase of waste and plastic productions, reduction measures and the quantities found at the surface and on shorelines remain unanswered. Potentially, important accumulation areas with high densities of debris are still to be discovered. It is now clear that managers and policy makers will need to better understand the distribution of litter in order to assess and evaluate precisely the effectiveness of measures implemented to reduce marine litter pollution.

Chapter 3

Below the surface: Twenty-five years of seafloor litter monitoring in coastal seas of North West Europe (1992–2017)

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ABSTRACT

Marine litter presents a global problem, with increasing quantities documented in recent decades. The distribution and abundance of marine litter on the seafloor off the United Kingdom's (UK) coasts were quantified during 39 independent scientific surveys conducted between 1992 and 2017. Widespread distribution of litter items, especially plastics, were found on the seabed of the North Sea, English Channel, Celtic Sea and Irish Sea. High variation in abundance of litter items, ranging from 0 to 1835 pieces km^{-2} of seafloor, was observed. Plastic items such as bags, bottles and fishing related debris were commonly observed across all areas. Over the entire 25-year period (1992–2017), 63% of the 2461 trawls contained at least one plastic litter item. There was no significant temporal trend in the percentage of trawls containing any or total plastic litter items across the long-term datasets. Statistically significant trends, however, were observed in specific plastic litter categories only. These trends were all positive except for a negative trend in plastic bags in the Greater North Sea - suggesting that behavioural and legislative changes could reduce the problem of marine litter within decades.

3.1 INTRODUCTION

Globally, marine litter has become a pollution problem, originating from a variety of land and sea-based sources. Ongoing surveys have demonstrated that man-made litter has now been documented to occur in almost every marine environment studied to date[18,53,161–163]. Marine litter consists mainly of plastic materials, both in numbers and by weight, with minor amounts of metal and glass contributing to the overall litter load. Typical plastic items such as bags, bottles and fishing related litter are omnipresent and indicative of a variety of anthropogenic pressures[2]. According to Jambeck et al.[163], population size and the quality of waste management systems largely determine where the greatest mass of uncaptured waste becomes plastic marine litter.

An ongoing challenge is in relation to reducing the amount of litter in the marine environment. This problem has been at the forefront of several international initiatives. In June 2012 at Rio + 20, the Global Partnership on Marine Litter (GPML) was launched. More recently, the Leader's Declaration of the 2015 G7 Summit acknowledged the global risks posed by marine litter, particularly plastics, to marine and coastal ecosystems and potentially human health. As such, marine litter generation and prevention are linked to a variety of human activities and policy areas operating at both national and international levels. Therefore, to address both the sources and impacts of marine litter, legislation and agreements need to relate to waste and wastewater management, product design, shipping, fisheries policies, consumption and behavioural patterns [164–166]. In Europe, specific legislation was introduced to deal with marine litter and its impact on the coastal and marine environment: the Marine Strategy Framework Directive (MSFD)[12]. The MSFD incorporates an indicator specifically in relation to litter and requires evidence that member states are moving towards Good Environmental Status (GES). More specifically, the MSFD operates by monitoring, amongst others, trends in the amount of litter deposited on the sea floor, including analysis of its composition, spatial distribution and, where possible, sources[12].

Globally, waste management legislation is seen in the broader context of enhanced resource efficiency, now a key cross-cutting policy goal[167]. As an example, the first jurisdictions where plastic bag reduction policies emerged and regulatory action was taken were in South Asia in the late 1990s and early 2000s, primarily based on concerns regarding human health and livelihoods[168]. Most northern industrialised countries have also seen attitudes shift in recent years[168]. In Europe, the first legislation against plastic bag use was introduced by Ireland and Denmark in 2002 and 2003 respectively. In Ireland, the effect of the tax on the use of plastic bags in retail outlets has been dramatic—a reduction in use of the order of 90%, and an associated gain in the form of reduced littering and negative landscape effects[169]. This tax on plastic shopping bags, previously provided free of charge to customers at points of sale, was adopted by other European member states in the

following years[169]. Since the plastic bag tax policy came into force in England in October 2015, the total number of carrier bags used at the UK's biggest retailers has fallen by an estimated 85%[170]. In the context of a European Circular Economy, a directive to reduce the use of thin plastic bags, many of which end up as waste in the marine environment was finally agreed on the 28th of April 2015[171].

In relation to marine litter from sea-based sources such as the fishing industry, legal and technical measures to ensure that littering from lost or abandoned fishing gear is minimised are provided by the Food and Agriculture Organization of the United Nations (FAO): Recommendations for the Marking of Fishing Gear[172] and Code of Conduct[173]. The abandonment of fishing gear is specifically prohibited by the International Maritime Organisation in its Convention for the Prevention of Pollution from Ships[174]. From a European perspective, the Common Fisheries Policy (CFP) states that measures should be taken to conserve resources and limit the environmental impact of fishing[175]. The European Commission also recognised the importance of the marking of fishing gear in 1994 and, more recently, in 2004[176]. Furthermore, the European Maritime and Fisheries Fund supports measures to remove lost fishing gears from the seafloor. This surge in marine litter related legislation has identified a requirement for long-term monitoring programmes, capable of assessing the effectiveness of newly implemented measures. To date, the majority of marine litter studies have focused on visible and easily accessible litter contamination, such as that along shorelines or floating on the surface of the water[53]. However, some litter sinks and almost all floating litter is expected to be cast onto a beach or to sink to deeper waters, eventually landing on the seafloor. This may be due to a variety of repeating processes such as degradation, fouling by marine organisms (e.g. bacteria, algae and sessile organisms), or ingestion and excretion by marine animals[145,177–181]. On continental shelves, fishing trawl surveys provide a practical way in which to monitor seafloor litter because they cover a wide area and collect a suitable quantity of litter for analysis[182]. Nevertheless, long-term datasets on marine litter on the seafloor are sparse[30,40]. Where studies are available they cover relatively short time series and have catalogued seabed litter using a variety of techniques such as snorkeling, SCUBA diving, trawl surveys, sonar and the use of submersibles and ROVs[33,40,47,49,141,183]. For example, the presence of large amounts of plastic litter has been reported in European continental shelf seas[23,39], including in the Baltic, North[184] and, Celtic Sea, the Bay of Biscay[22], the Barents Sea and Norwegian Sea[185], and the Mediterranean[21,31,126,186,187], Adriatic[188]and Black Sea[189]. Plastic litter items have been found in deep sea canyons of the French Mediterranean coast[126], the west coast of Portugal[143] and nearby to sea-mounts close to the Azores[39,139].

Since 1992, the Centre for Environment, Fisheries and Aquaculture Science (Cefas), a UK Government organisation, has been collecting seafloor litter data on environmental and fisheries stock assessment surveys. Such research provides spatial and temporal trend assessments of the abundance of seafloor litter within North West European seas and acts as a baseline against which litter reduction mitigation measures can be assessed. Here we present an assessment of 25 years of seafloor litter data (1992–2017), gathered during 39 scientific surveys at 2461 stations in the coastal seas of North West Europe. We divided the analysis in two main parts: an analysis of the trends of the major litter categories and plastic sub-categories during the 1992–2017 period and a spatial analysis in 2011, the last year in which all surveys took place, thus providing a comparison of the inshore (within 12 nm of land) and offshore (>12 nm) regions of the Celtic and Greater North Seas.

3.2 MATERIALS & METHODS

3.2.1 Survey data

Cefas undertakes several fish stock assessment and environmental trawl surveys. With respect to the current study the relevant ones are the International Bottom Trawl Survey (IBTS), the ICES Ground Fish Surveys (Q4SW) and the Clean Seas Environment Monitoring Programme (CSEMP) survey. Figure 3.1 shows the spatial coverage in 2011. The selected surveys used two similar types of otter trawls: CSEMP uses the Granton trawl, while IBTS and Q4SW use the Grande Ouverture Verticale (GOV) trawl.

Otter trawls derive their name from the large rectangular otter boards which are used to keep the mouth of the trawl net open; these boards act like a plough, digging up to 15 cm into the seabed. Both otter trawls have a mesh size of 40 mm at the cod end, but the GOV is considerably larger in size and volume than the Granton trawl. They are designed to trawl the seafloor and catch fish living on or near the seabed. The mean catch (either in weight or in numbers) per unit of effort or per unit of area is an index of the stock abundance (i.e. assumed to be proportional to the abundance) [190]. Similar assumptions can be made in relation to the number of litter items trawled.

These three surveys cover all waters surrounding the UK, including the Greater North Sea (GNS) and Celtic Sea (CS) as defined by the MSFD (Figure 3.1). In this study, we have combined data from the IBTS, Q4SW and CSEMP surveys from 1992 to 2017 in two main areas: the GNS and CS. Within these, we created two more sub-divisions - inshore and offshore - based on the 12 nm boundary. The three surveys did not all take place annually (Table A – supplementary information (SI)). Cruises between 1992 and 1999 were all in the IBTS series and only collected litter in the offshore GNS area from 72 to 150 stations. In 2000, the CSEMP cruise started gathering litter data in the inshore parts of the GNS and in/off- shore CS areas from 17 to 50 stations. Between 2009 and 2011, marine litter data collection was introduced within the Q4SW survey, covering the inshore and offshore CS area from 68 to 79 stations (Table A- SI); therefore, full coverage of the Celtic Sea is only available for these three years (Figure 3.1).

Different transects were trawled at each station every year. As haul lengths averaged 4 km (SD 1.4 km) across all trawls, each haul is effectively a point sample in the sea. The area sampled at each station was estimated from the width of the net multiplied by the assumed distance it had been in contact with the seabed and functioning. All historic data were translated manually from logbooks into the new database using the MSFD classification system. The IBTS data from 1992 until 2010 measured litter items by weight; this hampers our ability to accurately determine the number of items based on these weight determinations due to different weights of polymer types and processes such as biofouling and degradation. Therefore, these data were used as an indication of presence or absence only.

3.2.2 Marine litter and metadata collection method

For each survey, the following information was recorded: the definition and specification of the survey, the positions of stop and start of each trawl and its technical specification e.g. wing spread, mesh size of net, cod end and blinders. After each tow, fish were deposited in the fish pound or hopper before being sorted, then all litter items were manually picked from the hopper, net and cod end and classified according to the classification system in the guidance document on Monitoring of Marine Litter in European Seas[40]. The MSFD classification system is composed of six main categories of litter (Plastic, Metal, Rubber, Glass, Natural and Miscellaneous), each divided into sub-categories (39 in total)[40]. We defined two further sub-categories of plastic litter to reflect land-based household litter (Household) and fishing-based (Fishing) sources. The Household class is composed of the subcategories plastic bottles, sheeting and bags. The Fishing class comprises the subcategories fishing net, fishing line (monofilament/entangled), synthetic rope, cable ties, strapping band, crates and containers. The litter data from surveys prior to 2009 were collected using the same main categories as the MSFD classification system, although with fewer subcategories. Several plastic subcategories (caps, sheet, fishing line, crates, straps, cable ties, diapers and sanitary towels/tampons) were added in 2009 and thus trends for those were calculated based on data from 2009 onwards.

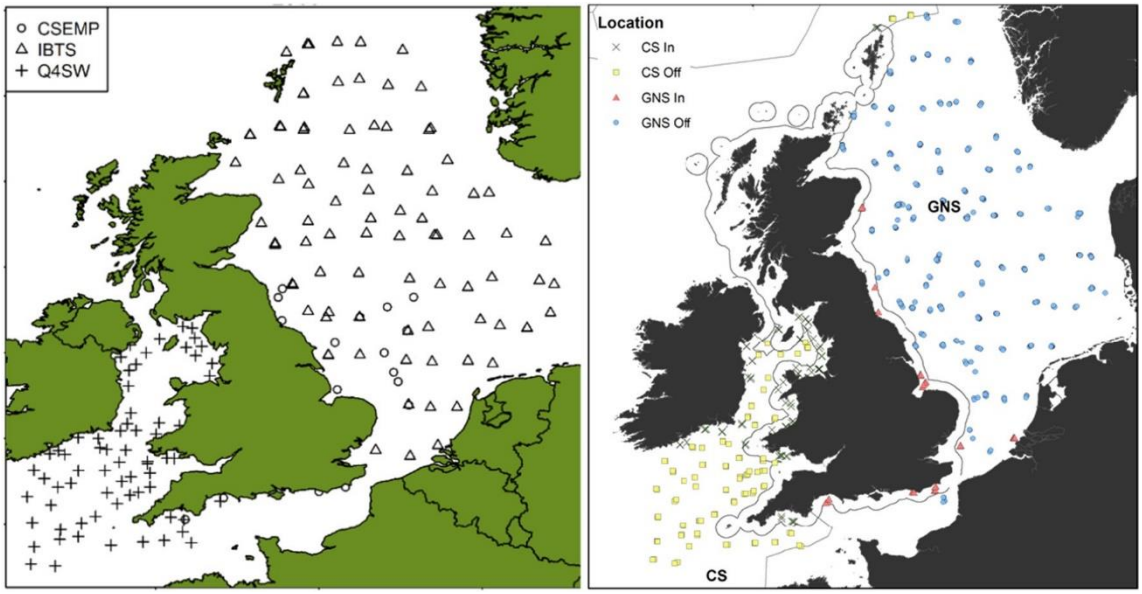

Figure 3.1. LEFT PANEL: Spatial coverage of the Cefas surveys in 2011: IBTS, International Bottom Trawl Survey; Q4SW, Quarter 4 Westerly Ground Fish Survey, CSEMP, Clean Seas Environment Monitoring Programme. RIGHT PANEL: Spatial coverage and divisions of the benthic marine litter stations in 2011. The black line surrounding the UK represents the 12 nm boundary. The black line in the Western Channel and starting near the tip of Shetland symbolizes the MSFD boundary for the Celtic Sea (CS) and Greater North Sea (GNS). Key to regional divisions: GNS- off, Greater North Sea offshore stations outside 12 nm; GNS-in, Greater North Sea inshore stations within 12 nm; CS-off, Celtic Sea offshore stations outside 12 nm; CS-in, Celtic Sea inshore stations within 12 nm. The two CS offshore stations at the top of the map were added to the GNS offshore region in the spatial and temporal analysis.

3.2.3 Data presentation and analysis

The IBTS data prior to 2010 reflects only presence or absence of litter items. Thus, to give a good representation of the extent of litter on the seafloor and to make correct comparisons across time, for each year, we have created the variable "percentage of trawls in which the litter item was recorded". While we are confident that the data generated by CSEMP correctly counted litter items, we have used the same per- centage variable to define litter for this survey as for IBTS above. This is partly for ease of comparison with IBTS which used a GOV otter trawl but also because the distribution of the number of litter items caught per trawl is often highly skewed. That is, generally observations are 0 or 1, but there are also some very high counts. These high counts could overly influence simple yearly means and transforming the data by taking natural logs prior to statistical treatment proved problematic due to the high proportion of zeros. For temporal trend analysis (1992–2017) the data are thus expressed as the percentage of trawls in which the litter item was recorded. In the spatial analysis (2011), the data are presented as abundance in number of marine litter items km^{-2} of seafloor.

To perform formal statistical evaluation of potential trends, the Mann- Kendall (MK) non-parametric test was used [191,192]. This was performed on the yearly means of the particular litter value being considered using the R [193] software and the emon package [194], with the function mannkendall two-sided tests were performed as there was no a priori knowledge of whether the trend might be positive or negative.

To look at temporal trends, due to the unbalanced nature of surveys and otter trawls (GOV and Granton) over this sampling period, the data were not integrated from different surveys. This allows temporal com- parisons to be made for the same survey. For the GNS, data from the IBTS surveys (1992–2000, 2005, 2008–2017) was included for the off- shore area and data from the CSEMP survey

for inshore waters (2000–2008, 2010–2014, 2016–2017). The CSEMP survey also covered inshore waters on the Celtic Sea side (2000–2008, 2010–2014, 2016–2017). The IBTS and CSEMP surveys are spatially consistent across years. There was a limited amount of long-term data covering the CS-offshore area, because the Q4SW survey collected marine litter data only from 2009 until 2011. Therefore, no attempt was made to carry out a temporal analysis for the inshore CS area.

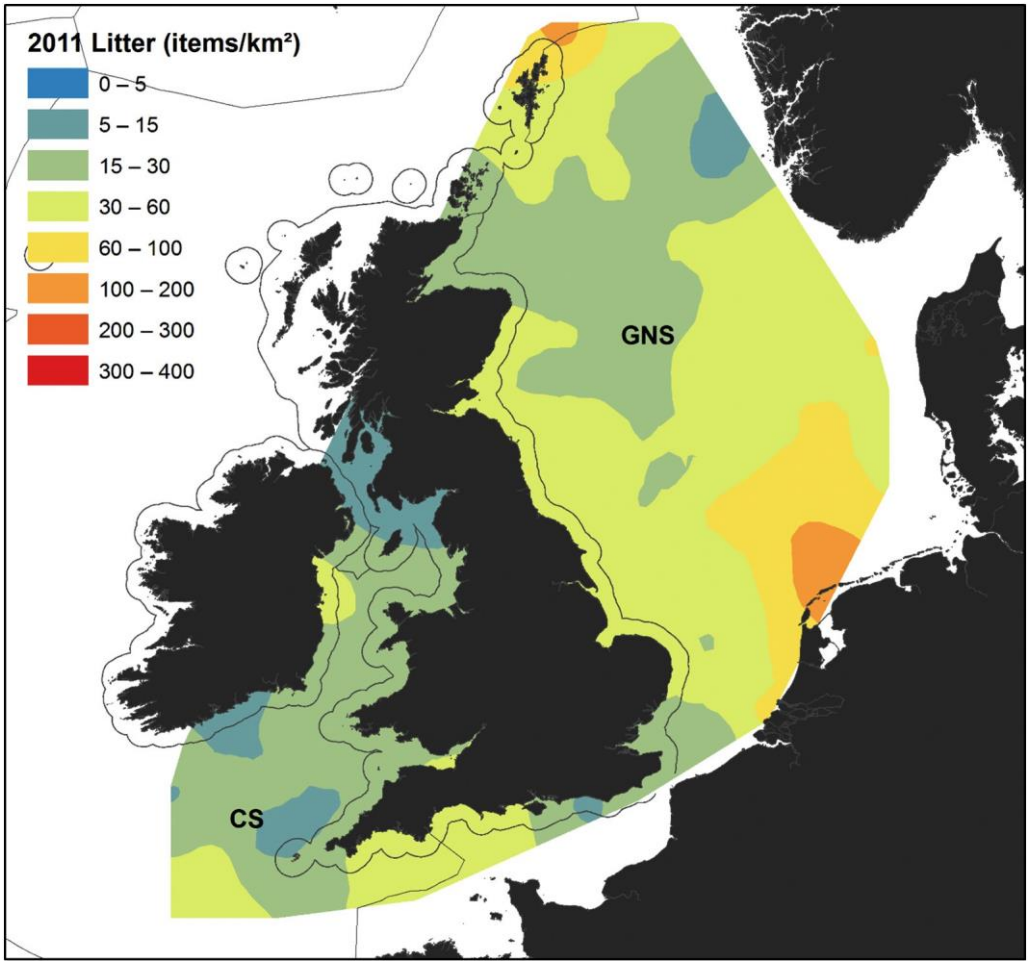

Figure 3.2. Marine litter abundance (litter items km^{-2}) on the seafloor in North West European Seas, all data from 2011 interpolated using R, Shiny and PostGIS. The black line surrounding the UK represents the 12 nm boundary. The black line in the Western Channel and starting near the tip of Shetland symbolizes the MSFD boundary for the Celtic Sea (CS) and Greater North Sea (GNS). Key to regional divisions: GNS-off, Greater North Sea offshore stations outside 12 nm; GNS-in, Greater North Sea inshore stations within 12 nm; CS-off, Celtic Sea offshore stations outside 12 nm; CS-in, Celtic Sea inshore stations within 12 nm.

Table 3.1. Total number of marine litter items km^{-2} of seafloor and percentage of trawls containing marine litter items km^{-2} of seafloor. Comparisons between the surveys using the Manly (2007) approach. Data are for the CSEMP, IBTS and Q4SW surveys in 2011. Key to regional divisions: GNS-off, Greater North Sea offshore stations outside 12 nm; GNS-in, Greater North Sea inshore stations within 12 nm; CS-off, Celtic Sea offshore stations outside 12 nm; CS-in, Celtic Sea inshore stations within 12 nm. N = number of stations.

2011	Mean	95% CI for mean	Median	N	% non-zero
CS-in	24.4	(13.0,35.8)	14.1	24	70.8
CS-off	21.7	(14.5,28.9)	14.1	44	59.1
GNS-in	49.0	(23.8,74.3)	40.0	10	100
GNS-off	40.5	(30.8,50.3)	28.4	95	78.9

For the spatial analysis, only data from 2011 were used to create identical areas to these previously used in the temporal analysis: CS- in, CS-off, GNS-in, GNS-off. For this analysis, mixed data was used to cover all four areas. Data were pooled based on their location (GNS/ CS) and distance from coast (in or outside the 12 nm boundary). The CS inshore area uses a mixture of inshore stations from the Q4SW and CSEMP surveys. The CS offshore area is made up entirely of Q4SW off- shore stations. Similarly, the GNS offshore area compromises mostly IBTS stations and some CSEMP offshore stations. The GNS inshore area included stations within the 12 nm boundary from CSEMP and IBTS (Figure 3.1). Comparisons between the different trawl areas of surveys within 2011 are made possible by transforming the litter counts to numbers per km^2. Baseline values of litter abundance (litter items km^{-2}) were calculated for all four areas; 2011 data were selected as it was the latest year with the largest spatial cover, and to synchronise with the start of the MSFD (initial assessment). A non-parametric randomisation test[195] using the function permute.groups in emon[194] was used to make comparisons between the UK areas. To generate a visual overview, our analysis interpolated the litter abundance data for 2011 to create a raster distribution map using Geostatistical Analyst extension from ArcGIS 10.1 [196] (Figure 3.2). The data exhibited a non-stationary distribution, the mean is not equal across the whole region, so a detrending surface with exponential function was applied in order to remove the existing trend[197]. A declustering and a Normal Score transformation was applied before interpolating the data. A simple kriging interpolation using a stable semivariogram model in the R package sgeostat was used to create the litter distribution surface. Using the semivariogram correlation distance parameters, the value in a non-sampled location was estimated, with a searching radius of 80 km an using a number of 5 maximum neighbours[198].

3.3 RESULTS

3.3.1 Litter distribution in the CS and GNS

In 2011, the inshore CS area contained statistically significantly less items km^{-2} than the inshore GNS region ($p = 0.01$, means are 24 and 49 respectively). Similarly, the offshore CS area contained statistically significantly less items km^{-2} than the offshore GNS region ($p = 0.04$), no statistically significant differences were observed in the number of items km^{-2} between the GNS inshore and offshore area ($p = 0.59$). All trawls conducted in the inshore GNS area contained litter in 2011 (Table 3.1). However, the two highest litter counts in the 22-year dataset, 1816 and 1835 items km^{-2} were observed in 2003 and 2004 at CSEMP Station 616 (Carmarthen Bay, 51.63 Lat; −4.59 Long), situated in the in- shore Celtic Sea area. High counts, more than a hundred marine litter items km^{-2}, were also detected in samples from parts of the English Channel, off the Dutch and Danish coasts, in the Irish Sea, the Bristol Channel and along the Devon and Cornwall coastline (Figure 3.2).

3.3.2 Litter composition in the CS and GNS

Many types of litter items were commonly detected in the trawls, especially pieces of plastic sheeting, bags and bottles, metallic objects, glass, and diverse materials including fishing gear. Items of natural

origin, like driftwood and branches were less prominent. Most litter items were partially degraded; although still recognizable, they were often functioning as a substratum and were populated by organisms e.g. bryozoans, hydroids, tunicates and bivalves. Like results for floating and beach litter findings, a high percentage of litter items detected on the seafloor were made of plastic. Around 38% (931) of all tows (2461) across all three surveys (CSEMP, Q4SW, IBTS) over the entire 25-year period (1992–2017) contained solely plastic litter items. In 2011, plastic items accounted for 77% (CS-in), 94% (CS-off), 65% (GNS-in) and 79% (GNS-off) of the total number of litter items (Table 3.2). Although, high proportions of plastic items km^{-2} were observed in the offshore areas of the CS, we did not find quantities to be significantly different (based on 2011 data only and using permutation tests) from the inshore ($p = 0.06$) and offshore area of the GNS ($p = 0.16$). Additionally, there were no statistical differences observed between the CS and GNS areas (inshore and offshore) in 2011 in terms of household or fishing related litter items km^{-2} (p N 0.05). High quantities of metal items were also found in the inshore parts of the GNS. Items made of rubber, glass and ceramics were absent in the offshore CS samples (Table 3.2). Table 3.3 expands on the information in Table 3.2 for plastic, household plastic and fishing-related plastic items. We can see, for example, that 90% of the trawls in the GNS-in region contained at least one plastic item.

Table 3.2 Mean number of items km^{-2} of seafloor by main litter categories in the four regions: CS-in, CS-off, GNS-in, GNS-off in 2011. Key to regional divisions: GNS-off, Greater North Sea offshore stations outside 12 nm; GNS-in, Greater North Sea inshore stations within 12 nm; CS-off, Celtic Sea offshore stations outside 12 nm; CS-in, Celtic Sea inshore stations within 12 nm.

2011	Stations	Mean litter items km^{-2}						
		Total	Plastics	Metal	Rubber	Glass/ Ceramics	Natural	Misc.
CS-in	24	24.3	18.8	1	0.4	0.6	2.9	0.6
CS-off	44	21.6	20.4	0.3	0	0	0.9	0
GNS-in	10	49.1	31.8	8.9	2.2	0.5	3.6	2.1
GNS-off	95	40.5	32.1	1.2	2.1	0.4	4.3	0.4

Table 3.3. Mean and median number of items km^{-2} and percentage of trawls containing AT LEAST ONE ITEM. The data are for plastic items, plastic household items and plastic fishing related items in the inshore and offshore regions of the CS and GNS in 2011. Key to regional divisions: GNS-off, Greater North Sea offshore stations outside 12 nm; GNS-in, Greater North Sea inshore stations within 12 nm; CS-off, Celtic Sea offshore stations outside 12 nm; CS-in, Celtic Sea inshore stations within 12 nm. N = number of stations.

2011	Mean	95% CI for mean	Median	Stations	% non-zero
1. Plastic items					
CS-in	18.8	(8.4,29.3)	13.4	24	58.3
CS-off	20.4	(13.6,27.3)	13.8	44	59.1
GNS-in	31.8	(16.3,47.3)	26.1	10	90
GNS-off	32.1	(24.5,39.7)	27.8	95	73.7
2. Plastic household items					
CS-in	10.5	(2.5,18.5)	0	24	37.5
CS-off	7.4	(3.8,11.0)	0	44	34.1
GNS-in	13.7	(5.2,22.1)	12.6	10	80
GNS-off	10.9	(7.7,14.1)	0	95	46.3
3. Plastic fishing related items					
CS-in	7.9	(1.1,14.7)	0	24	37.5
CS-off	9.9	(6.1,13.7)	0	44	45.5
GNS-in	15.4	(5.3,25.5)	13.9	10	70
GNS-off	17	(11.8,22.1)	13.7	95	54.7

3.3.3 Litter trends in the CS and GNS

Surprisingly, no significant temporal trends were detected in the percentage of trawls containing any litter and in almost all main litter categories (total plastic, metal, glass/ceramics, natural items) across the long-term datasets in the 3 regions (GNS-off, GNS-in, CS-in) (Table B – SI & Figure 3.3a–b). The category Rubber is decreasing in the offshore and inshore GNS (p = 0.01) (Figure 3.3c) and the category Miscellaneous is increasing in the inshore CS (p = 0.002).

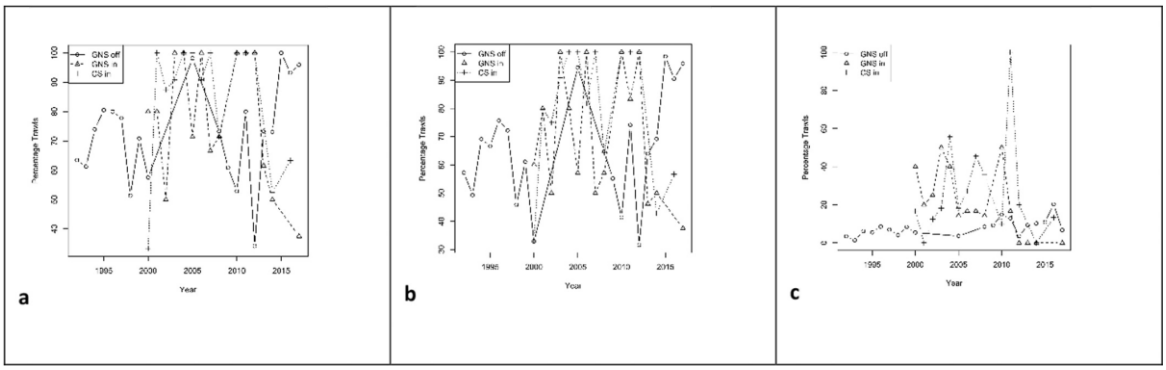

Figure 3.3. (a) Percentage of trawls containing any litter item for the three areas by year. (b) Percentage of trawls containing a plastic item items for the three areas by year. (c) Percentage of trawls containing a rubber item for the three areas by year. Key to regional divisions: GNS-off, Greater North Sea offshore stations outside 12 nm; GNS -in, Greater North Sea inshore stations within 12 nm; CS-in, Celtic Sea inshore stations within 12 nm.

Our analysis considered the plastic category in a greater level of detail by looking at trends in its components and in the two newly created categories, Household and Fishing (Table 3.4). No trend was detected in the proportion of household litter in all 3 regions (GNS-in, GNS-off, CS-in) assessed. The percentage of plastic sheeting (including packaging) showed an upward trend in all regions. A statistically significant upward trend was also detected in the proportion of fishing related litter in the offshore area of the GNS (p = 0.02). This was caused by upward trends in the plastic subcategories: fishing line (p b 0.001), cable tie (p b 0.001), cable strap (p b 0.001) and crates (p = 0.003). Plastic bags were the only category with a statistically significant downward trend in both the inshore (p = 0.05) and offshore (p = 0.01) regions of the GNS. The trend plots for plastic bags and the Fishing category are shown in Figure 3.4a–b.

Table 3.4. p-Values for trend as assessed by the Mann-Kendall test for percentage of trawls containing plastic litter categories by region. For p-value of 0.05 or less, the direction of the trend is shown. Key to regional divisions: GNS-off, Greater North Sea offshore stations outside 12 nm; GNS-in, Greater North Sea inshore stations within 12 nm; CS-in, Celtic Sea inshore stations within 12 nm.

Category	GNS-off	GNS-in	CS-in
Household	*0.39*	*0.81*	*0.70*
Bottles	0.30	0.35	0.43
Sheet	0.001 (+)	0.005 (+)	0.01 (+)
Bag	0.01 (-)	0.05 (-)	0.40
Fishing	0.02 (+)	0.81	0.71
Rope	0.63	0.34	0.06
Fishing net	0.09	0.52	1.00
Fishing line	0.001 (+)	0.10	0.02 (+)
Cable tie	0.001 (+)	0.67	0.17
Cable strap	0.001 (+)	–	0.71
Crates	0.003 (+)	0.08	0.11

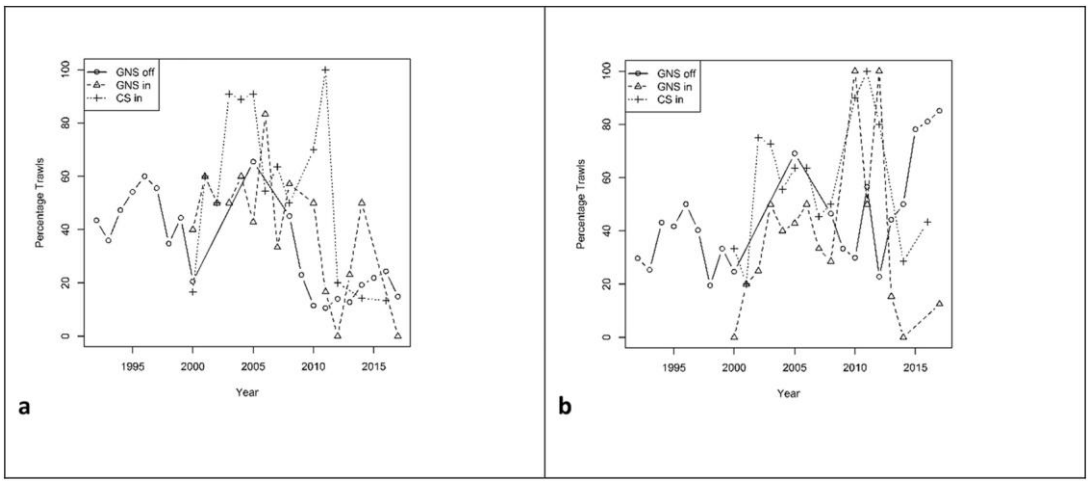

Figure 3.4. (a) Percentage of trawls containing plastic bags for all three areas by year. (b) Percentage of trawls containing plastic Fishing items for all three areas by year. Key to regional divisions: GNS-off, Greater North Sea offshore stations outside 12 nm; GNS-in, Greater North Sea inshore stations within 12 nm; CS-in, Celtic Sea inshore stations within 12 nm.

Our analysis compared the proportion of plastic bags prior to 2010 against the percentage from 2010 to 2017. All three regions (GNS-in, GNS-off, CS-in) demonstrated statistically significant reductions (p b 0.05) between the mean annual percentages. The actual mean percentages were (pre2010 vs 2010 onwards): GNS-off (43% vs 16%); GNS-in (53% vs 21%); CS-in (65% vs 24%).

3.4 DISCUSSION

3.4.1 Litter distribution in the CS and GNS

To date, centralized information on marine litter quantities and its distribution on the continental shelves of the North-East Atlantic is still fragmentary. The range of litter densities on the seafloor found at our study sites was within the same range as those reported in other parts of the Atlantic Ocean [23,33,39,133,139,143,186,199,200]. In 2011, between 13 and 74 litter items km^{-2} were detected at stations across the GNS and CS. The density of seafloor litter in the Barents Sea and Norwegian Sea is 202 and 279 items/km^2 respectively, and highest densities were found close to coast and in canyons [185]. Litter evaluations at deep sea sites (seamounts, banks, mounds, and ridges) in the Atlantic Ocean around Europe using a trawl or dive studies, indicated a density of 180 plastic items km^{-2} [39]. Other seafloor litter studies in the deep sea of the southern Indian Ocean and Atlantic Ocean, using a remote operating vehicle (ROV), found densities of 555 and 483 items km^{-2} [133]. In 2014, at the Arctic sea- floor, a mean litter density of 6566 items km^{-2} was measured with a tow camera system [201]. Similar litter densities were reported at canyons near Lisbon (6620 items km^{-2}) using video footage and still images from ROVs [143]. We found maximum values, reaching up to 1816 and 1835 items km^{-2}, at Carmarthen Bay in previous years (2003 and 2004). Based on material input, caused by a clockwise gyre, sediment accretion studies suggest a direct linkage between the Bristol Channel, a major river inlet, and Carmarthen Bay [202], which could explain the high abundance of litter at this location. Worldwide surveys in coastal waters have indicated an average seafloor litter concentration of 723 plastic items km^{-2} [199]. Two studies, using the same MSFD protocol [40], indicated a mean litter abundance of 16.8 items km^{-2} in the North Sea, 5.07 items km^{-2} in the Baltic Sea [184], 24 items km^{-2} in the Eastern Mediterranean and 1211 items km^{-2} in the Black Sea [189]. Although data obtained with similar sampling methods might permit some comparisons between studies, dissimilarities in the sampling sizes, techniques and equipment implies that the different results should be treated with caution when compared directly [53].

The heat map, using data from 2011 (Figure 3.2) reveals that predicted litter density ranges between 0 and 60 items km^{-2} with higher amounts of litter near shore. Similar to the findings in French coastal waters, accumulations of litter were observed around urban areas and major estuaries [23], indicating that rivers might be driving litter inputs. Worldwide, large rivers running through urban areas have been found to be major sources of marine litter [20,37,44,203,204]. One can clearly observe the effects of the Rhine ROFI (Region Of Freshwater Influence) [205] creating a significant input of buoyant water and probably floating litter from freshwater river sources, which seem to have important implications on the distribution of seafloor litter.

3.4.2 Litter composition in the CS and GNS

Similar to other seafloor litter studies [23,39], in most stations sampled in our study, plastic accounted for a very high percentage (between 65 and 94%) of the total number of litter items. The most prevalent plastic litter items were bags, plastic sheeting and derelict fishing gear items. Although no significant difference in the number of total plastic, plastic household or fishing items was observed between GNS and CS regions in 2011, the confidence intervals indicated the presence of more plastic items in the GNS inshore compared to the CS inshore. Similarly, no significant difference in fishing related litter items was found between the offshore GNS and CS ($p= 0.08$) area; however, the confidence intervals indicated a higher presence in the offshore GNS area. The North Sea is surrounded by many industrialised countries, an international fishing ground and contains some of the world's most important shipping lanes – all of which could explain this high abundance of litter. Foekema et al.[206] speculated that the higher frequency of fish with ingested plastics the southern North Sea resulted from higher, localised plastic pollution levels. In spite of existing regulations to prevent waste from maritime industry[207], several studies reported litter pollution from ships in the German Bight[94,208]. High amounts of litter were also reported on beaches and the seafloor in the southeastern North Sea[200,209]. These higher numbers of fishing related litter in the GNS compared to the CS could reflect fishing efforts, which are far greater in the GNS than in the Celtic and Irish Sea[210]. It seems that the pattern of accumulation and composition of the litter is determined by a complex range of environmental and anthropogenic factors.

3.4.3 Litter trends in the CS and GNS

In our study, Rubber (including tyres) and Miscellaneous were the only main categories which showed a significant trend in one or more of the assessed areas. For all other main categories, we were not able to detect a statistical change over time in any of the three regions (GNS-in, GNS-off, CS-in). Such absence of a clear or statistically significant trend with regard to variations in seafloor litter quantities was also reported by Galgani[199] when analysing seafloor litter data from French trawls undertaken between 1994 and 2014. Temporal trends indicated a stable situation in the Gulf of Lion and seasonal variations in the northern part of the Bay of Biscay[22,186].

However, across the entire dataset, we detected a clear downward trend in the percentages of trawls containing plastic bags in the inshore and offshore GNS area. Our results also showed a reduced proportion of plastic bags in all three regions (GNS-in, GNS-off, CS-in) from 2010 onwards. This could be the result of the implementation of measures against the use of plastic bags [168], changes in plastic bag composition and thus degradation rates [211] or underlying hydrodynamics [23,199].

Our results also indicated an increase in the proportion of fishing debris in the GNS. The following subcategories were rising: fishing line, cable tie, straps and crates. In the last two decades, specific actions and measures to target the loss of fishing nets have been introduced. Extensive seafarer training and specific industry actions might be useful to target some of these sea-based items.

3.5 CONCLUSIONS & OUTLOOK

The results of this work have indicated that large quantitative variations occur, and that the geographical distribution of litter could be affected by hydrodynamics, seasonal variation geomorphology and human factors. Moreover, fishing effort is not uniformly distributed and shifts locations over the years. Since the late 1990s, both fishing effort and trawling effort have decreased substantially in both the GNS and CS regions [210,212]. The combined effects of improved measures, changing inputs and shifting fishing pressures make it difficult to make firm conclusions in relation to marine litter.

Our data for 2011 did not show a clear difference in litter density between inshore and offshore areas, a pattern previously reported for European Seas[23,39] and off California [47]. In the Gulf of Lion, Galgani et al.[186] suggested that low litter density on the shelf was caused by strong water flow from the Rhone River, transporting litter to the south into deeper waters. The main inflow of water into the North Sea is from the North Atlantic into the northern basin where we observed rather low amounts of litter. Water also enters from the English Channel, the water movements and general circulation in the English Channel are presumably responsible for the dilution of the litter in the center of the Channel, pushing it towards the Southern North Sea[22] into the Skagerrak and along the Scandinavian coast into the Norwegian Sea and deeper canyons[213]. A similar situation occurs in Monterey Bay, California, where sediment and litter are being swept off the continental shelf down into Monterey Canyon[49]. This suggests that the amounts of litter on the seabed are not static and thus the observed marine litter abundance on the seafloor results from a dynamic equilibrium between continuous input and output. Some litter items will transfer into the deep or remote parts of the Atlantic Ocean. Several reports have indicated the presence of litter in deep sea trenches, canyons and at the poles [18,33,126,143,201,214]. We observed far lower litter densities on the seafloor of the GNS and CS compared to surveys at submarine canyons and deep sea locations in the North East Atlantic [139]. Plastic items and their breakdown fragments seem to dissipate into the wider North East Atlantic[39,127,139], Baltic [215] and Mediterranean Sea[24], which could cause trends in larger litter and plastic items km^{-2} of seafloor to remain stable in the GNS and CS despite increasing inputs. Controversially, the observed presence of a downward trend in plastic bags in the GNS indicates that we can influence the abundance and distribution of certain marine litter items over short time scales, within decennia.

The present study illustrated several opportunities and limitations of using trawl surveys to evaluate abundance, spatial distribution and qualitative composition of benthic marine litter. Seafloor litter data can easily be obtained from environmental and fisheries surveys using bottom trawls. Such monitoring occurs at several times a year with similar trawling equipment undertaken by several countries with adjoining sea borders. Therefore, international co-operation and data sharing, will facilitate regional assessments and improve the power of detecting trends in future years. The higher the power of a survey, the more accurately one can assess the effectiveness of marine litter measures.

3.6 ACKNOWLEDGEMENTS

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Chapter 4

Microplastics Baseline Surveys at the Water Surface and in Sediments of the North-East Atlantic

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ABSTRACT

Microplastic contamination was determined in sediments of the Southern North Sea and floating at the sea surface of North West Europe. Floating concentrations ranged between 0 and 1.5 microplastic particles/m^3, whereas microplastic concentrations in sediments ranged between 0 and 3,146 particles/kg dry weight sediment. In sediments, mainly fibers and spheres were found, whereas at the sea surface fragments were dominant. At the sea surface, concentrations of microplastics are lower and more variable than in sediments, meaning that larger sample sizes and water volumes are required to find detectable concentrations. We have calculated the widths of the confidence intervals (CI) for different sample sizes, to give a first indication of the necessary sample size for a microplastic survey at the water surface. Higher concentrations of floating microplastics were found near estuaries. In sediments, estuaries and areas with a high organic carbon content were likely hotspots. Standardization of monitoring methods within marine regions is recommended to compare and assess microplastics pollution over time.

4.1 INTRODUCTION

Marine litter accumulating in the marine environment may be one of the greatest threats facing the planet. The exact quantity of plastic in the ocean and volumes entering the ocean from waste generated on land is unknown. Recent studies estimate that 275 million metric tons (MT) of plastic waste was generated in 192 coastal countries in 2010, of which 4.8–12.7 million MT could have entered the ocean[163]. It has been estimated there are 5.25 trillion pieces of plastic debris in the ocean, of that mass, 269,000-ton float on the sea surface[157,216,217]. Due to UV radiation and mechanical forces, this plastic slowly breakdown into smaller and smaller fragments below 5 mm, also known as microplastics[218]. The origin of these fragments can be broken down fishing nets or lines, plastic films and bottles, remains of oxo-biodegradable plastic, industrial raw material like pellets, but also synthetic fibers from textiles as a result of washing clothes or other particular direct sources of microplastics, for example facial cleansers [18,93,161,162,219,220]. In Norway, they found that abrasion from tires and roadmarking was the biggest source of microplastics, followed by dust and particles from plastic based paint [221]. Next to breakdown, city storm water effluent and road runoff could thus be another major pathway for microplastics [222,223]. Some of these microplastics will escape water treatment [224] and can be transported via rivers downstream to estuaries and the marine environment [37,44,204,225]. In Brazil, the highest amount of microplastics was observed during the late rainy season, when the environment is under influence of the highest river flow, which induces the runoff of plastic fragments to the lower estuary [225]. Microplastic fibers can even be deposited by atmospheric fallout[226].

A large proportion of plastics normally float on the surface being less dense than seawater, however the buoyancy and density of plastics depend on polymer type and may change during their residence at sea due to weathering and biofouling and therefore spread across surface, water column and sediments [227,228]. Recent studies have demonstrated that pollution of microplastics, particles <5 mm, has spread at the surface of oceans, in the water column and in sediments, even in the deep sea [132]. Concentrations at the water surface range from thousands to hundred thousand of particles km^{-2}. Because of their size microplastics are available to a broad range of organisms and have already been shown to be ingested by several species [145]. The ingestion of microplastics by species at the base of the food web causes human food safety concerns as little is known about their effects and transfer across trophic levels [229]. Moreover, plastics can leach toxic additives and accumulate persistent organic pollutants (POPs) while residing in the marine environment. Some of these POPs are known to have endocrine disruptive and carcinogenic effects [230]. Furthermore, plastic particles create habitats for micro-organisms and other species, allowing potential invasive species to transfer to new areas of the ocean [179,231].

International attention is focusing more and more on the problem of marine litter, including microplastics. In Europe, marine litter and microplastics are included in the Marine Strategy Framework Directive (MSFD), specific information in relation to trends in the amount, distribution and, where possible, composition of micro-particles (in particular microplastics) is requested (criterion 10.1.3 of the MSFD) [232]. There are several other actions and measures directly related to microplastics and their sources e.g., microbead bans and thus baseline studies are urgently needed to produce appropriate regional baselines to monitor future amounts of microplastics and follow progress of action plans and where required assess potential impacts on the marine environment [40].

This study presents the outcomes of two baseline studies, looking at microplastics in sediments of the Southern North Sea and floating at the water surface in seas of North West Europe. Samples from the surface layers of the North Sea, Irish Sea, Celtic Sea, and Channel Area were analyzed and compared with sediment samples in approximately the same region. Even though sampling locations do not overlap exactly in terms of spatiotemporal scale, it is the first study in the North Sea region in which results from both matrices are compared. Since sediment is thought to be a sink [127,206,216,233] for microplastics, research on the occurrence and relationship between floating and deposited microplastics is paramount in understanding the physical processes acting on plastic particles and predicting hotspots for monitoring and clean-up [234].

4.2 MATERIALS AND METHODS

4.2.1 Sediment

Sampling took place on the Dutch continental shelf in 2014; on the Belgian continental shelf in 2013 and 2014; in the North Sea and English Channel area of the UK in 2013 and 2014 (Figure 4.1); and in the French part of the English Channel in 2014. In total, 27 locations were sampled (Table 4.1). The sample size differed per country; the UK had the smallest number of sampling stations (4 stations), whereas the Netherlands had the highest number of stations (11 stations).

Table 4.1. Overview of sampling details and number of microplastic particles at each location.

Location number	Country	Location name	IVM LIMS code	Latitude	Longitude	Sampling year	Total MPs/kg dry weight sediment
1	BE	MIC 1	14/0030	51◦17.944	002◦50.004	2013	252
2	BE	MIC 1	14/0031	51◦17.944	002◦50.004	2013	110
3	BE	MIC 3	14/0032	51◦26.400	002◦35.500	2013	54
4	BE	MIC 1	14/0562	51◦17.944	002◦50.004	2014	59
5	BE	WO2	14/0563	NA	NA	2014	330
6	BE	830	14/0564	51◦42.54	2◦27.03	2014	146
7	BE	OO harbor	14/0565	51◦14.277	2◦54.415	2014	3,146
8	UK	CSEMP475	14/0014	52	2.33	2013	0
9	UK	CSEMP536	14/0015	50.43	−3.12	2013	348
10	UK	CSEMP484	14/0016	50.97	1.03	2013	643
11	UK	CSEMP466	14/0017	51.5	1	2013	233
12	NL	NOORDWK 70	14/1180	052◦34’10.00’’	003◦31’53.00’’	2014	96
13	NL	NOORDWK 20	14/1179	052◦20’30.00’’	004◦10’30.00’’	2014	418
14	NL	NOORDWK 10	14/1178	052◦18’08.00’’	004◦18’09.00’’	2014	301
15	NL	NOORDWK 2	14/1177	052◦15’41.00’’	004◦24’22.00’’	2014	109
16	NL	GOERE2	14/1174	051◦50’49.00’’	003◦50’05.00’’	2014	0
17	NL	SCHOUWN 10	14/1173	51,950	2,667	2014	176
18	NL	WALCRN70	14/1172	051◦57’25.00’’	002◦40’45.00’’	2014	225
19	NL	WALCRN20	14/1171	051◦39’31.00’’	003◦13’14.00’’	2014	0
20	NL	WALCRN2	14/1170	051◦32’56.00’’	003◦24’39.00’’	2014	62
21	NL	LOSWLN	14/1175	NA	NA	2014	499
22	NL	TERHEIJ2	14/1176	52,052	4,160	2014	561
23	FR	BR 3	14/0525	N 48◦37’47.56	O 003◦50’51.79	2014	194
24	FR	BR 4	14/0526	N 48◦46’51.60	O 003◦00’46.69	2014	138
25	FR	BR 5	14/0527	N 48◦30’09.19	O 002◦40’47.43	2014	140
26	FR	BR 6	14/0528	N 48◦36’18.49	O 002◦01’51.08	2014	425
27	FR	BR 7	14/0529	N 48◦40’02.14	O 001◦51’41.22	2014	1,509

Sediment samples were collected from shallow (wadable) locations using a scoop (FR) and from deeper locations with a van Veen grab (NL, BE, UK). At those deeper locations, three sediment grabs were taken from which the upper 5 cm layer of sediment was collected and pooled into one sample. Samples were collected in 1l glass jars with plastic lids and cooled (4ºC). Any visible biota was removed. Upon arrival on shore, samples were frozen at -20ºC until further analysis.

Samples were analyzed by the Institute for Environmental Studies (Amsterdam, the Netherlands). Sediment samples were thawed and homogenized, subsamples were taken for microplastic analysis and determination of dry weight. To extract microplastics from sediments, a modified method of Thompson et al.[27], was followed. The sediment (25 g) was added to an Erlenmeyer with MilliQ water and a saturated NaCl solution (1.2 kg/L). The suspension was stirred for 2 min using a Teflon stirrer at the bottom of the Erlenmeyer flask. This allowed the sample material to suspend and enabled density separation of the sediment and particle material. Post-stirring, the suspension was left for 1 h, allowing the heavier sediment particles to sink while the lighter particles start to float on the saturated salt solution. The suspension was filtered over a 0.7 µm Whatman GF/C glass filter, followed by a rinsing step with hydrogen peroxide (30%) to remove any residual organic material. Alongside each batch of samples, two blanks, and two duplicate analyses were performed. The filters were examined using light microscopy and measured the length of the particles with MicroCamLab for Microsoft. Microplastics were counted and corrected for background levels determined by the blank samples. The dry weight of the sediments was determined gravimetrically after freeze-drying a 5 g subsample of the homogenized sample until a constant weight was observed. Microplastic concentrations were expressed as number of particles per kg of dry sediment and sorted into three categories “fibers/kg DW,” “spheres/kg DW,” and “fragments/kg DW.”

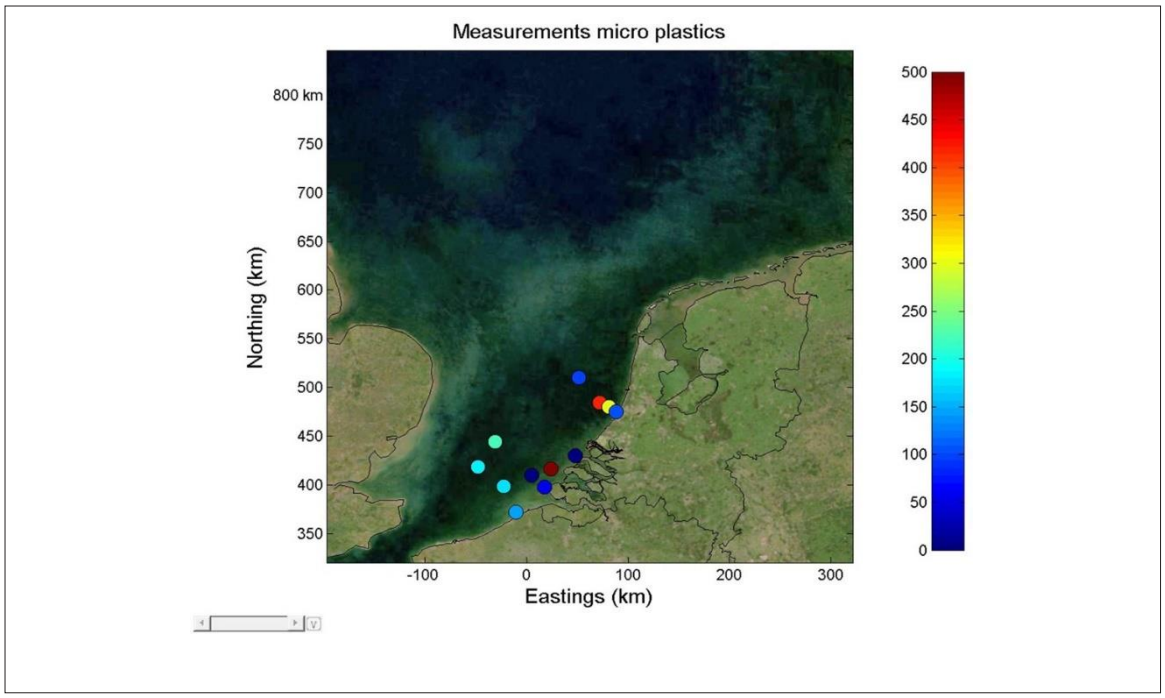


Figure 4.1. Visual representation of amounts of microplastic particles found per location/kg dry weight sediment.

Sediment organic matter or total organic carbon (TOC) on the upper layer sediment was measured using the “dichromate method” [235]. Carbonate content was measured on the same sediment fraction as “loss on ignition” [236]. Grain size distribution was calculated using laser diffraction particle sizing. All samples were analyzed by means of a Malvern Mastersizer 2000G hydro version 5.40 (ISO

13320:2009). Grain size fractions were determined as volume percentages according to the Wentworth scale [237]: clay (<4 µm), silt (4–63 µm), very fine sand (63–125 µm), fine sand (125–250 µm), medium sand (250–500 µm), coarse sand (500–1000 µm), very coarse sand (1–2 mm), and gravel (>2 mm). Throughout this study, the clay and silt fractions have been combined as clay/silt (<63 microns).

4.2.2 Sea Surface

Floating microplastic sampling was carried out during existing fisheries surveys in the UK Channel, North, and Celtic Sea area from January to March 2011 (Figure 4.5). Samples were collected from surface waters in between fisheries stations using a high-speed manta trawl with a rectangular opening 50 cm high by 15.5 cm wide, and a 4.5 m long 333 µm net with a 30 × 10 cm cylindrical collecting bag. Collection took place in wintertime, when low biomass facilitated sampling, during the following three Cefas cruises: Cend3/11, Cend4/11, and Cend5/11 (Table 4.3). In the Atlantic Ocean the water flow is predominantly from west to east driven by the northern and southern branches of the North Atlantic Drift. In the shelf areas currents are predominately generated by tides and wind, but the main water flow is from south to north [238]. The sea state on the Beaufort Scale remained between 1 and 3 for all sample sites. Water surface samples were only collected during calm sea conditions with wave heights below 50 cm.

The sampled transects were not equidistant, but sampling periods were each 60 min long. Coordinates of start and stop positions were registered, along with the number of rotations of the flow meter inside the lower part of the mouth of the manta trawl. The area sampled was calculated firstly by calculating the distance between start and stop coordinates and secondly by using the onboard knotmeter, which takes into account the ground speed and measures the number of nautical miles traveled over a defined distance, to measure the actual length of sea surface trawled in the 60-min period. These tow lengths multiplied by the width of the trawl mouth provided the area sampled, allowing for the particle abundance per square kilometer to be calculated in two different ways. Next to this, we also calculated the total number of particles by volume sampled as indicated by the flowmeter. The lower part of the manta trawl opening was fitted with a GO environmental flowmeter* with a standard speed rotor constant of 26,873 and 1 rotor revolution equaling 10 counts. *http://cce.lternet.edu/docs/data/methods/M2-1314e%20Mechanical%20flowmeter.pdf

The trawled distance in meters equals the count between rotation numbers multiplied by 26,873 divided by 999,999. Marks were made on the side of the high speed mantatrawl to visually estimate the depth of the opening during transects. The sample surface of the net is 15.5 by 50 cm but for the majority of the duration of the transects the net was only half submerged while operating as a result of the repetitive wave oscillation. Based on these observations, the net surface was calculated as 0.155 by 0.25 m. These assumptions allowed us to calculate the measured volume in cubic meters by multiplying the sample surface of the net in meters by the trawled distance obtained by the calculations above.

The manta net was rinsed from the outside with a hose to concentrate the sample in the cod end. The cod end was removed over a bucket, to prevent any spillage and the sample was transferred into a large bowl. The cod end was inverted and washed out from the outside using very little water. Leftovers were gently removed by using a long metal spoon which was rinsed into the bowl. Samples were put into a glass container and preserved in 10% formalin. A yellow waterproof label with the trawl number, date, and time was included in all containers. The lids were covered with aluminum foil and the lids labeled again with a waterproof marker from the outside of the sample container.

In the laboratory, samples were rinsed with filtered, distilled water and large floating plastic items were removed. The remaining items were separated on sieves in six size classes and stored in isopropyl alcohol. Size classes above 4.75 mm were hand picked out the sieve and the smaller fractions (>4.75) were sieved over five more sieves to retain ever smaller fractions (0.355–0.499, 0.500–0.709, 0.710–0.999, 1.00–2.79, 2.800–4.749 mm). The fractions were removed by gentle washing of the sieves and concentrated in Petri dishes. A dissecting microscope was used to sort through the remaining debris and organic material. Debris was sorted by category (plastics, non-plastics, plankton, and miscellaneous) and plastics were further categorized and counted (fragment, pellet, line, film, and foam). These size classes were then sorted and quantified into shape type (fragment, pellet, line, film, and foam). The color of each piece of plastic was also recorded (by size class) (BLACK/GRAY, BLUE/GREEN, BROWN/TAN, ORANGE/PINK/RED, TRANSPARENT/TRANSLUCENT, WHITE, YELLOW). Plastic, plankton, and plant material were weighed, then oven dried at 65 C for 24 h and weighed again. The selection of sieve sizes, plastic shapes, and color categories was based on available literature and existing studies [204,239,240].

4.2.3 Data Analysis

The statistical analysis and strength of correlations in the sediment microplastic data were calculated with a 2-tailed Pearson Correlation in SPSS (version 22). We analyzed the floating microplastic data and calculated the widths of CI for different sample sizes using the R package. The graphical representation of the sediment and floating data was produced with Microsoft Excel (2010), except for the histograms which were produced in R.

4.3 RESULTS

4.3.1 Sediment Samples

At all stations, apart from UK station (No. 74) and two Dutch station (No. 16 and 19), microplastic particles were found in the sediment. Both the highest and lowest number of microplastics were found in samples from Belgium, respectively at location 3 with 54 particles/kg DW sediment and location 7 with 3,146 particles/kg DW sediment. The overall average amount found across all areas was 421 particles/kg DW sediment. Remarkably, no plastic fragments, only spheres and fibers were observed at any of the locations. Furthermore, the amount of spheres/kg DW of sediment was higher on average across all stations compared to the amount of fibers.

The average amount of fibers/kg DW was the lowest (99 fibers) in the Dutch coastal sediment samples, whereas the highest average amount of fibers/kg DW was found in coastal sediment samples from Belgium (301 fibers). The sediment samples from the French coast of the English Channel had the highest amount of spheres/kg DW on average (350 spheres) while the Dutch samples had the lowest (123 spheres) amount of spheres/kg DW. In terms of the average number of total particles/kg DW, the highest amounts were found in marine sediments collected from coastal zones in Belgium (585 particles) and the lowest amounts in coastal zones from the Netherlands (222 particles). The average amounts of plastic particles/kg DW are in the same order of magnitude between the different countries, indicating that there are no marked differences between countries, however, more samples are required to obtain a clearer picture. In terms of percentage of dry weight of the sediment, samples from France had the lowest level (55%), and samples from the Netherlands had the highest level (76%). An overview of the results is given in Table 4.2.

Table 4.2. Average amounts of microplastics found per country in terms of number of samples, average fibers/kg dry weight sediment, average spheres/kg dry weight sediment, average fragments/kg dry weight sediment, average total particles, dry weight (% of wet weight), average median grain size of the sediment.

Country	Number of stations samples	Average fibers/kg dry weight	Average spheres/kg dry weight	Average fragments/kg dry weight	Average total particles	dw (% of ww)	Average median grain size (µm)
BEL	7	301 (445)	283 (695)	0	585 (1,114)	69 (21)	245 (140)
FR	5	131 (154)	350 (471)	0	481 (587)	55 (13)	62 (45)
NL	11	99 (110)	123 (136)	0	222 (198)	76 (3)	291 (98)
UK	4	121 (144)	185 (150)	0	306 (267)	70 (13)	260 (194)

Values between brackets represent standard deviations.

An indication of a relationship between the percentage Total Organic Carbon (TOC) and the number of plastic particles/kg dry sediment (R^2 = 0.616, p = 0.001), signifies that there are more plastic particles present with higher concentrations of TOC in the sediment (Figure 4.2). In all samples, an indication of a negative relationship between the median grain size of the sediment and the number the number of microplastic items was found (R2 = −0.492, p = 0.009), signifying that at locations with a smaller grain size, more plastic particles can be found (Figure 4.3).

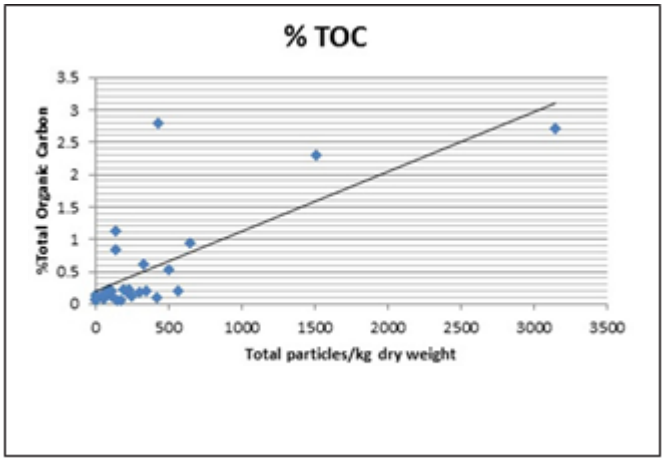


Figure 4.2. Relationship between Total Organic Carbon (TOC) and the total plastics amount found.

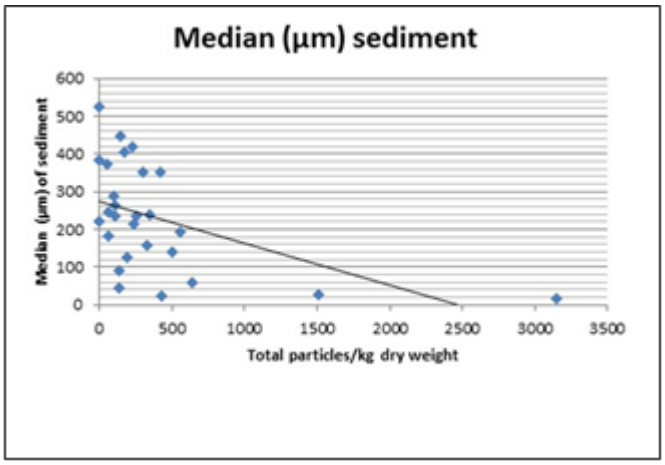


Figure 4.3. Median size of the sediment grains (in µm) in relation to the total amount of particles found per location/kg dry weight.

The microplastics particles make up a certain fraction in weight of the sediment. Here, the dry weight (DW) of the sediment was determined as a percentage of the wet weight. Similarly, an indication of a negative relationship can be found between DW and the total number of microplastics present (R2 = −0.796, p = 0.000), indicating that at locations with a lower DW, more plastic particles can be found (Figure 4.4).

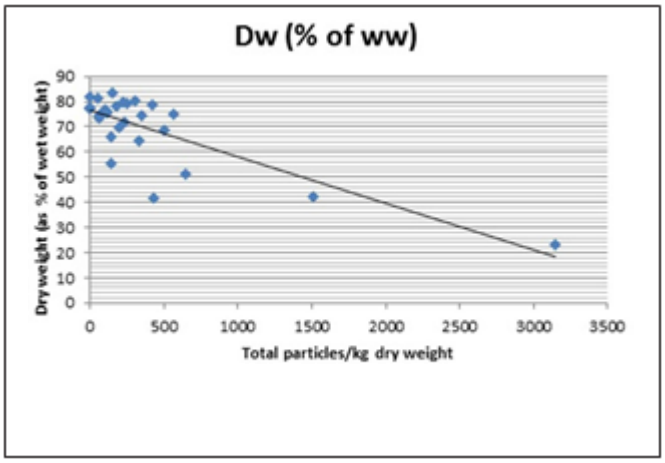


Figure 4.4. Dry Weight (as % of wet weight) in relation to the total amount of particles found per location/kg dry weight.

4.3.2 Water Samples

A total of 3,597 items were collected from 152 manta trawl transects in the Channel, North, and Celtic Sea with vessels speeds between 1.6 and 8.2 knots (Table 4.3). We were not able to sample the North-East part of the Channel and parts of the North Sea due to adverse weather conditions in 2011, leading to rough seas, complicating the sampling by manta trawl (Figure 4.5). Nevertheless, on almost all sampled locations, litter items were found, indicating a general presence of plastic items floating at the sea surface of both the North Sea and Celtic Sea.

Table 4.3. Selected surveys for manta trawl sampling.

Time	Cruise name	Cruise type	No. of stations Greater North Sea	No. of stations Celtic Sea
Feb-11	Cend 3/11	Nutrient	15	9
Mar-11	Cend 4/11	Fisheries	0	48
Mar-11	Cend 5/11	Fisheries	65	15

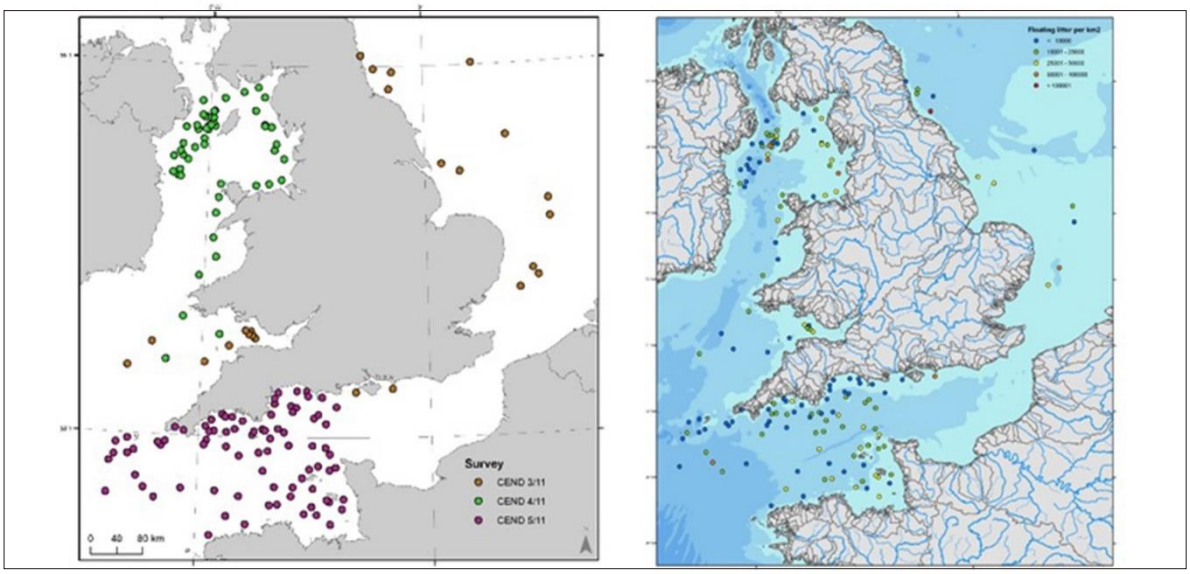
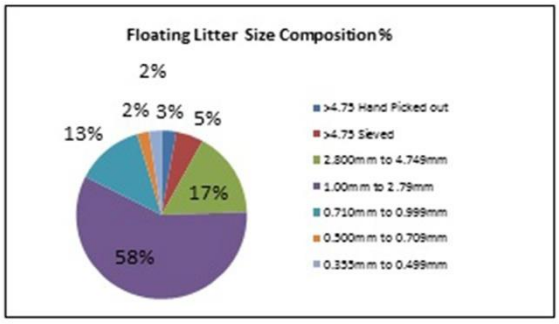
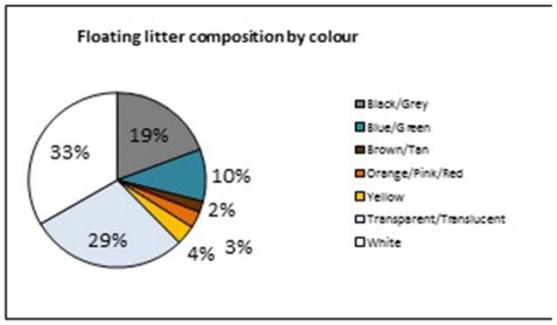


Figure 4.5. Spatial overview of manta trawl stations (left) and microplastic concentrations (right).

Geographical variations in microplastic abundance at the sea surface were observed (Figure 4.6). The different type of distance measurements available, allowed us to calculate the mean number of plastic items in a few different ways. We calculated the number of items present per trawled surface area and per volume (Table 4.4).





Number of Microplastics in UK waters	
Mean	19,237 items/km²
Median	11,881 items/km²
Max	15,6624 items/km²

Figure 4.6. Floating marine litter concentrations in the NW European seas based on the distance as calculated by vessel instruments, including a table with mean, median, and maximum values.

Table 4.4. Number of floating microplastics per surface area and per volume using different types of observations.

	Abundance lat/long (items/m^2)	Abundance knotmeter (items/m^2)	Abundance flowmeter (items/m^2)	Concentrations lat/long (items/m^3)	Concentrations knotmeter (items/m^3)	Concentrations flowmeter (items/m^3)
AVG	0.023360	0.019237	0.036623	0.093439	0.076947	0.146494
STDEV	0.029278	0.022878	0.045556	0.117114	0.091512	0.182225
MEDIAN	0.013146	0.011881	0.023183	0.052586	0.047525	0.092732
MIN	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
MAX	0.184727	0.156624	0.375854	0.738907	0.626498	1.503417

Lat/long, distance calculated based on coordinates; knotmeter, distance based on onboard equipment measuring speed; flowmeter, distance based on number of rotations.

From the three applied methods to measure distance, the flowmeter results were significantly different from the others ($p < 0.005$). Abundance ranged from 0 to 185,000 items per km^2 using the distance between coordinates, 0 to 157,000 items per km^2 when using the actual distance covered by the ship and 0–376,000 items per km^2 when using the distance as measured by the flow meter. Expressed as items per m^3, this equals to 0–0.7 items per m^3 when using coordinates, 0–0.6 items per m^3 using the knotmeter and 0–1.5 items per m^3 using the flow meter readings.

The size class 1.00–2.79 mm accounted for the highest proportion of microplastics. In terms of shapes, the most abundant types found were fragments (63%), followed by thin film (14%), pellets (10%), foam (8%), line (5%). The most prominent colour was white (33%), but also transparent (29%) and black (19%) The highest catch contained 283 items consisting out of 128 fragments, 28 pellets, 28 pieces of lines, 50 thin films, and 49 foamy items.

Our study did report wind data and indicates average wind speeds of 12.5 mph which only allows for a low amount of mixing[241]. No correlation between the measured wind speed and the observed concentrations was found ($R = -0.1497$; Figure 4.7).

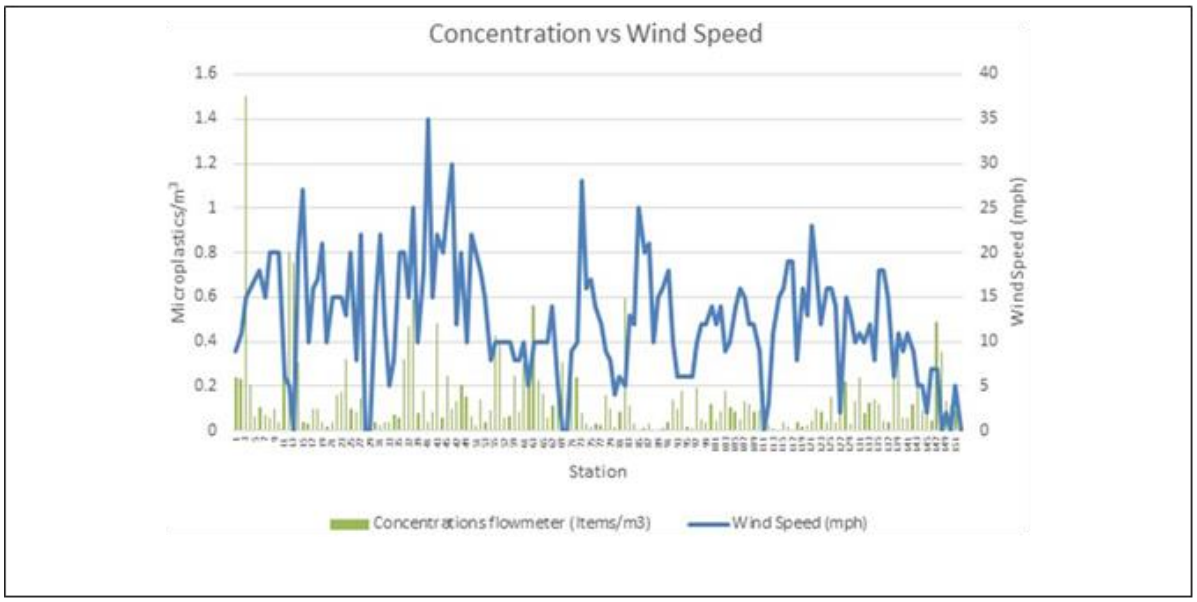


Figure 4.7. The concentration of microplastics on the water surface compared with the wind speed.

We have calculated the widths of the confidence intervals (CI) for different sample sizes so that the mean can be estimated with a certain precision of its value, giving a first indication of the necessary sample size for a microplastic survey at the water surface. From our 152 transects, only two returned with no microplastics. A histogram of the non-zero observations and the natural log of these values is shown in Figure 4.8. From this it seems reasonable to assume that the non-zero data follows an approximate lognormal distribution (i.e., that the natural log of the data is Gaussian). Thus, we modeled the data as a two-stage process. Firstly, we assumed that a proportion p (where p is estimated by 2/152 = 0.01316) of observations are zero and that the remaining data follows a lognormal distribution. N observations were simulated from this distribution and the width of the bootstrap 95% percentile confidence interval (using 1,000 replications) was calculated. The values of N were 20, 40, 60, 80, 100, 120, 140, 160, 180, 200. This whole process was repeated 500 times and a mean width was determined for each value of N. A plot of these mean widths against N is shown in the bottom left plot of Figure 4.8. This width represents the precision with which we have calculated the mean number of items per km^2. From the original data, the original mean was 19,237 items per km^2. Thus, with a sample size of n = 200, we achieve a confidence interval of width (8,000), almost 40% of this mean (Figure 4.8). Future monitoring programmes for microplastics at the sea surface in coastal waters of North West Europe should thus have a minimum of 200 stations so that the mean can be estimated with a precision of 40% of its value.

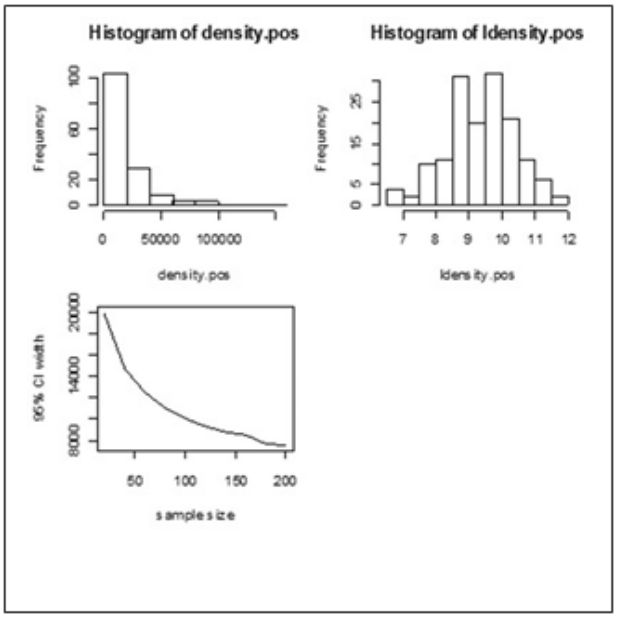


Figure 4.8. Histograms of non-zero and ln non-zero data (top row), confidence interval widths (bottom row).

4.4 DISCUSSION

4.4.1 Microplastics in the Sediment

Microplastics particles were found in 89% of the sediments (24 out of 27) collected from locations in the North Sea and Channel area between BE, NL, FR, and the UK. No plastic fragments were found, most observed plastic particles were spheres, followed by fibers. In the sublittoral zone of the Belgian Continental Shelf, part of the North Sea, an average concentration of 97.2 microplastics particles/kg dry sediment was found[146], lower than the findings in our study. In harbors, however, both studies found markedly higher amounts of microplastics compared with other locations[146]. The different amounts of microplastic particles reported by studies in nearby locations (Table 4.5) might be an indication of the heterogeneous nature of microplastics presence in marine sediments, temporal

changes, and/or result from differences in the analysis[242], we filtered over a smaller pore size filter. Apart from the harbour station (nr. 7), results are still in the same order of magnitude and might thus give an indication for the accumulation rate of microplastics at those sites. Results from a tidal flat in Germany showed concentrations ranging between 36 and 136 microplastics per 10 g of sediment[243], a result which falls within a similar range of our highest observations. In the sampled regions, however, due to the regular disturbance of the sediments by natural events such as storms[244] and/or anthropogenic activities such as trawling and dredging[245,246], the upper sediment layer is regularly mixed, making it difficult to link sedimentation rates with temporal microplastics accumulation[127].

Table 4.5. Comparison between microplastic numbers at the same stations between Claessens et al. (2011) and the findings in this study.

	Claessens et al., 2011	This study
Station	S5	MIC 1
Result (particles/kg dry sediment)	98.2	110–280 (2013) 59 (2014)
Station	S2	WO2
Result (particles/kg dry sediment)	115.8	330
Station	OO4	Ooh
Result (particles/kg dry sediment)	109.2	3,146

There is a large spread of values around the average, indicating a heterogeneous spread of microplastics in sea floor sediments. This inhomogeneity could mean that there are areas where microplastics settle in higher amounts. In the present study, we investigated if a correlation between sediment characteristics and microplastic abundance exists. Our research indicates a relationship between the amount of organic carbon and the amount of microplastics present in the sediment. This finding is supported by a Danish study[247] who found a correlation between the content of microplastics in marine sediments and %TOC. Although further research is required, similarities in densities and resulting sedimentation processes might be driving this correlation, %TOC could help to identify potential areas with high microplastic concentrations. From our findings, it seems sensible for future monitoring to target undisturbed patches of fine sediments.

4.4.2 Microplastics at the Sea Surface

The ubiquity of small floating litter items in the UK Channel, North and Celtic Sea is prominently illustrated in this study by the presence of microplastics in all samples except two. The abundance of microplastics appears to be still relatively low in surface waters of the North Sea and Celtic Sea compared to other regions e.g. Pacific gyre[248]. We observed some higher concentrations of microplastics near the coast and river estuaries. This might indicate the relative importance of inputs through rivers[249] or could be a result of higher inputs from industrialized and populated areas nearby[161,250]. Nevertheless, plastic particles were also commonly found at the sea surface of the North and Celtic Sea far away from land or potential sources. This could be a result of atmospheric deposition of microplastics[226]. Microplastic abundance at the sea surface has been shown to vary with wind speed due to vertical mixing[241,251]. Data from the eastern North Pacific suggest that the abundance of suspended plastic within 10–30 m of the sea surface averages two orders of magnitude less than that of surface[53]. We found no correlation between wind speed and microplastic concentrations.

The distances measured or calculated by different techniques such as coordinates, knot meter and flow meter result in large differences in reported microplastic concentrations. Our maximum values of 157,000 particles km^{-2}, calculated using the distance given by the onboard instrumentation, are similar to those reported on average in the Mediterranean[154], 116,000 particles km^{-2} and well below those measured in the Pacific Gyre[87], where densities of more than 300,000 particles km^{-2} were recorded in 1999. However, abundance based on flowmeter data equaled maximum abundance of 375,854 particles km^{-2}. This indicates the need for standardized marine litter protocols, methodologies and units worldwide. Internationally, various techniques, and principles have been applied to sample and analyse floating microplastics[242]. Consequently, available studies have been reporting marine litter abundance in diverse dimensions and scales, making direct comparisons extremely difficult, e.g., the number of microplastics by volume (particles/m^3) or by surface area (particles/km^2), smaller or bigger than 5 mm, analyzed with microscopes or spectroscopes[199].

We showed above that even within the same study, several ways of expressing microplastic quantities can be used depending on the initial calculation of trawled distance. Only using coordinates could easily lead to errors as it doesn't consider ocean currents and factual sampling distance. When available, using onboard instruments to precisely measure the vessels groundspeed while sampling gives a more accurate estimate of the trawled distance. The flow meter determines the distance based on the water flow through the net. However, there were significant differences between the first two methods and the flowmeter method. The flow meter registered a smaller distance than what was obtained by using coordinates or onboard instrumentation. This could be due to the bow wave effect which has been previously observed when trawling nets at high speeds or a result of the chopping through waves[252], meaning that a far lower volume will be filtered by the manta net compared to what one could calculate from less direct measurements such as coordinates and ship speed. Microplastics are vertically distributed within the upper water column due to wind and temperature driven mixing[241,253]. This suggests that microplastic concentrations could be significantly underestimated by traditional surface measurements. To allow for comparison, it is therefore recommended to sample in comparable conditions of calm sea state with low wind and wave intensity. The authors also propose to use flow meters and to report both units, items per km^2 and items per m^3, in future microplastic studies at the sea surface.

We listed microplastic concentrations from within the same geographical area, using comparable equipment for sampling microplastics (Table 4.6). Our average value, 0.14 items per m^3 and maximum value of 1.5 items per m^3, based on the flow meter data, is comparable to previous microplastic studies with manta nets in this region.

Table 4.6 Comparison of the current study results with results from research in the same region.

Location	Equipment	Particles/m^3	Sources
UK offshore waters	Manta trawl	0.14	Current Study
Offshore, Ireland	Underway sampling	2.46	Lusher et al., 2014
English Channel, UK	Plankton net	0.27	Cole et al., 2014
Bristol Channel, UK	Lowestoft Plankton Sampler	0–100	Morris and Hamilton, 1974
Portuguese coast	Neuston net/CPR	0.02–0.036	Frias et al., 2014
North Sea	Manta trawl	0–3.5	Mintenig, 2014

4.4.3 Comparison Water and Sediment Matrices

Our results indicated that sediments were more contaminated with microplastics, by number of items per volume, than surface waters. The transport of small particles to the seafloor and their deposition in the benthic sediments is facilitated by the colonization of the material by fouling organisms, which increase the density of the particles and force them to sink[84]. Plastics degrade very slowly resulting in

high persistence of plastic litter especially at the seafloor[84]. Several microplastics of a few micron were found in marine sediments with a rapid-screening approach based on fluorescent tagging with Nile Red, highlighting the role of marine sediments as a sink[10]. In our study, most of the microplastics found in the sediments were fibers and spheres, with spheres having the highest average amount/kg dry sediment. This is in contrast with the findings from the floating microplastics were mainly fragments were found. It seems that for the floating microplastic particles there is a potential influence from rivers. Rivers are both pathways and producers of microplastics[37,94]. A study on microplastics in European rivers indeed found that fragments (Po and Rhine) and fibers (Danube and Dalålven) were the largest part of the microplastics found[254]. Plastic fragments are breakdown products of larger plastic items via mechanical and/or UV-weathering[18], which occurs when exposed to the sun, wind and other mechanical stresses such as found in a river. Similarly, we observed thousands of fragments in the floating fraction in our study.

The fact that there are mostly spheres and fibers found in the sediments is not so surprising, many spheres and fibers are made from polystyrene and polyacrylamides which are often heavier than seawater and thus readily sink[224]. The shape of the particle and fiber could influence its settling velocity, however, few studies have been published on this topic. The high amounts of fibers in the sediments, could be a result of the degradation of fishing nets and dolly rope while dragging over the seafloor[255], from the continuous input via sewage and laundry or via the disposal of sewage sludge and dredged sediments[161] . Also, Brown shrimp, collected from the same study area, contained mainly fibers[255]. It is much more challenging to define the main sources of microplastics in sediment due to the wide variety of potential pathways[84], including atmospheric depositions[226].

Due to wind and currents, floating microplastics are more mobile compared to those found in sediments[241], which act as a stable sink[132]. In this study, microplastic concentrations in different surface transects varied between a few tens to a few thousands. Due to this variability, large sample sizes, above 200 stations, are required to ensure that the mean can be estimated with a precision of 40% of its value. North West European seas in the North-East Atlantic are periodically impacted by geologically significant storms, which have a marked influence on water circulation, but also affect terrigenous sediment supply, flood deposition, and long-term accumulation of fine-grained sediment on the continental shelf[256]. Also, fisheries activities disturb the sediment and homogenize the upper sediment layers by trawling[245]. So far, there are no studies considering the impact of these physical processes on microplastic distribution in water or sediment samples.

Our results indicated that the number of microplastics in sediment samples were less variable, especially at locations with high %TOC, in comparison to those found at the sea surface. To look at temporal trends, it seems sensible for future monitoring to target undisturbed patches of fine sediments with high %TOC. Such monitoring could be combined with the monitoring of hazardous substances, since these surveys are well established and targeting fine sediments (<64 microns) to monitor persistent organic pollutants[257].

4.4.4 Monitoring of Microplastics

This study is one of the first to determine baseline values for microplastics in North West European seas. Based on our findings, we see a potential for microplastics monitoring in combination with existing environmental surveys. Standardized methods resulted in a comparable outcome between the project partners of the Interreg 2 Seas MICRO IVa project[258]. The standardization of methods for collecting, processing, and analysis of samples is required to achieve comparable outcomes within one region. When counting microplastics, different types of equipment like regular microscopy or spectroscopy can be applied, causing under or over estimations which possibly influence the final numbers[88]. To monitor and compare spatial and temporal trends of microplastics, simple, cost-effective and standardized protocols, capable of efficiently and accurately sampling, and enumerating

microplastics in a variety of environmental matrices are recommended[10,259]. Without this it will remain impossible to make direct comparisons among studies and habitats, because such comparisons could be confounded with methods used[259].

The results of this study demonstrate that microplastics were present at the sea surface and in sediments of the UK Channel, North Sea and Celtic Sea. Different shapes and types of plastics were found in both matrices. Monitoring of both matrices had certain advantages and disadvantages which must be considered when designing future monitoring programmes. Microplastic monitoring in sediments can easily be combined with existing contaminant surveys sampling fine sediments. Water column and sea surface monitoring might be more appropriate for determining effect concentrations for certain marine biota. Because the concentrations of microplastics in the water are lower and the variability is higher than in sediments, more water must be sampled to achieve a comparable sample size to sediments or other seafloor indicators. We recommend installing a flow meter near the lower edge of the manta net frame to give additional information on the number of items per cubic meter.

Areas with high concentrations of floating microplastics were found in the estuarine and coastal areas. For the sediment, we observed high concentrations of microplastics in estuarine areas and in organic sediments, supported by the correlation with high total organic carbon content. Hotspot areas are thus likely situated in areas with fine muds since these generally contain high concentrations of organic materials and are made up of smaller grain sizes. The settling of microplastics might be following similar sedimentation processes as those observed in fine sediments. A previous study of microplastics in the deep sea suggested that aggregation of microplastics with organic matter, such as marine snow and fecal pellets of marine organisms, could play a role in the sinking processes[127]. This also indicates that benthic organisms burrowing and feeding in muddy environments, are likely exposed to higher concentrations of microplastics than benthic organisms in areas with a larger grain size and lower TOC. Pooled sampling, repeated over time, is advisable to determine trends while minimizing spatial heterogeneity. Determination of sediment characteristics will enlarge our understanding of underlying sedimentation processes and could help with the identification of potential microplastic hotspots. We suggest that future programs of monitoring continue to distinguish the type of microplastic particles as well as the sampled size fractions, and we advise to monitor microplastics in sediments with standard mesh sizes and equipment such as the van Veen grab to allow future spatiotemporal comparison of microplastic abundance across wider marine environments.

4.5 CONCLUSIONS

This study presents a baseline for the monitoring of microplastic in coastal sediments and surface waters of North West European seas. Floating concentrations ranged between zero and 1.5 microplastic per m^3, whereas microplastic concentrations in sediments ranged between zero and a few thousands per kg DW sediment. In sediments, mainly fibers and spheres were found, whereas at the sea surface fragments were dominant. For the water phase concentrations of microplastics are lower and more variable than in sediments, meaning that larger sampled water volumes are required to find detectable concentrations.

Future monitoring programmes for microplastics at the sea surface in coastal waters of North West Europe should have a minimum of 200 stations to estimate the mean with a precision of 40% of its value. Standardization of monitoring methods within OSPAR and EU is recommended to aid in the implementation of the MSFD and the assessment of the microplastics pollution of Northern European waters over time. High concentrations of microplastics in the water can be found in estuaries. For sediments, estuaries and areas with a high organic carbon content are likely hotspots.

4.6 ACKNOWLEDGEMENTS

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Chapter 5

You are what you eat, microplastics in porbeagle sharks from the North East Atlantic: method development and analysis in spiral valve content and tissue

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ABSTRACT

Researchers worldwide are studying the environmental distribution and impacts of manufactured or environmentally fragmented small pieces of plastics, so called microplastics (<5 mm). These microplastics eventually build up in the marine environment, threatening marine ecosystems. The magnitude, fate and effects of these microplastics across the food web are largely unknown. Here, we measured digested microplastics in a top predator and critically endangered species, the North-East Atlantic Porbeagle shark (Lamna nasus), and compared this with general health conditions. A method for quantifying microplastics in spiral valves of porbeagle sharks was developed. Microplastics were detected in all spiral valves, up to 10.4 particles per g wet weight (w.w.) content and 9.5 particles per g w.w. tissue. This equates to individual microplastics loads as high as 3850 particles per spiral valve, most likely a result of trophic transfer. No statistically significant correlations were found between the average number of plastic particles in spiral valve content and tissue and the Condition and Hepatosomatic Index of porbeagle sharks. The results of this research show that North-East Atlantic porbeagle sharks ingest and digest microplastics and that there is a potential for microplastic biomonitoring using this species. More research is needed to detect possible health effects of microplastic contamination in these apex predators.

5.1. INTRODUCTION

Concerns about plastics in the environment are mounting. Plastic items of all sizes end up in marine waters by inappropriate waste disposal and human behavior, resulting in ever-increasing marine litter concentrations[163]. The anthropogenic litter currently floating at sea, according to estimates, accounts for at least 5.25 trillion particles of plastic[157] and can be found in the most remote marine[260] and fresh water systems[261]. They have a wide range of sizes, causing impacts across the entire food web. The larger sized items, such as the macroplastics (> 20 mm diameter) and mesoplastics (5 – 20 mm), are known to cause entanglement and/or obstruction of the gut when ingested[206,262–264]. The smaller particles, microplastics (< 5 mm), are available to the smallest marine organisms, building up in food webs[265], causing amongst other things abrasion, blockage of digestive tracts, starvation[266] and death[267]. Laboratory studies have shown that plastic particles in the lower micro- and nano-sized range can cross cell membranes, causing tissue damage[6,268]. In addition, the easily ingestible microplastics can form a pathway in the entry of chemical contaminants. These chemicals are either leached from the plastic material itself (e.g. additives) or adsorbed to plastic from the surrounding water and thus taken up together with the plastic by the organism when ingested[269–271]. The chemical concentrations of these persistent pollutants accumulate in biota and are often higher at the top of the food chain[272–274].

Microplastic ingestion has been reported in a wide range of marine organisms, such as sea cucumbers, mussels, lobsters, amphipods, lugworms, barnacles, and zooplankton[6,218]. In addition, it has been reported in higher trophic levels, such as fish (e.g. herring), birds (e.g. Northern Fulmars) and marine mammals (e.g. whales)[43,206,263,275–279]. It is suggested that the larger marine animals obtain the microplastics directly via ingestion (e.g. filter feeding)[280] or indirectly by trophic transfer via fish which consumed microplastics[264,281].

Therefore, the impact of microplastics might be an additional problem for a critically endangered species, such as the North East Atlantic top- predator porbeagle shark[273,274,282]. Porbeagle sharks, Lamna nasus[283], are stout-bodied sharks with large black eyes and pointed snouts attaining a maximum length of about 355 cm (total length)[284]. They are mostly found in the cold-temperate areas in the upper pelagic zone of the North Atlantic, South Atlantic and South Pacific oceans. Their diet consists of small to medium-sized pelagic and ground fishes, such as lancet fish, herring, mackerel, lance, lumpfish, flounders, hake, and cod, but feed also on squid and invertebrate[285]. Their low fecundity, late maturation age and prolonged gestation period makes them susceptible to overfishing[286]. In addition, they are long-lived species and are therefore potential targets for

contaminant accumulation, such as mercury[273,274].Moreover, these sharks might be subject to direct or indirect plastic ingestion, as they reside in marine waters with reported high levels of plastic debris[89,133,139,287], including microplastics[217,288,289], and the presence of microplastics in their prey[3,206,263,277].

The concerns about the ecological impact of microplastics match the increasing trend in microplastic studies and publications of the last 4 to 5 years[290,291]. One of the main topics of these studies is the development of analytical techniques and detection methods for the presence of microplastics that can be applied to sediment, water and biota samples[292]. Differences in biota types, sample size and a range of other parameters (e.g. feeding behavior) have led to a variety of procedures for the analysis of microplastics in biota[292]. Different steps to clean-up biota samples without affecting the microplastics (i.e. removal of biological tissues, sediment grains, and other non-plastic organic fractions) make harmonisation difficult. A standard procedure that can be applied to different organisms is, therefore, not yet available[292]. Moreover, most research has been conducted on small amounts of soft tissue and opts to exclude particles of certain sizes[292,293]. Harmonisation in applied methodologies is needed to improve the comparison between studies[294], but methodologies should also be stretchable in order to obtain more knowledge of the magnitude, fate and effects of microplastic particles in marine organisms throughout the food web[292].

Macro debris ingestion and entanglement has been commonly observed in a range of sharks species[280,295–298]. There are only few published studies identifying plastics and/or microplastics in sharks[280,281,296,298–303]. Available microplastic studies mostly analysed small types of Elasmobranches where the entire stomach and intestines was dissected and/or digested for subsequent microscopic analysis[298,299]. Studies dealing with larger sharks are limited to stomach content analysis and report only macro litter items[280] and/or microplastics within the range 5-1mm[296]. There are many opportunities for bias in estimating diets from the stomach contents of elasmobranches in the field[304,305]. Predatory fish frequently regurgitate their stomach contents[305,306], potentially removing part of the plastic load. To reduce such monitoring bias and to focus on the ingested and digested microplastic fraction, we only analysed the spiral valve content and tissue.

In this study we aim to assess the impact of microplastics on the general health condition of marine top predators, Porbeagle sharks (Lamna nasus) from the North-East Atlantic Ocean. Existing studies focus on ingestion rather than digestion and use stomach, instead of spiral valve, contents. Different existing methods were adapted and tested to extract the spiral valve content and analyse this content for microplastics. The best performing method was then applied to test the hypotheses that i) porbeagle sharks ingest and digest microplastic and ii) the uptake of microplastics adversely affects the general health condition (as assessed by Condition Index and Hepatosomatic Index) of Lamna nasus sharks.

5.2. MATERIALS AND METHODS

5.2.1. Sampling and Study Design

5.2.1.1. General Health Indications and Observations

Cefas, the Centre for Environment, Fisheries & Aquaculture Science, received 53 dead porbeagle sharks provided by commercial fishers for research purposes. These specimens were captured as seasonal by-catch in Celtic Sea gillnet fisheries with nearly all fish captured between August and October 2014[274,307]. Individual specimens were frozen after being brought ashore and were subsequently transferred to Cefas (Lowestoft) for processing, where they were sexed, measured and weighed[307] (Figure 5.1 A-D).

Figure 5.1. A-D. Porbeagle sharks were sexed, measured and weighed and spiral valve dissected for microplastic analysis.

Body length was determined by the Total Length and refers to the length measured from the tip of the snout to the top of the upper lobe of the caudal fin in a flexed down position, with this measurement made in a direct line under the body[307]. After examination and opening of the internal cavity, samples of the spiral valve were tightened at the top and bottom[296] with cotton strings before cutting, bagged separately and stored in the freezer (Figure 5.2 A-D).

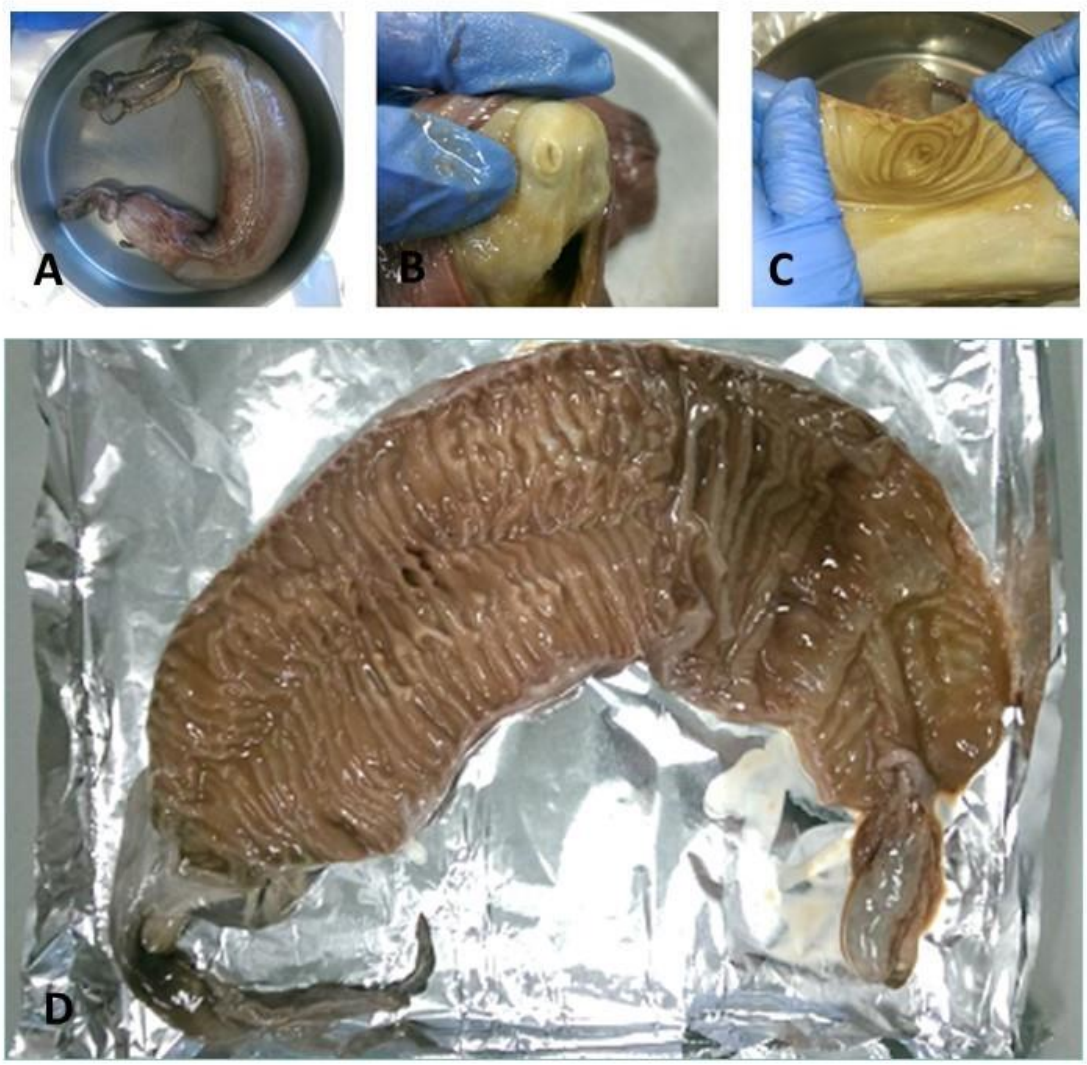

Figure 5.2. A-D. A: a spiral valve tight with strings, B: inside opening at the top of spiral valve (duodenum to spiral valve), C:lower part of the spiral valve cut open as seen from below, D: one of the test spiral valves cut open from bottom (right) to top (left).

The spiral valve, part of the intestinal tract, was made available for research on microplastics. Due to the large size of each porbeagle spiral valve, time and budget limitations, 13 randomly chosen frozen spiral valves were sent to The Vrije Universiteit Amsterdam for further microplastic analysis. The spiral valves were inspected, and measurements were taken while collecting the content and tissue. Background information on the digestive tract of the porbeagle shark is found in the supporting information (SI).

5.2.2. Method Development

5.2.2.1. Sample Collection

The frozen spiral valves were put in a lukewarm water bath for approximately 3 hours to speed up the thawing process, with 2 additional zip lock plastic bags to prevent the risk of leaking. The spiral valve was weighed (Sartorius CP2202S) before and after excess material was cut away (part of gonads, rectal gland and blood vessel remains). Other remaining material was removed from the exterior tissue with milliQ water.

To determine the presence of microplastics, the content of each spiral valve and the inside tissue of the valve were examined. Abnormalities, such as tissue damage and the presence of parasites, were also notedBefore subsampling, the valve content was homogenised by one-minute hand-stirring with a metal spoon. Spiral valve tissue samples were used to estimate the amounts of plastic particles trapped inside.

Four methods to collect the spiral valve content were explored (Figure 5.3 A-D) on 4 individual spiral valves, the best method was then used on the remaining 9 spiral valves. In the first two methods, the content material was recovered from the spiral valves by hanging the spiral valve vertically from a tripod. In the first method, the spiral valve was hung above 6 different sieves (1 mm, 710 µm, 500 µm, 355 µm, 200 µm and 100 µm) to separate the collected content immediately based on size. A glass funnel was used to keep the top part of the spiral valve open and 1 L of milliQ water was flushed through the spiral valve to rinse the remaining content from the spiral valve. In the second method, the spiral valve was hung on the tripod without the funnel and sieves. The content was forced out of the spiral valve by squeezing the spiral valve from top to bottom in downwards strokes, collecting the content in a glass bowl (Figure 5.4). In the third method, the spiral valve was cut open to scrape out the content with a metal spoon. For the fourth method, the spiral valve was cut open in a glass bowl with 200 mL of milliQ water to collect the content through washing. The collected content of all spiral valves was stored separately in glass bottles and the weight was determined. The bottles were covered with aluminum foil and stored in a freezer at minus 20 °C. The weight of the emptied spiral valve was determined afterwards.

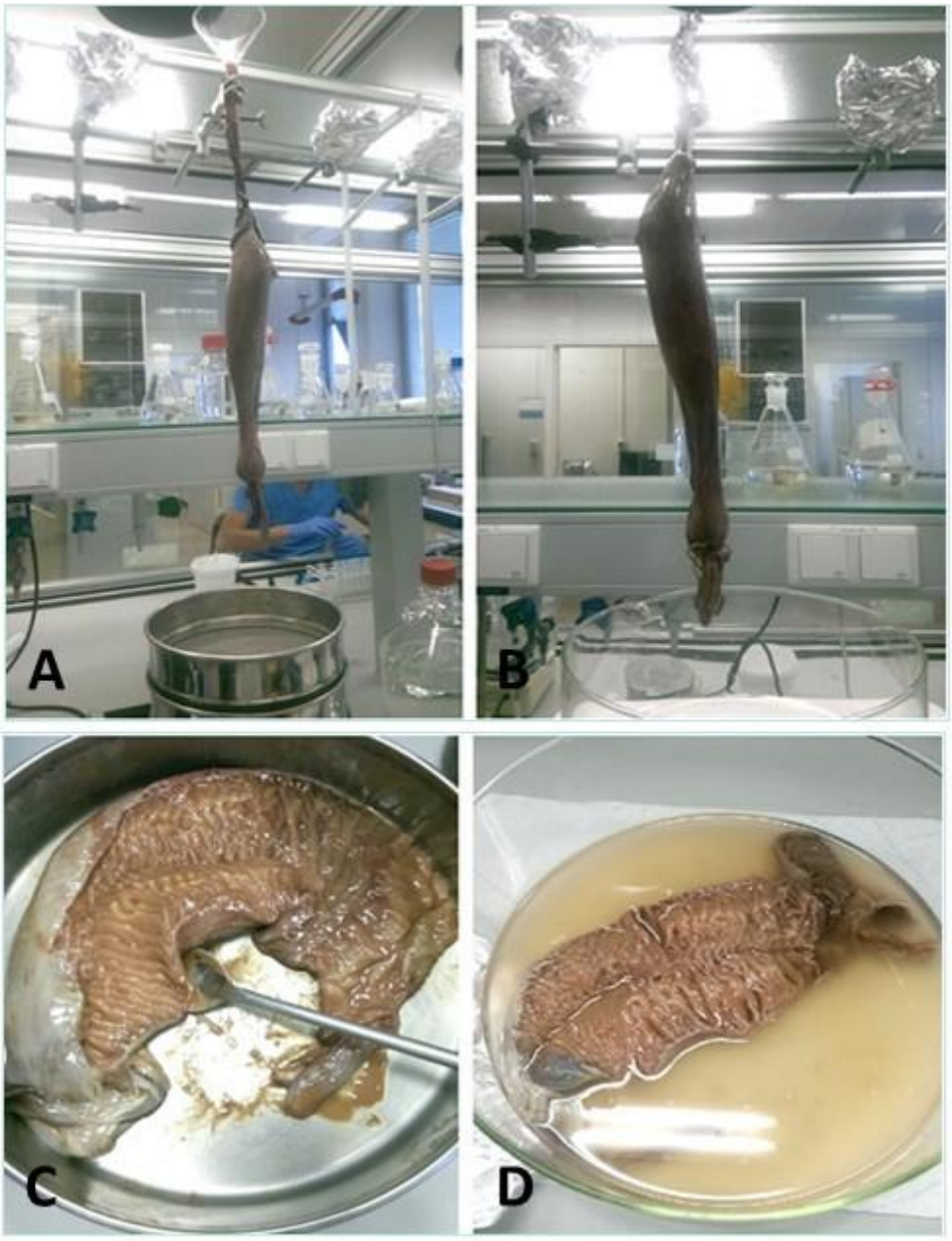

Figure 5.3 A-D. The four different methods for the content collection. A: spiral valve hung above sieves and held open with a glass funnel to wash out contents B: spiral valve hung above glass bowl, ready for massaging C: opened spiral valve in a metal bowl to scrape out the content, D: opened spiral valve in glass bowl filled with milliQ water to wash out content.

The emptied spiral valve was cut open with scissors from the rectum up to the top part of the spiral valve. Pieces of the inside tissue were collected by cutting small fractions of the top, middle (2x) and lower pleat-shaped chambers. The 1 g tissue samples were placed in separate 25 mL glass containers, covered with foil and stored in the freezer at minus 20 °C.

5.2.2.2. Digestion and Validation

Different methodologies for the digestion of spiral valve content and tissue were tested. The procedures tested included an enzymatic[294,308], acid[309] and alkaline[206,310–312] digestion. In addition to these selected procedures some further acid and alkaline digestion tests, using different sample volumes and/or digestion times, were performed. An overview of all digestion tests is given in Figure 5.4, more methodological details can be found in the SI. Different types of microplastic particles were also subjected to the different digestion methods to assess whether plastic particles could be recovered and/or were affected by the digestion procedures. After exposure to the acid and alkaline solutions, recovery and changes in colour and shape of the 12 tested plastic types were evaluated under the microscope. In addition, the best performing digestion procedure was further validated, by assessing the impact of the digestion procedure on the weight of the spiked plastic material (SI). All chemicals were purchased from Sigma-Aldrich unless stated otherwise.

Digestion method: (content)	Sample size:	Digestion time:
Enzymatic digestion Proteinase K	0.2 grams	Overnight
Acid digestion * HNO_3 + $HClO_4$	10 grams	Overnight
Acid digestion HNO_3 + $HClO_4$	5 grams	48 hours
	1 gram	16, 18, 24, 48, 148 hours
Acid digestion HNO_3	1 gram	16, 18, 24, 48, 148 hours
Acid digestion HNO_3 + $HClO_4$ (10%)	5 grams	> 1 week
Alkaline digestion KOH (10%)	10 grams	96, 168 hours
	1 gram	24, 48, 120 hours
	5 grams	48, 72, 96 hours, 18 days
	2 grams	48, 72, 96 hours
Alkaline digestion KOH (30%)	10 grams	96, 168 hours
	1 gram	24, 48, 120 hours
Digestion method: (tissue)	**Sample size:**	**Digestion time:**
Alkaline digestion KOH (10%)	1 gram	17 days

Figure 5.4. Overview of the different digestion methods, sample sizes and digestion time frames. * included boiling

Following the results of method testing, the alkaline potassium hydroxide (KOH, Riedel-de Haën, 10%) method[206,310–312] was adopted to extract microplastics from the spiral valve content and tissue. The removed content of each spiral valve was homogenized and five subsamples of 2 g were taken from each spiral valve. The subsamples were added into 25 mL glass containers and a 6 mL solution of 10% KOH was added. The samples were incubated in a fume hood at room temperature for 2 to 9 days. Of each spiral valve, four tissue samples of 1 g were placed into 25 mL glass containers and a 3 mL solution of 10% KOH was added. The tissue samples were incubated for 17 days. During the digestion procedures, parasites were included in the subsamples. When only one parasite was found in a spiral valve, the parasite was digested with one of the subsamples. When multiple parasites were found, they were homogenized with the contents and included in the samples. To provide some additional information on microplastics in parasites, the content of one rectal gland (1.2 g), where high amounts of parasites were observed, was incubated with a 3.6 mL solution of 10% KOH for 3 days and one sample with nine small parasites, individually picked from the spiral valves, was incubated with a 2 mL solution of 10% KOH for 2 days.

5.2.2.3. Filtration

With digestion completed, samples were vacuum filtered through a Whatman GF/F filter (pore size 0.7 µm, Ø 47 mm) to retain the microplastics[313]. The emptied glass containers were rinsed with milliQ water and vacuum filtered again. The effect of combining KOH with an additional alkaline solution (e.g. bleach or soap) to remove lipid residues was tested[310]. To simplify existing procedures[206,277,310], after filtration, the filter was covered with 2.5 mL 20% Extran® (MA 01 liquid, alkaline soap) for a maximum of 30 minutes to decrease the amount of digest. After adding the soap, the glassware was rinsed three times to make sure all sample was filtered through. A total of 50 mL of filtered milliQ water was used for every sample. Samples of both the content and tissue were filtered separately. When a filter became clogged, multiple filters were used. The difference between filters of various tests was determined by visual and microscopic inspection. Filters were stored and frozen in a petri-dish until further analysis.

5.2.3. Microplastic Analysis

5.2.3.1. Quality Control

An essential aspect of this study was the monitoring and prevention of contamination and an adequate recovery of microplastics from the sample matrix. Therefore, all tests were performed in a closed environment (e.g. fume hood), except for spiral valve weighing and the microscope analysis. The fume hood was cleaned regularly throughout the study with ethanol. A cotton lab coat was worn, and blue nitrile gloves were used during the sample collection, digestion and filtration procedures. Only sterile glassware was used and thoroughly rinsed three times with milliQ water prior to use. Metal spoons and scissors were acetone-washed prior to use and all equipment and samples were covered with aluminum foil or glass. In between filtrations, glassware was cleaned with milliQ water. In addition to these steps, it is important to note that all samples were processed by a single researcher. Although Quality Assurance and Quality Control (QA/QC) tools and certified reference materials are lacking for this emerging field[292], some additional precautionary steps were introduced to prevent and control contamination[314]. To eliminate any contamination from the chemical solutions, all solutions were filtered on GF/F filters (pore size 0.7 µm, Ø 47 mm) before use. Only GF/F (pore size 0.7 µm, Ø 47 mm) filtered milliQ water was used to make up solutions or clean glassware. The fume hood was cleaned before every procedure. Potential airborne contamination for processes in the fume hood was determined by leaving 3 clean GF/F filters (pore size 0.7µm, Ø 47 mm) in 3 open petri-dishes for 30 minutes (the maximum time needed to collect the content and tissue samples in one spiral valve). To determine air contamination during sample weighing, 3 GF/F filters (pore size 0.7 µm, Ø 25 mm) were exposed for 2 minutes (blank control samples). All filters were visually checked for microplastic contamination with a microscope after exposure. During the digestion and filtration steps, for every two spiral valves, blank samples were included for both spiral valve content and tissue

analyses. The concentrations of microplastics in spiral valve content and tissue were corrected for the average value of the blank samples. The Limit of Detection (LoD) was defined as 3 x the Standard Deviation (SD) of the blank results. The Limit of Quantification (LoQ) is defined as 3.3 x LOD or 10 x SD of the blank results. The LoD and LoQ are reported for the spiral valve content and spiral valve tissue separately (Table C – SI), they give an indication of the level of significance of the results (LOW <LoD, MEDIUM <LoQ and HIGH>LoQ).

5.2.3.2. Identification and Classification

In this study, microplastics were defined as plastic items between 10 µm and 5 mm in size. Based on the anatomy of the digestive tract of the porbeagle shark and their potential to regurgitate larger items, it was expected that items larger than 5 mm were not present in the spiral valve. All filters were analyzed under a light microscope (Olympus CX31 - 4x) to evaluate the performance of the digestion and the presence of microplastics. Following recommendations of Ferreira[315], microplastics were categorized according their physical characteristics: size, shape and color. Plastic particles were categorized in size bins of approximately 100 µm (20 – 100, 101- 200, etc.) and were measured using MicroCamLab for Microsoft Windows. Their shape was described as: fragments, pellets (spheres), fibers, plastic films, foamed plastic and granulates. A needle was used to carefully turn the particle and to help identify the shape when there was uncertainty. Results of the microscopic analysis were reported as number of microplastic particles per g w.w.

5.2.3.3. Validation

Raman spectroscopy (Renishaw® inVia Raman Microscope) was used to determine the polymer type[316]. The results of this technique were compared with existing references of (virgin) polymers and non-polymer particles. Only the distinguishable items, clearly visible under the microscope (>80µm), were selected for the Raman analyses. We did not analyse the particles below 80µm with Raman spectroscopy, because they proved extremely difficult to transfer from the GF/F to the edge filters for Raman spectroscopy without introducing contamination. In addition, the blue nitrile gloves and blue plastic from the filter packaging was analyzed with Raman to examine their potential role in contamination. Items were located with a magnification lens of 20x, illuminated with a red (785 nm) or green (532) laser at an intensity laser power from 0.5 up to 20%, and imaged with a Philips SP1030NC webcam.

5.2.4. Statistical Correlation Between Ingested Microplastics and General Health Indices

Plastic ingestion was defined as the average plastic particle per g w.w. content and the average plastic particle per g w.w. tissue of spiral valve. The general health condition of the porbeagle shark is determined by the Condition Index (K = fish body weight (g)/length (cm)3 x 100)[317]. In addition, the Hepatosomatic Index (IH = liver weight (kg)/body weight (kg) x 100)[317], also an indication of the status of energy reserve, was used as an additional general health estimate[317]. Higher numbers for both variables indicate a good fitness condition and general health. Correlations were controlled for the Fullness Index (IF = weight of spiral valve contents (kg) / weight of fish (kg) x 10,000), because it is hypothesized that a fuller spiral valve results in a higher amount of plastic. Simple statistics were performed in Microsoft Excel 2010. The correlation analyses were performed with IBM SPSS Statistics 21 and statistical significance was set at $p<0.05$. To determine the correlation between plastic ingestion and the Condition Index of the porbeagle shark, normal distribution was tested with Shapiro-Wilk test and Spearman's rho test was used on blank subtracted results from the microplastic analyses. To determine the correlation between plastic ingestion and the Hepatosomatic Index of the porbeagle shark, a Pearson correlation test was performed. To control the Fullness Index, only partial correlation tests were performed. By holding this third variable constant, the influence of the fullness of the spiral valve could be eliminated.

5.3. RESULTS

5.3.1. Sampling and study design

5.3.1.1. General Health Indications and Observations

Overall, the spiral valves and their contents looked similar. The dark brown/reddish colored content was a thick sticky substance and contained no large items (>5mm). However, some contained small gray unidentified fragments which may have been remains of bones. Parasites were found in 50% of the spiral valves (Table A - SI). They were assigned, with some uncertainty, to one tapeworm species, Dinobothrium septaria and one unidentified nematode species. The tapeworms were approximately 8 cm long and the nematodes were approximately 3 cm long (Figure E - SI). In addition, parts of arthropod exoskeleton and appendages of different organisms were observed during microscope inspection (Figure F - SI). An overview of the collected measurements during the sampling and during the sample collection is given in Table 5.1. Condition Index and Hepatosomatic Index, together with other general health indicators are included.

Table 5.1. Overview of measurements during sampling and sample collection. Maturity is defined as: A: immature, B: maturing, C: mature. All weights are in g, except for total body weight (kg). Parasites: ** multiple parasites found, *only one parasite found. Fullness Index: (weight of stomach contents * 10.000) / weight of fish). Condition Index: K = 100 x (Body weight/TL3). Hepatosomatic Index: IH = Liver weight/Body weight x 100.

Fish no.	1	2	3	4	5	6	7	8	9	10
Sex	M	F	M	M	F	F	F	M	F	M
Maturity	C	A	B	B	A	A	A	C	A	C
Total Length (cm)	218	170	144	113	139	119	183	194	221.4	216
Total body weight (kg)	74.2	43.1	24.1	11.2	22.6	15	46.2	65.8	84.5	77.6
Liver weight (kg)	6.376	5.382	2.156	1.35	1.89	2.158	2.888	6.852	10.42	8.15
Weight full spiral valve (g)	865.6	626.6	376.5	220.6	403.3	270.3	974.2	675.7	871.9	785.2
Wet weight spiral valve content (g)	370.2	240.7	128.2	89.1	167.6	120.2	424	254.9	273.6	278.7
Wet weight spiral valve tissue (g)	495.4	385.9	248.3	131.5	235.7	150.1	550.2	420.8	598.3	506.5
Parasites (Y/N)	yes**	no	yes*	no	yes**	yes*	no	no	yes**	No
Tissue damage (Y/N)	No	no	no	no	no	no	yes	no	no	no
Fullness Index	49.89	55.85	53.2	79.55	74.15	80.14	91.76	38.74	32.38	35.91
Condition Index	0.716	0.877	0.807	0,776	0.842	0.89	0.754	0.901	0.779	0.77
Hepatosomatic Index	8.593	12.49	8.946	12.05	8.363	14.39	6.251	10.41	12.33	10.5

5.3.2. Method Development

5.3.2.1. Sample Collection

Four spiral valves were used to test four different procedures[318–323] for the collection of spiral valve content. This included hanging of the spiral valve and collecting the content via sieves (1) or massaging into a glass bowl (2), scraping the content out with a metal spoon (3) or washing the content out with milliQ water (4) (Figure 5.3). Although most of the content could be collected with the use of milliQ water, one of the main issues with the use of milliQ water is that the w.w. of the content cannot be determined. An additional problem was that some water was residing in the spiral valve. Therefore, it seemed inappropriate to flush the spiral valve with milliQ water and wash the content out of the spiral valve. Multiple problems occurred when using the sieves. Most of the content stuck to the sieves and clogged the mesh. This made it impossible to collect the content from the sieves and separate fractions based on size. To remove the content from the sieves, large volumes of hydrogen peroxide (30%) were used and (wet) weight of the spiral valve content could again not be measured. The hanging of the spiral valve seemed the most effective, part of the content was collected passively using gravity. In addition, actively squeezing aided to retrieve the remaining content. Cutting open and collecting content by scraping or washing the spiral valve seemed to be ineffective because of the potential issues it caused in relation to content loss or contamination. Therefore, it was decided that the remaining spiral valves would be by hung of a tripod and massaging would be applied to retrieve the content. The glass bowl was replaced with a glass bottle with a smaller opening to decrease the risk of air borne contamination. The lower part of the spiral valve was placed in the glass bottle to prevent spilling of the content. An overview is shown in Figure 5.5. After collecting the spiral valve content, tissue samples were taken from the empty spiral valve. Any content that could still be retrieved was added to the already collected content with a metal spoon. The blank filters were analyzed with a microscope after the exposure. No microplastics were detected. Therefore, it was assumed that airborne contamination in the fume hood was of low concern while sampling and weighing spiral valve content and tissue.

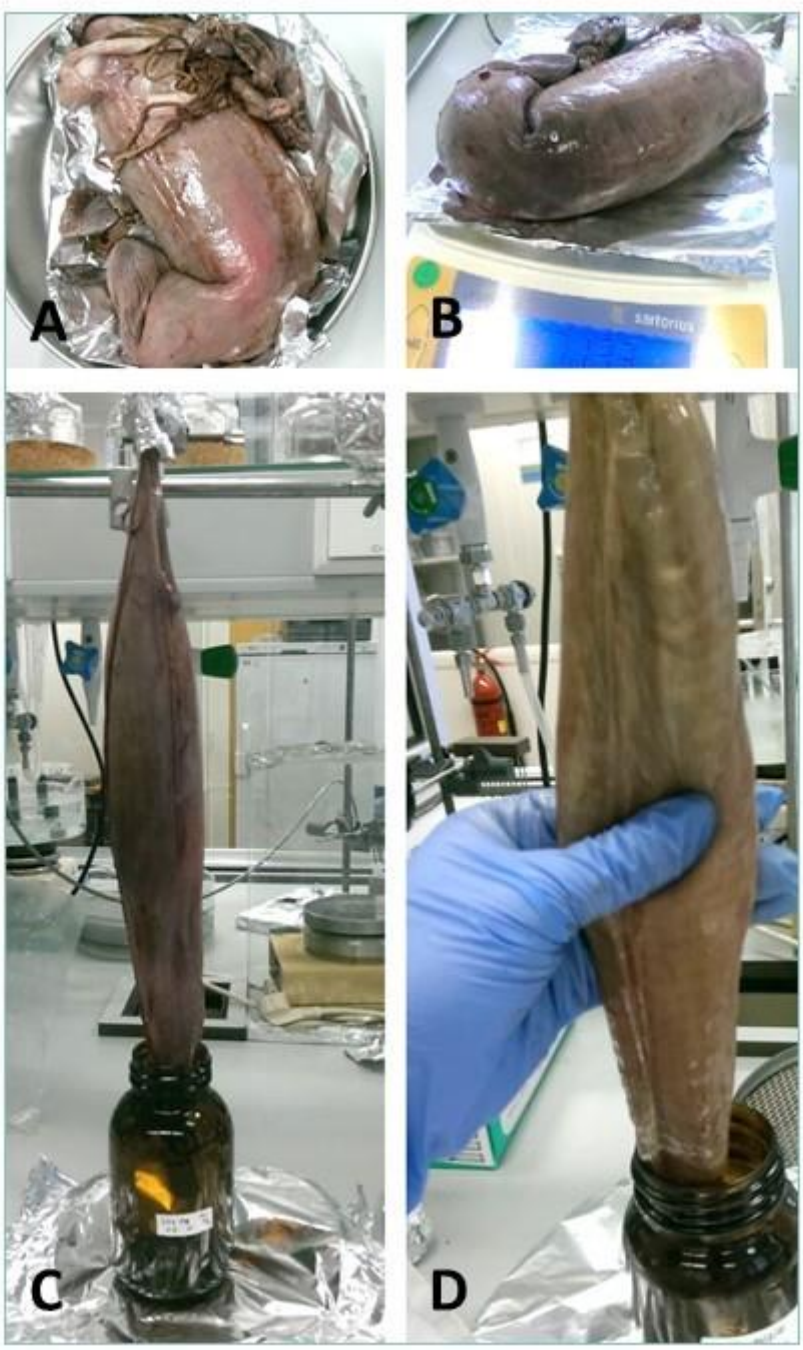

Figure 5.5. A-D. Pictures of one spiral valve and the applied massaging method.

5.3.2.2. Digestion and Validation

To extract microplastics from spiral valve content and tissue samples, different digestion methods, sample sizes and time frames were explored and compared. These included enzymatic digestion, acid digestion and alkaline digestion techniques (Figure 1.1 – SI). Their performance was determined by how well the sample could consequently be filtered through a glass fiber filter and how much residual content was observed by visual and microscopic inspection after filtration. The results are summarized below, more methodological details and pictures can be found in the SI (Figures 1.1 & 1.2 – SI). The filters using the acid digestions were covered with digest residue and could therefore cover possibly microplastics (Figure 1.2 – SI). The enzymatic digestion was an effective method for small samples, but

inappropriate for larger samples, such as spiral valve content and tissue, due to the high costs of Proteinase K. The 10% KOH solution seemed to perform best with a spiral valve content sample of 2g and a tissue sample of 1 g, leaving a minimal digest residue on the filter (Figure 5.6). Some filters were stained brownish and/or contained gray fragments, most likely dietary remains. The gray fragments were not considered a problem during the identification as they could be crushed into a powder with a needle, making them distinguishable from plastic. The filters were increasingly covered when using larger sample sizes. Still, larger volumes or even better, a large series of smaller replicate samples would provide a better representation of the total spiral valve contents which ranged from approximately 90 to 424 g w.w. (Table 5.1). Concerning the most efficient timeframe, there did not seem to be a clear improvement with longer digestion times. The results of the KOH digestion differed, however, between spiral valves. Most likely due to different diets and stages of digestion at the time of death. The composition of the remaining spiral valves was unknown, therefore, it was decided to digest the content samples of the next spiral valves slowly over a period of several days until contents were, homogenous and fluid, ready for filtration (Figure 5.7).

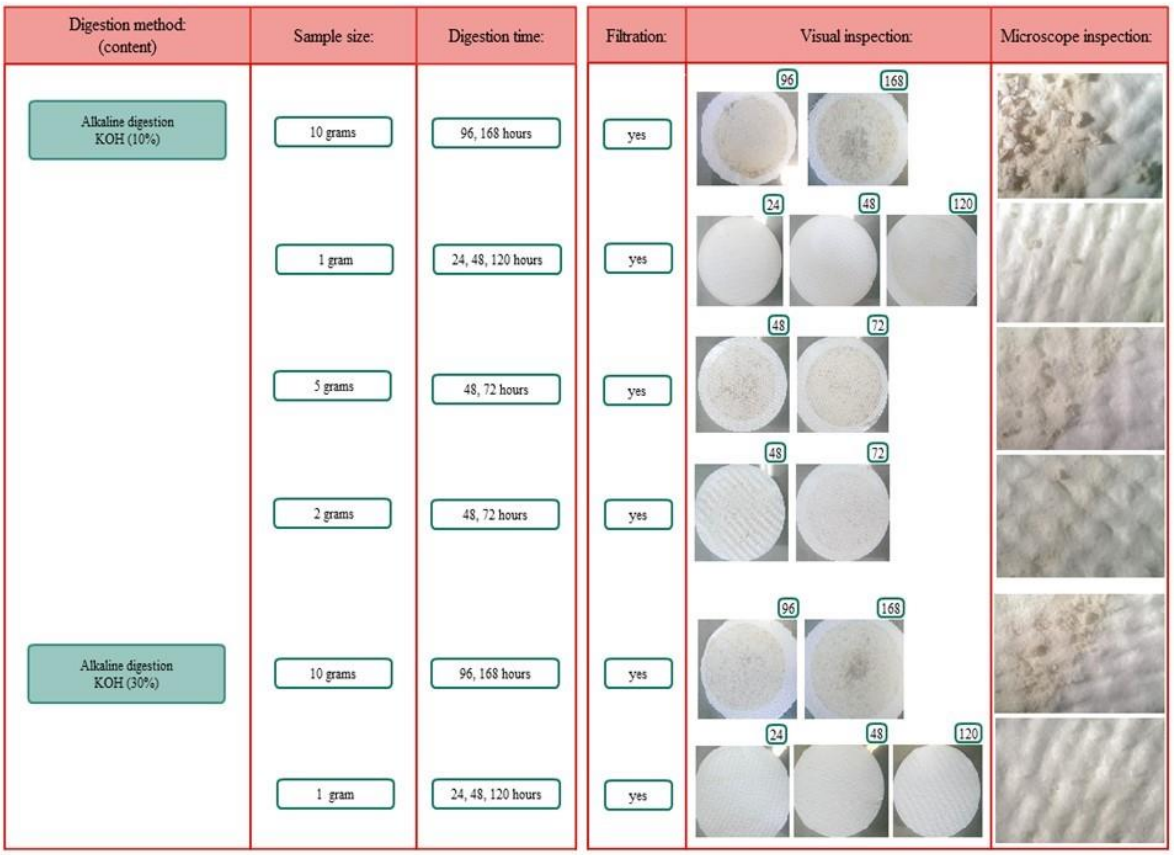

Figure 5.6. Alkaline digestion test results.

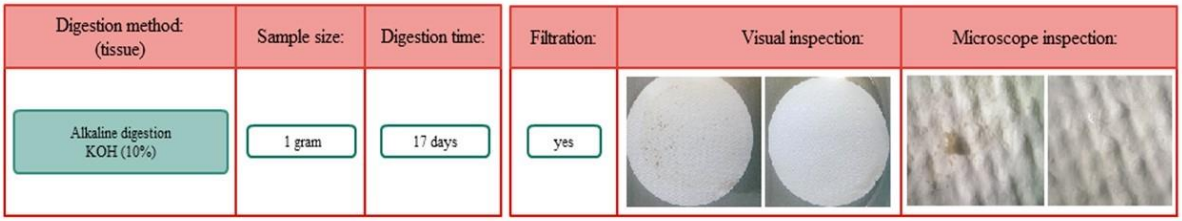

Figure 5.7. Alkaline test results for tissue samples.

Almost all tested particles (8 types of plastic, 12 particles each) could be recovered with the KOH content (94.8%) and tissue (98%) method. Unrecovered particles were attributed to the rinsing procedure (transparent plastics stuck on the glassware wall) and not to the digestion method. In addition, the spiked PVC particles were difficult to count and to filter as a result of their small size. No apparent changes in color and shape of the spiked plastics occurred as a result of the KOH digestion (Figure C - SI). Also, no particles were stuck together. In addition, the use of 10% KOH and extended digestion times (>24hours) was further validated on 100 spiked particles, by assessing recovery and weight disparities. The recovery for all plastics was 100%, except for PET (99%) due to the loss of one PET particle before weighing. The weight of the plastics increased at first due to the dried KOH residue on the particles. After a milliQ rinsing step, the average weight of the particles did not differ significantly (SI) from the weight before the digestion. Only the mean differences in weight for PA at all 3 exposures and PVC after 9 days exposure were significantly higher after the KOH digestion. The average weight difference of the spiked plastics before and after 10% KOH digestion together with the significance levels is shown in the SI (Table 6.1 – 6.4).

5.3.2.3. Filtration

Filters that were rinsed by covering them with a layer of 2.5 mL solution of 20% Extran® appeared visually cleaner than the ones which were only rinsed with milliQ water. Therefore, the use of the alkaline soap (prefiltered) was added to the filtration procedure. The difference between simply milliQ rinsed filters and milliQ with soap rinsed filters tested on 5 and 2 g samples can be seen in Figure D (SI).

5.3.3. Microplastic Analysis

5.3.3.1. Quality Control

The blank measurements showed contamination of mostly fibers and blue fragments but were not consistent. The average number of plastic particles in the content blanks was 6.6 ±6.5 particles and for the tissue 1 ± 0.7. Figure G (SI) illustrates also the range in number of particles per blank sample.
For spiral valve content, the LoD (3 x SD of the average plastic particle in the blanks) was 19.6 and the LoQ (3.3 x LoD) was 64.8. For the spiral valve tissue, the LoD was 2.1 and the LoQ was 7.0. Table C (SI) shows the LoD and LoQ described for 1 g and includes blue fragments, fibers, black fragments and other plastics.

5.3.3.2. Concentration of Plastic Particles

A total of 878 plastic particles were identified by visual identification. These were identified as fragments (65.9%), fibers (32.9%), pellets (0.9%), and films (0.5%). The most abundant colors were blue (44.8%), black (23.7%), red (9.6%) and transparent (6.5%). Other colors that were present were orange, brown, green, grey, yellow, purple, white, pink or multicolored (all <5%). Almost all particles were below 100 µm (fibers excluded) and the largest plastic particle identified was approximately 930 µm long (Figure H - SI). An overview of the average number of blue fragments, fibers, black fragments, other and total plastic particles is given in Table 2 and Figure 5.8. It also includes the standard deviation (SD) per spiral valve content and tissue, before and after correction with the blank values (adjusted per type). One spiral valve (1) was not included in the analysis due to the presence of large amounts of sand particles in its content which interfered with the detection of microplastics. Instead of analysing the entire sample, the total amount of microplastics per spiral valve was recalculated by multiplying the subsample concentrations with total weight of content and tissue and adding both together (Table 3). In the content of the rectal gland (1.2 g) three blue fibers were found, but after blank subtraction, no plastics can be reported. Also, nine parasitic nematodes were examined, but no plastic particles were discovered within. A raw data file is presented in the SI.

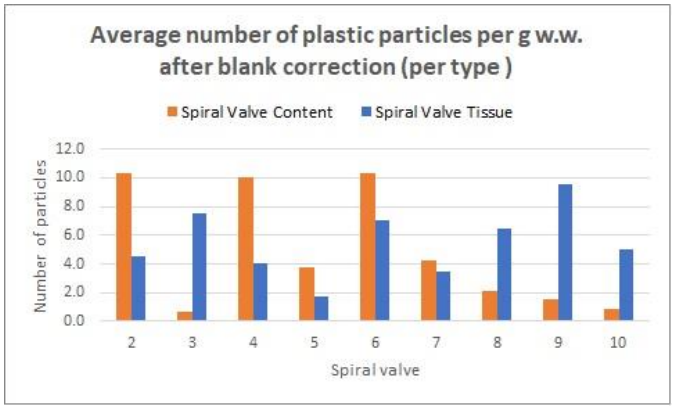

Figure 5.8. Average number of plastic particles per g ww measured in spiral valve content and tissue after blank correction.

Table 5.2. The number of plastic particles in spiral content and tissue, expressed as average* number of plastic particles per g ww before and after blank correction (per type); SD = Standard Deviation; NA = Not Available; *5 content and 4 tissue replicates per spiral valve

SPIRAL VALVE	BLANK CORRECTION	PARTICLES	1	2		3		4		5		6		7		8		9		10	
			Average	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD
Content	BEFORE	blue	NA	7.9	2.1	0.5	0.5	9.4	3.8	0.2	0.3	3.4	1.2	0.8	0.4	1.1	1.0	0.5	0.6	1.0	0.5
		fibre	NA	2.1	1.1	0.5	0.4	1.3	0.8	1.3	0.6	3.7	3.0	3.0	2.3	2.4	1.6	1.9	0.9	1.4	0.7
		black	NA	2.0	2.0	0.1	0.2	0.3	0.3	3.3	1.4	3.3	1.2	2.1	1.9	1.1	1.0	0.3	0.4	0.7	0.3
		other	NA	1.4	0.7	0.6	0.9	1.8	0.8	0.7	1.1	3.2	1.5	0.5	0.9	0.1	0.2	0.8	0.8	0.3	0.3
		Total	NA	13.4	3.6	1.7	1.0	12.8	3.9	5.5	2.6	13.6	4.1	6.4	3.8	4.7	2.9	3.5	1.9	3.4	1.1
	AFTER (PER TYPE)	blue	NA	6.4	2.1	0.0	0.0	7.9	3.8	0.0	0.0	1.9	1.2	0.0	0.0	0.2	0.4	0.0	0.0	0.0	0.0
		fibre	NA	0.8	0.8	0.0	0.0	0.2	0.4	0.1	0.2	2.2	3.0	1.9	1.8	1.0	1.5	0.6	0.5	0.2	0.4
		black	NA	1.8	1.9	0.0	0.1	0.1	0.1	3.0	1.4	3.0	1.2	1.9	1.8	0.9	0.9	0.2	0.3	0.4	0.3
		other	NA	1.4	0.7	0.6	0.9	1.8	0.8	0.7	1.1	3.2	1.5	0.5	0.9	0.1	0.2	0.8	0.8	0.3	0.3
		Total	NA	10.4	3.5	0.6	0.9	10.0	4.0	3.8	2.3	10.3	4.1	4.3	3.0	2.2	2.2	1.6	1.0	0.9	0.3
Tissue	BEFORE	blue	NA	1.8	1.3	5.3	2.8	1.0	0.8	1.3	1.9	0.0	0.0	0.3	0.5	2.0	2.2	1.5	0.6	2.0	1.8
		fibre	NA	2.8	1.3	2.0	2.4	2.5	1.7	0.8	1.0	5.5	4.4	3.3	1.7	3.5	0.6	5.3	2.6	3.0	2.2
		black	NA	0.5	0.6	0.5	0.6	1.0	1.4	0.0	0.0	0.0	0.0	0.3	0.5	1.0	0.8	2.5	1.9	0.0	0.0
		other	NA	0.5	0.6	0.3	0.5	0.5	1.0	0.3	0.5	2.5	2.1	0.8	1.0	1.0	0.0	1.3	1.3	1.0	1.4
		Total	NA	5.5	2.6	8.0	4.5	5.0	1.8	2.3	1.7	8.0	5.1	4.5	0.6	7.5	2.6	10.5	3.4	6.0	2.2
	AFTER (PER TYPE)	blue	NA	1.8	1.3	5.3	2.8	1.0	0.8	1.3	1.9	0.0	0.0	0.3	0.5	2.0	2.2	1.5	0.6	2.0	1.8
		fibre	NA	1.8	1.3	1.5	1.9	1.5	1.7	0.3	0.5	4.5	4.4	2.3	1.7	2.5	0.6	4.3	2.6	2.0	2.2
		black	NA	0.5	0.6	0.5	0.6	1.0	1.4	0.0	0.0	0.0	0.0	0.3	0.5	1.0	0.8	2.5	1.9	0.0	0.0
		other	NA	0.5	0.6	0.3	0.5	0.5	1.0	0.3	0.5	2.5	2.1	0.8	1.0	1.0	0.0	1.3	1.3	1.0	1.4
		Total	NA	4.5	2.6	7.5	4.0	4.0	1.8	1.8	1.7	7.0	5.1	3.5	0.6	6.5	2.6	9.5	3.4	5.0	2.2

Table 5.3. Total amount of microplastics in spiral valves of porbeagle sharks.

MICROPLASTICS IN PORBEAGLE SHARK SPIRAL VALVES *corrected for blank per type	1		2		3		4		5		6		7		8		9		10	
		SD		SD		SD		SD		SD		SD		SD		SD		SD		SD
Average # microplastics/ g ww content*	NA	NA	10.4	3.5	0.6	0.9	10.0	4.0	3.8	2.3	10.3	4.1	4.3	3.0	2.2	2.2	1.6	1.0	0.9	0.3
Average # microplastics/ g ww tissue*	NA	NA	4.5	2.6	7.5	4.0	4.0	1.8	1.8	1.7	7.0	5.1	3.5	0.6	6.5	2.6	9.5	3.4	5.0	2.2
total valve content ww (g)	370.2	NA	240.7	NA	128.2	NA	89.1	NA	167.6	NA	120.2	NA	424.0	NA	254.9	NA	273.6	NA	278.7	NA
total valve tissue ww (g)	495.4	NA	385.9	NA	248.3	NA	131.5	NA	235.7	NA	150.1	NA	550.2	NA	420.8	NA	598.3	NA	506.5	NA
total microplastics in spiral valve content*	NA	NA	2493.7	852.8	82.0	110.9	892.8	359.4	636.9	381.3	1238.1	496.3	1806.2	1260.9	550.6	550.1	432.3	275.2	250.8	76.3
total microplastics in spiral valve tissue*	NA	NA	1736.6	1021.0	1862.3	1003.5	526.0	240.1	412.5	402.5	1050.7	765.4	1925.7	317.7	2735.2	1113.3	5683.9	2043.6	2532.5	1094.2
total microplastics in spiral valve*	NA	NA	4230.2	1873.8	1944.3	1114.4	1418.8	599.4	1049.4	783.8	2288.8	1261.7	3731.9	1578.5	3285.8	1663.5	6116.1	2318.8	2783.3	1170.5

5.3.3.3. Validation

After the identification of all isolated particles by microscope, Raman spectroscopy was used as an additional tool to identify polymer types. There were on average 4.9±2.4 particles per g w.w. content and 5.5±2.7 particles per g w.w. tissue. From all filters, a total of 24 particles, at least one particle from each spiral valve, between 84 – 572um were examined by Raman spectroscopy for polymer identification purposes by cataloguing a collection of fingerprints. These included the five main observed types of microplastics: a black fiber and transparent, black, red and blue fragments. Pigment spectra and environmentally driven changes to surface properties, such as weathering and biofouling, hinder spectroscopic identification[324,325]. The process of degradation, autoxidation of hydrocarbon polymers, entails the formation of novel oxygen-containing groups in the main chain through a series of primary and secondary radical reactions that involve chain scissions and cross-linking of polymer backbone, the formation of polar carbonyls (C=O) and vinyl (CH2=CH) groups, and, finally, changes in the conformation and crystallinity of the polymer[326].

The spectra of the items showed typical characteristics of weathered synthetic polymeric material, but none can be assigned with complete confidence. The water solvent was easily identified at about 1640 cm^{-1} and used as an internal intensity standard. Although the spectra were not clear enough for full identification, significant signals were present in the OH/NH stretch region between 3000 and 3700 cm^{-1}. These signals were weaker relative to the C-H stretches than would typically be seen for natural carbohydrate-based polymers such as cellulose, carrageenan or chitin, or for proteinaceous material, suggesting that they are indeed synthetic. Although difficult to be certain, the transparent particles showed characteristic features of PET: the signals around 3500–3700 cm^{-1} and 1970 cm^{-1}, as well as the strong carbonyl signal at 1730 cm^{-1}. Some notable peaks of amide-like and ester character in the spectra in the 1400–1800 cm^{-1} region of some red particles indicate these may be polyamides. The C-C stretch, CH2 twist and CH2 bend in the spectra of the remaining fragments suggest these might be polyethylene. In addition, four blue fragments from the samples (Figure I - SI) were compared with the spectra of the blue nitrile gloves and the blue plastic from the filter packaging. The blue fragments in the samples gave a clear spectrum, comparable to the spectrum of the blue packaging. Since all blue fragments resulted in the same spectrum, it was concluded that this was not due to contamination: not the polymer spectra, but pigment spectra were obtained. Figure J (SI) illustrates the similarities in spectra.

5.3.4. Statistical Correlation between Ingested Microplastics and General Health Indices

The correlation between the average plastic particle per g w.w. content (4.9±2.4) and w.w. tissue (5.5±2.7) and the general health indices of porbeagle sharks were examined by comparing the corrected average microplastic concentrations with the Condition Index (0.82±0.06) and Hepatosomatic Index (10.6±2.5). Spearman's rho correlation showed no statistically significant correlation between the average particle per g w.w. content and the Condition Index (r=-0.008, p=0.983) and the Hepatosomatic Index (r=0.165, p=0.651). Pearson correlation tests showed no statistically significant correlation between the average particle per g w.w. tissue and the Condition Index (r=0.167, p=0.668). When controlled for the Fullness Index with partial correlation no significant correlation between the average plastic particle per g w.w. tissue and the Condition Index was found (r=0.151, p=0.722). Both tests were also performed for the Hepatosomatic Index and again no statistically significant correlation was observed (Pearson: r=0.597, p=0.09 and Partial: r=0.582, p=0.130). While no statistically significant correlation was found here due to the significance level set at 0.05, the p-value for the Pearson correlation was below 0.1 and had a moderately positive r value. This might mean that an increase in average particle per g w.w. tissue possibly relates to an increase in the Hepatosomatic Index. No clear difference was observed between the Pearson correlation and the partial correlation. Therefore, the fullness of the spiral valve did not influence the correlation between the average number of plastic particles per g w.w. tissue and the Hepatosomatic Index.

5.4. DISCUSSION AND CONCLUSION

A large part of the study focused on method development as standard techniques for microplastic analysis in large top predator sharks are missing. In a microplastic study using Blue sharks, stomachs were opened and the contents were washed through a 1mm metal sieve with pre-filtered water[296]. In this study, spiral valve content and tissue were used, representing digestion compared to ingestion. Studies investigating tapeworms or parasites presence in sharks, cut open the spiral valves and remove parts of the lumen tissue[320,321] or the contents via suspension, washing or shaking[318,327]. Different methodologies to extract spiral valve content and tissue were explored and the most appropriate method, a combination of gravity and massaging, was applied on the remaining spiral valves. Several studies report microplastic ingestion based on the examination of the whole or substantial parts of the digestive tract[206,263,280,296,298,328]. Most laboratory equipment was not readily available for the rather large volumes of porbeagle spiral valve content we encountered (e.g. one porbeagle spiral valve content measured 424 g w.w.). Hence, only subsamples of the spiral valve content were analysed.

This study supports the use of a 10% KOH solution to digest spiral valve content and tissue. The (lipid) content of the spiral valves left fatty residues on the filter after digestion. To improve results, the filters were washed with alkaline soap to decrease the amount of residue. The success of this mixture aligns with previous findings which suggest that a combined alkaline digestion with KOH and NaClO are useful compounds when digesting biota and/or gastrointestinal content for microplastic analysis[308,310,329]. This method, using a post soap wash step, proved to be safe, cost effective, with less procedural steps, without affecting any of our spiked microplastics. Although good results were obtained with the alkaline soap, it was decided to harmonise procedures by adopting the combined KOH:NaClO digestion for future biota analysis in our laboratory.

To exclude non plastic particles during the microscopic analysis, a step to determine the consistency of the larger particles (>100µm) was added. Fish bone remains were easily distinguished from plastics by carefully touching the item with a needle. On applying pressure, the remains of prey broke down into a powder following alkaline digestion[330]. One spiral valve contained a large amount of sand, making visual microplastic analysis impossible, although this was not an issue in the other spiral valves, it would be useful to introduce a density separation and simplify microplastic detection (e.g. Nile red[10]) by introducing additional steps after the digestion procedure. This shows that while certain steps of the protocol can be harmonized (e.g. KOH digestion & microplastic quantification), small adaptations might be required to make protocols species or case specific.

Although no values were reported for spiral valve 1, due to the presence of sand, plastics particles were detected in both the content and tissue of all spiral valves. Fibers, blue fragments and black fragments were most prevalent. Remarkably, all spiral valves and some of the blanks contained blue fragments. It was suspected that these fragments were a result of contamination due to the high numbers of particles with this specific combination of shape and colour. Although Raman spectroscopy was applied, there is still some uncertainty in terms of the origin of these fragments. At the moment, certified reference materials are unavailable and polymer identification with spectroscopy is wrought with challenges, especially in this small size range of weathered and pigmented particles detection[331,332]. The Raman spectroscopy picked up the spectra of the blue pigments more easily than polymer spectra and confirmed that the blue pigment in the packaging of the filters was comparable with some blue fragments in the samples. This could be due to resonance enhancement, where signals from the pigment are enhanced, but signals from the polymer are hidden. The pigment is known as copper phthalocyanine and is used in multiple applications. Several studies mentioned blue plastic fragments in environmental samples and attribute the Raman spectra to this pigment[127,294,333,334] which makes source tracking rather difficult. It indicates that non-plastic materials, including filter packaging, should be used, wherever possible, to minimize contamination

and/or when in doubt, screening for plastic contamination should take place prior to usage. Extra precautions should be sought to further lower the blank values. Only a small portion of samples were higher than the LoQ. This may be partly due to the low number of blanks used. It is therefore recommended to increase the number of procedural blanks and to repeat this over time as contamination might be day dependent.

The high numbers of microplastics in the spiral valves imply that plastic is ingested and digested by porbeagle sharks. In 4 out of 9 spiral valves, more plastic particles per g w.w. were found in tissue samples than in spiral valve content. This indicates that taking samples from solely the content might not represent the actual amount of microplastics in the spiral valve. Moreover, the results of the lipid content and tissue analysis demonstrate that the sample collection via the massaging method might not be enough to collect all content (SI). This was already observed during the tissue sample collection, small amounts of spiral valve content were stuck to the surface of the tissue. Therefore, it is uncertain whether and how many microplastics in the tissue samples were resulting from the small amounts of content in the tissue samples or if the microplastics were absorbed into the 3D structured lining of the tissue where nutrients are absorbed into the shark's body. Eventually, this might result in adverse effects on the shark's health if other organs and tissues are exposed[218,268,301,335]. Due to this uncertainty both spiral valve content and tissue should be analysed and reported jointly for monitoring purposes. Parasites were present within the spiral valves but contained no plastic particles. This could be due to visual cut off point of 10 µm. The collected parasites might ingest particles much smaller than the particle size examined in this study. In addition, the parasites of only one spiral valve were examined and therefore, no conclusions about microplastic ingestion in parasites can be drawn.

To examine if the porbeagle shark's health was adversely affected by microplastic ingestion, we looked for a correlation between the average plastic particle per g w.w. in spiral valves content/tissue and several general health indices. In this case, the Hepatosomatic Index seemed to be the most relevant health indicator in relation to microplastic ingestion , maybe because it relates more directly to toxic effects[336]. Some microplastics will be excreted together with the rest of the spiral valve content. A portion gets stuck within the tissue and could potentially exchange chemicals[270,337] during their extended stay in the digestive tract. Previous studies reported chemical concentration levels in the liver[273] and muscles[274] of porbeagles and concluded that current levels were mostly low or undetectable. To preserve these and other vulnerable marine top predator species, examining both microplastics and concentrations of toxic chemicals adsorbed or leaching from microplastics and in specimens itself would be recommended to provide more insights in the toxicity of microplastic particles and associated chemical equilibriums[337]. Moreover, additional ecosystem variables should be considered, such as the presence of parasites and a range of environmental stressors. Although no statistically significant correlation between the average plastic particle per g w.w. tissue and Hepatosomatic Index was observed, the correlation was moderately positive. An explanation could be that liver weight increases due to pathological changes[336] as a result of the increased residence of microplastics in the gut, causing a higher Hepatosomatic Index. All other correlations were not statistically significant (all $p>0.05$). This could be due to the small sample size in the analyses ($n=9$). However, it is likely that the found concentrations of microplastics in the spiral valve of the porbeagles did not cause effects measurable by general health condition indexes such as the Hepatosomatic Index. Similarly, some fish studies reported no statistical significant relation between plastic ingestion and their condition[206,338].

The presence of microplastics in porbeagle sharks is most likely the result of the contamination of their food supply[206,263] (indirect) and/or internal fragmentation of the larger plastics they ingested[297] (direct). DNA analysis did not allow us to ascertain what the porbeagle sharks diet consisted of and whether prey was the source of microplastics (SI). Earlier studies indicated potential accumulation and trophic transfer of microplastics across parts of the foodweb[264,265,339]. Studies looking at microplastics

in fish from the North Atlantic region report around one microplastic per fish[3,206,263]. When recalculating the total amounts of microplastics in the spiral valves of porbeagle sharks, rather large numbers were observed (Table 3). Concentration went up as high as 6116 microplastics per individual spiral valve, suggesting that microplastics are accumulating across the food web, potentially harming critically endangered North East Atlantic porbeagle sharks.

The present work emphasizes the potential for top predator application in microplastic monitoring. Porbeagle sharks are apex predators feeding on a wide range of organisms, including teleosts and cephalopods[284,285,340] and play a key role in controlling ecosystem dynamics[284]. Distances of over 1,000 nautical miles (nm) were recorded by tagged porbeagle sharks, though over 90% of the 143 sharks tagged moved less than 500 nm from their original tagging location[341,342]. Although one porbeagle has been recorded crossing the Atlantic[284], tagged sharks in the Celtic Sea mostly remained in that area[342]. The spatial distribution of incidental porbeagle bycatch reported by the participating vessels confirm that porbeagles are widespread within the Celtic Sea[307]. There may be a separate North Atlantic stock off Iceland[343], this North-East Atlantic stock is generally considered to be distinct from those in the North-West Atlantic and Mediterranean[344,345]. As such, porbeagle sharks could be an ideal species for integrated monitoring across a wider (sub)region. In future, it would be recommended to target spiral valve content in by-caught Elasmobranches, such as the porbeagle shark, for microplastics monitoring purposes. This avoids potential bias from gastric evacuation[304,305,346,347] and might give a better link to probable impacts and pollutant loads as it relates to digestion[304]. To overcome temporal changes in bycatch rates of porbeagle sharks in gillnets[286,307,348] and to support an appropriate microplastics monitoring programme, the analysis should be expanded to other by-caught top predator species.

5.5. ACKNOWLEDGEMENTS

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Chapter 6

The world is your oyster: low-dose, long-term microplastic exposure of juvenile oysters.

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ABSTRACT

Bivalve filter feeders, such as oysters, filter large volumes of water and are particularly exposed to microplastics (MP). Consequently, these animals digest and assimilate high levels of MP in their bodies that may likely impact their physiology, and potentially affect shellfish stocks, benthic habitats and, indirectly, the health status of the marine ecosystem and human consumers. In this study we exposed juvenile oysters, *Crassostrea gigas*, to 3 different MP concentrations (10^4, 10^5 and 10^6 particles L^{-1}), represented by 6 µm polystyrene (PS) microbeads, compared to a control treatment receiving no MP. The study ran for a period of 80 days to test for the impacts of MP on growth, Condition Index and Lysosomal Stability. From histological analysis, microbeads were detected in the intestines of exposed oysters and in the digestive tubules, but no cellular inflammatory features were observed over time. Weight and shell length remained comparable between the different treatments and control. We found that Condition Index in the highest concentration increased initially but significantly reduced over time. The oysters in the highest MP exposure also showed the lowest mean Lysosomal Stability score throughout the experiment. Lysosomes play a vital role in the cells defense mechanisms and breakdown of constituents, crucial for the oysters' wellbeing. Most importantly, we detected an increased mortality in those oysters who were chronically exposed to the highest loads of MP.

6.1 INTRODUCTION

It is well established that the marine environment is widely polluted with MPs (< 5 mm) and that this issue poses a serious threat to marine biota[218,349]. Bivalve filter feeders living in coastal waters, such as oysters, are particularly exposed to MPs because of their feeding mode and enormous filtration capacity; individual oysters can filter ~ 5 - 25L of seawater h^{-1} [333,350,351], making them likely to ingest MPs[350,352]. Many specimens have been found to contain high loads of MPs in the field[218]. Microplastics in oysters are directly related to the population density within the watershed. Hooded oysters, Saccostrea cucullata, along the Pearl River Estuary (China) near urban areas contained statistically significantly more MPs than those near rural areas[353]. Bivalves ingest and assimilate high levels of MPs in their bodies that may likely impact their physiology, and potentially affect both shellfish stocks, habitats and, indirectly, the health status of the marine ecosystem and human consumers[291,333,354–356]. Bivalves are recommended as ideal sentinel species in several marine monitoring programmes, including those supported by international bodies such as ICES and OSPAR[357]. As a result, bivalves have been recommended as a bioindicator for monitoring MP pollution[358]. They are typically chosen for exposure experiments due to their important role in the economy and the ecosystem. Several experimental studies have shown cellular responses (e.g. loss of lysosomal membrane integrity, oxidative stress, DNA damage) or negative effects on feeding, growth and reproduction of adult bivalves, such as oysters, mussels and clams, after exposure to relatively high concentrations of certain types of MPs, mostly PS spheres[268,352,359–361]. Yet another study found no statistically significant effect on development or feeding capacity of Pacific oyster larvae (Crassostrea gigas) after ingestion of micro- and nanoplastics[362]. More evidence is needed, as previous studies mostly used acute and subchronic treatments and exposure concentrations exceeding environmental concentrations, and thus being of indirect relevance[218,363]. The Pacific oyster is the most cosmopolitan of all oyster species and a successful aquaculture species. They have a wide global distribution, are hardy and grow rapidly, and thrive in temperatures ranging from 8-22°C with a salinity between 24 and 28 ppt[364]. As a result, the Pacific oyster has also become the leading species in world shellfish culture, with an estimated production of 573 617 t in 2016[365]. They are relatively straightforward to culture and handle in the laboratory and bioaccumulate toxins by filtration, making them an ideal species for studying biological processes. In this study, we tested the hypothesis that a model MP (fluorescently stained spherical PS; 6 µm) impacts the physiology and health of juvenile Pacific oysters during their growing phase. To detect impacts of 6µm PS microplastics in juvenile oysters we opted for three different MP concentrations and a long-term treatment of 80 days. To demonstrate exposure to and effects of PS microbeads, a set of generic biomarkers and endpoints, showed to be responsive

in earlier laboratory studies with bivalves, were used. These were: Condition Index (CI), Lysomal Stability (LMS) and growth. In addition, we performed a histological analysis to identify the distribution of PS microbeads in digestive tissues and to screen for potential pathology.

6.2 MATERIAL & METHODS

6.2.1 Tested organism

The oysters were supplied from Guernsey oyster hatchery and were considered healthy and uncontaminated by biological agents other than normal flora. The oysters were sent directly from the hatchery and transferred in crates to Cefas' Weymouth laboratory. To avoid biological contamination, the study was conducted in a room where no further studies with other bivalve species were taking place. The seed oysters were held in 15L flow-through glass tanks. Oysters were rinsed and acclimatised for 1 day prior to the start of the study. All oysters were fed a diet consisting of live algae (Tetraselmis suecica & Isochrysis galbana mixture from Guernsey Sea Farms delivered weekly) supplemented with artificial food (SD1800 - Shellfish Diet 1800 from Reed Mariculture) at predefined feeding times. They were dosed with 6 µm PS microbeads once daily. The uptake of the microbeads was optimised by feeding the oysters algae mixtures within a similar size range as the microbeads. During weekdays, oysters were fed twice a day, once with the live algae mixture supplemented with preserved algae (SD1800) and once with pure preserved algae (SD1800). During the weekend, oysters were fed only once a day, with a mixture of live and preserved algae. Food concentrations were calculated as: 5% wet weight of live algae and 8% dry weight of preserved algae per g dry weight of oyster tissue. The body weight used in this feeding calculation was increased weekly, with 5% as a measure of predicted growth in the absence of real data during the first 10 days and revised body weight predictions after each sampling point using collected data. The tanks in the study were bespoke glass aquaria, semi static 15 L tanks, all of which could be easily emptied via a bottom valve. Daily, the seawater in the tanks was drained and refilled with clean seawater. All used seawater was UV treated and filtered via a series of three sequential ceramic filter units (20µm, 10µm, 0.2µm – Deltaqua International). The PS microbeads were added to the tanks and then corresponding live algal suspensions were added. Further details of the feeding regime can be found in the supplementary information (SI). The oysters were supplied from Guernsey oyster hatchery and were considered healthy and uncontaminated by biological agents other than normal flora.

6.2.2 Tested MP

The MPs used in this study are chemically inert 6µm Red Fluorescent PS Microbeads (Fluoresbrite Polysciences Cat #19111-2 Lot 653002 (day 0-58) & Lot # 660155 (day 59-80)). Fluoresbrite particles are routinely used in a wide range of applications, including as tracer particles and in phagocytosis assays. The initial stock solution, $1x10^6$ particles L^{-1}, was made according to Table A (see SI) using the manufacturer's supplied solution. The MPs stock, as supplied by the manufacturer and stored in the fridge, was removed in the morning and sonicated in a water bath for 5 minutes prior to use to disperse any aggregates formed. Solutions for the $1x10^5$ and $1x10^4$ particles L^{-1} were prepared by serial solutions (1:10) of the stock solution in reverse osmosis water (rH2O). PS microbeads were added to filtered seawater and suspended in the water column by using a filtered air lift.

At two different stages, water samples were taken during one full cycle (0h, 1h, 4h, 12h & 24h) from tanks with different concentrations to improve our understanding of the actual exposure conditions and processes involved within the tanks. These samples were analysed for PS microbead concentrations using a fluorescent cytoflow counter. Two additional tests were run in duplicate tanks, one set containing seawater and PS microbeads, the other set containing seawater, PS microbeads and algae. A $1x10^{-4}$ dilution of the Fluoresbrite polychromatic 6.0 µm Microspheres (Polysciences) was prepared to identify the position of the bead cluster on the cytogram. This cluster reference was used in further analysis to identify the number of beads in the samples. Each water sample was placed in an ultrasonic bath for 10 minutes and homogenised before being passed through a 200 µm mesh and

a 20 µm mesh, removing excessive organic matter prior to the flow cytometer analysis. Each sample was run using the following cytoflow counter settings: Forward scatter; Trigger level of 25mV; Maximum flow speed; 10-minute runtime. Forward scatter was selected as trigger level and used to remove noise from the cytogram. Ten minutes of runtime was allocated to analyse the maximum number of beads/volume.

6.2.3 Experimental design and treatment

In this study we exposed C. gigas to 3 different concentrations of MPs (10^4, 10^5 and 10^6 particles L^{-1}; PS microbeads; 6µm) compared to a control treatment receiving no plastics for a period of 80 days, to test for the impacts of MP on growth, CI and LMS. We reviewed available microplastic field concentrations[366] in combination with model outputs[293,367] to select three concentrations for this size of microplastics, representing potential short-term and long-term environmental exposure scenarios. Histology was conducted at the start and during the sampling points to locate the PS microbeads in the oyster tissue. Samples (growth, CI, LMS and histology) were taken on days 0, 10, 20, 40 and 80.
Each of the 4 treatments was replicated 12 times (48 tanks in total) (Figure 6.1). Each tank contained 30 oysters, 2 glass strips to which 15 juvenile oysters were attached. All 1440 oysters were weighed and measured at the start. At each sampling day (10, 20, 40 and 80) all the animals were removed from the 3 replicate tanks for each treatment and processed (Table B – see SI).

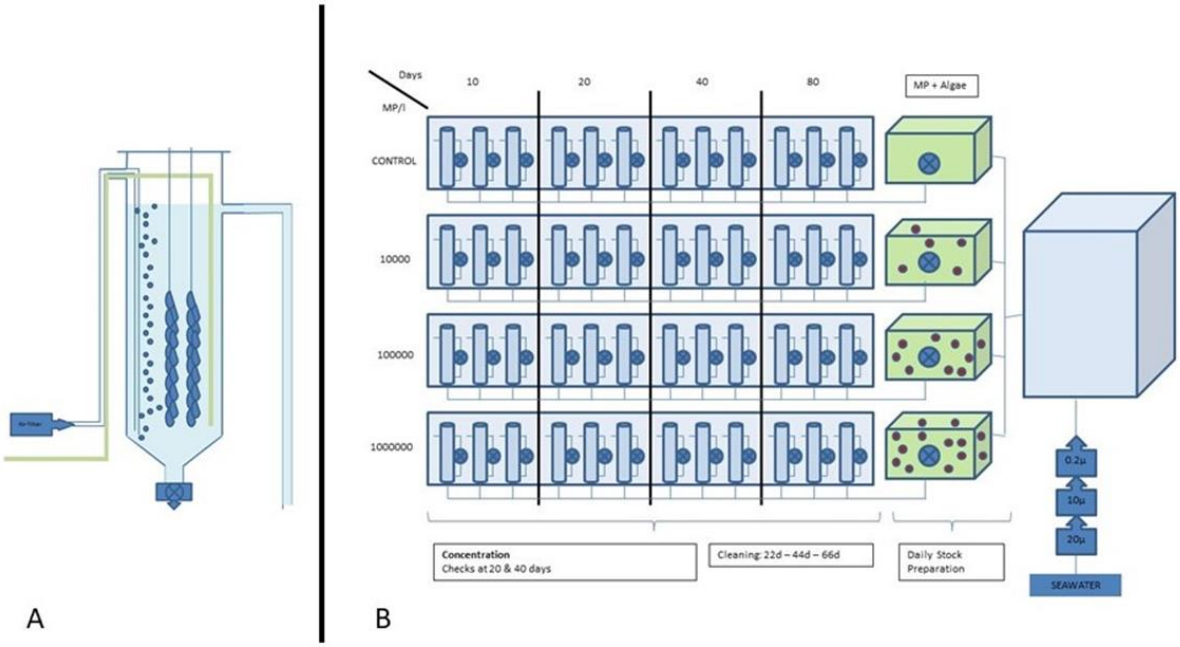

Figure 6.1. (a) Individual tank setup with 30 juvenile oysters (2 glass rods x 15 oysters) and airlift (filtered). (b) Experimental setup showing the 4 treatments (Control and 3 different PS microbead concentrations) in triplicates for each sampling date (4) (total of 48 tanks), the green tanks represent the stock preparation made daily for each exposure concentration (Algae and 3 different concentrations of PS + Algae). All seawater was UV treated and ran through three sequential ceramic filtration units (20µm, 10µm, 0.2µm) before use. PS concentrations were checked at day 20 and 40 over 24h. Cleaning occurred at 22d-44d-66d.

The water temperature was dependent on ambient air temperature, around 18 +/- 2°C throughout. The oysters were acclimatised in the experimental tanks, under these conditions, for a minimum of 1 day before the start of the study. The following parameters were logged to ensure consistency between the tanks during the entire experiment: temperature (daily), pH (twice weekly; AM Monday & PM Friday), DO2 (twice weekly; AM Monday & PM Friday), Salinity (twice weekly; AM Monday & PM Friday). Light levels were set on a 12h day cycle and total Lux was measured at the end of the study.

All tanks were covered during the entirety of the experiment, access to the laboratory was limited and appropriate laboratory ware (cotton) was worn to avoid contamination from the air. The tanks were manually cleaned on day 22, 44 and 66. Biofilm scrapes from the side of the tank and pseudofaeces were collected and smeared on microscopic slides to determine microbead presence. The wastewater was sand filtered and treated by the facility Ozone plant before discharge. Any other whole animal or tissue waste was discharged as clinical waste and incinerated.

6.2.4 Sampling procedure

A specific bench area in the biocontainment experimental tank facility was prepared and cleaned before and after sampling. Sampling of oysters was done inside this area to avoid contamination. Oysters were weighed and measured prior to fixing them onto the glass rods. On the selected sampling dates (10, 20, 40 and 80) the oysters were weighed and measured again, after which CI was determined on a subset of 15 oysters (Figure 6.2). Another sub-selection of 10 oysters was taken to determine LMS (Figure 6.2). Live samples were taken to the postmortem room and prepared for biomarker assays on site. Tissues were frozen for biomarker analysis, fixed for histology or dried for final dry weight. The digestive gland was removed, embedded in OCT in cryotomes and frozen in liquid nitrogen, after which the blocks were stored at -80C for later analysis. The remaining 5 oysters were used for histology (Figure 6.2); for this, 8-10µm soft tissue slides were made and stored at -80C.

	A			B	
1	☐	LMS	**1**	☐	LMS
2	☐	G - CI	**2**	☐	G - CI
3	☐	Histo	**3**	☐	Histo
4	☐	LMS	**4**	☐	LMS
5	☐	G - CI	**5**	☐	G - CI
6	☐	G - CI	**6**	☐	G - CI
7	☐	LMS	**7**	☐	LMS
8	☐	G - CI	**8**	☐	G - CI
9	☐	Histo	**9**	☐	Histo
10	☐	LMS	**10**	☐	LMS
11	☐	G - CI	**11**	☐	G - CI
12	☐	G - CI	**12**	☐	G - CI
13	☐	LMS	**13**	☐	LMS
14	☐	G - CI	**14**	☐	G - CI
15	☐	Histo	**15**	☐	G - CI

Figure 6.2. Oyster number and sampling procedure, length and wet weight were measured for all oysters, before the experiment and at all sampling points. The abbreviations stand for: LMS= wet weight, shell length, flesh removed and cryopreserved for further lysosomal stability analysis; G- CI = Wet weight, length, dry shell weight, dry tissue weight for Condition Index determination; Histo = Wet weight, shell length, shell removed and tissue fixed in Davidsons for further histology analysis.

6.2.5 Biological parameters, biomarkers and histology

6.2.5.1 Shell Length & Weight

Shell height, the maximum dimension from hinge to growth edge, is commonly referred to as shell length, which will be used to describe this dimension here. The shell length of every oyster was measured to the nearest mm. Additional dimensions were measured to account for irregular oyster shapes (e.g., long and thin). All measurements (±1.0 mm) were taken using a digital calliper system that enabled the rapid recording of data. In the weighing technique, oysters were air dried at room temperature for 5 minutes and weighed to the nearest 0.0001g. Oyster meat was oven dried to constant weight (68C for 48 hours) and then meat and shell were weighed separately to the nearest 0.0001g, after a short cooling period.

6.2.5.2 Condition Index

The CI of bivalves is measured by relating either the weight or volume of the meat to some aspect of the shell. In the current study, oyster shell length and weight measurements were standardized using the following formula: Condition Index = (dry meat weight in g) * 100 / (shell weight in g). This widely-used condition index, because of the nature of the measurements involved, is easily standardized and is thus used globally[368]. In addition, the use of dry tissue weights eliminates the bias due to water content fluctuations of whole tissue. A low value for this index indicates that a major biological effort has been expended, either as maintenance energy under poor environmental conditions or disease, or in the production and release of gametes. Thus, as an indicator of stress, or sexual activity, this index gives meaningful information about the physiological state of the animal[369].

6.2.5.3 Lysosomal Membrane Stability

A series of solutions and reagents were used to test LMS. A lysosomal membrane labilising buffer (Solution A) was made with 0.1M Na-citrate Buffer - 2.5% NaCl w:v (pH 4.5). The substrate incubation medium (Solution B) consisted of 20 mg of N-Acetyl-β-hexosaminidase (Sigma, N4006) or Napthol AS-BI phosphate (Sigma N2125), dissolved in 2.5 mL of 2-methoxyethanol (Merck, 859) and made up to 50 mL with solution A. This solution contained 3.5 g of collagen-derived polypeptide (POLYPEP, P5115 Sigma) as low viscosity polypeptide to act as a section stabiliser. This solution was prepared 5 minutes before use. The diazoniumdye (Solution C) contained 0.1M Na-phosphate buffer (pH 7.4) containing 1 mg mL^{-1} of diazonium dye Fast Violet B salts (Sigma, F1631). The fixative (Solution D) was made from Baker's calcium formol containing 2.5% NaCl (w:v). An aqueous mounting medium (Vector Laboratories H1000, Kaiser glycerine gelatine, Difco, Sigma) was used. The lysosomal membrane stability was cytochemically determined using N-Acetyl-β-hexosaminidase [370–372]. Cryostat sections were cut at 8-10µm (in duplicate on the same slide) and left in the cryostat chamber until just before use. Seven slides were prepared in this manner. Solution A was placed into a water bath at 37 °C to acclimatise. The slides were placed into pre-treatment solution A so that each slide had a different pre-treatment time of 30, 25, 20, 15, 10, 5, and 2 minutes i.e. slide 7= 30 minutes, slide 6 = 25 minutes, slide 5= 20 minutes, etc. Following pre-treatment, slides were transferred to solution B for 20 minutes at 37 °C in a staining jar in a shaking water-bath. The slides were rinsed with a saline solution (3.0% NaCl) at 37 °C for 2 to 3 minutes. The slides were then transferred to solution C at room temperature for 10 minutes. Following this, slides were rinsed rapidly in running tap water for 5 minutes. Sections were fixed for 10 minutes in Solution D pre-cooled to 4 °C. Finally, slides were rinsed in distilled water, mounted in aqueous mounting medium and analysed. The labilisation period (LP) is the time of pre-treatment required to labilise the lysosomal membranes fully, resulting in maximal staining intensity for the enzyme being assayed. The staining intensity was assessed visually using microscopic examination. The labilisation period can be effectively measured by microscopic assessment of the maximum staining intensity in the pre-treatment series, a microdensitometer is not completely necessary for accurate determination. All assessments were carried out on duplicate sections for each digestive gland at each pre-treatment time. Lysosomes will stain reddish-purple due to the reactivity of the substrate with N-acetyl-ß-hexosaminidase. The LP for each section corresponds to the average

incubation time in the acid buffer that produces maximal staining reactivity. LP for the other replicate is similarly obtained. Finally, a mean value of LMS of the sample was calculated utilizing the data obtained from the 10 animals analysed [370]. Determination of the LP is usually quite straightforward, but a complicating situation occasionally arises in which the pre-treatment series shows two peaks of staining intensity, possibly due to differential latent properties of the subpopulations of lysosomes. In this situation, the first peak of activity was used to determine labilisation period, as it is the most responsive to staining [370].

6.2.5.4 Histology

Histological analyses were conducted on paraffin-embedded tissues sectioned at 8-10µm thickness and stained using a pentachrome staining procedure to determine the prevalence and intensity of the fluorescent PS microbeads by histological examination. Slides were examined using a Nikon Eclipse E800 microscope equipped with fluorescent filters. Images were captured using the Lim Lucia G Screen Measurement™ image analysis system (Nikon, UK) and Nikon DXM1200F video camera. The microbeads used in this experiment are suitable for fluorescence microscopy and yield intense fluorescence. Microscopic viewing using a 475-490nm filter shows an extremely bright red fluorescence, while use of a 545-610nm filter yields a yellow fluorescence with excitation maxima of 491nm and 512nm and emission maxima at 554nm. The main aim was to confirm the uptake and presence of the microbeads but, where possible, the occurrence and extent of tissue pathologies, and the intensity of anomalities were recorded using quantitative or semi-quantitative measures. Measures of prevalence or occurrence, however, do not give a true indication of the health of an organism[373].

6.2.6 Statistics

The statistical importance of the apparent difference of Condition Index, Shell Length and Shell Weight were tested by fitting linear mixed models. These were fitted using the lmer function in the R package lme4. Details of the precise models fitted are shown in the Results section. When investigating the dead oysters, because of the low numbers, the deaths were not modelled with mixed models as above. Instead, Fisher's exact test[374] was used based on two-way contingency tables of treatments vs the control.

Mixed models, as used for the CI analysis, were not used for the LMS data because the LMS scores could take only one of eight different values (including zero for the dead ones). In addition, oysters within a tank often had similar LMS scores and so a normally distributed random error – or indeed a tank random effect - wasn't appropriate. A priori, a central interest is in comparing the LMS for the control and the treatment groups. Thus, we performed our comparisons by comparing the tank means of the control against each of the three treatment levels. This allowed us to compare groups where each contained ten tank means. We performed two-sided, non-parametric randomisation tests of the mean levels, using the permute.groups function in the R library emon[194].

6.3 RESULTS

6.3.1 PS MP concentrations:

All experimental parameters remained stable and within acceptable limits for optimal oyster cultivation over the entire period: daily temperature (18 +/- 2°C), pH (8.1), DO2 (~7.0 mg L^{-1}) and Salinity (32-35‰). Light levels mimicked a normal day cycle. All the collected water samples, biofilm glass tank scrapes, pseudofaeces and faeces contained PS microbeads. Microplastic concentrations in the water column appeared to be much lower than expected ($1x10^4$ particle L^{-1}, $1x10^5$ particle L^{-1}, $1x10^6$ particle L^{-1}). On average, the detected concentrations of microplastics in the water were a factor 10 lower from the start onwards and dropped to about 1000 times lower 24 hours later across all exposures. A similar effect, although much lower, was observed in the tanks containing no oysters (concentration dropped on average with a factor 100 after 24 hours) and almost no difference was

observed in the tanks containing only seawater and PS microbeads (concentration dropped on average with a factor 10 after 24 hours). These concentrations drops are most likely the result of the removal via the oysters, algae and biofilms and the static interaction between microbeads. Microbeads were clearly present in the scrapes from the glass ware and in the pseudofaeces and faeces.

6.3.2 Uptake of PS microbeads

From histological analysis, microbeads were detected in the intestines of exposed oysters (Figure 6.3a) and in the digestive tubules (Figure 6.3b). No cellular inflammatory features, including granulomas were observed in exposed animals. No microbeads were observed in control oysters.

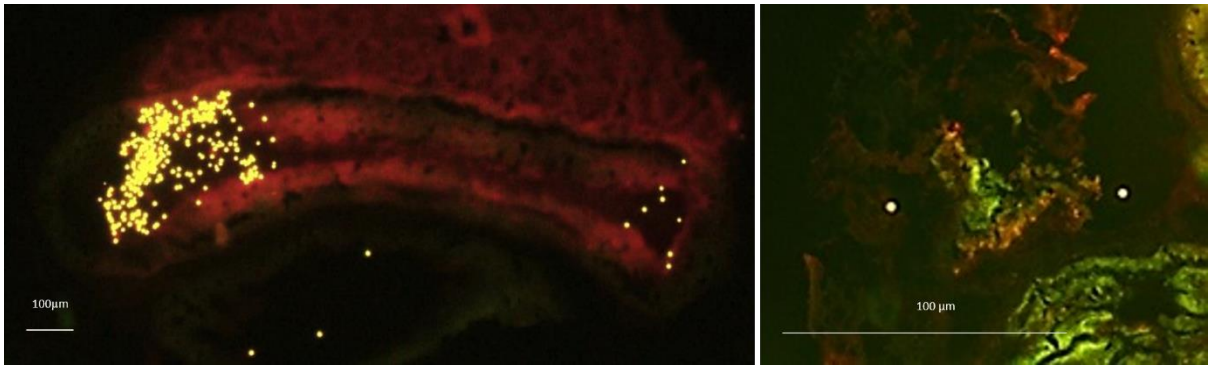

Figure 6.3. histology slides of the oyster intestines (a) and digestive tubules (b) showing the fluorescent PS microbeads (yellow) excited at 491 & 512nm.

6.3.3 Effects of Exposure to PS microbeads

The effects of a range of microbead concentrations on growing juvenile oyster across the 80-day period were determined using various measurements and endpoints: Condition Index, Shell Length, Weight, Lysosomal Membrane Stability and Mortality.

6.3.3.1 Condition Index:

Four observations were excluded because of missing information. This left 716 observations. Of these, 22 were for dead oysters and 694 for live ones. For the first part of the analysis below, only the live oysters were used. The numbers of dead oysters were analysed separately.

The mean CI was plotted by treatment and day. This is shown in Figure 6.4a. The plot suggests that there is little noticeable difference between the means for the control and two lowest MP exposure concentrations. However, the mean of the highest exposure concentration is initially the highest (days 10 and 20) but then becomes the lowest (days 40 and 80). This is perhaps even more clearly illustrated in Figure 6.4b, which has the CI transformed by square root (this transformation will downplay the influence of some of the extreme, high CI values).

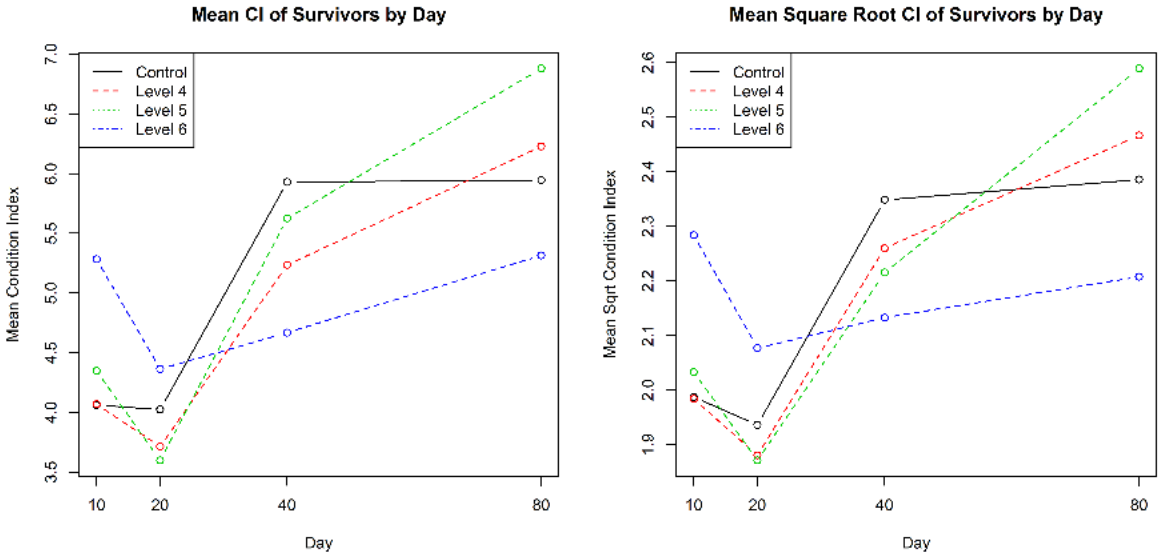

Figure 6.4. (a) Plot of CI means by treatment and day (b) Plot of CI means of square root CI, by treatment and day

The statistical importance of the apparent difference between the highest exposure concentration and the other ones was tested by fitting linear mixed models. Initially, the full model was fitted

$$SCI = \alpha + TREAT + DAY + TREAT.DAY + TANK + ROD|TANK + \varepsilon \quad (1)$$

where SCI=CI0.5, TREAT is a factor representing a fixed effect of microbead concentration, DAY is a factor representing a fixed effect due to the duration (0, 10, 20, 40 , 80 days) of the experiment, TANK is a random effect and ROD|TANK is a random effect of ROD, nested within TANK. For model comparisons, parameter estimates were obtained by maximum likelihood.

A new treatment factor (TREAT2) was created which contained a single value if the treatment was Control, 10^4 particles L^{-1} or 10^5 particles L^{-1} and a second value if it was 10^6 particles L^{-1}. Thus, the new factor has two levels: 10^6 particles L^{-1} and 'the other exposure concentrations'. A similar model to (1) was fitted of the form

$$SCI = \alpha + TREAT2 + DAY + TREAT2.DAY + TANK + ROD|TANK + \varepsilon \quad (2)$$

but with TREAT2 replacing TREAT. When comparing the fit of models (1) and (2) using a likelihood ratio test we obtain a p-value of 0.88, suggesting little difference between the models. Thus, our modelling suggests that the lowest three levels of MP are behaving similarly.

We now turn to assessing whether the two treatment levels defined by TREAT2 are different. We do this by fitting a model without a treatment effect and then comparing its fit with model (2). That is, how important is it to distinguish the two treatment levels or can we assume that there is no difference between the treatments?

$$SCI = \alpha + DAY + TANK + ROD|TANK + \varepsilon \quad (3)$$

When models (2) and (3) are compared using a likelihood ratio test we get a larger difference in the log-likelihood than before and a p-value of 0.006. Thus, this gives statistical evidence that highest exposure (10^6 particles L^{-1}) is acting differently to the other exposures. From observation of Figure

6.4a and 6.4b, this difference manifests itself in increased growth for the highest exposure treatment for days 10 and 20 but then reduced growth for days 40 and 80.
The analysis above was done on oysters that were alive, however, we also found some dead oysters at the different sampling points. Table 6.1 below shows the number of deaths by day and treatment. It should be noted that these numbers are all relatively small compared to the 716 oysters analysed for CI. However, the results are interesting in that they show that there were more deaths for the highest MP concentration and, perhaps not surprisingly, there were more deaths on day 80 than the other days.

Because of the low numbers, we did not model the deaths with formal models as above. However, we did consider two-way contingency tables of highest MP concentration vs the control (Table 6.2a) and highest concentration vs the other treatments (Table 6.2b). As with the modelling above, we need to be careful with implicit multiple comparison tests because we have, to some extent, used the data to guide our testing. Having said that, a priori, we might expect to be comparing the highest levels of MP against either the control or the lower treatment levels.

Table 6.1. Number of deaths by day and treatment (out of 716 oysters)

	DAY				
TREAT	10	20	40	80	**Total**
Control	2	0	1	2	5
10^4 particles L^{-1}	1	0	2	0	3
10^5 particles L^{-1}	0	1	1	0	2
10^6 particles L^{-1}	1	3	1	7	12
Total	4	4	5	9	22

For the two-way contingency tables, Fisher's exact test was used to investigate whether there were more deaths from the 10^6 particles L^{-1} concentration than there were from the (i) control and (ii) control, 10^4 particles L^{-1}, 10^5 particles L^{-1} treatments (Tables 6.2a and 6.2b). For comparison (i), p=0.13 if we assume an alternative hypothesis that 10^6 particles L^{-1} is different to the control and p=0.07 if we assume that 10^6 particles L^{-1} would result in greater deaths than the control. For (ii), corresponding p-values are 0.004 and 0.002 respectively. Thus, whilst there is a suggestion that there are more deaths for 10^6 particles L^{-1} than for the control, the small numbers mean that any difference is not quite statistically significant. However, when comparing the 10^6 particles L^{-1} with the larger group of treatments, we easily attain the 5% level of statistical significance – suggesting a greater probability of death at 10^6 particles L^{-1} than for the other three treatments.

Overall, analysis of the CI data provides strong evidence that the highest PS microbead concentration is having a different/adverse effect on oysters.

Table 6.2. Number alive and dead oysters

a: Number alive and dead by treatment 10^6 particles L^{-1} and control

	10^6 particles L^{-1}	Control	Total
Dead	12	5	17
Alive	167	174	341
Total	179	179	358

b: Number alive and dead by treatment 10^6 particles L^{-1} and the other three treatments

	10^6 particles L^{-1}	Control, 10^4 particles L^{-1}, 10^5 particles L^{-1}	Total
Dead	12	10	22
Alive	167	527	694
Total	179	537	716

6.3.3.2 Analysis of Shell Length and Weight

Only live oysters were analysed. There were originally 2,873 data points, 1,440 length and weight values from the oysters at the start of the experiment and 360 data values for the length and weight of the oysters at each of days 10, 20, 40 and 80 (7 values were excluded due to data oddities). At the end of the experiment, 27 oysters were found dead and 1,406 alive.

The plot of the shell length means is shown in Figure 6.5a. There is no obvious pattern amongst the treatments – apart from a reduction for the mean shell length for the highest concentration at day 40, but this is not continued at day 80. Using mixed models of the form in (1) and with the square root of shell length as the dependent variable, likelihood ratio tests confirm that there is no statistically significant interaction between DAY and TREAT ($p=0.18$), that there is a statistically significant effect of DAY ($p<0.001$) and the effect is close to statistical significance for TREAT ($p=0.052$).

The plot of the weight means by day and treatment is shown in Figure 6.5b. Formal statistical modelling suggests that both the DAY by TREAT interaction, the DAY effect and the TREAT main effect are statistically significant ($p=0.007$, $p<0.001$ and 0.015 respectively).

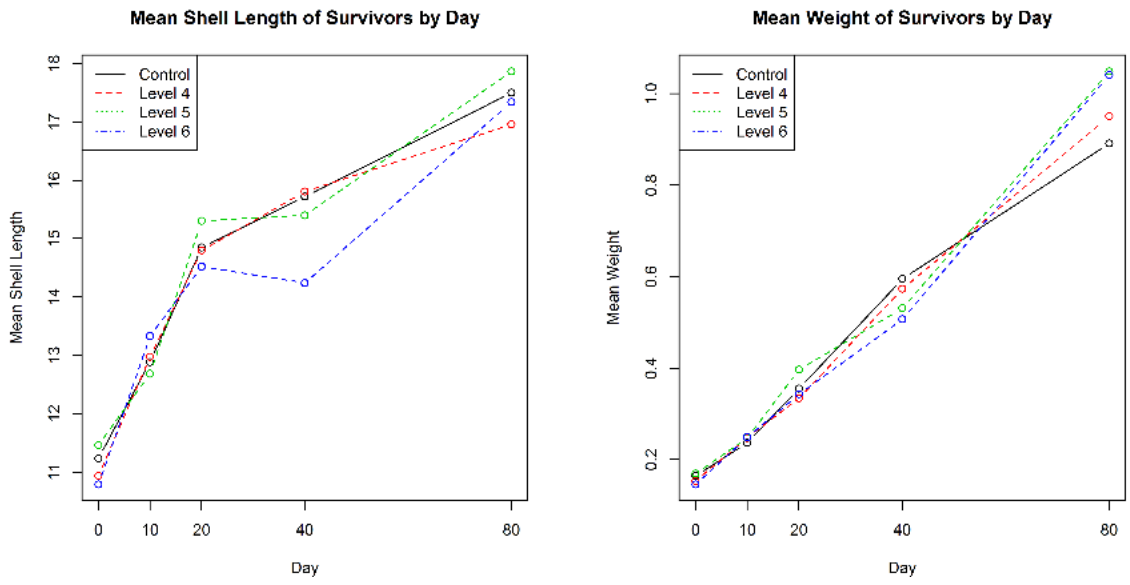

Figure 6.5 (a) Plot of means of shell length by treatment and day; (b) Plot of means of weight by treatment and day (the figures are for survivors only).

6.3.3.3 Analysis of Lysosomal Membrane Stability

An LMS score was obtained for 342 out of the 480 oysters, there were 14 dead oysters and 124 oysters for which no LMS score could be obtained due to sampling or analytical issues. If we consider that the 14 confirmed dead oysters should have a score of 0, then there are scores for 356 oysters.

Figure 6.6a shows the mean LMS scores for the 342 alive oysters by treatment and day. There is no obvious pattern with respect to treatment dose here. It is interesting to see that the mean for the highest microbead exposure is always less than the control mean and that the mean for the highest microbead exposure has the lowest mean for the first three measured periods. Figure 6.6b shows a similar plot to that in Figure 6.6a, except that in Figure 6.6b the dead oysters are included, with their LMS scores of 0. This figure perhaps creates a clearer picture in that the mean for the highest MP exposure has the lowest mean score throughout the experiment, tentatively suggesting that the higher dose of microbeads is having a detrimental effect on the lysomal membrane stability.

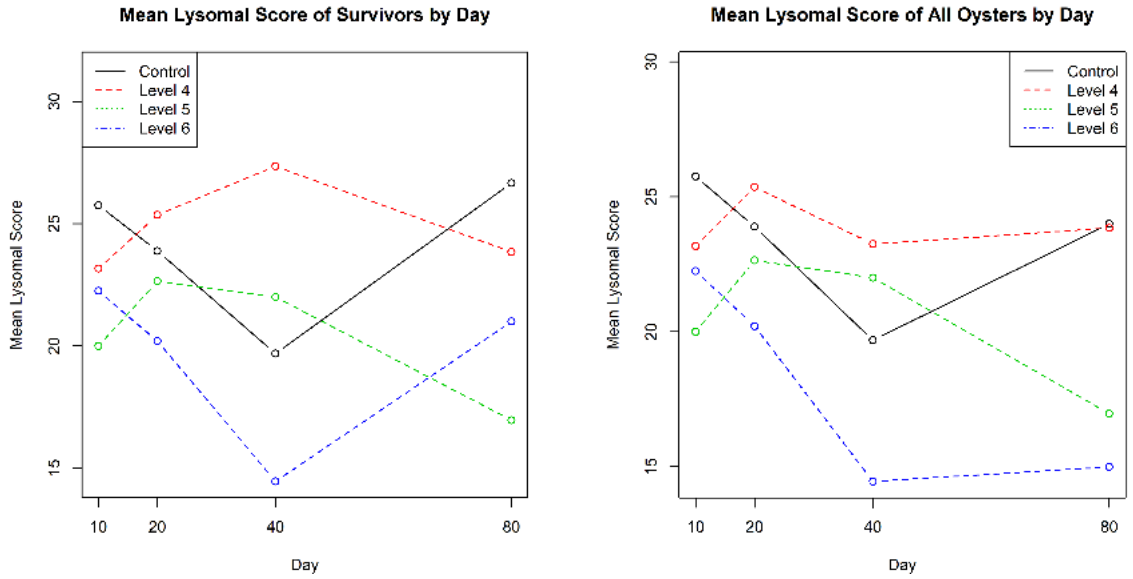

Figure 6.6. (a) Mean LMS score of survivors by day (b) Mean LMS of all oysters (dead & alive) by day

For the analysis excluding and including the dead oysters, the comparison results are shown in Table 6.3. These confirm the impression given by Figure 6.6, that the tanks with the highest MP concentration have lower LMS scores than the control tanks (p-value=0.017).

Table 6.3: P-values for comparing LMS score tank means for control and each level of treatment dose. Comparisons have been done twice, without and with the 14 dead oysters.

Comparison	p-value: Alive oysters only	p-value: Alive and 14 dead oysters
Control vs Dose 4	0.86	0.74
Control vs Dose 5	0.29	0.35
Control vs Dose 6	0.34	0.017

6.4 DISCUSSION

Our results showed that oysters will accumulate MPs from the water column. Shellfish containing MPs consumed whole not only pose concern for human exposure[255,291,333,355,358], but also for the animals themselves. The highest PS microbead concentration in our study has been found to increase mortality amongst juvenile oysters. Other studies have shown that exposure to relatively high densities of MPs

alter the respiration rates, immunology, reproductive capacity and filtration rates of bivalves [359,375–378]. Owing to their role as ecosystem engineers (e.g. reef formation, benthic – pelagic coupling, biodepositioning), such effects are likely to permeate beyond the individual organism into benthic and pelagic food webs[377]. If MPs alter the ability of these filter feeders, there may be wider impacts on their associated communities and on the functioning of coastal ecosystems[376]. More studies are urgently needed to determine the effects of MPs on these key marine species and habitats.

In this study, the MPs deployed were hard and smooth microbeads; however, actual MPs can have any form or shape. To date, there are few peer-reviewed publications on suspected microbeads collected in the field. We could not immediately observe a practically meaningful effect of PS microbeads on the length and weight of the individual oysters. In the same way, Cole et al.[379] found no measurable effects on the development or feeding capacity of oyster larvae exposed to plastic concentrations exceeding those observed in the marine environment. The condition index data provided evidence that the highest PS microbead concentration resulted in lower mean CI levels when compared to the lower treatment levels. These data also showed that a disproportionate number of oysters in the highest dose group die compared to the other groups.

Our results also indicate that the highest dose of microbeads is having a detrimental effect on the lysomal membrane stability. Lysosomes are responsible for the breakdown of all the constituents of the cells and macromolecules derived from the extracellular space via endocytosis[380]. They are also involved in cell defense mechanisms, in the protection against the toxic agents and infection by viruses and bacteria[380,381]. The physicochemical modifications which lead to the loss of the integrity of the membranes of diverse components of the lysosomes are almost always associated with cellular dysfunction, inflammatory and degenerative diseases as well as apoptosis and cell death[380,382]. These findings suggest that environmental concentrations of MPs are harmful to the wellbeing of oysters in the long term.

We found no histological evidence of damage to the digestive tissue structures, suggesting that these low concentrations of PS microbeads do not provoke any inflammatory reactions. It is difficult to define the exact underlying mechanisms from the selected endpoints in this study, but other studies have highlighted that PS microbeads in high-dose, short-term experiments caused feeding modifications and reproductive disruption in oysters, with significant impacts on offspring. Dynamic energy budget modeling, supported by transcriptomic profiles, suggested a significant shift of energy allocation from reproduction to structural growth, and elevated maintenance costs in exposed oysters, which is thought to be caused by interference with energy uptake. Molecular signatures of endocrine disruption were also revealed, but no endocrine disruptors were found in the biological samples[359]. In a study by Ribeiro et al.[360] the effects of PS MPs were assessed in tissues of the clam Scrobicularia plana. Clams were exposed for 14 days to 4 particles mL^{-1}, comparable to our lowest (10^4 particles L^{-1}) concentration, followed by 7 days of depuration. The results revealed that MPs caused reduced antioxidant capacity, DNA damage, neurotoxicity and oxidative damage[360]. A two-month study of the black-lip pearl oyster, P. margaritifera, has shown that environmental concentrations of PS microbeads significantly impact the assimilation efficiency and more broadly the energy balance, with negative repercussions on reproduction. Gonads may have provided the missing energy to maintain animals' metabolism through the production of metabolites derived from germ cells phagocytosis[361].

Our data shows little noticeable difference between the CI of the oysters in the control and those in the two lowest MP concentrations (10^4 particles L^{-1} & 10^5 particles L^{-1}). Similar results were observed in other bivalve studies using low concentrations of PS microparticles (4 particles mL^{-1}), where no statistically significant changes were observed between control and exposed clams (S. plana) after 14 days and in the 7day elimination period. A low value for this index indicates that a major biological

effort has been expended, either as maintenance energy under poor environmental conditions. The oysters dosed with the highest concentrations (10^6 particles L^{-1}) were, however, in a better condition than the oysters in all other treatments for days 10 and 20 but then their condition plummeted for days 40 and 80. We can only speculate on the reasons for this. Initially, the oysters seem to be boosted by MPs and it is only later in time that adverse health effects due to the high MP diet may manifest. This might be related to a higher filtration rate. The European flat oyster, O. edulis, exposed for 2 hours per day to MPs filters more algae h^{-1} than without MPs[377]. Likewise, an increase in filtration rates of the Pacific oyster was found in response to constant exposure to 6 µm PS microbeads[359]. This suggests that oysters filter more in response to plastic particles.

A study in clams, Atactodea striata, has shown that ingestion and retention of MPs were limited by the production of pseudofaeces and faeces[383]. We detected similar stress effects and found pseudofaeces containing high amounts of microbeads, a mechanism known to be a cleaning mechanism, preventing the gills being blocked by particulate matter[384], and as a rejection mechanism for inedible particles[385]. We found rather low amounts of PS microbeads in the faeces. In a similar study, the detoxification of PS MPs in clam tissues was inefficient for the 7 day duration tested[360]. Although this indicates that oysters have the ability to egest MPs via faeces, there is still potential for accumulation and trophic transfer [339,360] and/or effects of long-term exposure[386].

Continuous augmented filtration without improved food uptake may lead to biomass losses in the long term. Green et al.[376] reported that the biomass of the peppery furrow shell clam, Scrobicularia plana was ∼1.5 times lower in mesocosms with the high dose of MPs compared to controls. This indicates that repeated exposure to high concentrations of MPs may lead to “MP fatigue” in oysters, altering the condition of important ecosystem engineers and the formation of benthic assemblages.

All polymer particles with a diameter between 0.1 µm and 5mm are defined as microplastics. This creates several issues in relation to microplastic sampling, analytical and reporting procedures. Most field studies include only particles in a narrower range as microplastic, often determined by their sampling methodology or the detection limit of devices they used[293]. A commonly used lower limit due to mesh size lies between 300 – 800 mm, while the upper limit is often set between 2.16 and 4.75mm or up to 5mm[44]. Likewise, studies differ as to whether all particle shapes are included, distinguishing between fragments, spheres, sheets, pellets, ropes and fibers[293]. The present literature also reports highly variable metrics of concentration, such as averages, medians, maximum averages, average maxima and maxima[293]. To make matters worse, several studies report microplastics in different units. Microplastic concentrations are variably reported as mass or as particle numbers per mass, per volume or per surface area of water or sediment, or even per study site[293]. These differences in units and lack of complete quantification make it difficult to determine realistic concentrations. It makes comparisons between field observations very difficult and limits their usefulness for ecotoxicological experiments. Globally, the highest reported microplastic concentrations in the water column using a mesh size of ~ 300 µm is 10^2 particles L^{-1} [293,387] , measured near a harbour, close to a polymer production plant. Up to 100 000 times higher concentrations of small plastic fibres were retained on a 80µm mesh compared to a 450µm mesh[387]. Estuarine studies in South Korea reported high MP concentrations up to 23 particles L−1 between 0.2 and 1 mm in contaminated regions[388]. Applying smaller mesh sizes will retain a larger fraction of MPs[151]. The limits set in these field studies thus result in microplastic numbers being underestimated compared to the definition. Furthermore, concentrations of microplastics in the water column are known to be very heterogenous and variable[389]. For example, the abundance of plastic particles in the water column increased 6-fold shortly after a storm in California coastal waters[390]. Taking into account that amounts of microplastic are also underestimated by up to a factor of 30 when based on surface sampling[391], microplastic concentrations, especially the smallest fraction, might be much higher in reality and present a risk to the most sensitive species at hotspot locations in near-shore regions. Microplastics in sediment are

also expected to affect organisms feeding in the water column, via resuspension or transfer through the food chain[293]. Due to increased water turbulence or defouling, originally settled plastic particles are expected to become resuspended in the water column (especially in shallow and near shore environments) and lead to exposure of organisms feeding of the water column[392]. Considering size distributions of particles, it is clear that abundance increases with a decrease in size probably due to fragmentation processes[157]. Just based on mass conservation principles, fragmentation of spherical microplastic particles with a size of >0.1 µm – 5mm into 100nm nanoplastic particles would lead to particle concentrations that are ultimately $>10^{14}$ times higher than the currently found microplastic particle concentrations[293]. Detecting these smaller fractions of microplastics (<10µm) proves rather problematic and costly with current methodologies and are thus often overlooked and unreported.

Considering the above, we exposed juvenile oysters for almost three-months to what the authors believe to be a series of potential environmental concentrations of a prototypical MP of that size. The concentrations of 6µm PS microbeads in this study, although seemingly high, could well represent a range of potential scenarios for current, short-term and long-term concentrations of microplastics to which invertebrates might get exposed in the environment. Furthermore, the PS microbead concentrations in our tanks quickly dropped off, especially in the presence of oysters (about a factor 1000 lower after 24h), leading to a steady state concentration over 24h, the situation where the overall input of MPs is fairly in dynamic equilibrium with their elimination via the oysters uptake and removal in the form of faeces and pseudofaeces, interaction with algae and biofilms and/or static clumping.

Most importantly, we observed an increased mortality in oysters exposed chronically for 80 days to 6µm PS microbeads, dosed at concentrations of 10^6 particles L^{-1}. Such concentrations are currently not frequently reported in the marine environment but could be found near inputs such as harbours[387], rivers[367], sewage outlets[393] or estuaries[388]. The biological responses and increased mortality, however, seem rather specific to MP and less distinguished in bivalves exposed to suspended sediment plumes[394]. More research, detailing diverse experimental setups, testing different endpoints in a wide range of marine key species and ecosystems, including studies combining realistic mixtures of polymer types and different stressors (e.g. temperature increase, ocean acidification, contaminant & microbiological load) are all needed to allow for future comparisons and greater insights.

6.5 ACKNOWLEDGEMENTS

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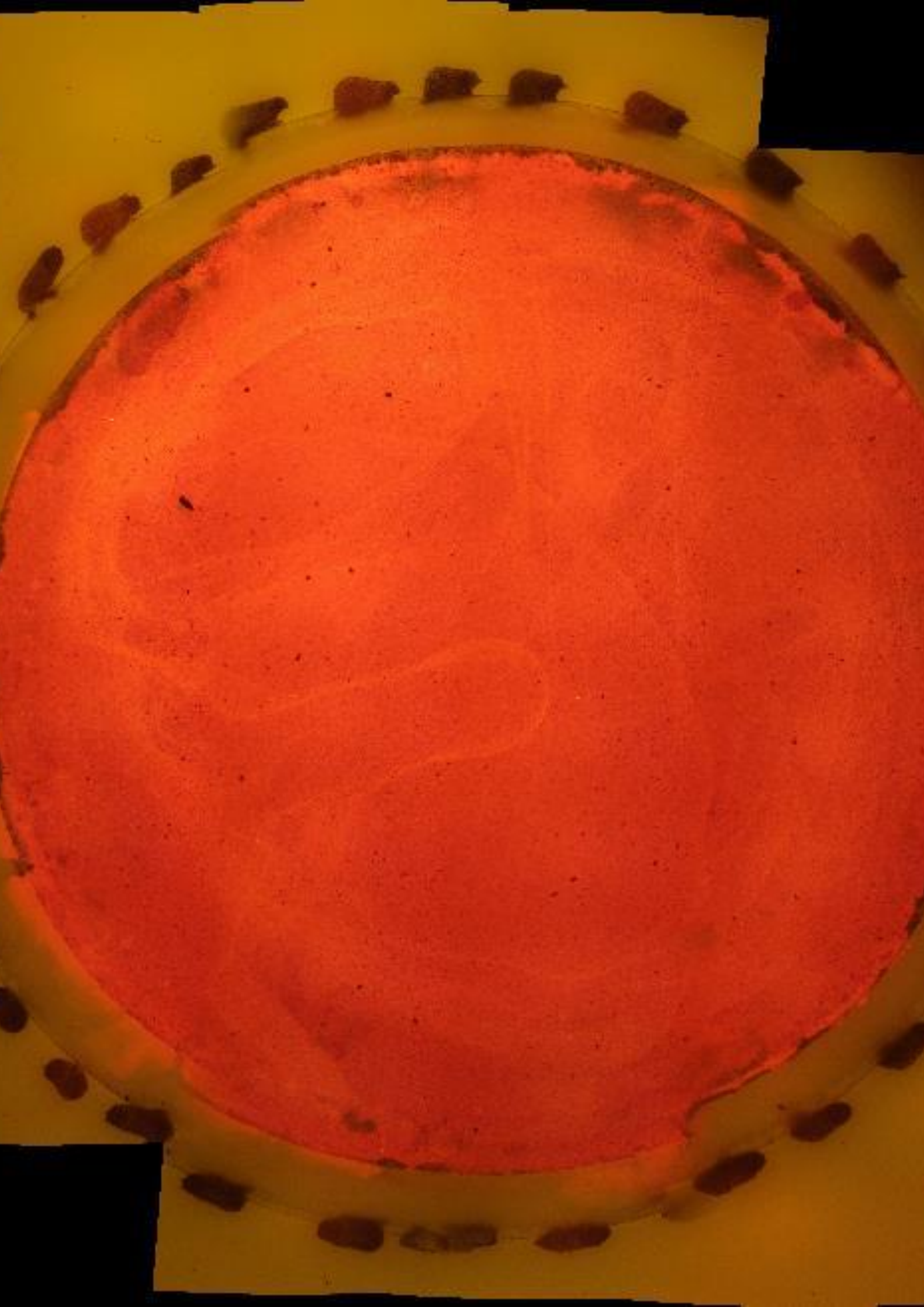

Chapter 7

A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red

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ABSTRACT

A new approach is presented for analysis of microplastics in environmental samples, based on selective fluorescent staining using Nile Red (NR), followed by density-based extraction and filtration. The dye adsorbs onto plastic surfaces and renders them fluorescent when irradiated with blue light. Fluorescence emission is detected using simple photography through an orange filter. Image-analysis allows fluorescent particles to be identified and counted. Magnified images can be recorded and tiled to cover the whole filter area, allowing particles down to a few micrometres to be detected. The solvatochromic nature of Nile Red also offers the possibility of plastic categorisation based on surface polarity characteristics of identified particles. This article details the development of this staining method and its initial cross-validation by comparison with infrared (IR) microscopy. Microplastics of different sizes could be detected and counted in marine sediment samples. The fluorescence staining identified the same particles as those found by scanning a filter area with IR-microscopy.

7.1 INTRODUCTION

Plastic litter, both at the macro and micro scale, is widespread and has accumulated worldwide in the marine environment. Due to ultraviolet (UV) radiation, oxidation and mechanical forces, plastic items break down into increasingly smaller microplastic fragments, below 5 mm in diameter[18,145]. Micro-sized fragments such as synthetic fibres from textiles, facial cleansers and many other products also introduce microplastics directly[145,161,219]. This has led to a build-up of microplastics of varying sizes, composed of different polymer types, across a wide array of marine habitats. Because of their size, microplastics are available and ingested by a broad range of organisms[43,177,255,263,264,335,350], possibly threatening ecosystems and even human health[229]. The risks that microplastics pose to marine life and humans are widely recognized and have been included in national and international marine protection strategies, policies and legislation (e.g. EU Marine Strategy Framework Directive[232]). Knowledge of plastic concentrations, spatial and temporal changes, sizes, polymer distributions and fragmentation dynamics are a prerequisite for understanding fate and impact of microplastics. To monitor spatial and temporal trends of microplastics, simple, cost-effective and standardized protocols, capable of efficiently and accurately enumerating microplastics in a wide variety of environmental matrices, need to be developed. Various floatation and density approaches have been described for microplastic studies in sediments[395]. Using the density increase caused by added salt solutions, microplastics float so they can be separated, filtered and analysed. Water column studies can use density separation or direct filtration methods for sample recovery. Biota studies will need to separate microplastics from the surrounding tissues after which they can be processed similar to water or sediment samples[308]. Such approaches lead to many filters containing various materials, including the putative microplastic fragments, which need to be identified and counted. For larger microplastics (0.3 to 5 mm) visual sorting is an accepted approach and one of the most commonly used methods for the identification of microplastics (using type, shape, degradation stage, and colour as criteria), but it still requires expert knowledge and judgement while being rather time consuming. In addition to visual quantification, recent studies have applied chemical and physical characterisation[151], vibrational spectroscopy[177,388,395–398] or electron microscopy[399–401]to reduce the risk of false positive/negative misidentification, to determine polymer types and to introduce automated routines[400]. Fourier transform infrared (FTIR) and Raman microspectroscopy have been used to allow polymer identification of particles down to a few µm[402]. There are several recent publications on automating IR-microscopy procedures for microplastic identification[398,403,404]to make it less labour intensive, but the techniques are not routinely applied for monitoring, because they are limited by slow speed, high cost and poor spectral resolution, which makes processing of larger sample sets by micro-spectroscopy challenging. IR microscopy requires technical expertise and assignment of individual particles from their spectral finger- prints is error prone, especially for small particles (<20 µm) where microscope resolution inevitably includes spectral signals from the surroundings (i.e. other adjacent particles or the filter itself). Polymers collected from the marine environment may have been exposed

to UV induced photodegradation, thermal degradation and biodegradation, altering the original polymer composition[405]. Bacteria within the coastal and marine environment can rapidly colonize microplastics, forming successional plastisphere-specific bacterial assemblages[325]. These degradation processes and biofilms, in combination with polymer additives, will further complicate spectroscopic analysis due to spectral changes and insufficient reference spectra for polymer degradation products[88]. This problem becomes more apparent for small particles, where the high surface to volume ratio makes the signals from surface material more significant. Many particles will thus fall into an unidentifiable category which is difficult to distinguish from natural polymers such as lignocellulose, chitin etc. Despite these shortcomings, the above-mentioned spectroscopic methods are the methods of choice for most studies of microscopic plastic particles, currently the only available approaches. To carry out the kinds of spatial and temporal studies necessary for emerging monitoring requirements, as well as addressing new research questions arising from increased awareness of the microplastics problem, much cheaper, faster and more easily applied methods urgently need to be created. Fluorescence staining methods provide a simple and sensitive approach to highlighting specific objects or structures in biological and medical studies. Andrady[406]proposed the use of a lipophilic fluorescent dye, such as Nile Red (NR) to stain microplastics in surface water samples, facilitating visualisation under a microscope, but this observation has not been followed up to date. NR is a lipid soluble fluorescent dye which allows the in-situ staining of lipids. It has been frequently employed to evaluate the lipid content of animal cells and microorganisms, such as mammalian cells, bacteria, yeasts and microalgae[407,408]. Furthermore, NR is solvatochromic, so its fluorescence emission spectrum shifts depending on the polarity of its environment. This behaviour might allow microplastics to be categorised into types based on their general hydrophobicity e.g. polyolefin, polyaromatic, polar (polyesters/nylons), or it could provide a useful indicator to evaluate residence time via temporal changes in surface properties due to oxidation or biofouling in the environment. In this manuscript, we present a detailed development and evaluation of this approach for the rapid screening of sediment samples for microplastics.

7.2 RESULTS

Multiple dyes (Oil red EGN, Eosin B, Rose Bengal, Hostasol Yellow 3G and NR) were tested for their ability to adsorb to plastics. NR was adopted, since it was the most effective in terms of adsorption and fluorescence intensity. The optimum dye concentration (between 1 and 1000 µg mL^{-1}) and incubation time (between 5 minutes and 66 hours) for visibility was determined. Using higher dye concentrations increased the fluorescence intensity of the dyed particles, but also increased the background signal from the Whatman filters. A working concentration of 10 µg mL^{-1} gave a good balance between visibility, speed and background signal. Fluorescence intensity increased rapidly with incubation time, but plateaued after 30 to 60 minutes and remained constant up to 66 hours. Incubation times longer than 30 to 60 minutes led to gradual aggregation of the unadsorbed dye (which has low water solubility) and stronger colouring of the filters, especially in the presence of higher concentrations of zinc chloride used to increase density. For most studies, incubation with 10 µg mL^{-1} NR for 30 minutes was adopted for staining. Different concentrations of ZnCl2 (from 0 to 1.8 g/g water) were trialed to determine the best density to cause microplastics to float, while ensuring that the vast majority of inorganic mineral particles and other potential interfering material sedimented during centrifugation[401]. A density of 1.37 g mL^{-1} provided a good compromise between maximising recovery and minimising interference from excessive unwanted particulates. Most common plastics have a density well below this value[409], while it is close to the density of PVC and PET (an important subset of frequently observed marine microplastics), hence only a very few unusual plastics (e.g. fluoropolymers) or dense composites would potentially be removed by sedimentation. Crab claw fragments, which showed a dull orange/red fluorescence, might give false positives in the counting. However, they are heavily mineralised with calcium carbonate, have higher density than plastics and are sedimented under the conditions of extraction (Supplementary Information (SI) Figure 6). Results of staining spiked particles of various polymer types in coarse and fine marine sediments

(30 particles in each sample) are shown in Table 7.1, with an image in Figure 7.1. The plastic particles fluoresced and could be counted easily (>100 µm). On average a 96.6% recovery rate was obtained. Samples with >100% recovery may have had additional microplastics present from the original sediment. This was confirmed from three unseeded control samples for each sediment. Control samples contained some very small fluorescent "dots", but also on average about 2 larger fragments per 1 g sediment. This represents microplastic in the control sample and/or a degree of contamination from labware and solutions, since at this stage no precautions were taken to avoid such contamination. This was addressed later by washing all equipment with filtered water (0.22 µm) and pre-filtration of all solutions through 0.22 µm filters (Whatman cellulose nitrate membrane filters or PTFE syringe filters) prior to use. A moistened wooden cocktail stick was used to collect any fluorescent fragments from the samples. Analysis of some of the small fluorescent "dots" from the control sediment by Raman microscopy gave strong bands indicative of calcium carbonate (see SI Figure 7), but the fluorescence staining suggested they were organic and hydrophobic in nature. These were most likely small fragments of mineralised chitin, which could potentially cause false positives. Chitin fragments are not buoyant under the conditions used for actual sample processing (see above) so they are separated from the microplastics and are unlikely to cause significant problems when using the proposed method, which gave very high (>97%) recovery in coarse sand, but a lower recovery of 85–88% in fine silt. This is probably due to a degree of entrapment and burial of microplastics and should be considered when reporting microplastic loadings.

Table 7.1. Recovery of seeded microplastics from sediment samples by direct counting of NR-stained fragments after NR staining with or without inclusion of the density separation step. The mixed polymer sample contained a total of 30 microplastics, 5 each of: nylon, PS, PVC, PET, PE and PP.

Protocol	**No extraction step**		**With extraction**	
Matrix amount	0.5 g	1.0 g	5.0 g	5.0 g
Microplastic type	Mixed polymers	Mixed polymers	nylon	PE
Number seeded	30	30	20	20
Sample	**CAP1 coarse sand**		**LIT 7C coarse sand**	
Replicate 1	32	27	20	17
Replicate 2	29	27	20	21
Replicate 3	31	30	19	20
Mean	31	28	20	19
S.D.	1.5	1.7	0.5	1.7
Recovery %	102	93	98	97
Sample	**SPI 6 fine silt**		**LIT 81C fine silt**	
Replicate 1	28	28	17	16
Replicate 2	29	32	20	20
Replicate 3	30	28	14	17
Mean	29	29	17	18
S.D.	10	2.3	2.4	1.7
Recovery %	97	98	85	88

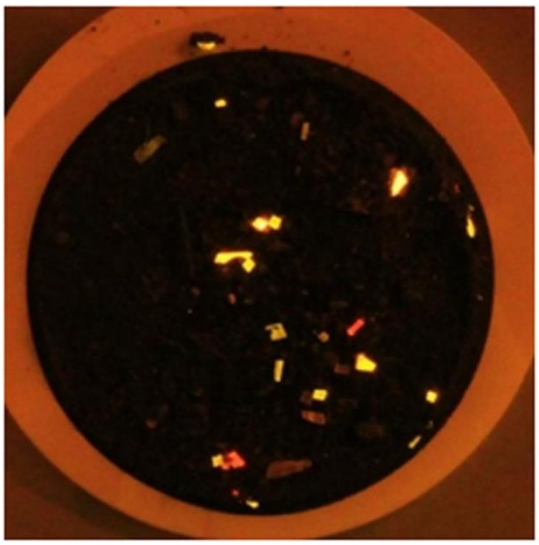

Figure 7.1. 1g of marine sediment (SPI 6) spiked with microplastics of six different polymer types, dyed with Nile Red (1000 µg mL^{-1}, 30 minutes), then filtered onto a 47 mm diameter membrane filter. Photograph taken with a blue light (Crime Lite: 450–510 nm) and orange filter (529 nm).

From these initial tests, it was also apparent that the different types of plastic displayed different fluorescent colours when stained with NR (Figure 7.1). NR is solvatochromic and its fluorescence emission spectrum red-shifts markedly as the polarity of the solvent increases (see SI Figures 9–11 for spectra and images demonstrating this). To investigate the potential application of this solvatochromic response, particles of individual known plastics were stained and imaged. The images were processed using Image J to determine the average RGB intensities from the image areas containing the stained plastic fragments. From the values, a simple "fluorescence index" was calculated as (R+G)/R. This equation normalised the overall intensity of the fluorescence and maximised the differences in colour, producing a single value that could be used to represent the "polarity" of the polymer surface.

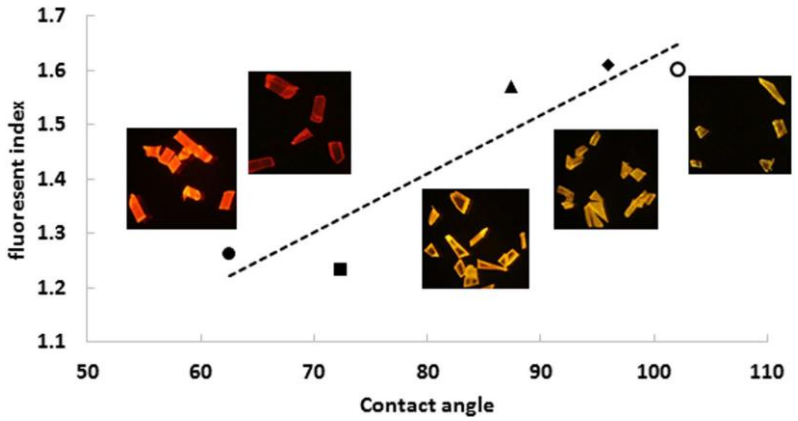

Figure 7.2. Fluorescent index, represented by (R+G)/R, plotted against published static contact angle values (a measure of the surface polarity). The actual images are inset to show the clear colour variations.

These values are plotted against literature values for static contact angle measurements (an easily-measured proxy for polarity) for these polymers in Figure 7.2, where the images of the actual colours observed are inset for reference. The graph shows a clear trend, confirming the relationship between polymer surface polarity and NR fluorescent colour. It was possible to group the polymers into "polar" (nylon, PET) and "hydrophobic" (PE, PP, PS) and this might be a useful distinction for general particle

counting and categorisation. Identification of individual polymer types using this approach is unlikely, but it offers promise (with further validation) for general particle categorisation, which might be useful for comparing proportions of different types of plastics with production or usage data to determine behaviour, fate, degradation etc. of plastics in the marine environment. Alternatively, it might provide an interesting tool to assess surface oxidation or biofilm adsorption onto plastic particles in relation to exposure time and conditions, in order to understand better the temporal changes that take place to particle surface properties.

When marine sediment samples were processed using the density extraction procedure, a certain amount of debris (organic material, black carbon fragments, small mineral grains etc.) usually floated to the top of the tubes, along with any microplastic fragments. The amounts and texture of this debris varied greatly depending on the nature and source of the sediment. A typical filter is shown in Figure 7.3 (sample 805). The white light image shows numerous particles on the filter surface, but the reconstructed fluorescence image of the whole filter demonstrates that only a few larger fluorescent particles are present in this sample. To detect smaller particles, it is necessary to zoom in and analyse the filter tile by tile. For method development, a 9 × 6 array of images was used, each one covering approximately 8 mm × 5.4 mm of the filter area, collected using the automated rig (see SI section 1 for details). A single pixel of the 5148 by 3456 pixel image array at this magnification thus represents about 1.5 µm, making it theoretically possible to image particles down to about 5 µm (assuming adequate optical resolution and taking at least 9 connected pixels to represent a real bright object, rather than random noise). Potentially, even smaller particles could be addressed, at the cost of time and effort, by zooming in further and using more tiles to cover the filter. Alternatively, for routine screening, a 7 × 5 array significantly reduces the number of images with little real decrease in the size limit of detection and this has now been adopted for our routine work.

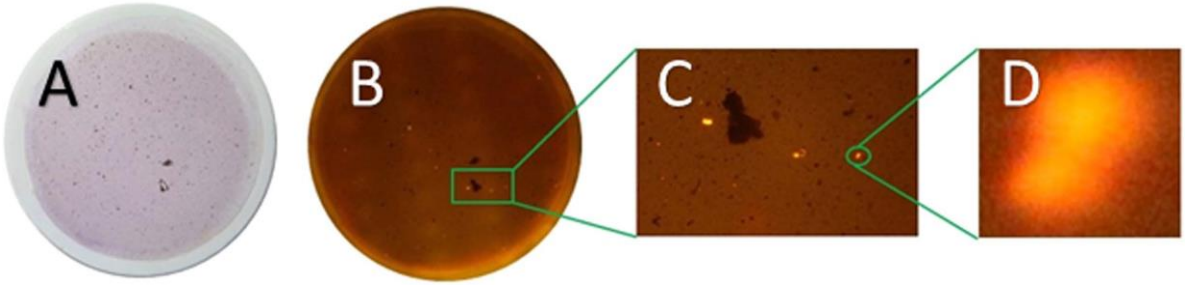

Figure 7.3. Filter images from processed sediment sample number 805. (A) white light, showing a variety of extracted debris; (B) Autostitch reconstruction of the 54 tiled images taken using a blue light and orange filter, (C) expansion showing three bright spots of fluorescently tagged microplastics and (D) close-up of one larger particle, approximately 100 µm across. Several bright spots much smaller than this are also clearly visible in image (C).

A typical result from a tiled filter image is shown in Figure 7.4, where part of a filter is shown, reconstructed from its individual tiles using free software Autostitch[410]. In Figure 7.4, three larger fluorescent particles were observed. These were sampled with a moistened cocktail stick and transferred to a clean Anopore filter for analysis by infra- red microscopy. The corresponding IR spectra are superimposed. This allowed the microplastics to be identified as polyethylene, polypropylene (fibre) and polyester (fibre) respectively. (More details from the IR microscope are shown in SI Figures 12–14). Careful analysis of this filter image, however, also shows at least an additional 25 small bright spots, which are also putative microplastics. These were too small to pick up and transfer reliably, however, so for the very small fragments an alternative approach was taken to validate the fluorescence staining result and demonstrate that these small fragments are indeed microplastics. A sample of sediment 295 was extracted using our method and filtered directly onto a

47 mm Anopore filter. This was observed and photographed under white and blue light and an area where a few very small bright spots could be seen was identified. An approximate 1 cm square was marked in the filter surface by scratching with a metal point, then the filter was fluorescence-imaged using 35 tiles in the normal way. The scribed area was reconstructed from the images. The filter was then transferred to the IR microscope and the whole scribed square scanned in rapid-scan mode. The various stages of this experiment are depicted in Figure 7.5 (with larger versions of the spectra available in SI Figures 15–20). The IR data were filtered for C-H stretch signals between 2800 and 3000 cm−1 to identify any organic material. Many particles were highlighted (Figure 7.5c), but inspection of the spectra at most of these locations (>100 were checked) indicated a consistent fingerprint of partially-oxidised carbonaceous material, which did not correspond with any common plastic. This is most likely "black carbon" material arising from decay of organic matter and it is clear from the fluorescence image that this material was not labelled with NR. Five locations were identified, however, with significantly different spectra.

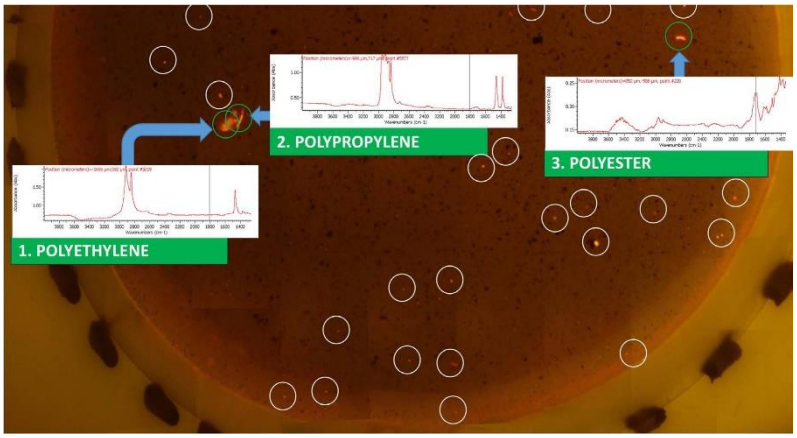

Figure 7.4. Part of a filter image from sample 805, reconstructed from individual tiles, showing fluorescent particles and, superimposed, the IR spectra obtained by picking the three larger particles and transferring them to an Anopore filter. This allowed them to be identified as common microplastics. Note also, the many additional small bright particles (25 have been ringed for clarity), which were too small to transfer reliably.

Spectra from highlighted locations 1–5 emphasise the problem of accurately identifying small plastic particles. These areas correspond with bright spots on the fluorescence image. Four notably different spectra are present and undoubtedly originate from polymeric material, but none can be assigned with complete confidence. Significant signals are present in the OH/NH stretch region between 3000 and 3700 cm−1, but these are weaker relative to the C-H stretches than would typically be seen for natural carbohydrate based polymers such as cellulose, carrageenan or chitin, or for proteinaceous material, suggesting that they are indeed anthropogenic. Particle 3, identified in Figure 7.5, has characteristic features of PET, in particular, the signals around 3500–3700 cm−1 and 1970 cm−1, as well as the strong carbonyl signal at 1730 cm−1. There are also notable differences between the spectra in the 1400–1800 cm−1 region, which indicate that particle 2 (Fig. 6.5) may be a polyamide, but particles 1 and 4 both have (different) balances of amide-like and ester character, which are difficult to characterise with confidence. This most likely results from heavy weathering and/or biofouling, introducing a complex balance of chemical functionality into the spectrum. Uncertainties over precise assignment notwithstanding, it appears that the fluorescent particles 1–5 identified by the staining method are indeed microplastic particles, providing validation that the method is robust and accurate in identifying microplastics. Inspection of the IR spectra around location 5 identified a single spectrum that had a form similar to particle 1. Since a 25 µm aperture was used in the IR spectral

imaging, this indicates that the particle must be very small, despite the quite bright spot on the fluorescence image. This indicates that even small microplastics are being picked up by the method.

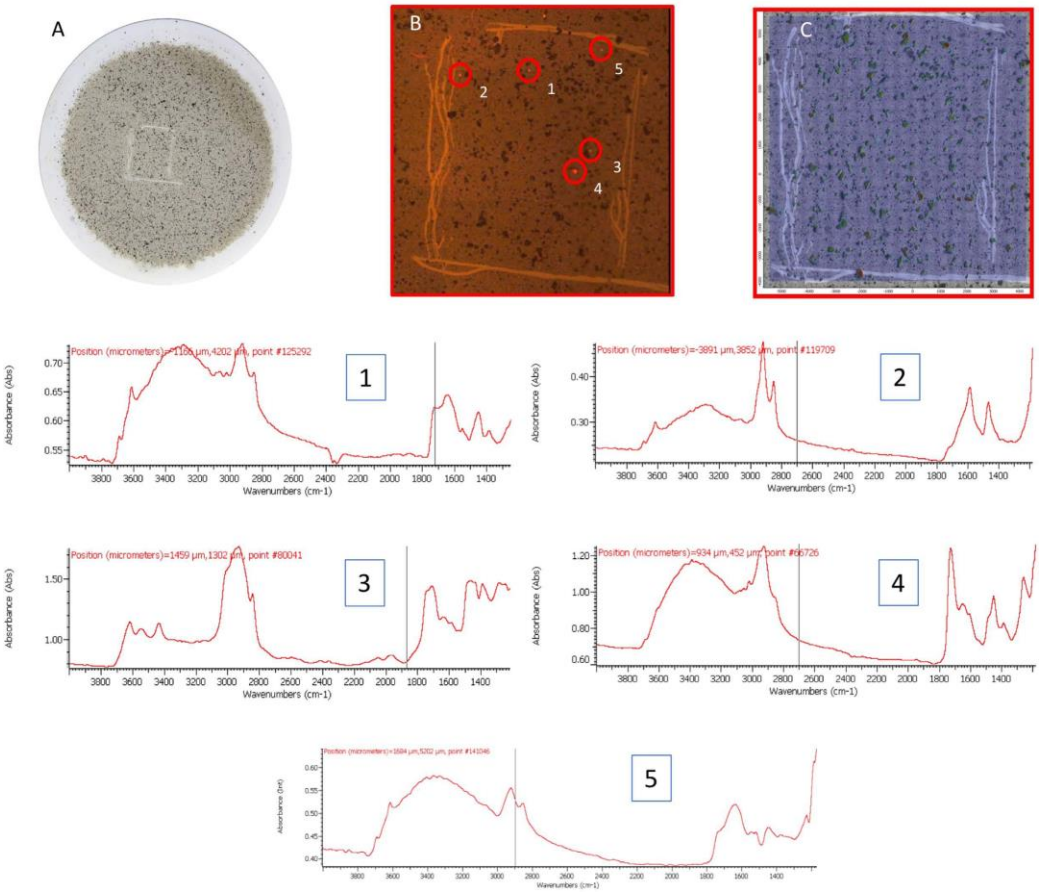

Figure 7.5. Image of the filter in white light showing (A) the scribed area; (B) expansion of the scribed area under blue light, photographed through an orange filter, reconstructed from tiled images showing the bright fluorescent objects identified, (C) tiled white-light image from the IR microscope overlaid with a C-H filtered IR spectral map to highlight organic material and below, IR spectra from the 5 locations ringed and numbered in panel (B).

The possibility that algae might stain using NR and hence produce false positives in the method was an important consideration due to their prevalence in the marine environment. No fluorescence was observed for any of the three algae cultures tested for interference using the lighting and optics used for microplastic identification (see SI Figures 20–22). NR staining of oil droplets in Tetraselmis has been widely reported, however, so this observation was explored further. Imaging with a fluorescence microscope (see SI Figure 20) showed that the algal cells were indeed stained, but high excitation intensity and long integration times for imaging were required, compared with those needed for microplastic fragments under the same microscopic imaging conditions.

7.3 DISCUSSION

This fluorescence staining method, in combination with density separation, provides a simple and sensitive approach to highlighting most common polymer fragments in marine sediments. The plastic types used in this study cover roughly 75% of annual European plastics demand and hence represent most plastic fragments likely to be found.

Validation required a demonstration that common materials and structures likely to be present in marine samples did not give false positives. From algal staining studies, we showed that while some algae may indeed be stained by NR, our protocol is quite inefficient for algal staining, lacking the higher levels of organic solvent usually used to enhance dye penetration into the algae, hence their fluorescence is weak in comparison to polymer particles, and they are not observed when imaged on the filter analysis rig. Similarly, other organic detritus, such as seaweeds, wood, feathers and various types of mollusc shells were shown to stain either very weakly or not at all, suggesting that the method has good selectivity for plastics under the conditions applied. Further discussion can be found in the SI.

The preliminary results showed that the solvatochromic behaviour of NR generated distinctively different colours for fragments from different types of polymers. This allowed microplastics to be grouped by polymer polarity and offers the potential to do basic polymer typing in the future. There is a need to further validate this "colour typing", however, to assess more fully the effects of intrinsic plastic colouration, weathering and biofouling. Indeed, it may provide a simple and effective tool for following these processes during environmental exposure, so further exploration of this behaviour would be valuable. While we have only tested a selection of polymers, they represent a wide range of polarity and surface functionality. Given the mode of interaction of NR with polymer surfaces (mainly van der Waals interaction with additional dipole interactions in some cases) there is no reason to suppose that it would not adsorb to any given polymer surface, including hard plastics, rubbers, resins etc.

Microplastics of different size fractions were observed in marine sediment samples using the described method and subsequently validated by FTIR microscopy. As a result of the fluorescent staining, microplastic fragments of a range of sizes and polymer types became clearly visible in blue light, which allowed them to be differentiated from other debris, making it much easier to sort samples and assess microplastic abundance.

The results showed fluorescing microplastics on the filters, with sizes from several hundred µm down to a few µm. The observed fluorescent particles in marine sediments indicates that microplastics have been settling down from the water column. Microplastics in the low µm range have rarely been reported, due to analytical issues and/or detection limits[3]. Our preliminary results indicate that microplastic abundance in sediments might have been underestimated previously. Further details of this work will be published elsewhere.

Depending on the required accuracy/certainty of analysis, the technique presented here can be used as a standalone technique for microplastic counting or in combination with existing FTIR or Raman instrumentation to speed up the process of object selection. The very small amounts of NR adsorbed on the particles did not interfere with IR or Raman spectroscopy. For instance, the white-light imaging optics in a FT-IR microscope could be easily adapted to excite with a blue LED and image through an orange filter to provide a fluorescence picture of a filter area, which could guide the operator directly to the microplastic fragments for IR imaging. As a stand-alone technique, the basic staining method allows for the detection and counting of particles down to a few microns using the described methodology, making it easy and inexpensive to apply globally in laboratories with basic equipment while providing a minimum standard operating procedure for microplastic quantification.

Very small objects down to a few micrometres could be detected on images of higher quality and thus the size limit of detection is defined by magnification and optical resolution. Already at this stage, sufficient microplastics were detected to complicate visual counting. Further improvements to the visual analysis are currently being developed, with automated image recognition/counting/measurement and RGB characterisation algorithms based on the polarity index.

Additional future developments are also envisaged by combining this approach with other image-based analytical methods to allow identification of the individual types of plastic. This would provide an even more powerful analytical approach, though the current method as described provides a simple and effective staining method to visualise microplastics. With appropriate alterations to the protocol, filtration steps to reduce volumes for water samples or digestion/solvent extraction methods generally applied for biota, the method should also be applicable to other matrices in which microplastic analysis is desirable, lowering cost and speeding up quantification processes.

7.4 METHODS

7.4.1 Materials and instrumentation

NR and acetone (AR) were purchased from Sigma Aldrich (Gillingham, UK). Zinc chloride (Acros, SLR) was purchased from Fisher Scientific (Loughborough, UK). Water used was 18 MΩ analytical grade. Whatman 25 mm and 47 mm diameter cellulose ester (0.22 µm), cellulose nitrate (0.45 µm) and Anopore (0.22 µm, aluminium oxide) filters were supplied by GE Healthcare. Glass membrane filtration apparati were used for all filtration operations, aided by vacuum from a KNF laboport pump. Photographs were recorded with a Canon EOS 600 or EOS 1200 digital SLR camera. For excitation, a high powered blue LED light source was used (Crimelite 450–510 nm, Foster and Freeman, Evesham, Worcestershire U.K). Fluorescent images were recorded through an orange filter (Kobo or Foster and Freeman, 529 nm) to exclude the incident blue light. FT-IR reference spectra and spectra to identify beach-found plastic litter were recorded on a Perkin Elmer Spectrum BX with a SensIR single pass diamond ATR attachment (16 scans; 4 cm−1 resolution). Infrared microscopy was carried out on a Thermo Scientific, Nicolet iN10MX infrared imaging microscope using a variety of settings and imaging modes. Raman spectra were recorded on a WiTec confocal Raman system with 532 nm laser excitation. Individual particles were dried onto gold-coated glass substrates for measurement and laser power was adjusted manually to give the best quality spectra. Fluorescence microscopy was done using a Zeiss SteREO Lumar V12 system comprising Axiocam camera, 2× ILL2500 LCD and an EXFO X-cite series 120. The microscope was fitted with an 80 mm NeoLumar Lens. Samples were placed on clean glass slides, covered with glass coverslips and imaged using transmitted light. Settings for GFP were used, with the installed GFP filter set. Samples were centrifuged in a Heraeus Biofuge Primo centrifuge with 6 × 50 mL rotor, using disposable plastic 50 mL centrifuge tubes (polypropylene tubes with blue polyethylene screw caps, supplied by Fisher Scientific). Marine sediment samples (supplied by Cefas) were collected from various locations around the UK coast. Each sample was dried in a vacuum oven to constant weight, using a bleed of filtered air to remove moisture from the oven while avoiding contamination from ambient dust. The samples used in this study are shown in Table 7.2.

Table 7.2. The samples used in this study.

Sediment	Texture	Latitude	longitude	Region
SPI 6	Fine silt	54.98610	–1.25050	Greater North Sea
CAP 1	Coarse sand	54.98200	–1.25000	Greater North Sea
LIT 79C	Coarse sand	50.42255	-2.86462	English Channel
LIT 81C	Fine silt	50.53553	-3.19052	English Channel
805	Mixed sand/silt	54.06000	-3.87970	Celtic Sea
295	Sandy	54.73330	-0.88330	Greater North Sea

7.4.2 Filter Imaging

The automated filter-scanning rig used a commercial micro-milling machine (Sanven, China) to provide automated XYZ motion, combined with a trinocular microscope head and a photo-adaptor to connect the Canon EOS camera. Further details can be found in the SI (section 1). The camera was operated via USB using the Canon remote shooting software. The camera was first focused using white light and

manually changing the Z-axis of the milling machine. The blue light was then used for fluorescent imaging. A G-code routine was written to control X-Y scanning (listed in SI section 8) and a series of slightly overlapping photographs were taken to cover the whole filter area. The demonstration version of AutoStitch33 was used to generate panoramic image stitching by automatically recognising matching images.

7.4.3 General method development

Nile Red (NR) stock solution was prepared at 1 mg mL^{-1} in acetone and filtered using a 0.22 µm PTFE syringe filter into a clean glass screw-top vial and used for all the staining experiments. Zinc chloride solutions were made up in analytical water at varying concentrations and filtered through 0.22µm cellulose nitrate filters into clean glass storage flasks with ground glass stoppers. Analytical water was filtered in the same way and used for suspension of microplastic samples and sediments.

Microplastic fragments (typically 0.1–0.5 mm) were prepared using a sharp scalpel to scrape fragments from blocks of virgin plastic, consumer plastic items identified through their recycling symbols or waste plastics picked from the tideline on Lowestoft beach. U.K. The identity of all test materials was confirmed by FT-IR measurement prior to use. The plastics used were polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (polyester – PET), polyvinylchloride (PVC) and polyamide (nylon 6).

Staining was carried out by adding NR stock solution in acetone to give a final concentration of 1, 10 or 100 µg mL^{-1} in the suspension of microplastics or sediment, with or without zinc chloride, depending on the experiment. Adsorption time varied between 5 minutes and 66 hours in the optimisation study, at varying concentrations. For most work, 10 µg mL^{-1} and an exposure time of 30 minutes was used.

7.4.4 Method validation.

Specificity in relation to polymer type

For initial spiking experiments, 1 g of dried sediment was weighed and spiked with a known number of microplastic fragments of six different polymers: nylon, PS, PVC, PET, PE, PP. The sediment was suspended in 5 mL water, dyed with 50 µL NR stock and incubated on a Heidolph Rotamix shaker at 100 rpm for 60 minutes. The sediment was then vacuum filtered (Whatman 47 mm cellulose nitrate filter membrane 0.22 µm). The samples were viewed under a blue light (Crime Lite: 450–510 nm) through an orange filter (529 nm) and seeded microplastics were counted. The filters were also photographed.

To investigate solvatochromism of the adsorbed NR, images containing nylon, PS, PVC, PET, PE and PP fragments were analysed. The fluorescent particles were identified in the images and their RGB values extracted using Image J (https://imagej.nih.gov/ij/). These values were then transformed into a "fluorescent index" value, (R+ G)/R, using the 8-bit colour intensity values in the red (R) and green (G) channels to provide a simple comparison value, which can be related to the polymer type.

Density separation for microplastic extraction from sediments

For microplastic separation, zinc chloride solutions with differing densities were prepared gravimetrically from a freshly opened bottle of zinc chloride and the densities measured by weighing a fixed (100 mL) volume. Values are given in SI Table 1. Microplastics of different known composition were tested for floatation in the various solutions under centrifugal conditions, along with samples of different types of coarse and fine marine sediment. A density of about 1.35 allowed floatation of all the polymer types tested (along with small amounts of sediment material), while most of the sediment material settled to the bottom of the centrifuge tube. Some sediment material remained buoyant under these conditions. The density could be reduced to decrease the fraction of floating material, but with the risk that some denser microplastics might be missed due to sedimentation.

Recovery of spiked microplastics

To test the recovery rate of seeded microplastics from marine sediments, a fine sediment, LIT 81C and a coarse sediment LIT 79C were chosen. Triplicates (3 × 5 g) for each sediment type were weighed and seeded with 20 nylon and 20 PE NR-dyed microplastics. The seeded sediments were slowly added to the zinc chloride solution (30 mL, 1.37 g mL^{-1}), mixed, then centrifuged at 3900 g for 5 minutes with a braking speed of 9. The fluorescent microplastics were collected from the top of the solution with glass Pasteur pipettes using blue/green incident light (450–510 nm) through an orange mask (529 nm) to visualise them. The samples were then made back up to volume with zinc chloride solution and resuspended, then centrifuged and extracted again. This was repeated to give a total of three extractions for each sample. The recovered particles were combined, filtered, photographed and counted.

Cross validation and confirmation with FT-IR

For validation of the fluorescent method compared with imaging FT-IR, real unspiked sediment samples were processed as above, then filtered onto 47 mm Anopore filters (0.22 µm; aluminium oxide). Large fluorescing fragments were handpicked using a wooden cocktail stick moistened with ethanol, resuspended in approximately 1 mL ethanol, and filtered onto a small Anopore filter (25 mm) and analysed by FT-IR microscopy in transmission mode, using bare filter to set the background.

Further analysis of even smaller fragments on the 47 mm Anopore filter was carried out by marking an area of about 1 cm by 1 cm. This area contained fluorescing particles as observed under blue/green incident light (450–510 nm) through an orange mask (529 nm). The filter was then photographed using the automated fluorescence scanning rig and the scratched area identified and reconstructed from tiled images. The same area was imaged using the FT-IR microscope in transmission mode, using bare Anopore filter as the background (25 µm× 25 µm pixel size, 1 scan at 16 cm−1 resolution)). Putative microplastic particles were identified by filtering the spectral data array for C-H stretch signals around 2800–3000 cm−1 (indicating likely organic material). Regions containing significant C-H signals were further analysed to provide spectra for identification. The IR map of the imaged area was compared with the fluorescence image to check for coincidence of the fluorescent particles and the microplastic fragments identified from the IR.

Specificity/selectivity in relation to biological materials

A possible drawback of this staining approach is the possibility that false positives might be introduced because of staining biological organisms such as marine algae. These can be found in a wide range of sizes and forms. It is well known that some (though not all) of these organisms can be stained with NR31, and indeed this has been developed as a screening assay for algae that produce lipid droplets32, due to the interest in this area for biofuel production. In general, algal staining protocols include a water-miscible organic solvent (typically acetone, DMF or DMSO) to improve dye penetration into the organism. Our plastic staining method has a low solvent concentration (1% acetone, introduced from the NR stock, com- pared with 25% DMSO in an optimised algal staining method) so it is rather inefficient at staining algae. The protocol was tested on three marine algae representing different classes, morphologies and size scales – *Diacronema lutheri* (4–6 µm), *Tetraselmis suecica* (10–15 µm) and *Skeletonema sp.* (filamentous, diameter 2–20 µm). Once stained, the samples were filtered and imaged as for microplastics. Samples of dyed algae and microplastics were also investigated using a Zeiss fluorescence microscope with GFP filter set and settings optimised for green fluorescent protein analysis. A wide range of other organic materials that might be found in sediments (wood, seaweeds, common whelk egg cases, feathers, cotton fibres, paper, crushed shells, crab and shrimp claws etc.) were also tested for NR staining.

7.5 ACKNOWLEDGEMENTS

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Chapter 8

Shades of grey: Marine litter research developments in Europe

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ABSTRACT

European research efforts to address concerns in relation to increasing levels of marine litter and potential effects on ecosystems and human health have been launched. We assessed a total of 52 European projects which researched or contributed to the implementation of European marine litter legislation. These projects ranged from national initiatives, to large scale programmes involving multiple EU member states. The best represented topics within those European projects were 'Policy, Governance and Management' and 'Monitoring'. Comparatively 'Risk Assessment', 'Fragmentation' and 'Assessment Tools' were underrepresented. The analyses showed that West-European countries have contributed more to marine litter research and therefore received more funding. As a result, thematic hotspots were present, and scientific capacity is concentrated by topic and countries. The results indicate the need to continue to support initiatives to cover clearly identified gaps, either geographic or thematic, to deliver risk assessments and recommendations to address the marine litter issue.

8.1 INTRODUCTION

Manmade waste in the marine environment, or 'marine litter', is a global concern, affecting all the oceans of the world with clear effects across marine systems. Every year, millions of tonnes of litter, particularly plastic-based litter, originating from a variety of land and sea- based sources end up in the ocean worldwide[163]. Recent studies have estimated that 275 million metric tons (MT) of plastic waste was generated in 192 coastal countries in 2010, of which 4.8–12.7 million MT could have entered the ocean[163]. In total, it has been estimated there are 5.25 trillion pieces of plastic debris in the ocean[217]. European seas are no exception: large quantities of litter have been documented on beaches[199], the seafloor[411] and floating at the surface[389]. Some of the highest densities of marine litter recorded were in enclosed seas such as the Mediterranean or Black Sea[126,189]. Due to UV radiation and mechanical forces, plastic litter in the oceans slowly breaks down into smaller fragments, which are known as microplastics when these fragments are smaller than 5 mm^3. Like macro items of plastics, microplastics can be found worldwide and have been reported in all four regional seas of Europe[145,199,234]. Observations of biota suffering from entanglement and ingestion are commonly observed in Europe[266].

Impacts of marine litter range from wildlife entanglement and ingestion, to habitat damage. Marine litter is also an eyesore, degrading the beauty of the marine and coastal environment, with the potential to further impact tourism and associated ecosystem services[412]. These impacts pose substantial economic costs to commercial fish stocks, the tourism industry, and other ecosystem services[413]. There has also been growth in public attention to marine litter: since the mid- 1990s, various organisations have sought to educate the public regarding the impact of man-made waste entering the sea. This is also reflected in peer-reviewed journals. Publications relating to marine litter sources, pathways and effects numbered at less than 40 per year in 1995, which increased to over 200 in 2013[7]. Policy is following suit and administering various national and international instruments, most notably, a dedicated legislation was introduced to deal with marine litter and its impact on the coastal and marine environment[167]. In Europe, this legal framework was enacted with the Marine Strategy Framework Directive (MSFD), introduced in 2008. The MSFD incorporates an indicator specifically in relation to litter (Descriptor 10: 'marine litter does not cause harm to the coastal and marine environment') and requires evidence that member states are moving towards Good Environmental Status (GES)[12]. A dedicated task group (TG ML) was created to address the scientific and administrative needs, currently known as the MSFD task group TG 10 (2010) and the GES-Technical Subgroup on Marine Litter (2011- to date). The main aim of the MSFD TG ML is the Common Implementation Strategy (CIS). Activities are based on a mandate and work programme agreed within the CIS and approved by the EU Marine Directors. The group reports through the Good Environmental Status (GES) group. The TG ML serves for information exchange, as discussion forum, providing guidance and facilitating science-policy interface. The TG ML report[41] provided a synthesis of the

current research needs to understand the mechanisms and processes associated with litter at sea. The following research strategy was defined in the 2011 report:

- Clarify any fundamental research gaps required to link quantities of litter and associated harm in the context of GES.
- Within the MSFD context, research must be conducted at the region/ sub region level to give a scientific and technical basis for large scale monitoring.
- Research must define priority (highly affected) areas.
- Harmonisation and coordination of common and comparable monitoring approaches are required.
- Research will support guidelines to assess GES on a regional/ European scale.

The 2011 TSG-ML report[41] identified various short-term research priorities to support the start of monitoring by 2014. These priorities comprise: the mechanisms, fate and impacts of marine litter in relation to marine resources; the standardisation of methodologies, baselines and protocols for monitoring, and; use of modelling and automation to facilitate monitoring and management.

The TG10 assessment indicated there was an urgent need for harmonisation and research collaborations[30] across Member States (MS). Harmonisation will require coordination by relevant representatives from each MS. This process will lead to common and comparable monitoring approaches, recommendations and guidelines to assess GES over a regional scale[40]. Furthermore, there is a clear need to improve monitoring, helping to expand the coverage, increase current understanding of baselines and allow scientists to examine the effectiveness of existing or new governance arrangements. Further research will need to include a much-improved knowledge concerning: impacts on marine life; degradation processes at sea; the study of litter-related microparticles; the study of chemicals associated with litter; the factors influencing the distribution and densities of litter at sea (human factors, hydrodynamics, geomorphology etc.); the normalisation of methods, and; the determination of thresholds. The assessment and monitoring of socio-economic harms has also been highlighted as an area, where targeted research needs to be done [30,232,414].

This study aims to review the EU funded projects in relation to marine litter, with the view to provide an overview of the funded effort to undertake research activities to document and assess marine litter in EU waters. The results of which will help to assess the strength of current understanding of the input, fate and interactions of marine litter with the environment, economy and human populations. This will be done through the provision of recommendations for future research areas, informed by the gaps identified in the project review. This will help to harmonise research efforts across different institutions and member states, to support some of the issues highlighted under the EU MSFD, Descriptor 10 targets.

8.2 MATERIAL & METHODS

A literature review of all funded European projects, specifically those relating to marine litter, was conducted under the umbrella of the Columbus project. The assessment was informed by communication with other experts. The overview of projects was synthesised in a dossier containing the relevant projects[415]. The main sources for this review were: The EurOcean Marine Knowledge Gate, the COLUMBUS assignment of projects to its structure of Competence Nodes, the COLUMBUS deliverable 5.3 'Overview of FP7 projects relevant to major Marine and Maritime Regulations: MSFD, MSPD and CFP and Blue Economy activities', the STAGES Project Deliverable 'State of the Art Report - Theme 3 Disturbances', 'The EurOcean Marine Knowledge Gate, CORDIS, LIFE Programme repository and JPI-OCEANS project database.' In addition to this, the list includes national initiatives funded by Member States that specifically tackle issues related with litter in their own and neighbouring marine environments. Over fifty projects relating to Marine litter within the European Union (including

countries with 'Associated Country' status) were reviewed. The rationale for the project selection was based on several factors such as: the projects contribution to implementing the MSFD descriptor 10. Most of these projects focused solely on marine litter, however, some projects had multiple points of focus. These projects were mostly large scale, focusing on building capacity to attain GES in reference to the MSFD. In order to approximate the amount of funding dedicated to marine litter research within these projects, the total funding for these projects was divided by the proportion that marine litter represents within the MSFD, i.e. 9% (1 descriptor out of 11). Where more detailed information was available, funding was defined by the number of deliverables or work packages dedicated to marine litter per country or coordinator in the absence of country specific details.

Three independent reviewers sorted through the projects and placed the projects into previously defined research categories. The reviewers recorded project duration, participating countries, and funding granted by country. Where more than one institution in a country was involved in a project, the funding amount was totaled and recorded as total funding per country. Each project was then reviewed to examine the extent of how they contributed to specific marine litter research topics and the significance of their results. To ensure consistency, definition statements were compiled for 13 prevalent research categories in marine litter (Table 8.1). Projects were then scored at three levels: blank, '+' and '++'; based on the following two criteria:

Criteria 1: Does the project have a research output that is clearly relevant to the research category in relation to its respective definition statement?

- No – The project row is left blank in the scoring matrix
- Yes – The project is marked '+' in the scoring matrix

Criteria 2: Is the research output a significant contribution to the research category such that it is the or one of the principal outputs of the project?

- No – The project remains marked as '+' in the scoring matrix
- Yes – The project is marked '++' in the scoring matrix

This methodology helped to ensure an objective framework to generate results that are as unbiased as possible. The scores (blank = 0, '+' = 1, '++' = 2) for each category were totaled (Figure 8.1), to display the contribution of projects against the various research categories. The scoring allowed us to identify the areas of marine litter research that have been well established, and the areas in which there are still knowledge gaps, which could be addressed by future research projects. In this study, we applied the MSFD definition of micro litter, particles below 5 mm[30]. The research category scoring results are displayed in the supplementary information (SI).

Table 8.1. Summary of definitions for each of the research categories.

Category	Definition
Analytical method development	Outputs which comprise a novel method of monitoring and/or assessment
Assessment tools	Protocols, technologies, or techniques which are designed to advance and optimise assessment of issues related to Marine litter
Bioaccumulation	Research which concerns the uptake and magnification of pollutants/litter substances throughout escalating trophic levels
Education and outreach	Outputs which seek to educate, inform and raise awareness of either public or industrial audiences/stakeholders.
Fragmentation	Research which studies the partitioning of macroplastics down to microplastics.
Impact and effect	Projects which explicitly test the impact of a phenomenon and the effect that it produces or does not produce.
Modelling	Outputs which use modelling as a mechanism or develop innovative modelling tools
Monitoring	Projects which actively monitor prevalence, distribution and/or composition of litter, or projects which actively research optimal methods for monitoring. Projects which use data from previous monitoring projects have not been scored for this category
Policy, governance and management	Any output that explicitly informs policy development or researches alternative governance/management schemes, as its primary aim.
Reduction	Any measure or strategy that aims to mitigate future marine litter, by reducing demand for prevalent types of litter.
Removal	Projects which concern the removal of litter that is already in the marine environment.
Risk assessment	Research which informs or designs the framework of a risk assessment for marine litter.
Socio-economics	Projects which assess the impacts that marine litter has on society (for example, projects which assess public perceptions of litter).

The information collected from the projects was used to summarise the results for each criterion examined. Heatmaps were created to measure the contribution of various countries to the research categories. These data were then further explored in two timelines: one Gantt style timeline examining the duration of the 52 projects in relation to whether they focused on litter size (Figure 8.2), and; one bubble style timeline examining the trend of research category development, measured against key events relating to marine litter (Figure 8.3).

8.3. RESULTS

There was a broad distribution of scores for each project, the trends of which are assessed in the Discussion section. The highest scoring categories (highest number of projects) were 'Policy, Governance and Management' ('PGM') and 'Monitoring', which were represented by 24 and 23 projects respectively. Comparatively 'Risk Assessment', 'Fragmentation' and 'Assessment Tools' were represented by less than 7 projects each (Figure 8.1).

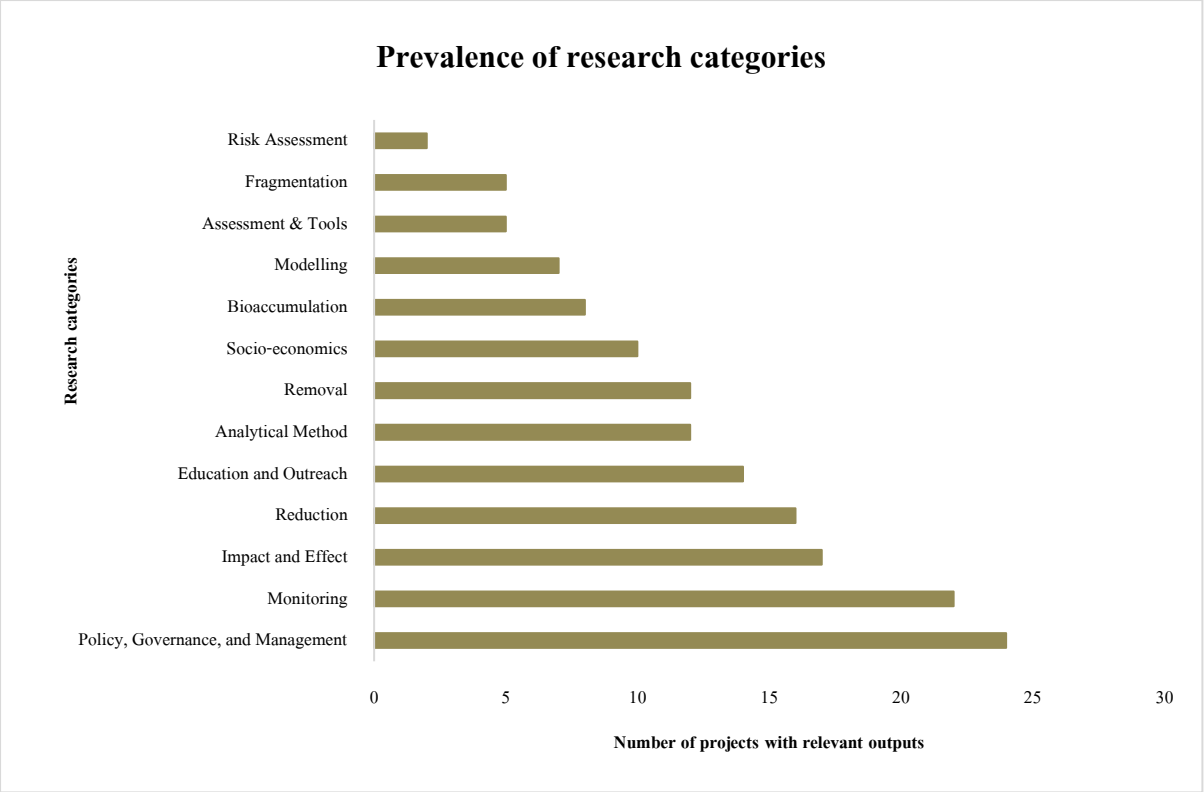

Figure 8.1. Summary diagram displaying the prevalence of research categories across Europe.

Additionally, we also classified each project on whether they focused on plastic waste management and then whether they involved microplastics or macro items of plastics (Figure 8.2). From the 52 revised projects, 23 projects involved only macro litter, 12 involved only microparticles, and 17 involved both micro and macro litter. Most of the research projects in progress between 2008 and 2010 and in 2016 focused on micro litter only, otherwise, macro litter was the dominant research focus. Most projects (40/52) involved plastic waste management to some extent. Projects which did not concern plastic waste management focused on discarded fishing gear (10), cigarette butts (1), and shipping containers (1).

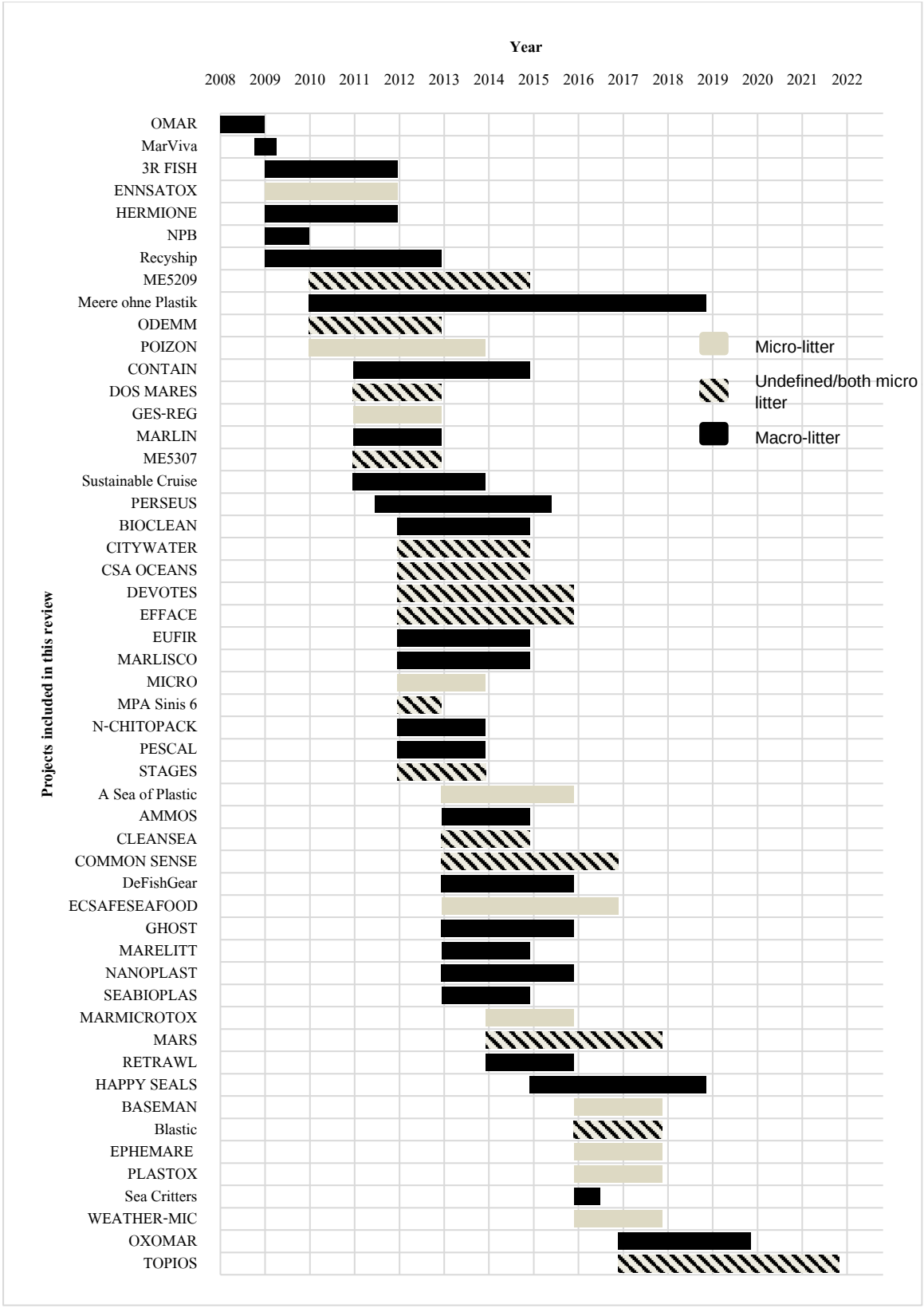

Figure 8.2. Overview of projects categorised by their focus on micro-litter, macro-litter, and both or undefined.

Marine litter research in Europe is driven by legislative demands such as the MSFD (Figure 8.3). We also calculated the total contributions for each research category and associated country and displayed these data on an individual heatmap by category (Figure 8.4 and further details provided in SI). Distinctive patterns of research categories across Europe appear clearly and support numerical evidence, i.e. there is a broad range of research contributions for Monitoring (1–24 projects), whilst `Fragmentation' has a low range of contributing countries (0–2 projects).

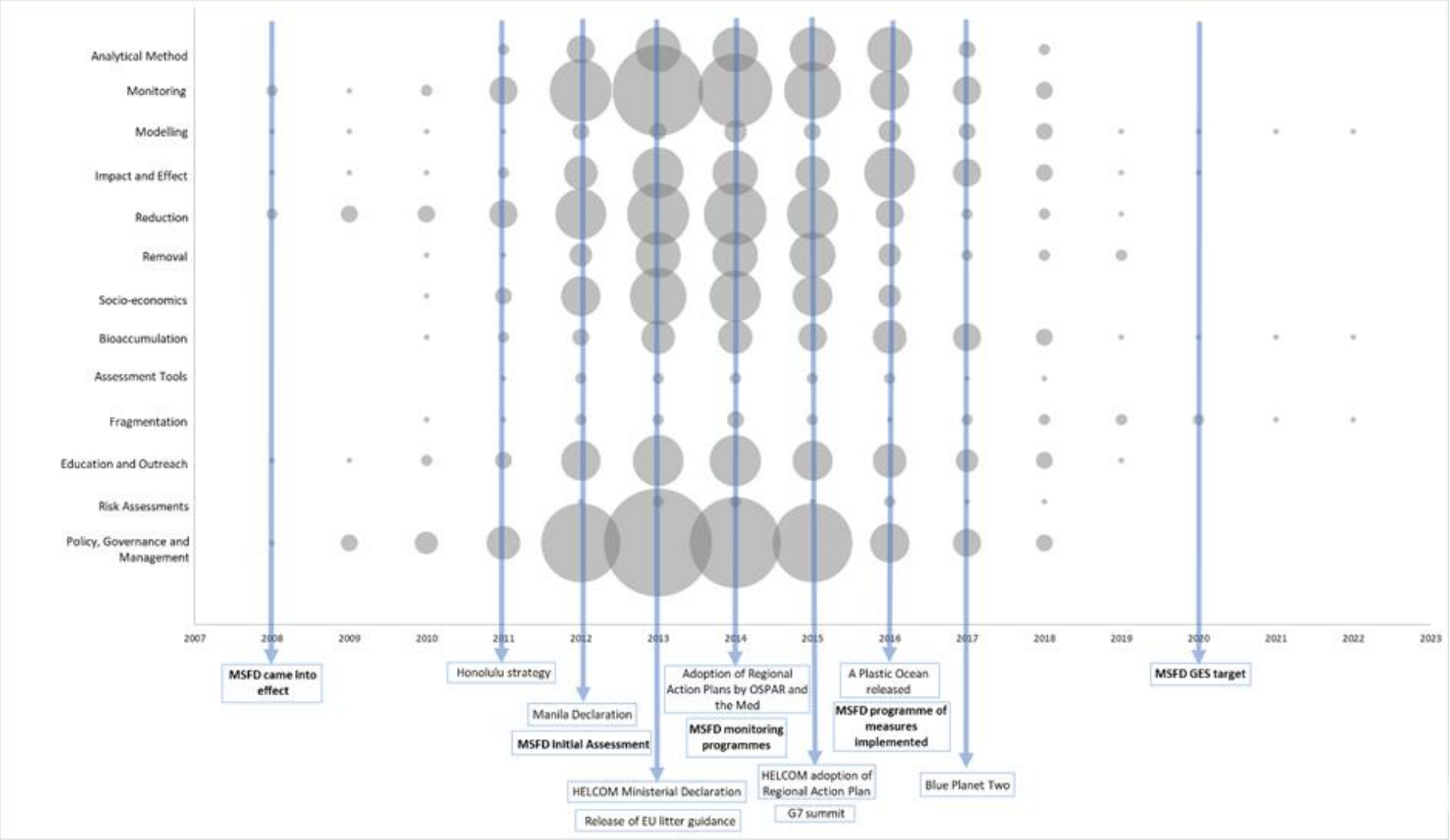

Figure 8.3. Timeline displaying current legislation drivers across several research objectives of the different projects.

Figure 8.4. Compilation of heatmaps (see more details in SI, displaying contribution by countries to: i) Reduction; ii) Analytical Method Development; iii) Monitoring; iv) Impact and Effect Studies; v) Education and Outreach, and; vi) Policy, Governance and Management. The darker the country, the larger the contribution.

Information in relation to project funds were mostly publicly available (either on project websites or on the European Commission's CORDIS website), however, information for 26.9%, which represented 14 of the projects were either undiscoverable or were no longer publicly accessible. Projects with some focus on marine litter (and for which funding information could be located) were awarded a total of €141 M funding, of which approximately 75% was EU funded. Most of these projects focused solely on marine litter, ranging from ship recycling, to plastic litter and lost fishing gear. Eight projects were identified as having multiple points of focus, these were COMMONSENSE, CONTAIN, DEVOTES, ECSafeSeafood, HERMIONE, MARS, ODEMM and PERSEUS. When applying the MSFD proportion (9% - 1 descriptor out of 11) and considering specified marine litter deliverables, total funding for marine litter solely comprises €64.6 M.

Looking at overall funding for programmes which to some extent study marine litter (€141 M – 75% EU funded), Italy (€13.2 M), the UK (€12.9 M) and Spain (€10 M) received the highest amounts of funding, comprising 36% of all funding combined. Liechtenstein, Luxembourg, Malta and Slovakia did not participate in any of the projects in this review, whilst most other landlocked countries such as Macedonia (€90 K), Hungary (€228 K) and the Czech Republic (€345 K) received some of the lowest quantities of funding. Especially countries of the North Sea and Mediterranean Sea Basins seem to have received the greatest amount of funding (€40 M each), closely followed by countries bordering the North Atlantic. Some projects focused specifically on areas such as the Baltic and Adriatic Seas, however, approximately half of all projects related to pan-European aims and objectives. As such, it was not possible to delineate exact funding according to Sea Basin (Figure 8.5a–b). Whilst these results may give direction to future funding pathways in future, they should not be considered wholly representative and are intended to be an estimated indication of research effort placed on marine litter in Europe.

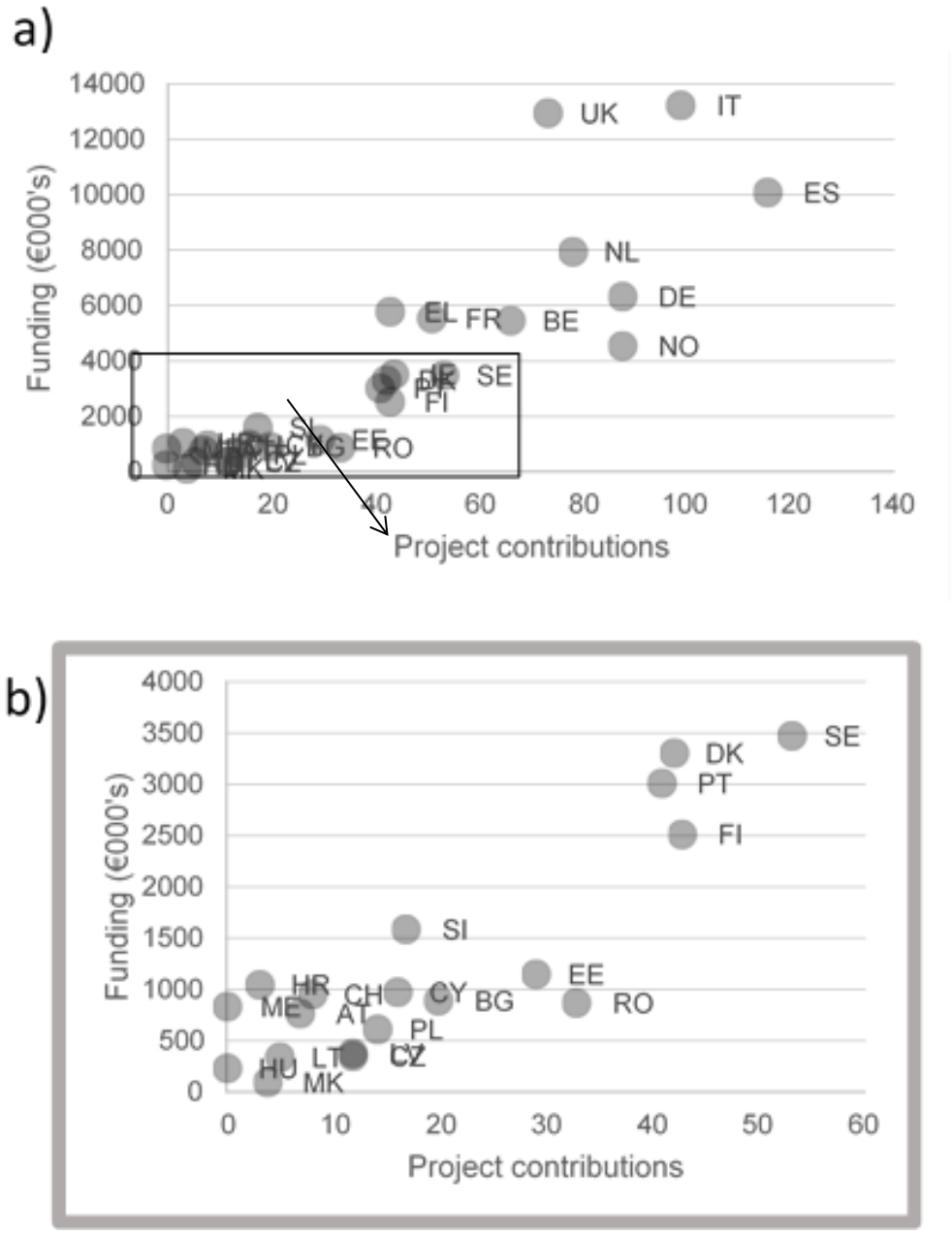

Figure 8.5. (a)Scatterplot of country (ISO 3166-1 alpha-2 code) involvement in marine litter projects, project values plotted against country representation (number of partners) (b) zoomed in section from Figure 8.5-a.

8.4 DISCUSSION

From our analysis, it becomes clear that the research categories of 'PGM' and 'Monitoring' were represented in the largest number of projects, with Italy acting as one of the lead countries for these categories. Conversely, the category of 'Risk Assessment' was included in the least number of projects (less than five), suggesting further research into this area is necessary. The projects were also categorised based on whether they focused on macro or micro litter, or a combination of the two. This gave some indication that micro litter research has increased since 2008 although macro litter research has generally remained the more represented type of litter.

There was some variety in the scale of projects that had outputs related to 'PGM', such that there were projects which focussed on a single country's national plan, those which concerned macro regions such as the Baltic and the Adriatic Sea, and those which concerned frameworks of international regulation across various countries and macro regions. Whilst it is encouraging to see that there are still major research opportunities for marine litter, research organisations are simultaneously developing the necessary 'PGM' tools to action marine litter threats (Figure 8.3). 'PGM' project outputs ranged from pilot management schemes to collect and recycle discarded fishing gear[416], to projects which built regulatory and policy standards for shipping container surveillance[417], and large-scale projects aimed at facilitating an effective research governance frame- work[418]. 'PGM' is multi-faceted and there is no significant positive skew in research effort, however, there remains various areas which still require further investment and development.

Many of the 'PGM' outputs comprised preliminary management schemes to coordinate litter removal and management at ports, beaches etc. Others concerned the establishment of research and policy frameworks. There was little output concerning risk assessment or 'PGM' schemes and projects to combat the reduction of items that would become marine litter. Whilst the latter is currently high profile amongst concerned consumers and various private bodies, there lacked any galvanised policy research tools to build on this concern. For example, available evidence indicates that consumer focused litter reduction projects (e.g. plastic bag taxes, bottle return schemes) can be effective and consumers responsive[167,411,419]. There is only a limited amount of European countries to date that do operate such schemes and it would be an interesting opportunity to pilot Europe-wide government subsidised schemes to assess whether this is effective over large scales[419]. The European Strategy for Plastics in a Circular Economy adopted on January 2018 will aim to change the way plastic products are designed, used, produced and recycled in the EU. This strategy should increase litter reduction projects on a European scale. Considering that the development of the marine litter research framework is still on its infancy, it would follow that 'Monitoring' is well-represented within the projects included in this review. Monitoring projects involve primary data collection, act as a foundation for many management strategies, and allow for scoping reviews. Many of the categories such as 'Risk Assessment' and 'Reduction' require data from enough monitoring prior to their design and implementation, which may contribute to the prevalence of monitoring studies observed in this review. Whilst the large body of monitoring research represented in this review indicates a progressive trend, it should be noted that the marine environment is dynamic, therefore, repeated monitoring will be required to continually assess levels of litter[420]. To build a successful framework of tackling marine litter on a global scale, all geographic areas will require considerable monitoring effort. A further step to ensure an effective monitoring scheme will have to go hand in hand with harmonisation and normalisation of existing techniques to develop a European assessment. There is a clear need to invest in the development and insertions of these three aspects, which are largely lacking.

'Impact and Effect', 'Reduction', and 'Education and Outreach' were the next highest scoring research areas in this review. "Impact and Effect" studies pair well with the prevalence of monitoring projects in this review, as they are necessary to evaluate effect concentrations, foster pilot projects, and contribute to more specialised research areas. Thus, the prevalence of 'Impact and Effect' projects

indicates the well roundedness of research outputs in this review, such that rather than solely gathering monitoring data, many projects actively tested hypotheses. Research concerning litter reduction strategies were mostly correlated with 'Education and Outreach' projects, identifying and piloting schemes to mitigate the production of marine litter. 'Education and Outreach' projects had a variety of targets ranging from the general public to industry, whilst reduction strategies were mostly targeted at the fishing industry. An example of the former comprises methods to combat the disposal of cigarette butts in the Mediterranean Sea, where a mobile app was used to engage beachgoers in helping to monitor cigarette butts and educate them as to the environmental fate of them[421] whilst an example of the latter comprises coordinated efforts to manage fishing gear in the Adriatic Sea[422]. This correlation is likely because outreach and education were used as tools to engage the project audiences, as such reduction strategies often face the challenge of concern from the user, and so effective engagement strategies are essential[423].

The remaining categories ('Analytical Method Development', 'Removal', 'Socio-economics', 'Bioaccumulation', 'Modelling', 'Assessment and Tools', 'Fragmentation', and 'Risk Assessment') were represented in less than 25% of the reviewed projects (Figure 8.2). 'Analytical Method Development' was relatively well represented across a broad range of projects. This is understandable given the body of preliminary research projects that involved monitoring or observation studies. This might also explain the relatively poor representation of 'Modelling' outputs, as baseline data and observation studies are a prerequisite for accurate models. Further, modelling often requires expert knowledge and can be expensive to undertake due to software requirements and licences. However, with the progression of marine litter research in the next decade, it is likely that knowledge of marine litter modelling will improve and therefore more accurate modelling outputs will become available.

The category of 'Socio-economics' was represented by twelve of the projects. There was an increase in projects undertaking socio-economics research with regards to marine litter in 2012 to 2016, which could have been influenced by progress made in research categories that contribute to socio-economic processes (e.g. 'Impact and Effect' and 'Education and Outreach'). With development in these areas the public have more information on the impacts of marine litter to their own health[424]. Large projects such as MARLISCO[425] had key outputs concerning socioeconomics in the scope of education, whilst local projects such as AMMOS[421] generated public perceptions of specific types of marine litter (cigarette butts). Both are exemplary that baseline data is not necessarily required for socio-economic outputs, and that public perceptions can be an important first step for public engagement, which is integral to many mitigation strategies.

The research categories 'Assessment Tools', 'Fragmentation', and 'Risk Assessment' feature in less than 10% of reviewed projects combined. A potential explanation for this is that data collection and analysis in the form of monitoring studies, and lab-based 'impact and effect' studies, are a prerequisite to these more focused research areas. For example, the research in other contributing categories (e.g 'Monitoring' and 'Modelling') needs to be developed further before being input into a risk assessment, especially as our understanding on sources and fates of marine litter, such as riverine inputs, are still developing[261,426]. This likely represents why 'Assessment Tools' was poorly represented in this review, as these tools and strategies often require baseline information and would perhaps be a good indicator of later-developed research, i.e. research based on data gathered in the monitoring and observation stages. Similarly, studies that concern fragmentation may not have been prevalent to date, as they require background data on the nature of marine litter, and common types of marine litter. Also, it may previously have been thought that information on the fragmentation of litter is not integral to the reduction of marine litter, which is a primary focus of MSFD descriptor 10. However, it is becoming apparent that a large majority of the microplastics found within the oceans are a result of fragmentation of larger macroplastics[3], therefore it is something that should be considered further in future. To consider additional measures to limit microplastics from all sources, including those

intentionally added in products and other main sources (textiles, tyres, plastic pellets, and artificial turfs) more information on sources, pathways and fragmentation rates is urgently required. Whilst there were some research outputs which might inform the construction of risk assessments in the future, there was little acknowledgement in many projects of the potential risks that marine litter could impose. This is likely because much of the research focus has concerned the removal or reduction of marine litter, potentially due to the immediate visible benefits. Whereas, MSFD descriptor 10 states that, 'properties and quantities of marine litter do not cause harm to the coastal and marine environment'. Thus, there is a clear need for more 'impact and effect' studies, to further assess the threats from marine litter. This type of research would also allow more appropriate prioritisation of the types of marine litter that are most harmful to certain receptors and help to provide a better understanding of the long-term effects on the marine and coastal ecosystem under differing scenarios of marine litter prevalence.

Research focused on macro litter was more prevalent in the projects evaluated compared to that focused on micro litter. This is reflected in the identified knowledge gaps of 'Bioaccumulation' and 'Fragmentation', as bioaccumulation relates primarily to micro litter, and fragmentation is concerned with the transition from macro to micro litter. It is understandable that most research outputs would concern macro litter due to the relative ease, simplicity and cost-effectiveness when compared with microparticles, particularly with regards to monitoring programmes. In addition, macro litter can also be more impactful when conducting education and outreach projects as it is easier for the general public to understand the impacts, particularly given the opportunity for use of emotive visuals such as plastic strangulation of marine mammals and seabirds[427]. However, the highly publicised microparticle regulations in cosmetic and household products across several European countries, although not the largest source of microplastics, indicate that general users are becoming more familiar with and more responsive to microplastic threats[287,428]. Although macro litter has historically been the focus of marine litter research, the number of projects focusing on micro litter appears to have increased in recent years. For example, of the six projects starting in 2016, four focused solely on micro litter, and only one focused solely on macro litter. This suggests that the general focus of marine litter research is shifting to incorporate the emerging threat of micro litter.

A geographically broad distribution of research effort was observed. Organisations from West-European countries contributed to more projects than other countries. They also acted as lead partner on most projects. This reflects the range of project scales, i.e. national initiatives to EU-wide schemes. When this is assessed on a category basis, however, there is a less clear trend. There is variation when analysing research output for each category by country. More economically developed countries are the dominant researchers concerning projects requiring sophisticated technology, such as bioaccumulation and fragmentation studies, though the low overall number of research outputs for these categories might dispute the reliability of this result. Similarly, well-established scientific organisations in western Europe lead the way in modelling outputs. A clear geographic trend is the prevalence of regional or sub-regional research projects. Many of these focussed primarily on monitoring, education and outreach. Examples of this include fishing gear management in the Adriatic Sea, parts of the Mediterranean, litter removal and reduction projects across the Baltic Sea. This is encouraging from a marine litter perspective as it provides a baseline of community engagement upon which effective management strategies can be built. An influencing factor here could be that many of the countries in these regions have important tourist economies, and as such, there is an economic incentive to keep their coastlines and seas aesthetically pleasing.

It is evident that the production, mechanisms and effects of marine litter are still being understood. The production of high profile macroplastics such as plastic bags and single-use products is understood by various stakeholders and consumers as posing a threat to increased volumes of marine litter[427]. Similarly, the usage of products containing microparticles has been the focus of various campaigns

and research programmes in relation to their potential to contribute towards marine litter[429]. Yet, the level of research effort for some components of marine litter research currently does not reflect the potential of some stressors to contribute towards the volume of marine litter. A realigning as such would allow the opportunity for research to monitor both prevalence of microplastics, and long-term studies to generate an idea of how much fragmentation contributes to the prevalence of microplastics and how different types of litter can be absorbed or ingested by marine life. Baseline data of this type will be essential in constructing accurate and specialist models, which will help to develop effective policy and management and provide the framework for regulations concerning risk assessment of various hazards as a result of marine litter[430]. These will be integral in informing and facilitating the legislation and management of litter. Funding and research effort will be best placed in these areas once enough monitoring data has been collected.

With this study we clearly recognise the importance of EU financial instruments (e.g. INTERREG, LIFE, Horizon 2020) to support large scale environmental and nature conservation projects. Such mechanisms not only improve cooperation and harmonisation across wider regions, they also allow to share the burden and capacity. Considering the variety of needs, it seems that there remains a large body of research to be undertaken. The status of marine litter research is certainly in its adolescence. To work towards Good Environmental Status by 2020 and its legacy beyond (e.g. EU Plastic Strategy), research will be needed to close the gaps identified by this paper and to improve current materials and redesign alternatives. Better design of plastic products, higher plastic waste recycling rates, more and a better quality of recyclable material will stimulate the market for recycled plastics. In order to develop the strategy and achieve future EU objectives it will be important to focus projects around 'Assessment Tools', 'Fragmentation', and 'Risk Assessment' to collect the underpinning evidence for existing and new products. Notably, the fragmentation of plastics over time, the environmental exposure routes and the wider impacts across marine and coastal ecosystems. It is therefore essential that research engages with the existing public interest to direct attention to lesser researched areas of marine litter. This is reflected by the prevalence of 'Education and Outreach' projects in this review, where engagement strategies proved essential to the success of the projects. These efforts must be maintained and built upon so that users of marine space, consumers, and other stakeholders are actively involved, recognise the potential threats, and understand their role in the management of marine litter[430] .

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Chapter 9

General discussion

The research described here aims to improve the scientific understanding of the marine litter issue, including microplastics, in the North-East Atlantic ecosystem. The research does so by addressing standardisation of marine litter and microplastic monitoring methods, analytical method development, field exposure of microplastic in both abiotic and biotic matrices, and laboratory exposure and chronic toxicity of microplastic to marine species (**Chapter 1**).

Marine litter, especially plastic, is omnipresent and enters from a multitude of land and sea-based sources. An estimated 19 to 23 Mt, or 11%, of plastic waste generated globally in 2016 entered aquatic ecosystems, this includes the amount that accumulates in lakes and rivers in addition to the plastic that escapes to the ocean[431]. Globally, floating marine litter ranges from 0 to beyond 600 items km^{-2}, on the seabed densities range from 0 to >7700 items km^{-2} and on beaches up to 5,058 items m^{-2} were found after flooding events (**Chapter 2**). Microplastic concentrations in sediments, water surface and biota vary widely depending on the matrix and location. Microplastic pollution in marine environments is concentrated most highly in coastal habitats, especially fjords and estuaries[8]. In terms of distribution and quantities, proper global estimations based on standardised approaches are still needed before considering efficient management and reduction measures. Spatial trends are driven by factors such as proximity of urban activities, shore and coastal uses, wind and ocean currents. Temporal trends are not clear with evidences for increases, decreases or without changes, depending on locations and environmental conditions (**Chapter 2**).

We have demonstrated that macro plastic pollution was ubiquitous on the epeiric seabed of the European continental shelf (**Chapter 3**). We developed a monitoring methodology to evaluate seafloor litter, using litter data obtained by existing trawling surveys. Over the entire 25-year period (1992–2017), 63% of the 2461 trawls from 39 independent scientific trawling surveys in seas surrounding the UK contained at least one plastic litter item. The monitoring methodology is currently used by several countries around the North East Atlantic to report status and progress under the MSFD (D10)[432] and to assess progress against the OSPAR North East Atlantic strategy and regional action plan[1]. To address the scientific and practical requirements, the International Council for the Exploration of the Sea (ICES) created the Working Group on Marine Litter[433]. This collaborative and large-scale approach aims to create a harmonised dataset to follow up marine litter related measures more precisely.

In the 25-year seafloor litter study, statistically significant decreasing trends were observed in the presence of plastic bags in the Greater North Sea following the implementation of measures to limit plastic bag usage e.g. plastic bag tax or bans (**Chapter 3**). The plastic bag taxes or bans across Europe that reduced single use plastic bag use by 90% per year in 5 years in some cases[434], correlates to a decrease in detection of the plastic bags on the seafloor in seas surrounding the UK. This suggests that the input of new plastic bags to the seafloor litter was reduced concomitantly with the implementation of policy to reduce single use plastic carrier bag applications. Design and retail policy decision-making may have led to these reductions in the seafloor litter monitored within a relatively short period of implementation, indicating that behavioural and legislative changes (e.g. plastic bag tax or ban) could reduce the problem of marine litter within decades. Although statistically significant local trends were observed in specific plastic litter categories in the 25 year seafloor litter study, recent global models indicate that current marine litter legislation is still not having large enough effects, it has been estimated that implementing all feasible interventions will only reduce plastic pollution by 40% from 2016 rates and 78% relative to “business as usual” in 2040[435].

While plastic inputs are continuous and rising, there was no significant temporal trend in the percentage of trawls containing any or total plastic litter items across the long-term datasets (**Chapter 3**). There are potential causes for this absence of trends and in reality, this is probably caused by multiple reasons. Macro litter seafloor monitoring, piggybacking on fisheries surveys, is far from ideal and rather monitors a part of the process and not a final sink of marine litter. The fragmentation of

macro plastic litter items to microplastic litter items is not a linear process and dependent on several factors such as polymer type and pathway, but it is inevitable that plastic will fragment sooner or later in the environment. Marine plastic litter gradually crumbles down into ever smaller fragments due to physical, chemical and biological degradation, continuously changing the particle densities[436,437]. Floating plastic fragments, if not ingested or beached, gradually start to sink, caused by biofouling and continuous breakdown processes, they will gravitate throughout the water column[9] and eventually settle out like sediments[8]. Therefore, when plastic litter decays into fragments smaller than our mesh size[27] (4 cm), it escapes the bottom trawls. It is however unlikely that this option alone is responsible for the rapid removal of a large part of the input, the absence of UV and oxygen make fragmentation processes slow or absent on the seafloor[396,438,439]. Although some of the sunken items could get buried deep into the sediments and go uncounted, it is more likely that intensive trawling and regular storms on the continental shelves dig and stir up the seafloor[245] which (re)moves a proportion of seafloor litter. Submerged items remerge (e.g. the decline of biofouling), and eventually strand somewhere on a beach, where UV radiation and physical battering breaks them more rapidly down into microplastics[440] which are washed back out to sea. Some of the sunken plastic items slip down submarine canyons[23,39,126,186] into the deep waters of the Atlantic Ocean where we have limited or no trawling data. These sunken litter items are driven by strong bottom currents in the deep-sea[441] and dependent on their density[24], get trapped and accumulate against ridges, in dead zones, in canyons [23,24,39,47,49,124,126,130,139,143,186,442,443] and in the Arctic[33,131,201] . The processes listed above, continuously (re)move macro items of seafloor litter, limiting the functionality of seafloor litter monitoring using fisheries surveys on the continental shelves.

The overall pattern of microplastic pollution mirrors the pathways of natural sediment accumulation in which most and heaviest material is deposited close to its input source. Microplastic pollution accumulates greatly in coastal areas and estuaries[8]. Our results confirmed this, hotspots for microplastics were found in sediments, estuaries and areas of the Celtic Sea, Greater North Sea and English Channel with a high organic carbon content (**Chapter 4**). Sea surface microplastic concentrations (0 and 1.5 microplastic/m^3) were low compared to the concentrations found in the sediment samples (0 and 3,146 particles/kg dry weight sediment). At the sea surface, fragments were dominant. In sediments, mainly fibers and spheres were found. Higher concentrations of sea surface microplastics were found near estuaries. The surface water of estuaries and sediments with a high organic carbon content were hotspots (**Chapter 4)**. Monitoring microplastics in fine sediments near estuaries allows for measurements close to the sources and can be easily combined with existing contaminant monitoring programmes. The high microplastic concentrations make detection more frequent and trend analysis more robust due to the increased statistical power of detecting trends at higher frequency of detection. To detect inputs or point source monitoring, e.g. sewage pipe, harbour or river inlet, (nearby) microplastic monitoring in the water column is more appropriate. Manta nets prove to be cumbersome for point or water column sampling. While the use of manta nets can be useful for long transects during calm sea states, for point source monitoring, a pump proves far more workable in such environments and also limits human interference during the subsequent cleaning and handling of the sample[444].

Different sampling techniques make direct data comparison difficult; human error, and issues of repeatability and reproducibility of sampling techniques make it nearly impossible. Some monitoring programmes are monitoring transitory pathways while others examine marine litter sinks. To complicate matters, different studies reported in the literature have used different units to express microplastics in sediment (items/g dw or ww), water (items/m^3 or items/m^2) and biota (items/g dw or ww). Ideally, microplastic concentrations in sediment should be reported normalised to dry weight. Water concentrations are best reported in terms of mass as well, when the mass of the sample is weighed during analysis. When water is not weighed, but filtered, the volume of the sample is the only measured data point to use to calculate the concentration. Due to the need for extremely large sample

intakes to reach limits of detection for microplastic analysis at the sea surface (typically on the order of thousands to tens of thousands of litres of water sample intake per analysis), it is impossible to determine the mass of the sample, and the volume is more often estimated than measured. For biota, gut content analysis requires different reporting units (concentrations per individual gut) than tissue content analysis (concentrations per g tissue). Concentrations in tissue of contaminants are sometimes normalised to dry weight, lipid weight or wet weight. For microplastics, such normalisation lacks the rationale of partitioning to lipids or proteins that applies to hydrophobic organic chemical when measuring and interpreting body residues. Microplastics are particles made up of macromolecules and behave differently than hydrophobic micromolecules in biological systems[229]. Hence, we recommend to avoid the use of wet weight units to report microplastics in sediment and biota, where possible these should be normalised to dry weight. Microplastic concentrations in the water should be expressed in items per mass or volume to connect with exposure studies. However, plastic mass balances urgently need to be determined to progress and validate plastic distribution models. There is thus a need to look into more detail into plastic fragmentation processes and pathways to understand how the plastic mass relates to the observed number of particles in each fraction.

Increased environmental data is necessary to further guide exposure studies measuring biological effects across a range of relevant species. More and more evidence about the harmful effects of microplastics are emerging and thus we investigated claims around potential food web accumulations. The presence of microplastics in top predators and an already endangered species, the North-East Atlantic porbeagle shark (*Lamna nasus*) was assessed, along with parameters for fitness/health (**Chapter 5**). We developed an appropriate method to quantify the concentration of microplastics in spiral valves of porbeagle sharks to test the hypotheses that i) porbeagle sharks ingest microplastics and ii) the ingestion of microplastics adversely affects their general health condition. Microplastics were detected in 9 out of 10 spiral valves `in high concentrations (>1000 microplastics/spiral valve), a potential sign of bioaccumulation. No statistically significant correlations were found between the average number of plastic particles in spiral valve content and tissue and the Condition and Hepatosomatic Index of porbeagle sharks (**Chapter 5**). The results of this research show that North-East Atlantic porbeagle sharks ingest microplastics, most likely indirectly via its prey but further research is needed to confirm and detect possible health effects of microplastic accumulation via the foodweb. Several other top predators[264,278,445] (e.g., seals, whales, sharks) have been found to accumulate microplastics via their food, showing their potential as indicator species for integrated monitoring across their feeding grounds, although related long term health risks for the individual species remain unclear. The microplastics present in the spiral valve may pose less of a particle toxicity risk than those microplastics lodged into intestinal tissue. Measuring plastic particles (<2.5 µm) in shark tissue and organs could elucidate whether the plastics in the gut are being transferred to the rest of the body. Adequate analytical tools to sample, isolate, detect, quantify, and characterize small microplastics (<10 µm), especially nanosized plastic particles, are urgently needed[446].

Our sewage (unpublished) and estuarine monitoring work indicated that high concentrations of microplastics were discharged directly into UK estuaries nearby oyster farming areas. As such, microplastics of different types and sizes become available for ingestion to a wide range of organisms. The particle toxicity, the toxicity of chemicals and the biological load of the plastic particle can all cause harm to an organism once consumed[447]. Our findings indicated that repeated exposure to high concentrations of MPs may lead to "MP fatigue" in oysters, altering the condition of important ecosystem engineers (**Chapter 6**). Bivalve filter feeders, such as oysters, filter and clean large volumes of water (up to 200 litres/day)[448] and are particularly exposed to microplastics[362]. Consequently, these animals can consume and accumulate thousands of MPs/day[353] that may impact their reproduction and physiology[359], and potentially affect shellfish stocks[291], benthic habitats[376,377] and, indirectly, the health status of the marine ecosystem[449] and human consumers[333]. To investigate this impact from

microplastics on bivalves in more detail, we designed a long-term laboratory exposure study with juvenile oysters, *Crassostrea Gigas*, using realistic estimates of concentrations of 6 µm polystyrene (PS) microbeads (**Chapter 6**). In the histological analysis, microbeads were detected in the intestines of exposed oysters and in the digestive tubules, but no cellular inflammatory features were observed over time. This absence of inflammation from the exposure of PS beads has been reported previously[359] and might be due to the smooth surface and spherical shape of the chosen particles[335]. Interestingly, weight and shell length remained comparable between the different treatments and control. Similar to other studies, microbeads were present in the faeces and pseudo-faeces, which could indicate that these spherical beads are rapidly excreted[359,379]. Although it did not seem to influence the external shell length and overall body weight, the Condition Index of animals in the highest exposure concentration trials initially increased, but then decreased significantly before the end of the test. The oysters in the highest concentration may have been investing energy (fat tissue) in the elimination of the microbeads, leading to a reduced overall tissue weight. Previous microplastic exposure studies on Pacific oysters, using much higher concentrations (0.023 $mg \cdot L^{-1}$), showed that dynamic energy budget modelling, supported by transcriptomic profiles, suggested a significant shift of energy allocation from reproduction to structural growth, and elevated maintenance costs in exposed oysters, which is thought to be caused by interference with energy uptake[359]. In our study, the oysters in the highest microplastic exposure also showed the lowest mean Lysosomal Stability score throughout the experiment. Lysosomes play a vital role in the cell's defence mechanisms and breakdown of biological waste products, bacteria and viruses, and as such are crucial for maintaining the oysters' wellbeing. Most importantly, increased mortality was detected in those oysters which were chronically exposed to the highest concentrations of microplastics, suggesting that current microplastic concentrations are indeed likely to cause an effect in marine bivalves and their function in benthic assemblages[376,377].

Standardisation of macro and microplastic monitoring methods within marine regions is urgently recommended to compare and assess plastics pollution over space and time. At the moment, microplastic sampling and analysis is disorganised due to the multiple approaches and recommendations from a variety of organisations with different aims and backgrounds[41,292,420,450]. Without harmonisation, it will be impossible to compare, assess results and effectiveness of measures as required by drivers such as the UN SDG, EU MSFD, OSPAR RAP and other national initiatives. To facilitate this process, different techniques could be grouped according to a set of defined criteria, for example the question they answer (e.g., numbers, polymer types, weight) and degree of accuracy they deliver (e.g., limitations, blanks, QA/QC schemes). Some techniques are likely to be investigative, addressing specific scientific gaps, while other types will rather be suitable for compliance monitoring, needed to create spatial and temporal trends. A global or regional database to collate data from all different techniques for each type of need will allow to collect and compare methods, which facilitates further selection and definition of reporting units and techniques. There is not one technique and the method selection for sampling or analysis will ultimately depend on the objectives and a series of factors like equipment, costs and time. When analysing microplastics in sediments, water or biota, we noticed that there was an urgent need for a cheap, accurate and rapid analysis tool for microplastics (**Chapter 7**). For example, there were challenges to overcome heterogeneous distribution of MP in environmental samples, the task of taking representative numbers of samples and analysing trace levels, a high number of samples or high sample volumes or masses need to be analysed[53]. Hence, we developed a new approach, based on selective fluorescent staining using Nile Red (NR), allowing plastic particles down to a few micrometres to be detected under blue light and categorised based on surface polarity characteristics (**Chapter 7)**. The fluorescent technique can be used to detect microplastics independently or in combination with other techniques such as microscopy or spectroscopy to speed up the analysis[451]. Due to these cross cutting aspects, it provides a common approach and one of the potential recommended techniques for the OSPAR candidate indicator for microplastics in sediment[452]. Owing to its simplicity, low costs and high analytical output, the

technique has been introduced in several Commonwealth countries as part of the Commonwealth Litter Programme (CLiP), allowing those countries to setup microplastic monitoring programmes[453]. Using the Nile Red technique, microplastics were detected in water, biota and sediments from Vanuatu and the Solomon Islands[454], in commercially important small pelagic fish species, in harbour water and sediment samples from Durban port in South Africa[444,455].

Cross collaborative and multi-disciplinary studies and evaluations are needed to improve our understanding and ability to tackle this plastic pollution problem. Several European research efforts to address concerns in relation to increasing levels of marine litter and potential effects on ecosystems and human health have been launched. One of these projects was the Colombus project[456], which capitalised on the European Commission's significant investment in marine and maritime research by ensuring accessibility and uptake of research outputs. To determine which marine litter research gaps have been addressed and to guide future research we used the mapping exercise of the Colombus project to assess a total of 52 European projects which researched or contributed to the implementation of European marine litter legislation in this review (**Chapter 8**). The analysed projects ranged from national initiatives, to large scale programmes involving multiple EU Member States. The best represented topics within those European marine litter projects were 'Policy, Governance and Management' and 'Monitoring'. Comparatively 'Risk Assessment', 'Fragmentation' and 'Assessment Tools' were underrepresented (**Chapter 8)**. To set criteria and thresholds for maximum allowable concentrations of marine litter and microplastics or achieve concepts such as "good environmental status" defined by the EU MSFD[12], more research is urgently required to determine fate, distribution and pathways, but also to define environmental risk assessments and eventually harm[266]. More extensive monitoring data will lead to the development of spatial and temporal trends, these, together with thresholds based on impacts and effect, could be used to follow progress against newly formed measures[40]. In order to build a complete understanding of the properties and quantities of marine litter which cause harm to the coastal and marine environment in Europe (MSFD Descriptor 10) it will be necessary to endorse projects looking at those underrepresented topics in the next decade. The analyses showed that Western European countries have contributed more to marine litter research and received more EU funding. As a result, thematic hotspots were present, and scientific capacity is concentrated by topic and countries. These knowledge hotspots could hamper harmonisation and assessments due to a lack of progress and funding in certain regions. Our results clearly identified technical or financial gaps, either geographic or thematic, that would need to be filled in order to deliver risk assessments and recommendations to address the marine litter issue on a wider scale across Europe. These findings were fed into different European science reviews and presented to the European expert group on marine litter. The science gaps, as identified by this study, became part of the selection criteria for project funding in the second Joint Programme of Investigations (JPI Oceans) call on microplastics[457].

The plastic problem is not different from of all our other environmental problems, some big obstacles will have to be tackled: unregulated industry, a globalised world, and our own unsustainable way of life. Our personal goals should be to achieve zero plastic waste by taking actions across your life and business to use less plastic, recycle more and support innovations to improve plastic waste reduction systems.

SUMMARY

The central aim of this thesis was to gain a better understanding of marine litter, including microplastics, in the North-East Atlantic ecosystem. We addressed several knowledge gaps in relation to the standardisation of marine litter and MP monitoring methods, analytical method development, field exposure of MP in both abiotic and biotic matrices, and laboratory exposure and chronic toxicity of MP to marine species.

No significant temporal trend was observed in seafloor litter around the UK for the past 25 years (Chapter 3)

We demonstrated that existing fisheries surveys using trawls can be used to monitor seafloor litter. Macro litter on the seafloor is widespread but patchy within the seas surrounding the UK, ranging from 0 to 1835 pieces km^{-2} of seafloor and dominated by plastics. Over the entire 25-year period (1992–2017), 63% of the 2461 trawls contained at least one plastic litter item. There was no significant temporal trend in the percentage of trawls containing any or total plastic litter items across the long-term datasets. Statistically significant trends, however, were observed in specific plastic litter categories only. These trends were all positive except for a negative trend in plastic bags in the Greater North Sea - suggesting that behavioural and legislative changes (e.g., plastic bag tax or ban) could reduce the problem of marine litter within decades.

Microplastics accumulate in North Sea sediments with a high organic carbon content (Chapter 4)

Microplastics are present in sediments of the Southern North Sea (0 - 3,146 particles/kg dry weight) and at the sea surface of North West Europe (0 - 1.5 microplastic particles/m^3). The highest concentrations of microplastics were found in estuaries and in sediments areas with a high organic carbon content. Sediments act as sinks for microplastics (mainly fibers and spheres), they are less heterogenous and contain higher concentrations of microplastics compared to surface waters (mainly fragments). Smaller sample sizes and volumes are required for microplastic monitoring in sediments. Sampling for marine sediments is already ongoing, is less prone to error and allows for more precise measurements compared to trawling nets. Standardization of monitoring methods within marine regions is recommended to compare and assess microplastics pollution over time.

North East Atlantic Porbeagle sharks digest microplastics but the health impact is unclear (Chapter 5)

Microplastics are present in high concentrations in top predators living in the North East Atlantic, up to 10.4 particles per g wet weight (w.w.) content and 9.5 particles per g w.w. tissue. This equates to individual microplastics loads as high as 3850 particles per spiral valve. These high concentrations might deliver a first indication of bioaccumulatution. We developed a method for quantifying microplastics in spiral valves of porbeagle sharks. No possible health effects of microplastic contamination were found. There is a potential for microplastic biomonitoring using this species.

Long-term microplastic exposure has adverse health effects on juvenile oysters (Chapter 6)

Juvenile oysters, Crassostrea gigas, exposed for a period of 80 days to 106 particles L^{-1}, represented by 6 µm polystyrene (PS) microbeads, showed an increased death rate compared to a control treatment receiving no microplastics. Weight and shell length remained comparable, but the Condition Index of the oysters in the highest concentration reduced significantly towards the end. The oysters in the highest MP exposure showed the lowest mean Lysosomal Stability score throughout the experiment. Microbeads were detected in the intestines of exposed oysters and in the digestive tubules, but no cellular inflammatory features were observed.

Microplastics in sediments detected using forensic science methods (Chapter 7)
The selective fluorescent staining using Nile Red (NR), followed by density-based extraction and filtration allows for rapid analysis of microplastics in sediments using simple photography through an orange filter at low cost. Image-analysis allows fluorescent particles down to a few micrometres to be identified and counted. The solvatochromic nature of Nile Red also offers the possibility of plastic categorisation based on surface polarity characteristics of identified particles.

Europe underfunds marine litter research on marine litter risks (Chapter 8)
The past decade, the best represented topics within European marine litter projects were 'Policy, Governance and Management' and 'Monitoring'. The underrepresented topics were 'Risk Assessment', 'Fragmentation' and 'Assessment Tools'.

From the evidence we gathered, it remains difficult to create a complete understanding of the marine litter issue in the North East Atlantic, much more work is required. What is clear, is that we found marine litter, including microplastic across all investigated sites and samples. In our 25-year study, seafloor litter presence remained constant, although waste types and inputs differed. This suggest that seafloor litter is moving through the marine environment, accumulating where we don't monitor, or escaping our nets under the form of smaller (micro)plastics. Without strong action to stem plastic production and usage, together with improved waste management, quantities of marine litter and microplastics will increase further, eventually leading to concentrations causing ecosystem impacts and population effects across marine biota. Our oyster and porbeagle shark study indicated that microplastics are taken up and moving through the food chain, affecting marine wildlife and thus potentially also us. Current concentrations of microplastics near point sources are certainly high enough to cause mortality in bivalves as shown in our exposure study. The collected evidence suggests that current properties and quantities of marine litter in the North East Atlantic are expected to cause a significant impact on the ecosystem. If we don't stop plastic inputs into our oceans, it will only be a matter of time before critical concentrations will be observed more widely. To address this rapidly growing issue, to advice future investments and to steer science needs across Europe, we suggest speeding up the harmonisation of methods, looking into more detail into plastic fragmentation processes and pathways, delivering more risk and life cycle assessments for new and existing products and developing (assessment) tools for each stakeholder. In future, cross collaborative and multi-disciplinary studies and evaluations are needed to improve our understanding and ability to tackle this plastic pollution problem.

SAMENVATTING

Marien zwerfvuil is een groeiend probleem voor zeeën en oceanen, en wordt beschouwd als een aanzienlijke bedreiging voor het milieu. Marien zwerfvuil wordt gedefinieerd als elk vast materiaal dat door de mens werd vervaardigd en direct of indirect, opzettelijk of onopzettelijk terechtkomt in het mariene milieu. Het overgrote deel bestaat uit plastic afval dat zowel afkomstig is van activiteiten op zee als op land. Plastics zijn synthetische stoffen met lange levensduur, samen met een continue toevloed van plastic afval leidt dit tot een opeenhoping in het milieu die nog heel lang in het milieu aanwezig zal blijven. Om de problematiek van marien zwerfvuil beter te kunnen inschatten, onderzochten we aantal wetenschappelijke vraagstukken in meer detail: standardisatie van monitoring; ontwikkeling van meet methodes; veldstudies in sediment, water en dieren; blootstelling in het laboratorium.

Geen significante temporele trend werd waargenomen in een 25 jarig onderzoek van afval op de zeebodem rond het Verenigd Koninkrijk (Hoofdstuk 3)

We hebben aangetoond dat bestaande visserij onderzoeken met sleepnetten kunnen worden gebruikt om afval op de zeebodem te monitoren. Macro afval op de zeebodem is wijdverbreid, maar gefragmenteerd in de zeeën rond het Verenigd Koninkrijk, variërend van 0 tot 1835 stuks km^{-2} van de zeebodem en gedomineerd door kunststoffen. Over de gehele periode van 25 jaar (1992-2017) bevatte 63% van de 2461 sleepnetten ten minste één plastic object. Er was geen significante temporele trend in het percentage sleepnetten dat plastic afval bevat in de gegevenssets op lange termijn. Statistisch significante trends werden alleen waargenomen in specifieke categorieën plastic afval. Deze trends waren allemaal positief, behalve een negatieve trend in plastic zakken in de Noordzee - wat suggereert dat gedrags- en wetswijzigingen (bijvoorbeeld plastic zak belasting of -verbod) het probleem van zwerfvuil op zee binnen tientallen jaren zouden kunnen verminderen.

Microplastics hopen zich op in Noordzee sediment met een hoog organisch koolstofgehalte (Hoofdstuk 4)

Microplastics zijn aanwezig in sedimenten van de Zuidelijke Noordzee (0 - 3.146 deeltjes/kg droog gewicht) en aan het zeeoppervlak van Noordwest Europa (0 - 1,5 microplastic deeltjes/m^3). De hoogste concentraties microplastics werden aangetroffen in estuaria en in sediment met een hoog organisch koolstofgehalte. Sedimenten fungeren als opslag voor microplastics (voornamelijk vezels en sferische partikels), ze zijn minder heterogeen en bevatten hogere concentraties microplastics in vergelijking met oppervlakte water (voornamelijk fragmenten). Kleinere monster volumes zijn nodig voor microplastic monitoring in sedimenten. De bemonstering van mariene sedimenten is al aan de gang, is minder vatbaar voor fouten en maakt nauwkeurigere metingen mogelijk in vergelijking met sleepnetten in het water. Standaardisatie van methoden in mariene regio's wordt aanbevolen om de vervuiling van microplastics te vergelijken en te beoordelen.

Noordoost-Atlantische haringhaaien verteren microplastics, maar de gevolgen voor hun gezondheid zijn onduidelijk (Hoofdstuk 5)

We ontwikkelden een methode voor het kwantificeren van microplastics in spiraalkleppen van haringhaaien, *Lamna nasus*. Microplastics zijn aanwezig in hoge concentraties in deze mariene roofdieren, die in het noordoostelijke deel van de Atlantische Oceaan leven, tot 10,4 deeltjes per g nat gewicht (w.w.) van de inhoud en 9,5 deeltjes per g w.w. in het weefsel. Dit komt overeen met individuele microplastic hoeveelheden van 3850 deeltjes per spiraalklep. Deze hoge concentraties leveren een eerste indicatie van bioaccumulatie. Er zijn geen verdere effecten op de gezondheid van de haaien ten gevolge van de microplastic besmetting gevonden. Er is een potentieel voor microplastic biomonitoring met behulp van deze soort.

Langdurige blootstelling aan microplastic heeft nadelige gezondheidseffecten op jonge oesters (Hoofdstuk 6)

Jonge oesters, Crassostrea gigas, blootgesteld voor een periode van 80 dagen aan 106 deeltjes L^{-1}, 6 µm polystyreen (PS) microbeads, toonde een verhoogd sterftecijfer in vergelijking met een controle behandeling die geen microplastics kreeg. . Gewicht en schaal lengte bleef vergelijkbaar, maar de condition index van de oesters in de hoogste concentratie verminderd aanzienlijk tegen het einde. De oesters in de hoogste blootstelling vertoonden de laagste gemiddelde Lysosomale Stabiliteit score gedurende het hele experiment. Microbeads werden gedetecteerd in de spijsverterings kanalen van blootgestelde oesters, maar er werden geen cellulaire ontstekingen waargenomen.

Microplastics werden ontdekt in sedimenten met behulp van wetenschappelijke forensische methoden (Hoofdstuk 7)

De selectieve fluorescerende kleuring met behulp van Nile Red (NR), gevolgd door een op dichtheid gebaseerde extractie en filtratie laat een snelle analyse van microplastics in sedimenten toe tegen lage kosten. De gekleurde microplastics lichten op met behulp van eenvoudige fotografie en een oranje filter. Beeldanalyse maakt het mogelijk fluorescerende deeltjes tot een paar micrometer te identificeren en te tellen. De solvatochromische aard van Nile Red biedt ook de mogelijkheid om kunststoffen te categoriseren op basis van hun oppervlakte polariteit.

Europa onder-financiert onderzoek naar zwerfvuil op zee (Hoofdstuk 8)

De afgelopen tien jaar waren 'Beleid, bestuur en management' en 'Monitoring' de meest vertegenwoordigde onderwerpen binnen Europese zwerfvuilprojecten op zee. De ondervertegenwoordigde onderwerpen waren 'Risicobeoordeling', 'Fragmentatie' en `Beoordelings instrumenten`.

Uit het bewijsmateriaal dat we hebben verzameld, blijft het nog steeds moeilijk om een volledig beeld te creëren van de kwestie van zwerfvuil op zee in het noordoosten van de Atlantische Oceaan, er is nog veel meer werk nodig. Wat wel duidelijk is, is dat we zwerfvuil op zee hebben gevonden, inclusief microplastic op alle onderzochte locaties. In onze 25-jarige studie bleef de aanwezigheid van zeebodemafval constant, hoewel afvaltypen en -invoer verschilden. Dit suggereert dat het zwerfvuil op de zeebodem zich door het mariene milieu beweegt, zich ophoopt waar we niet monitoren, of ontsnapt aan onze netten in de vorm van kleinere (micro)plastics. Zonder krachtige maatregelen om de productie en het gebruik van plastic tegen te gaan, samen met een beter afvalbeheer, zullen de hoeveelheden zwerfvuil op zee en microplastics verder toenemen, wat uiteindelijk zal leiden tot concentraties die effecten in mariene biota en ecosystemen veroorzaken. Uit onze oester en haringhaai studie blijkt dat microplastics worden opgenomen en door de voedselketen bewegen. De huidige concentraties microplastics in de buurt van puntbronnen zijn hoog genoeg om sterfte in oesters te veroorzaken, zoals blijkt uit onze blootstellingsstudie. De verzamelde gegevens wijzen erop dat de huidige hoeveelheden plastic zwerfvuil op zee in het noordoosten van de Atlantische Oceaan naar verwachting een aanzienlijke impact op het ecosysteem zullen hebben. Als we niet stoppen met plastic input in onze oceanen, zal het slechts een kwestie van tijd zijn voordat kritische concentraties worden waargenomen op grotere schaal in verschillende soorten die eventueel al verzwakt zijn door andere stressoren. Om dit snel groeiende probleem aan te pakken, toekomstige investeringen te adviseren en de wetenschappelijke behoeften in heel Europa te sturen, stellen we voor de harmonisatie van methoden te versnellen, meer in detail te kijken naar fragmentatie processen, trajecten voor plastic doorheen het milieu te bepalen, meer risico- en levenscyclusbeoordelingen voor nieuwe en bestaande producten te leveren en voor elke belanghebbende partij instrumenten te ontwikkelen om de toestand te beoordelen. In de toekomst zijn multidisciplinaire samenwerking en evaluaties nodig om ons begrip en vermogen van dit plastic probleem aan te pakken te verbeteren.

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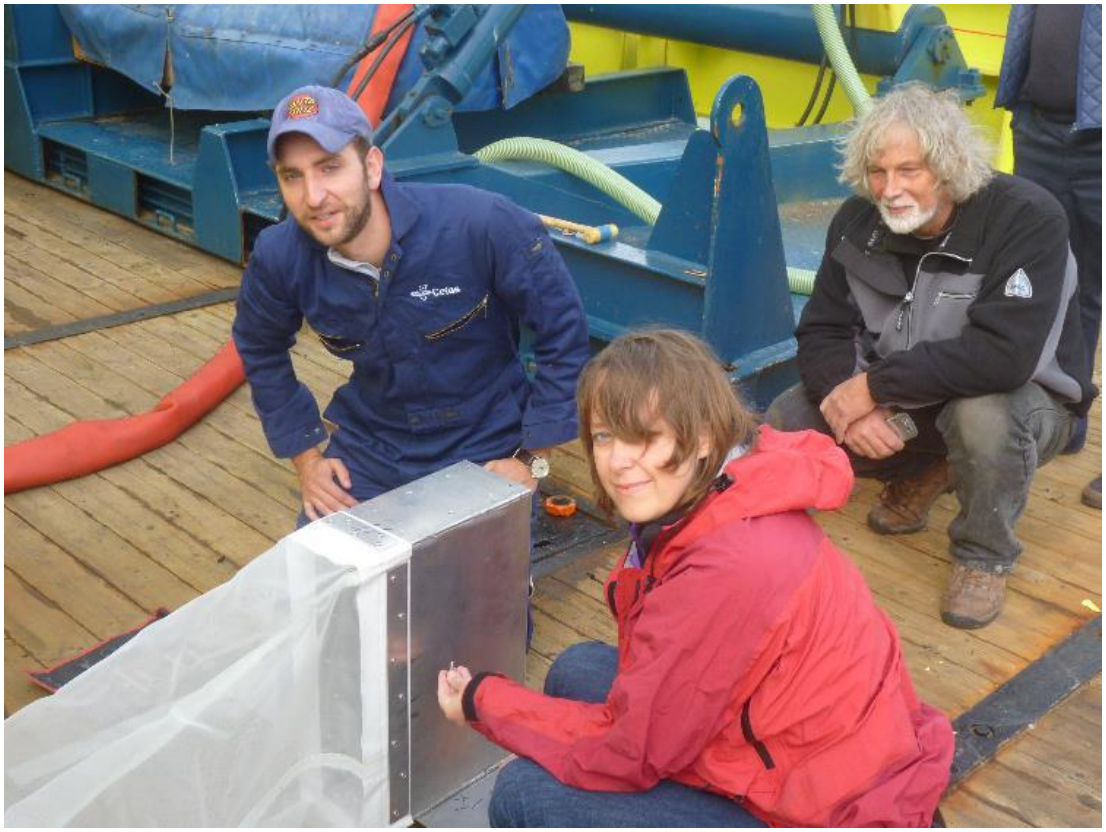

Thomas, Heather & Dick on board of the RV Zirfea in 2014

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PROFILE

Thomas is an excellent communicator in different languages. He has a wide educational background, including pre-MBA, civil engineering, terrestrial & marine biology. He started his international career as an environmental manager on large development projects for the dredging company Jan De Nul (JDN) e.g. the Palm Islands, Ras Laffan Gas Terminal, Sfax Taparura Remediation, Port Hedland Harbour.

From there, Thomas moved on to the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) in the UK, becoming a Principal Scientist leading a large portfolio of projects and a team of marine experts. He coordinated several UK national and international marine monitoring programmes for EU Directives and other regional drivers. Thomas also provided advice to the UK Government and some of their most senior members on issues related to human pressures such as contaminants, waste and other pollutants. He directly supported UK ministers and royals at international meetings, audits and visits.

In 2020, Thomas joined GRID-Arendal, a collaborating centre of UNEP with highly qualified staff that specialises in complex environmental issues. To tackle the challenge of pollution and waste, Thomas supports global policy development through the UN frameworks, including UNEA and the Basel Convention. Thomas also assists developing countries with capacity building and research on the impact of waste and marine litter, their sources and pathways. He works closely with the Regional Seas Conventions and other UN agencies to find regional and global solutions to tackle pollution issues.

Thomas has an extensive knowledge of relevant environmental institutes and organisations and maintains an impressive global network. He attends, chairs and facilitates several committees, political debates, working groups and national/international expert meetings. The topics that Thomas specialises in are: marine litter, microplastics, POPs, nutrients, hazardous substances, ecotoxicology, circular economy, waste, waste management, waste trade, waste water.

Thomas operates between science and policy and develops products to inform policy-makers. He has a growing list of peer reviewed publications and reports. He regularly gives keynote speeches and presentations in relation to pollution at international fora.