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Investigation of microplastic debris in marine surface waters using different sampling methods

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Investigation of microplastic debris in marine surface waters using different sampling methods

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Abstract

‘Microplastics’ are pieces of plastic debris <5mm in diameter. They are introduced into the marine environment directly for example via release of small pieces of plastics used as abrasives and indirectly through the fragmentation of larger items. The European Marine Strategy Framework Directive 2008/56/EC (MSFD) regards microplastics as an emerging issue of concern and calls for more data on the quantity, distribution and composition of this debris. This thesis examines the amount, composition and distribution of buoyant microplastic debris in marine waters using different sampling devices and methodologies.

To investigate the spatial distribution, abundance and composition of microplastic debris between nearshore and offshore marine subsurface waters a subset of samples from the Continuous Plankton Recorder (CPR) survey were examined. Abundance was generally higher in nearshore coastal waters than the offshore oceanic samples, with the highest mean concentrations observed in the UK’s coastal waters of the northeast Atlantic and the southern North Sea.

To validate the accuracy of the presence/absence of microplastic debris reported in the Continuous Plankton Recorder (CPR) samples by analysts at the Sir Alister Hardy Foundation for Ocean Science (SAHFOS) a subset of data was formally analysed using Fourier Transform Infrared (FTIR) spectroscopy. This analysis indicated a good level of accuracy (~66%) in the ability of the

SAHFOS' analysts to detect visually microplastic fragments during their normal processing of plankton samples.

To compare quantity and composition of buoyant microplastic debris collected by two different approaches (CPR vs. Manta net) samples were collected from a similar body of coastal waters. The results showed significantly higher abundance of microplastic in samples collected by the manta net per unit of distance but no significant difference once the results were standardised per cubic volume of water.

To assess the susceptibility of each method to 'procedural contamination', repetitive controlled tests were conducted to quantify the amount and composition of contaminants before, during and after the sampling programme. In both methods the highest number of contaminants was found on the net and prior to the deployment to sea. The semi synthetic Rayon fibres were the most abundant type of contaminants in both cases.

To compare the abundance and composition of buoyant plastic debris in estuarine waters according to daily and lunar tidal cycles a mensurative experiment was conducted in a macrotidal Estuary. Microplastics comprised 82% of the debris and there was a significant difference in size frequency distribution between the spring and neap tides with more fragments of larger size observed during spring tides.

In conclusion, this study shows further evidence of the spatial heterogeneity of microplastic debris distribution in marine waters and therefore also highlights the need for more comparable data from different marine habitats using standardised methodologies.

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Author's declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Committee. Work submitted for this research degree at the Plymouth University has not formed part of any other degree either at Plymouth University or at another establishment

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    - Sadri , S., Edwards, M., Thompson, R. C. Using the Continuous Plankton Recorder to determine the abundance of microplastic debris in the marine environment.

Contribution to public education:

- September 2012 - Contributed to the organisation and presentation of the educational material and activities on marine debris in the Science and Technology Showcase at the Marine City Festival in Plymouth.
- February 2013 – Appeared and helped prepare for sampling and laboratory analysis of microplastic samples from Tamar estuary which was presented in the BBC's Bang goes the Theory science programme related to microplastic debris on March 4th, 2013.
- September 2013 – Contributed to the content of My Sea Book on the marine debris theme published by Marine Biological Association for official launch during the European Marine Science Educators Association 2013 Conference in Plymouth.

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Chapter 1.General introduction

1.1 Background and Rationale

Plastic debris has been the subject of many scientific studies and there is now a strong body of evidence describing the ecological, social and economic problems associated with this debris in the marine environment worldwide. In particular, the presence of small pieces of plastic known as ‘microplastics’ is a matter of growing concern. Microplastic debris is introduced to the marine environment directly via release of small pieces of plastics used in personal care products and abrasives and indirectly through the fragmentation of larger items.

The European Marine Strategy Framework Directive 2008/56/EC (MSFD) lists specific indicators for assessment of Good Environmental Status (GES) of the European marine waters. Indicator 10.1.3 regards micro-particles, in particular microplastics as an issue and calls for research on the amount, composition, distribution and where possible trends in this debris. However, knowledge of the distribution, accumulation and temporal trends of this debris are incomplete and in order to more reliably assess the level of microplastic contamination and better understand its wider impact there is a need for comparable data within and between member states based on standard methodologies.

This thesis provides new information on the distribution, quantity and composition of buoyant microplastic debris in marine surface waters by comparing different methods of collection. Chapter one will start by providing a background to the problem of plastic as the most common type of debris in the marine environment and describes some of the sources, sinks and associated

harmful effects as well as the processes affecting the degradation of plastic. This is followed by a more detailed review of the distribution, abundance and consequences of microplastic debris. The chapter concludes by looking at current microplastic sampling and processing methods and an outline of the specific aims and objectives of the thesis.

1.2 Plastic debris in the marine environment

United Nations Environment Programme (UNEP) defines marine debris as *'as any persistent, manufactured or processed solid material discarded, disposed of, or abandoned in the marine and coastal environment'* and marine litter as such *'items that have been made or used by people and deliberately discarded into the sea or rivers or on beaches'* and plastic composes the largest proportion of this (GEF, 2012). Due to the lack of adequate waste management facilities in many countries, coupled with irresponsible disposal, a large amount of plastic waste is finding its way into the marine environment (Thompson *et al.*, 2009a). Our knowledge of the sources and sinks of this debris is not complete but it is believed that the majority have land-based sources such as poorly managed landfills, riverine transport, untreated sewage, manufacturing facilities with inadequate controls and recreational use of coastal areas by tourists (Barnes *et al.*, 2009; UNEP, 2011).

Other sources of plastic litter are offshore and include fishing and recreational vessels, merchant shipping and oil and gas platforms (Ryan *et al.*, 2009). Abandoned, Lost or Otherwise Discarded Fishing Gear (ALDFG) is the main concern in terms of larger debris (STAP, 2011) and plastic-based ALDFG could threaten not only marine habitats and fish stocks but also human health

(MacFadyen *et al.*, 2009). Figure 1 shows schematic diagram of the sources and movement pathways of plastic debris in the marine environment.

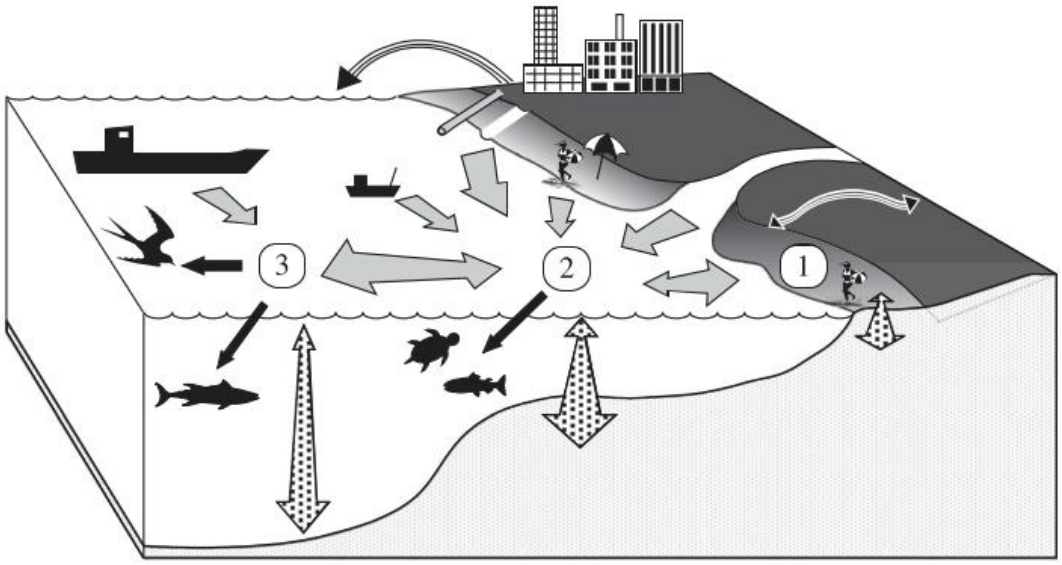


Figure 1) Diagram showing main sources and movement pathways of plastic debris with sinks occurring (1) on beaches, (2) in coastal waters and their sediments and (3) in the open ocean. Curved arrows depict wind-blown litter, gray arrows water-borne litter, stippled arrows vertical movement through the water column (including burial in sediments) and black arrows ingestion by marine organisms. (Source: Ryan, 2009)

More than 70% of marine debris on continental shelves and slopes of Europe have been identified as plastic (Galgani *et al.*, 2000) and there are reports of plastic debris in all zones of the marine environment including coastlines (Colton, Knapp & Burns, 1974; Morris, 1980) sea floor (Galgani *et al.*, 2000; Katsanevakis & Katsarou, 2004; Mordecai *et al.*, 2011) and sea surface (Colton, Knapp & Burns, 1974; Dixon & Dixon, 1983; Law *et al.*, 2010; Moore *et al.*, 2001; Morris, 1980; Thompson *et al.*, 2004). The occurrence of larger plastic items is especially evident on the coastlines with presence of plastic litter now a

common sight on most beaches around the world. The majority of plastic materials entering the marine environment are buoyant and once afloat their distribution is influenced by natural factors such as coastal currents, wind, tidal flow and the coastline geography (Moore, 2007; Andrady, 2011). Some debris is washed up on shorelines while others drift out into the open oceans with reports of their presence even in remote seas far away from any population centres such as Antarctica (Barnes *et al.*, 2009).

In the open oceans, floating marine debris becomes subject to large scale currents and studies indicate that debris can accumulate in circular oceanic gyre systems such as those in the North Pacific and Atlantic oceans (Moore *et al.*, 2001; Law *et al.*, 2010) and more recently confirmed in the South Pacific tropical gyre (Eriksen *et al.*, 2013). Satellite-tracked drifter data and probabilistic models have been used to study the pathways by which marine debris travels and have identified five main areas of debris accumulation in subtropical waters with predictions in close agreement with those observed by Law *et al.* (2010) in the North Atlantic (Maximenko, Hafner & Niiler, 2012). This study also indicated that the distribution of marine debris on small scales (<100 km) is more influenced by local oceanic eddies and fronts rather than large scale oceanic gyres but the interaction between these is not fully understood.

Some plastic debris are denser than seawater and will sink but even those that float initially may eventually become weighed down by fouling such that they sink to the seafloor (Stefatos *et al.*, 1999; Galgani *et al.*, 2000; Barnes *et al.*, 2009; Keller *et al.*, 2010). Some consider the seabed to be the ultimate sink for marine debris (Goldberg, 1997). The abundance and composition of marine benthic debris was investigated in shallow coastal areas of Greece where accumulation rates were shown to be higher in the shallow coastal areas

such as bays with weaker currents and less wave actions compared to continental shelves and deep seafloor (Katsanevakis & Katsarou, 2004).

Research into the deeper seabed on European continental shelves shows that similar to the quantities found at the sea surface the Mediterranean seabed has high densities of debris, possibly as a result of densely populated coastlines, heavy shipping activities and limited water exchange (Galgani et *al.*, 2000). Recent studies of deep sea debris in Monterey Canyon, USA using a 22-year video annotation database recorded by Remotely Operated Vehicles (ROVs) has shown that the highest relative frequencies of plastic is below 2000 m, suggesting that submarine canyons maybe acting as sinks for debris from shallower coastal habitats (Schlining *et al.*, 2013).

The problem of marine debris is global and requires a combination of regionally coordinated measures. Improvement of waste management facilities and design of environmentally friendly products as well as management of the 'discarding behaviours' are effective ways of reducing the input at the source points (Cheshire, 2009; STAP 2012).

1.3 Harmful effects of plastic debris

The harmful impacts of plastic debris in marine environment are manifold and can be categorised into three groups: ecological, social and economical (Piha *et al.*, 2011; Hall K., 2000). Most of our knowledge on the ecological impacts of debris is at individual level and from larger marine organisms such as seabirds, sea mammals and turtles (Derraik, 2002; Gregory, 2009; Jacobsen, Massey & Gulland, 2010; Lazar & Gracan, 2011). According to a recent report by UNEP as many as 663 species have been impacted by marine debris with

over half of these as a result of entanglement in and ingestion of marine debris (GEF, 2012).

Furthermore, with the increasing amount of durable, synthetic and non-biodegradable debris there are concerns that the dispersal rates and prospects for transport of aggressive invasive species could be enhanced as the hard surfaces of pelagic plastic debris could provide a suitable substrate for epibiota such as barnacles, worms and coralline algae (Barnes, 2002; Gregory, 2009; Goldstein & Goodwin, 2013). Examples of some invasive species are: the Indo-Pacific oyster, *Lopha cristagalli*, found on plastic rope on remote beaches in Fiordland, New Zealand (Winston *et al.*, 1997; Gregory, 2009); the intertidal anemone, *Diadumene lineate* native to Japan found on derelict trawl nets in the lagoon of Pearl and Hermes Reef in the north-western Hawaiian Islands (Zabin *et al.*, 2004) and dispersal of harmful microalgae species to the Catalan coast in north-western Mediterranean via pelagic plastic debris (Maso *et al.*, 2003). However, the relative importance of marine debris as a transport medium for invasion compared to other routes such as ballast waters from the haul of the vessels is not known.

There is also growing concern that plastics may pose a risk to human health. For example potentially harmful chemicals such as nonylphenols (NP) and polybrominated diphenyl ethers (PBDE) that are incorporated into plastics as additives during manufacturing in order to enhance their properties (e.g. durability, colourfulness and safety) and these could leach from the plastic either while in use or when it becomes debris. Ingested plastic could provide a route for the accumulation of these chemicals in body tissues (Oehlmann *et al.*, 2009; Talsness *et al.*, 2009). Additionally, evidence also suggests that small

plastic debris could facilitate the transport of persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloro ethylene (DDE) from contaminated seawater to marine organisms (Moore & Jones, 2007; Browne *et al.*, 2013; Gassel *et al.*, 2013; Rios; Rochman *et al.*, 2013; Bakir *et al.*, 2014). Such chemicals are known to have endocrine disrupting, carcinogenic and immunotoxic effects (Mato *et al.*, 2001; Rios, Moore & Jones, 2007; Teuten *et al.*, 2007; Teuten *et al.*, 2009; Andra, 2013).

Other undesirable but less widely recognised socio-economic impacts of plastic litter include hazards to shipping, fisheries and maritime activities (Nash, 1992). For example in UK removing beach litter is costing municipalities approximately €18 million per year (Mouat *et al.*, 2010).

1.4 International/EU conventions on marine litter

Marine litter has been the focus of many international and regional agreements and conventions. The following lists some of these in chronological order as described by the United Nations Environment Programme.

- The International Convention for the Prevention of Pollution from Ships (MARPOL (1973/78 and Annex V): an international convention for the prevention of pollution of the marine environment from the shipping industry.
- UNEP Regional Sea Programme (1974): provides a set of comprehensive actions and aims to engage the neighbouring countries to protect their shared marine environment through sustainable management and use.

- The Basel Convention (1992): a global environmental agreement to control the trans-boundary movements of hazardous wastes and their disposal.
- United Nations Convention on the Law of the Sea (UNCLOS, 1994): sets out the legal framework for all activities in the oceans and seas through its General Assembly Resolutions.
- Global Programme of Action (GPA, 1995): an intergovernmental programme for protection of marine environment from land-based activities. It also covers the linkage between freshwater and coastal environment.
- The London Dumping Convention and its 1996 Protocol: a global agreement to control pollution of the sea by dumping of hazardous and harmful wastes.
- Global Partnership on Marine Litter (2011): a UNEP-led coordinating platform for managing the marine litter problem based on the Honolulu Strategy - a global framework for tackling marine litter backed by governments, members of the plastics industry, scientists, NGOs and other groups.

In Europe some of the problems of marine litter are now also considered by the Marine Strategy Framework Directive (MSFD) where marine litter has been defined as ‘items that have been made or used by people and deliberately discarded or unintentionally lost into the sea or coastline including such materials transported into the marine environment from land by rivers, drainage or sewage systems or wind’. The overall aim of the framework is to provide clear criteria and methodological standards in order to promote a more

consistent and effective approach towards protection of marine environment across Europe with a goal of achieving a Good Environmental Status (GES) in EU marine waters by 2020.

The first step towards achieving this goal is to assess the current state of marine environment for member states to provide clear environmental targets and monitoring programmes (Hanke *et al.*, 2013). In the UK *Charting Progress 2* (CP2) is the comprehensive report on the state of the UK seas and Chapter 4 of this; “Clean and Safe Seas” addresses the issue of marine litter. Marine litter is one of the 11 qualitative descriptors that will be used for assessment of Good Environmental Status under the EU Marine Strategy Framework (DEFRA, 2011).

1.5 Plastics: A general overview

Plastics are synthetic polymers (large molecules) made of repeating chemical units called “monomers”. These are the basic units of plastic production which are extracted during the refinery of crude oil through the processes of “distillation” and “cracking” where heavy oil compounds are broken into smaller hydrocarbon molecules. These small hydrocarbon monomers are then linked together during a process known as “polymerisation” to form the polymer chains used in production of plastics (Plastics Europe, 2013; Saldivar-Guerra & Vivaldo-Lima, 2013). Plastics can be classified according to their chemical composition as *‘carbon-based’* or *‘heterochain’* polymers (Saldivar-Guerra & Vivaldo-Lima, 2013; Environment Agency, 2001).

Most of the common plastics are made of chains of carbon-based monomers. The main plastic types in this group are: polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (solid PS and

expandable PS), polyethylene terephthalate (PET) and polyurethane (PUR). These are lightweight, versatile and strong plastics which have a wide range of applications especially in the packaging sector and together account for nearly 39% of the overall demand for plastics in Europe (Plastic Europe, 2013). Table 1 shows some of their applications and uses.

Table 1) Common plastics and some of their applications

Plastic type	Applications and uses
Low and high density polyethylene (LDPE, HDPE)	Carrier bags, bin liners (LDPE). Milk, shampoo and detergent bottles (HDPE)
polypropylene (PP)	Food containers such as margarine and yogurt pots
polyvinyl chloride (PVC)	Cable, hoses and window frames
polystyrene (solid PS and expandable PS)	Vending cups and packaging materials
polyethylene terephthalate (PET)	Water and fizzy drink bottles

Heterochain polymers are made of monomers containing other elements such as oxygen, nitrogen, sulphur as well as carbon. These are also known as ‘engineering plastics’ and have had the highest growth in 2011 (Plastics Europe, 2013). They have enhanced properties such as heat resistance, mechanical strength and chemical stability and are mainly used in manufacturing of more specialised products in areas such as electronics and automotive and compared to the more conventional plastics. For example, Acrylonitrile

butadiene styrene (ABS) is used to manufacture car bumpers, dashboard trim and Lego bricks, Polycarbonate (PC) is used in motorcycle helmets and Polyamides (nylons) are used for skis and ski boots and some fishing nets (Rosato, 1996).

Plastics may also be categorised according to their physical properties into *thermoplastic* and *thermosetting* plastics. Thermoplastic polymers, such as polyethylene, and polystyrene can be reprocessed through heating while the thermosetting plastics cannot be reprocessed by heating as their monomer chains have been interlocked during polymerisation. Examples of thermoset plastics are: Epoxy resin and Urea formaldehyde which are mainly used in electrical insulators, melamine formaldehyde used in tableware and laminating of work surfaces and Polycarbonate used in spectacle lenses and crash helmets (Environment Agency, 2001; Dodiuk and Goodman, 2013; Plastics Europe, 2013).

The main drivers for the widespread use of plastic are its enhanced chemical and physical properties and low cost of production which makes plastic suitable for a wide range of applications from food containers to automotive, household goods, aircraft parts, sports and medical equipment. Global production of plastic has been rising by almost 5% per year over the past 20 years and currently despite the current uncertain economic forecasts; its production increased by 2.8% since 2011 to a total of 288 million tonnes in 2012 (Figure 2). The packaging sector comprises the highest (39.4%) of the total European plastics demand followed by building and construction (20.3%) and electrical and Automotive (8.2%). One area of potential growth is predicted to be the rapid developing Asian markets where the current use of about 20 kg

plastic per year per person is estimated to rise to 36 kg by 2015. Examples of increases in Asia are apparent in countries such as China which, with its over-average growth rate and production capacity, accounted for the highest (23.9%) of the world's plastic material production in 2011 (Plastics Europe, 2013).

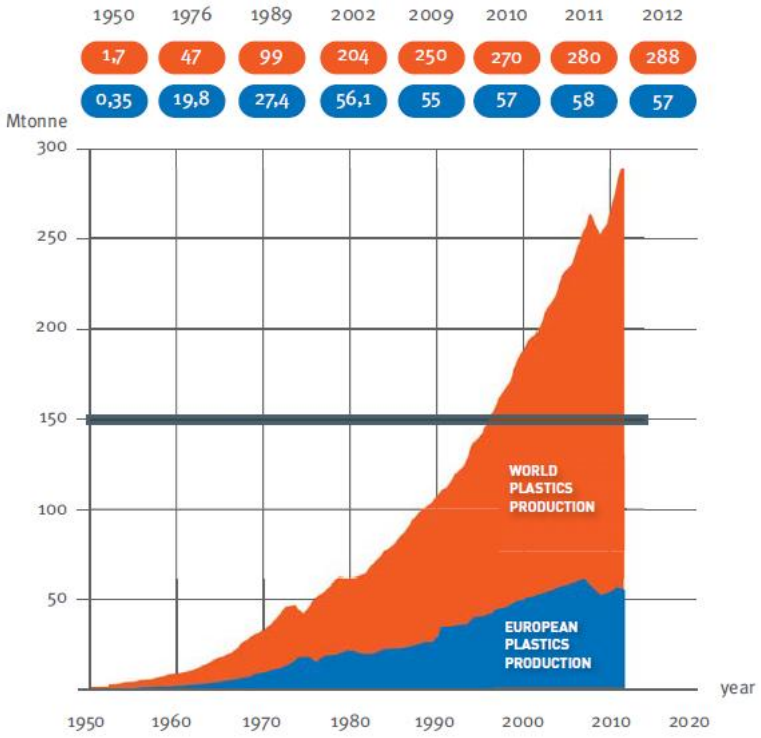


Figure 2) Plastic global productions in 2012 (Plastic Europe, 2013)

The majority of plastic packaging is made from one of six resin types: polyethylene terephthalate (PETE); high density polyethylene (HDPE); polyvinyl chloride (PVC or vinyl); low density polyethylene (LDPE); polypropylene (PP); or polystyrene (PS) and at the end of their useful lives most commodity plastic items can be recycled. The Society of the Plastics Industry, Inc. (SPI) introduced a resin identification coding system in 1988 as means of identifying the resin content of bottles and containers commonly found in the residential waste stream (Appendix 1).

However, despite recent improvements within the developed countries, the recycling and re-use efforts remain low and at best variable (UNEP, 2011). In Europe 51.3% of the total 24.9 million tonnes of post-consumer plastics were recovered of which only 5.3 million tonnes (10%) were recycled (EuPC, 2010). In the UK a large (48%) portion of the 5 million tonnes of plastics used in 2010 was from plastic packaging items such as plastic bottles, pots, tubs, trays, films and plastic bags (BPF, 2010). The recent collection and consumption data from a UK household plastic packaging collection survey by the RECycling Of Used Plastics limited (RECOUP, 2013) indicates a recycling rate of 58% for plastic bottles 19% for pot, tubs and tray and 37% for rigid plastic packaging.

The main aim of the household waste management programmes are to divert plastics from landfills but the immediate challenge seems to be the lack of efficient infrastructure capable of separating different types of plastics for recycling and energy recovery (Barnes et *al.*, 2009). In the UK a recent study by the Waste & Resources Action Programme (WRAP) has assessed four different waste management options: Recycling, Incineration, Landfill and Pyrolysis for end of life plastic using four specific indicators: depletion of natural resources, global warming, energy demand and water consumption as drivers. The report suggests the mechanical recycling as the most and landfill as the least environmentally friendly options (WRAP, 2010) and considers the lack of adequate domestic recycling infrastructure in UK where around two-thirds of the packaging plastics recovered from the waste stream are exported overseas, a source of negative environmental impacts.

1.6 Degradation of plastic in the marine environment

Degradation of plastic can be described as any change which has adverse effects on its chemical, physical or functional properties and often in the context of environmental studies the degradation process is referred to as “ageing” or “weathering” (Shashoua, 2009). Plastic is considered as one of the most durable synthetic materials available and some estimates suggest that plastics may persist in the marine environment for centuries (Gregory, 1996). Eventually all plastics will degrade due to the action of chemical, physical or biological factors. The rate and extent of the plastic degradation depends on the chemical composition of plastic and intensity of the degrading agents (Singh & Sharma, 2008) but timescales are likely to be considerable.

Andrady (2011) has classified different types of plastic degradation in the environment according to the agent causing it as follows:

a. Biodegradation – action of living organisms;

b. Photodegradation – action of light;

c. Thermooxidative – action of slow oxidative breakdown at low temperature; and

d. Hydrolysis – reaction with water.

In the marine environment most plastic debris will slowly break down as a result of prolonged exposure to solar ultraviolet, UV-B (315 – 280 nm wavelength) light and physical abrasion on the shorelines and beaches where the sunlight intensity and wave action are the strongest (Barnes *et al.*, 2009; Corcoran, Biesinger & Grifi, 2009; Cooper & Corcoran, 2010). In seawater, however, due to lower temperatures and reduced oxygen concentration the effect of UV radiation is reduced; therefore slowing the degradation process but

once degradation is initiated it can progress even without further exposure to UV radiation as long as oxygen is available (Andrady, 1994; Barnes *et al.*, 2009; Andrady, 2011). In addition, biota could grow on the floating plastic debris and cause it to sink deeper in the water column where its degradation rate will further be reduced due to the lower levels of UV light, oxygen and temperature (Andrady, 1994; Ye & Andrady, 1991). The degradation rate of plastic debris could also be reduced due to the formation of a biofilm on the plastic surface thus reducing exposure to sunlight (Muthukumar *et al.*, 2011).

Biobased plastics known also as biopolymers are reletaviely new materials that may have the potential to reduce the negative impacts of plastic debris on the marine environment, however, the term “bio” should not be confused with biodegradability as it only refers to the carbon source of these plastics. Most “biodegradable” plastics available today are designed to degrade under industrial conditions such as those in composting plants at high temperature and humidity are unlikely to degrade fully or within an acceptable time frame in the natural environment, hence they are of limited effectiveness in reducing the impacts of marine debris (O'Brine & Thompson, 2010). A 40 weeks study of two different oxo-biodegradable, a biodegradable and standard polyethylene bags showed that compostable plastics degraded relatively quickly compared to oxo-biodegradable and conventional plastics (O'Brine & Thompson, 2010).

Reddy *et al.* (2013) have classified the biobased plastics in 3 main groups according to their production sources as follow:

1) *Renewable-sources:* made from plants and animals and includes starch, cellulose, proteins and poly lactic acid (PLA).

2) *Petroleum-sources:* made from petroleum resources but are biodegradable at the end of their functionality. Polycaprolactone (PCL) and poly (butylene adipate-co-terephthalate)(PBAT) are examples in this category.

3) *Mixed sources:* made form combination of biobased and petroleum monomers and includes polymers such as poly (trimethylene tereph-thalate) (PTT), bio-thermosets and biobased blends.

Poly β-hydroxybutyrate (PHB), poly ε-caprolactone (PCL), poly ethylene succinate (PES), poly butyrene succinate (PBS) and poly lactic acid (PLA), are more examples of biopolymers and studies have identified that certain species of bacteria found in the deep-sea waters are able to degrade some of these biopolymers such as poly ε-caprolactone (PCL) (Tokiwa & Calabia, 2004; Bobek *et al.*, 2009). However other studies have also shown that PHB, PBS and PLA could not be fully degraded by the deep-sea microorganisms (Sekiguchi *et al.*, 2009). The ANIMPOL project is an initiative funded by the European Commission with an aim to use waste streams (e.g. hearts, livers, lungs) from slaughterhouses as a source of lipids and nitrogen that are needed for the production of polyhydroxyalkanoates (PHAs), a group of biopolymers and biodegradable polyesters (Kettl *et al.*, 2011).

Biobased plastics such as Polyethylene furanoate (PEF) are now available in products such as beverage bottles, yogurt pots and haircare packaging (PlasticEurope, 2012) but only account for around 1% of global plastic production. One study has estimated that the substitution potential of

biobased plastics replacing petrochemical plastics could be as high as 90% and highlights the potential of the biobased plastics in reducing the dependence on the currently mass produced and environmental unfriendly petrochemical plastics (Shen *et al.*, 2010). Another rrecent research has demonstrated the transformation of edible vegetable waste such as parsley and spinach stems into 'bioplastics' with a wide range of mechanical properties but higher degradability rate (Bayer *et al.*, 2014).

1.7 Microplastic debris

"Microplastic" is a relatively new term in the field of marine debris. It was first used by Thompson *et al.* (2004) referring to microscopic pieces some as small as 20 µm in size found in the Continuous Plankton Recorder (CPR) and sediment samples from the northeast Atlantic ocean. Microplastics were subsequently defined by the National Oceanic and Atmospheric Administration (NOAA) Marine Debris Program as plastic pieces typically in the size range of 0.3-5mm (Arthur et *al.*, 2009) in order to recognise the 333 µm mesh size used as the lower bound in most neuston nets used in sampling of floating debris. Gregory & Andrady (2003) refer to smaller size (~0.06–0.5 mm in diameter) pieces as "micro litter". There is no agreed minimum size for microplastics but it is likely that even smaller manufactured plastic nanoparticles used in consumer products are introduced directly into the oceans via runoff (Maynard, 2006; Andrady, 2011).

Microplastics pieces come in variety of shapes, sizes and colours (Figure 3) and on the basis of their sources could be classified in two groups:

1) Primary microplastics: produced for direct use in products such as spherules in personal care products like facial scrubs (Murray, 1996; Fendall & Sewell, 2009) and also as precursors to other products such as pre-production plastic pellets and powder raw material (Gregory, 1978; Mato *et al.*, 2001). Other sources into marine waters include accidental losses through runoff and sewage sludge dumping grounds at sea (Ryan *et al.*, 2009; GESAMP, 2010; Browne *et al.*, 2011).
2) Secondary microplastics: formed from the breakdown of larger plastic debris as a result of weathering processes such as photo oxidation and mechanical abrasion (Thompson *et al.*, 2004; Moore, 2008; Barnes *et al.*, 2009; Andrady, 2011).

Figure 3) Microplastic debris of various sizes, shapes and colours collected from the surface water of the North Pacific Ocean by the SUPER expedition in 2008 (source: C-MORE, http://cmore.soest.hawaii.edu/)

The sources and sinks of microplastics are poorly understood but it is likely that secondary microplastics (those formed through fragmentation of the larger plastic items) comprise the major part of the total abundance of this debris in the marine environment (Thompson *et al.*, 2004; Barnes *et al.*, 2009; Andrady, 2011) and therefore linking prevention measures for microplastics to

the broader issues of solid waste management, plastic waste recovery and recycling (GESAMP, 2010).

The occurrence of primary microplastics in the form of pellets and polystyrene spherules of the size range now described as microplastics found in the plankton net hauls from the north-western Atlantic Ocean were first reported in 1970s (Colton, Knapp & Burns, 1974) and have since been regularly reported worldwide on sediments (Gregory, 1978; Gregory, 1983; Ng & Obbard, 2006; Browne *et al.*, 2010; Costa *et al.*, 2010; Hirai *et al.* 2011; Browne *et al.*, 2011; Claessens *et al.*, 2011; McDermid & McMullen, 2004; Reddy *et al.*, 2006; Vianello *et al.*, 2013) and water column (Moore *et al.*, 2001; Thompson *et al.*, 2004; Law *et al.*, 2010; Collignon *et al.*, 2012; Desforges *et al.*, 2014).

In intertidal habitats near Plymouth, UK small (<5mm) plastic pieces comprised ~10% of the samples by weight (Browne *et al.*, 2010). In the northeast Atlantic Ocean, the multi-decadal (1960s-1990s) plankton records shows that the quantities of microplastics in the water column have increased in line with the production of synthetic fibres (Thompson, 2004). It is envisaged that even if the input of plastic debris were to reduce today; the abundance of microplastics would still continue to rise as the result of weathering of the existing plastic debris (Barnes *et al.*, 2009; Thompson *et al.*, 2009b). Nearly 88% of a subset of samples from the North Atlantic Ocean subtropical gyre were <10mm in size and most showed signs of physical deterioration such as brittleness and rough edges (Law *et al.*, 2010; Morét-Ferguson *et al.*, 2010).

Most of studies and media attention have been focused on macro size plastic debris as their effects are easier to detect and more visible to the public eye. However a growing number of studies have recognised microplastic debris

as an important emerging contaminant (Thompson *et al.*, 2004; Ng & Obbard, 2006; Andrady, 2011; Barnes *et al.*, 2009; Fendall & Sewell, 2009; Thompson *et al.*, 2009a; Frias *et al.*, 2010; Sutherland *et al.*, 2010; Browne *et al.*, 2011; Claessens *et al.*, 2011; Cole *et al.*, 2011; Harrison *et al.*, 2011; Hirai *et al.*, 2011; Karp, 2011; Bakir *et al.*, 2012; Ivar and Costa, 2014). In comparison with larger plastic debris our knowledge of small particles is poor (STAP, 2011) and in its latest report on marine litter United Nations Environmental Programme (UNEP) acknowledges the accumulation and potential harmful impacts of microplastic particles in the marine environment and calls for further research (UNEP, 2011).

A recent study by Browne et *al.* (2011) used samples from 18 shorelines around the world and found pieces of microplastics in all of them. Polyester, acrylic and polyamides (nylon) fibres were amongst the most abundant types and greater concentrations of this debris was found in areas near to the urban centres suggesting these be major source of these synthetic fibres into the marine environment (Browne *et al.*, 2011).

Due to their larger surface area to volume ratio, smaller pieces of plastic may have increased levels of contaminant uptake and release to the food web (Mato *et al.*, 2001; Thompson *et al.*, 2004; Teuten *et al.*, 2007; Teuten *et al.*, 2009; Thompson *et al.*, 2009a; Frias, 2010; Hirai *et al.*, 2011). Furthermore because of their buoyant and persistent properties they are dispersed widely by the ocean currents and other hydrodynamic processes and hence may become available to a broader range of organisms (GESAMP, 2010). Boerger et *al.* (2010) were first to quantify the ingestion of microplastic debris by the lower trophic level planktivorous fishes in the North Pacific central gyre and found that the average size of ingested plastic to be 1.00–2.79 mm with a positive

correlation between the average number of pieces and the size of the fish, however, further research is needed to establish the extent to which fish retain the ingested plastic (Boerger *et al.*, 2010). Microplastics has also been found in stomach contents of the commercially important lobster, *Nephrops norvegicus,* in the Clyde Sea where 83% of the samples contained strands (mostly filaments) of plastic (Murray & Cowie, 2011). This study also showed that lobsters fed with fish seeded with polypropylene strands retained some of the ingested plastics rising concerns over the potential impacts. A recent report by United Nations Environment Programme (UNEP) reveals that as much as 10% of the encounters between organisms and debris are with microplastics (UNEP, 2012).

Laboratory experiments have demonstrated ingestion by a range of invertebrates including deposit feeding lug-worms, filter-feeding barnacles and suspension-feeding sea cucumbers (Thompson et *al*., 2005; Browne *et al.*, 2008; Graham & Thompson, 2009; Goldstein & Goodwin, 2013). Browne *et al* (2008) used the mussel, *Mytilus edulis*, to investigate ingestion, translocation, and accumulation of microplastics and showed evidence of accumulation in the gut as well as subsequent translocation of microplastics to the circulatory system where it persisted for over 48 days. This suggests that as plastic fragments into smaller sizes, the potential for accumulation in the tissues increases. A laboratory experiment by Graham & Thompson (2009) has provided evidence of selective ingestion of plastic fragments by three species of sea cucumber. More recent studies has shown the potential of zooplankton taxa to ingest plastic: Cole *et al.* (2013) demonstrated the capacity of as many as 13 taxa of zooplankton to ingest 1.7–30.6 µm polystyrene beads and also showed that the adherence of this debris to appendages of at least one of the exposed zooplankton, *Centropages typicus,* significantly decreasing its algal feeding

implying that microplastic debris could negatively impact the function and health of zooplankton. Setälä *et al.* (2014) have recently shown the ingestion of polyestyrene microspheres by differen Baltic Sea zooplankton taxa such as mysid shrimps, copepods, cladocerans, rotifers, polychaete larvae and ciliates.

As well as its direct physical effects microplastics could also affect the wellbeing of organisms indirectly by altering the physical and biogeochemical properties of their habitats. A recent examination of sediment samples from beaches of Hawaiian Islands has shown that, compared to sediments with less or no plastic fragments, those with higher concentration of plastic fragments are more permeable and retain water for a shorter length of time and hence are warmed more slowly (Carson *et al.*, 2011). This study shows that even a small amount of plastic (1.5%) could decrease the maximum temperature of the sediment by 0.75 ˚C and authors argue that these changes could have disturbing effects on some beach organisms such as turtles with a temperature-dependant sex-determination mechanism in their eggs. A multi-decadal study of the microplastic debris from surface waters of the North Pacific Ocean Subtropical gyre has shown a positive correlation between the increase in the egg densities of the pelagic insect *Halobates sericeus* and microplastic and emphasises the potential ecological impacts that microplastic could have on pelagic ecosystems (Goldstein *et al.*, 2012) and a recent study by Wright *et al.* (2013) indicated that microplastic ingestion decreases energy reserves in marine worms due to a combination of reduced feeding activity resulted from longer gut residence time of ingested plastic and inflammation.

Monitoring is important in assessing the effectiveness of any measures employed to reduce the abundance of plastic debris. However, this has mostly been carried out on beaches and focused on larger items of debris (Ryan *et al.*

2009). Monitoring microplastics in particular at sea, is a much more challenging task and requires large sample sizes in order to detect any spatiotemporal trends.

1.8 Methods of sampling and processing microplastic debris in marine waters

Microplastic sampling and processing methods vary considerably and there is a need for standardisation (GESAMP, 2010; Piha *et al.*, 2011). In sedimentary environments the majority of work has been from sandy beaches with samples taken from different tidal levels using varying methods and equipment. Sea surface and water column samples are mostly taken by plankton nets (e.g. manta and bongo nets) with different mesh sizes and at varying depths and speeds. A recent review of 68 studies by Hidalgo-Rez et *al.* (2012) described the main sampling strategies and processing as follows:

Sampling strategies

- Selective sampling: collecting microplastics directly from the environment.
- Bulk sampling: extracting microplastics from the entire sample volume in the laboratory.
- Volume-reduced sampling: extracting microplastics from the sieved sediment or filtered water samples in the environment.

The selective strategy is more time consuming and could also underestimate the true abundance of microplastics since not all the microplastic pieces are located at the surface and even if they are this

approach could be biased towards the larger pieces as the smaller items are not easily detectable by the naked eye.

Processing steps

- Density separation: to separate low density microplastic from sediment samples.
- Filtration: to separate suspended microplastic from bulk water or solution samples.
- Sieving: to separate microplastic from sediment and water samples
- Visual sorting: to separate microplastic by naked eye or dissecting microscope

The extent and types of reported information such as units of abundance, colour, size, and shape is also in need of harmonisation. Size in particular is an important feature that could influence the level of potential harm and is also one that is directly affected by the sampling and processing methods. NOAA's classification of microplastics as pieces <5mm in size represents a step forward towards standardisation of quantitative methods (Arthur et *al.*, 2009); however this was to recognise the fact that most particles are captured during net based biological sampling and there is currently no consensus on what is to be considered as the smallest size (GESAMP, 2010). However microplastics down to around 20 µm have been caught on some plankton nets with larger mesh sizes and subsequently identified using Fourier Transformed Infrared (FT-IR) spectrometry (Thompson *et al.*, 2004).

In order to assess levels of microplastic contamination worldwide; there is a need for comparable data across marine environments that are based on

standard methodologies (Hidalgo-Ruz *et al.*, 2012). Nets traditionally used to sample zooplanktons have also most commonly been used to sample the pelagic (sea surface and water column) plastics. For near surface samples a device known as manta net is towed behind the ship normally from a boom away from the bow wave of the ship and samples the topmost layer normally 10-25 cm in thickness (David , 2009). Bongo nets are usually paired mesh nets designed to sample deeper waters (down to 200m depth.) and are normally towed obliquely (in a V shape) so that all depths are sampled twice. Water enters through the net's mouth also known as 'aperture' and particles larger than the mesh size (~300 µm) are collected at the end of the net into receptacles, called cod ends. There are flow meters in the mouths of the nets so that the volume of water filtered can be calculated accurately (Wiebe and Benfield, 2010).

Archived samples from Continuous Plankton Recorder (CPR) survey run by the Sir Alister Hardy Foundation for Ocean Science (SAHFOS) have been used in past and shown the prevalence of microplastic in the Northeast Atlantic (Thompson *et al.*, 2004) during the past fifty years (Figure 4). With its long-term archived data, consistent methodology and broad scale sampling programme the CPR survey could provide a unique opportunity for spatiotemporal study of microplastic debris in marine surface waters. However, its use as a standard platform needs to be evaluated.

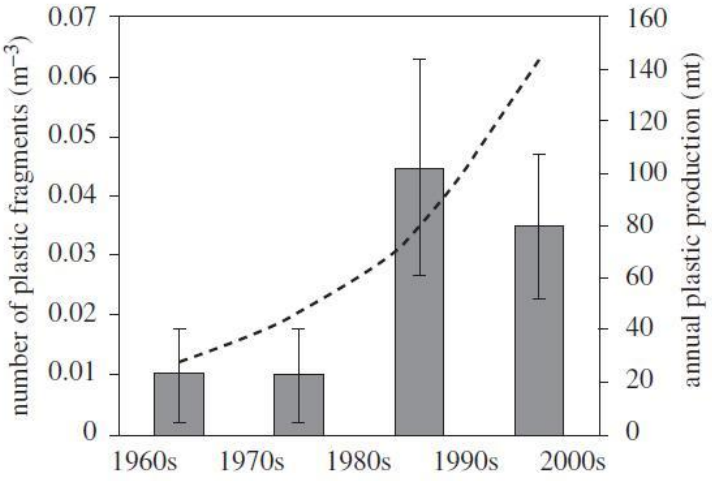


Figure 4) Microplastic in CPR samples showed significant increase in abundance (Source: Thompson et *al., 2004)*

1.9 Aims and Objectives

The primary aim of this research was to evaluate the use of the CPR to sample buoyant microplastic debris in marine waters, and to compare the abundance and composition of plastic debris collected by CPR with that collected by manta trawl. This aim was accomplished through completion of the following objectives and is reported in the following chapters.

- Chapter 2: To compare the spatial distribution, abundance and composition of microplastic debris between the nearshore and offshore marine subsurface waters as captured by the Continuous Plankton Recorder (CPR).
- Chapter 3: To validate the accuracy of the presence/absence method of reporting microplastic debris in the Continuous Plankton Recorder (CPR) samples by the SAHFOS's analysts and to quantify and formally identify this debris. And to investigate and quantify the levels of

‘procedural contamination’ by conducting inspections at different stages of the sample preparation and analysis.

- Chapter 4: To compare quantity and composition of buoyant microplastic debris collected by two different approaches (CPR vs. Manta net) providing new data on the amount, composition and distribution of buoyant plastic debris in water column using different sampling devices.
- Chapter 5: To compare the abundance and composition of buoyant plastic debris in estuarine waters according to daily and lunar tidal cycles using Manta net and to investigate and quantify the levels of ‘procedural contamination’ by conducting inspections at different stages of the sample preparation and analysis.
- Chapter 6: The final chapter summarises main findings of this study and introduces some guidelines for standardisation of microplastics sampling and processing methods by looking at gaps and shortcomings of current strategies.

Chapter 2. Distribution and composition of plastic debris captured by the Continuous Plankton Recorder (CPR)

2.1 Abstract

‘Microplastics’ are pieces of plastic debris <5mm in diameter. They are introduced into the marine environment directly for example via release of small pieces of plastics used as abrasives and indirectly through the fragmentation of larger items. The full impacts of this debris are largely unknown but they have been reported in many parts of the world and known to be ingested by marine organisms. The European Marine Strategy Framework Directive 2008/56/EC (MSFD) regards microplastics as an emerging issue of concern and calls for more data on the quantity, distribution and composition of this debris. Reports by the analysts at the Sir Alistair Hardy Foundation for Ocean Science (SAHFOS) on presence of microplastic debris in Continuous Plankton Recorder (CPR) samples indicate there is a wide distribution of this debris in marine surface waters. This study examines the content of CPR plankton samples were and confirms the presence of synthetic polymers in samples collected from European waters (including North Sea, Irish Sea, English Channel and the Northeast Atlantic Ocean). Man made semisynthetic rayon fibres were the most abundant (62%) type of polymers followed by synthetic polymers: Polyester (20%), Polyethylene terephthalate (10%), Nylon (3%), Polyvinyl Chloride (2%), Polypropylene (2%) and Acrylic (1%). Total abundance was significantly higher in samples from nearshore coastal waters compared to that of the offshore waters, with the highest concentrations observed in UK’s coastal waters of the northeast Atlantic Ocean and the southern North Sea at 0.53/m^3 seawater. This may be attributed to sources of debris from urban centres and riverine input as well as the modulating effects of oceanographic features such as frontal zones on distribution of flotsam in coastal waters.

2.2 Introduction

Global plastic production has rapidly increased from 5 million tonnes in 1950s to 288 million tonnes in 2012 with the packaging sector alone accounting for nearly 40% of the total demand (Plastics Europe, 2013). Due to the lack of adequate waste management facilities in many countries and irresponsible disposal a large proportion of the plastic waste is finding its way into the marine environment (Thompson et *al.,* 2009a, b). The extent of the problem is global and the presence of plastic litter is now a common sight on most beaches around the world with plastic debris even reported floating in remote locations far away from any population centres such as Antarctica (Barnes *et al.*, 2010). Most of our knowledge on the biological impact of this debris is at the individual level and from larger marine organisms such as seabirds, sea mammals and turtles through ingestion or entanglement (Derraik, 2002; Gregory, 2009; Jacobsen et *al.*, 2010; Lazar & Gracan, 2011; Votier *et al.*, 2011).

There is growing evidence that as a result of weathering processes such as photo-oxidation and mechanical abrasion large items of plastic debris are fragmenting into “microplastic” pieces and therefore increasing the surface area and possibility of their interaction with a wider range of biota along the food chain (Colton *et al.*, 1974; Thompson *et al.*, 2004; Andrady, 2011). A recent review by the United Nations Environment Programme (UNEP) indicates that around 10% of the reported encounters between organisms and debris are with microplastics (UNEP, 2012). Beside fragmentation some microplastics such as those used in plastics pre-production, abrasive products and clothing are regularly discharged into oceans via runoff and sewage effluents (Gregory, 1996; Ryan *et al.*, 2009; Browne *et al.*, 2011). The environmental consequences of this debris is not fully understood but laboratory experiments have shown

evidence of ingestion by a range of invertebrates including filter feeders, deposit feeding worms, detritivores and zooplankton (Thompson *et al.*, 2004, 2005; Browne *et al.*, 2008; Cole *et al.*, 2013).

There are also growing concerns that microplastics might present a toxological hazard if these particles facilitate the transport of harmful persistent organic pollutants (POPs) such as PCBs, DDE, nonylphenols and phenanthrene from the contaminated seawater (Mato *et al.*, 2001; Derraik, 2002; Teuten *et al.* 2007, 2009; Bakir *et al.*, 2012; Koelmans *et al.*, 2013; Lee *et al.*, 2014). Furthermore, studies have shown that some of the additive chemicals used during the production of plastics to enhance its properties can have endocrine disturbing, carcinogenic and immunotoxic effects and could leach out of the ingested plastic (Mato *et al.*, 2001; Endo *et al.*, 2005; Rios *et al.*, 2007; Oehlmann *et al.*, 2009; Tanaka *et al.*, 2013).

Most commodity plastics such as Polyethylene (PE), Polypropylene (PP) and Polystyrene (PS) are less dense than seawater and once afloat in the marine waters their movement is influenced by currents, eddies and gyres on a large scale and by the density of sea water on a smaller scale (GESAMP, 2010). Larger items of plastic debris have been shown to accumulate in large oceanic zones such as gyres (Moore et *al.*, 2001; Law et *al.*, 2010; Morét-Ferguson *et al.*, 2010) and also in nearshore coastal waters of the North Sea (Dixon and Dixon, 1983; Thiel *et al.*, 2011) but in spite of several studies (Colton, Knapp & Burns, 1974; Moore *et al.* 2001; Thompson *et al.,* 2004 ; Ng & Obbard, 2006; Costa *et al*., 2010; Browne *et al.,* 2011; Collignon *et al*., 2012) there is no evidence that smaller plastic debris follow a similar path and current knowledge of the sources, pathway and fate of microplastic debris in marine waters remains limited. Recent studies have highlighted wastewater from washing

machines as an important source of microplastic fibres such as polyester and rayon entering the marine waters through sewage-discharges (Browen *et al.*, 2011). A study of microplastic in 10 species of fish from English Channel found the rayon fibres as the most common semi-synthetic material ingested by fish (Lusher *et al.*, 2012).

A worldwide study of microplastic debris from shorelines has shown greater concentrations of this debris in areas closer to the urban centres suggesting that they could be a major source of these synthetic fibres into the marine environment (Browne *et al.*, 2011). Similarly spatiotemporal studies of floating objects in coastal waters around Chile found the sources of floating marine debris (mainly plastic objects and Styrofoam) to be mainly local (Hinojosa *et al.,* 2011).

Archived CPR samples were first used to evaluate microplastics abundance in subsurface waters of the northeast Atlantic (Thompson *et al.*, 2004) and subsequently SAHFOS decided to record on a presence/absence basis which samples they considered to be contaminated with pieces resembling microplastics (Richardson *et al.*, 2006).

This chapter presents data on the distribution and composition of microplastics debris found in CPR samples and specifically examines the:

- Spatial distribution of microplastic debris in CPR samples based on formal analysis of the presence/absence reports by the SAHFOS analysts.

- Comparison of the microplastic concentrations between near-shore coastal waters and offshore oceanic waters in the North Sea and Northeast Atlantic Ocean.

- Quantity, composition and characteristics of the microplastic debris present in the CPR samples.

2.2.1 Hypothesis tested

H0: the quantity of microplastic debris captured by CPR is similar in nearshore coastal waters and offshore oceanic waters.

2.3 Material and Methods

2.3.1 Continuous Plankton Recorder Survey

The Continuous plankton recorder (CPR) survey is the longest plankton recording program of its kind in the world and its aim is to regularly record the subsurface plankton community. It is run by the Sir Alister Hardy Foundation for Ocean Science and has a network of over 50 routes (Figure 5) currently sampling over 10,000 nautical miles of water every month from the North Sea, North Atlantic Ocean and Pacific Ocean.

The CPR device is approximately 1 meter long and is towed behind commercial vessels at a speed of up to 25 knots (Warner and Hayes, 1994) at a depth of approximately 10 meters where the seawater passes through an entrance aperture of about 1.27 cm x 1.27 cm and plankton are filtered onto a slow-moving band of silk (270 micrometre mesh size) and then covered by another layer of silk. The silks and plankton are then spooled into a storage tank containing formalin (Figure 6). Once back in the laboratory, the silk roll is removed from the mechanism and divided into individual sections (each section

is referred to as a sample, approximately 15 x10 cm in size) each representing 10 nautical miles of tow and approximately 3 m^3 of water (Richardson *et al.*, 2006).

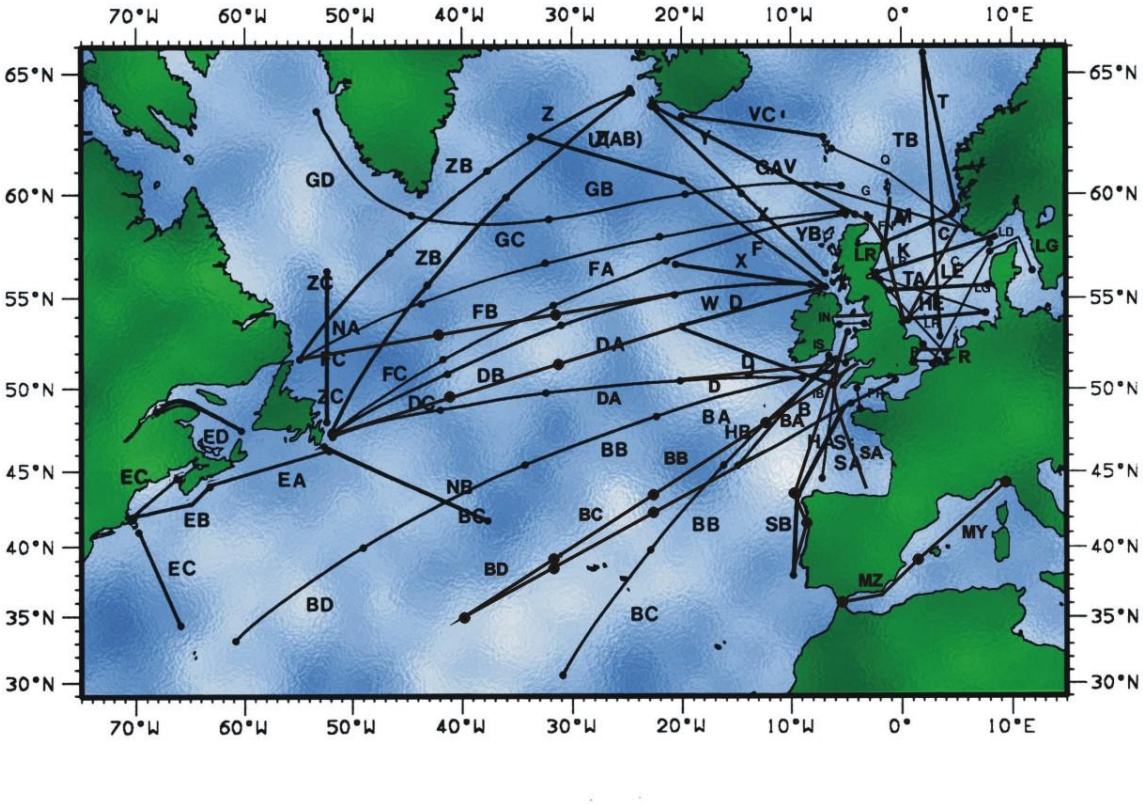


Figure 5) North Atlantic routes used by the Continuous Plankton Recorder (CPR) (Source: SAHFOS)

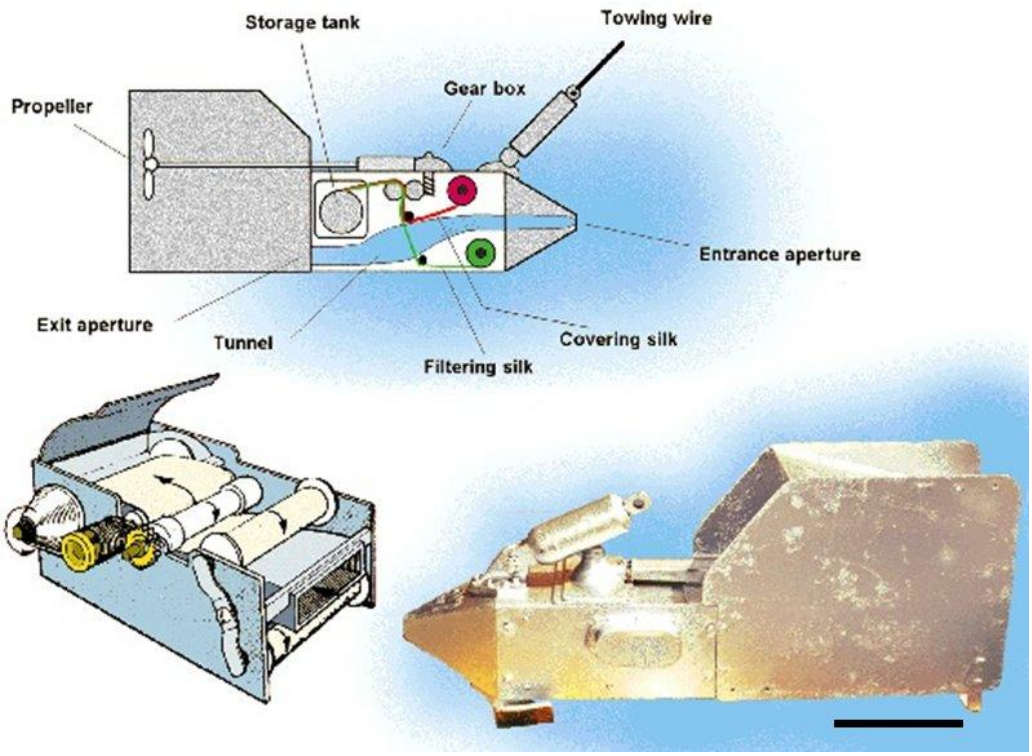


Figure 6) Continuos Plankton Recorder device. Scale bar represents 20 cm (Source: SAHFOS)

2.3.2 Study area and sample selection

The study area included samples from nearshore and offshore waters in order to investigate the possible effects of land based sources and hydrographical features such as rivers and coastal fronts on abundance of microplastics. Since 2004 SAHFOS analysts have been looking for and reporting the presence of microplastic debris in the CPR samples during their standard analysis of plankton samples. As the geographical location, time and date for each silk sample is known; this could allow mapping of the microplastic distribution. For this study to map the distribution of the microplastic we used 35739 CPR samples that were processed between 2004 and 2011, about 5% (1994 samples) of which were reported to contain fragments that could potentially be microplastic debris (Figure 7).

Although CPR survey has broad scale spatial coverage with routes in almost all the oceans; in this study for comparison of microplastic abundance between the nearshore and offshore samples due to some of the inherent inconsistencies (described in Section 2.3.3) we only included samples from the northeast Atlantic Ocean and the North Sea which had the most complete and consistent temporal dataset. A total of 130 CPR silk samples from 3 areas with different hydrographical regimes were selected in order to compare the concentration of microplastic debris between the nearshore and offshore waters. These included 60 silk samples from the CPR-V route (between Sule Skerry, Scotland 59°10'N 04°20'W and South East Iceland 62°30'N 18°00'W) in the northeast Atlantic Ocean, 35 silks samples from the CPR-HE route (between Cuxhaven, Germany 54°00'N 08°07'E and Immingham, UK 53°33'N 00°14'E) in the southern North Sea and 35 silks from the CPR-M route (between Aberdeen, Scotland 57°08'N 02°02'W and Tananger, Norway 58°41'N 05°25'E) in the northern North Sea.

All samples were selected from the winter months to exclude seasonal effects and were equally distanced along the sampling routes. For this study samples within 50 nautical miles of land were considered as the '*nearshore'* and those further away (> 50 Km) as the '*offshore'*. This distance was selected in order to ensure that the influence of any discharge from land as well as the effects of the coastal currents and frontal systems was included. Figure 8 shows the location of the selected samples in the study areas along the selected CPR routes.

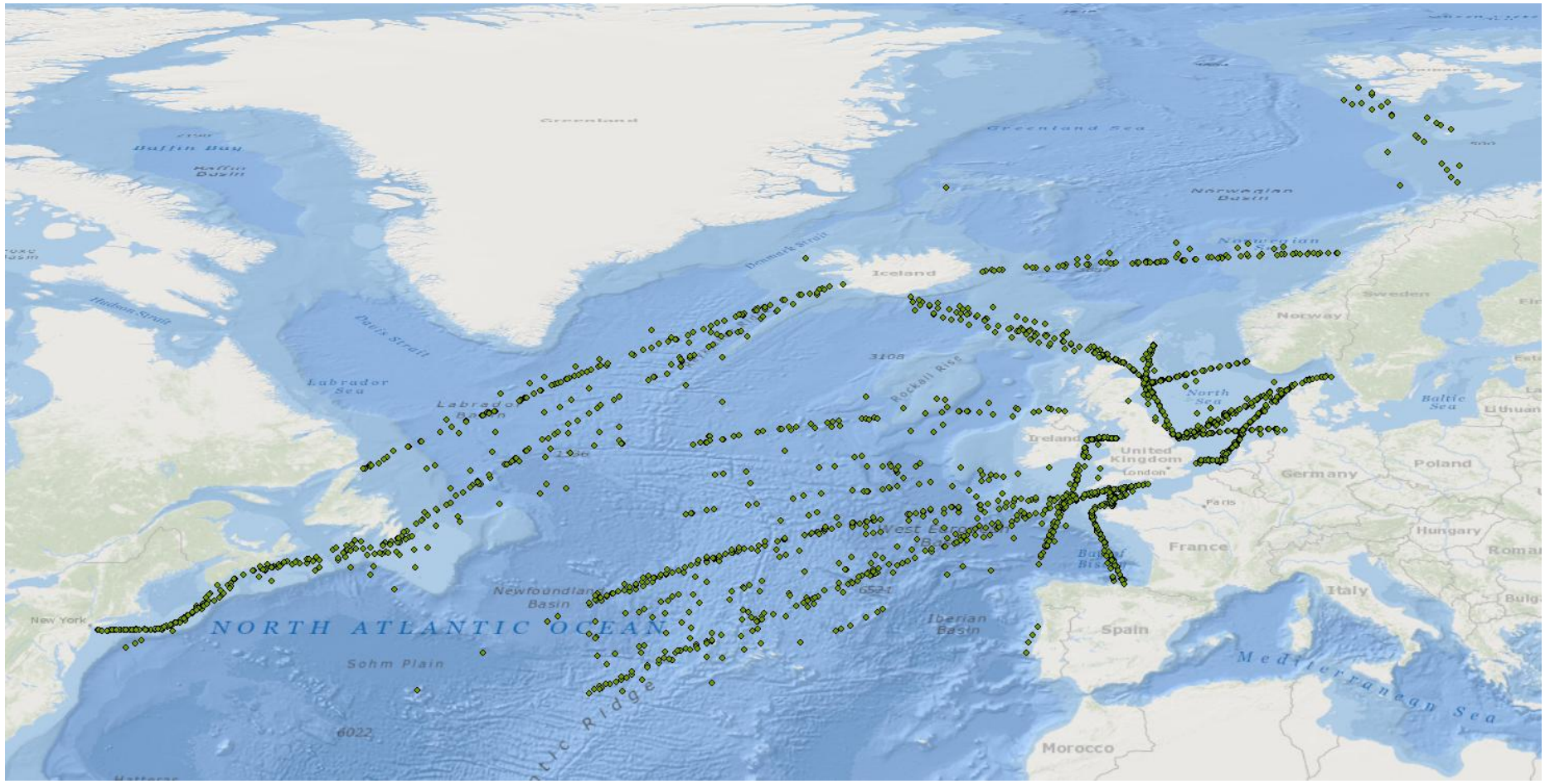


Figure 7) Spatial distribution of CPR samples reported to contain microplastic debris in the North Atlantic Ocean and the North Sea. Each dot represents a CPR sample equivalent to 10 nautical miles of tow (~3 m³ of seawater).

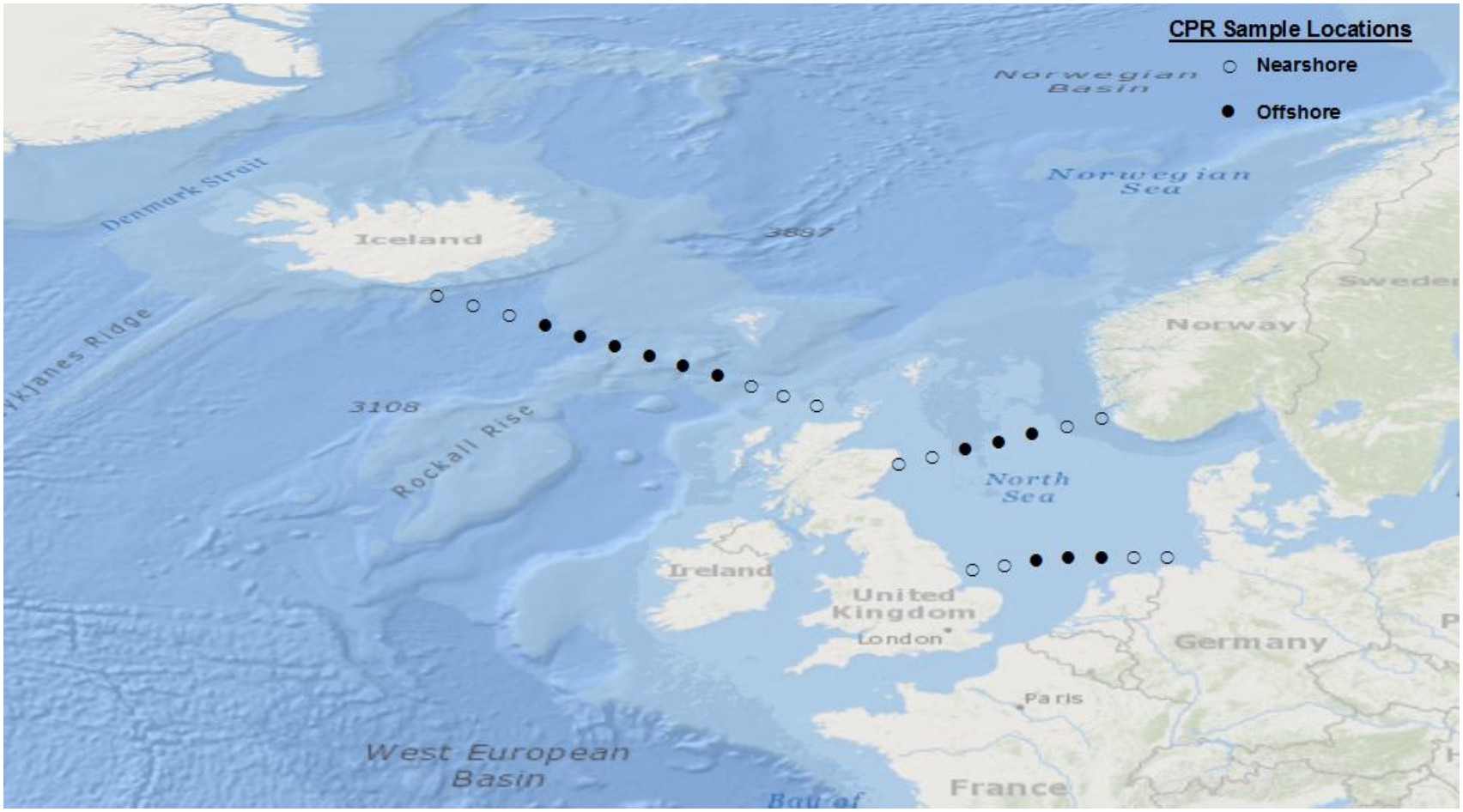


Figure 8) Location of CPR samples analysed for spatial patterns in the northeast Atlantic Ocean and North Sea

2.3.3. Data Reduction

Closer examination of the database that used to produce the distribution map in Figure 7 revealed high variability in the analysts' report of microplastics both in time and also between the individual analysts with over 80% of all samples that were reported as containing plastic being since 2008. As the total number of samples processed in each year and also by each analyst was similar, this increase in reports suggested improved ability of the analysts in detecting microplastic fragments in plankton silk samples as a result of experience. Therefore, to produce a map of frequency of occurrence for microplastic debris in the North Atlantic Ocean (Figure 9), the data were reduced to include only samples from 2008, which was then normalised for the sampling effort and mapped in ArcGIS (ESRI 2011, ArcGIS Desktop: Version 10). However, even after this reduction; there still remained some considerable variability in the number of plastic reports amongst the analysts which was not easy to eliminate. To reduce any influence from these inconsistencies, samples were selected from those prior to the inclusion of microplastic in CPR sample processing protocol (e.g. pre-2004).

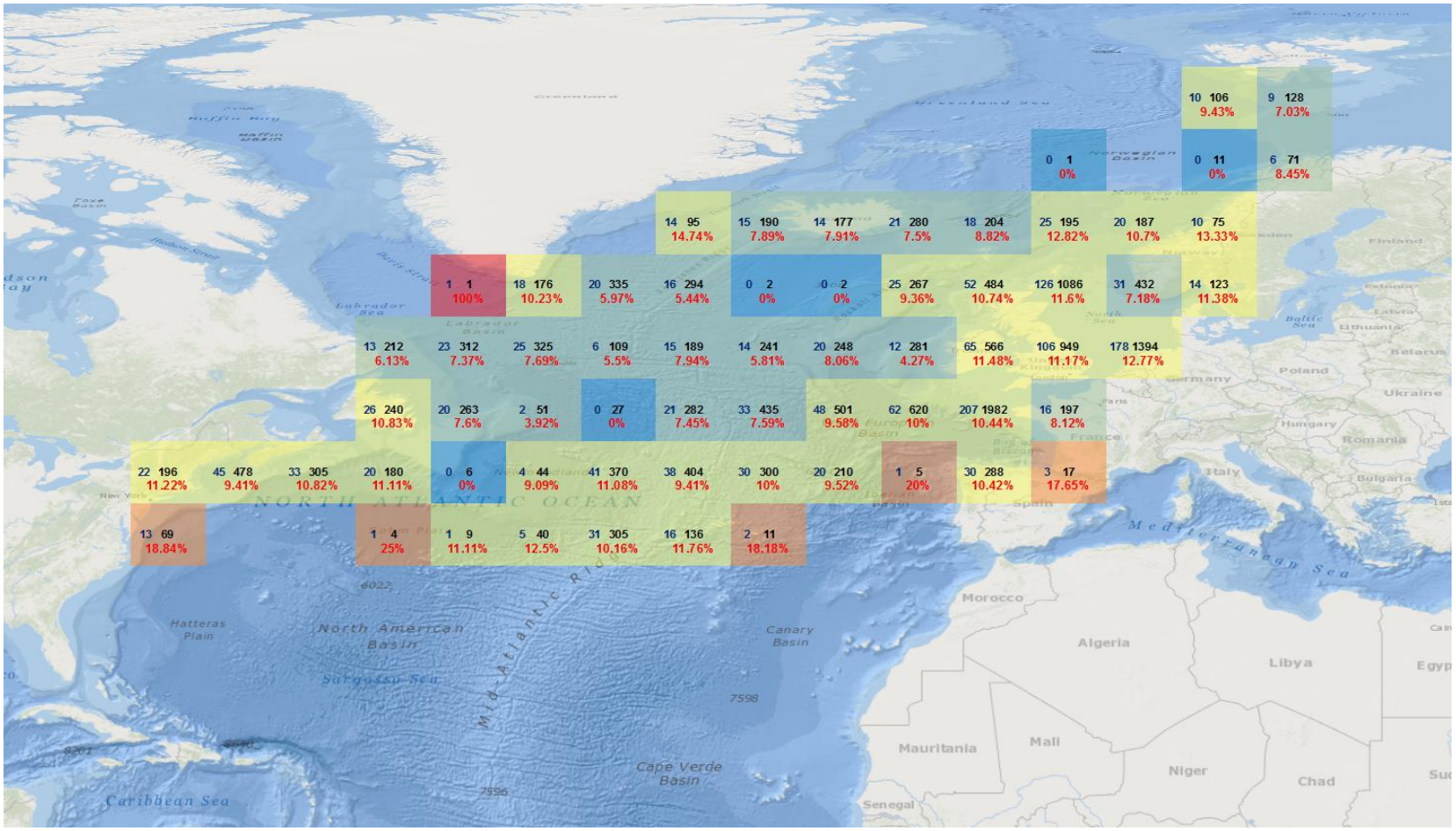


Figure 9) Frequency of occurrence of microplastic debris in CPR samples (2008-20011) normalised for sampling effort and presented in a 5˚x5˚ gridded map. Numbers in each grid cell represent (red): percentage of plastic, (blue): total number of plastics and (black): total number of samples examined.

2.3.4 Sample processing protocol

Each CPR sample was then taken out of its protective plastic cover and placed on a mobile sliding glass stage and examined inside a ventilated fume cupboard under a binocular dissecting microscope at x50 magnification (Figure 10). This magnification was used for the initial identification and gave a field of view of approximately 2mm. Both “covering” and “filtering” silks were examined in a systematic manner using a longitudinal top to bottom traverse method starting from top left hand corner (Figure 11).

Similar to the analysts’ approach, the initial discrimination of plastic fibres or fragments was mainly based upon basic physical features such as colour and form. Exceptionally bright hues of colours, that are not typically present in planktonic organisms or natural particulates, were selected for further examination. Fibres and fragments suspected of being plastic were manipulated using forceps and a fine needle mounted on an inoculation loop handle to better distinguish them from the naturally occurring material such as plants and soft gelatinous animal parts. These unknown, but potentially plastic pieces were then transferred on to labelled filter papers and kept covered in petri dishes before being taken for identification by Fourier Transform Infrared Spectroscopy (FT-IR).

Figure 10) Analyst examining CPR samples under the specialised microscope (Source: SAHFOS)

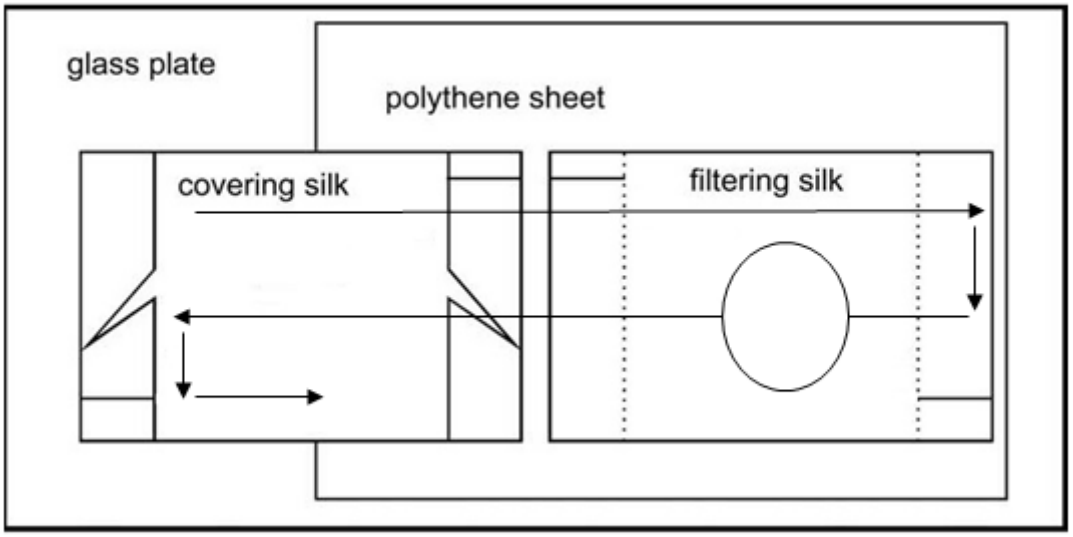


Figure 11) Diagram showing the method used in examining CPR samples (Adapted from Richardson, 2006)

2.3.4.1 Minimising operator contamination

Due to the nature of the investigation particular care was taken to avoid introduction of any external synthetic material into samples. Tiny synthetic fibres used commonly in clothing (e.g. polyester, rayon and nylon) could easily detach, become airborne and contaminate the samples. To avoid this cotton laboratory coat and latex gloves were worn at all times and handling of samples was kept to the minimum necessary. Samples were also stored in covered petri dishes at all times to reduce their exposure to open air.

2.3.5 Plastic Identification

Fourier Transform Infrared Spectroscopy (FT-IR) is the most reliable method for identifying the types of plastic found in the environment. In infrared spectroscopy, samples are exposed to IR radiation (4000-200 wavelength per centimetre) causing chemical bonds to vibrate as specific frequencies where some of the radiation is absorbed by the sample and some of it is passed through (transmitted). FT-IR is a more advanced form of infrared spectroscopy in which an *infrometer* is used to determine the absorption level at all wavelengths simultaneously (Shashoua, 2009). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample (Figure 12).

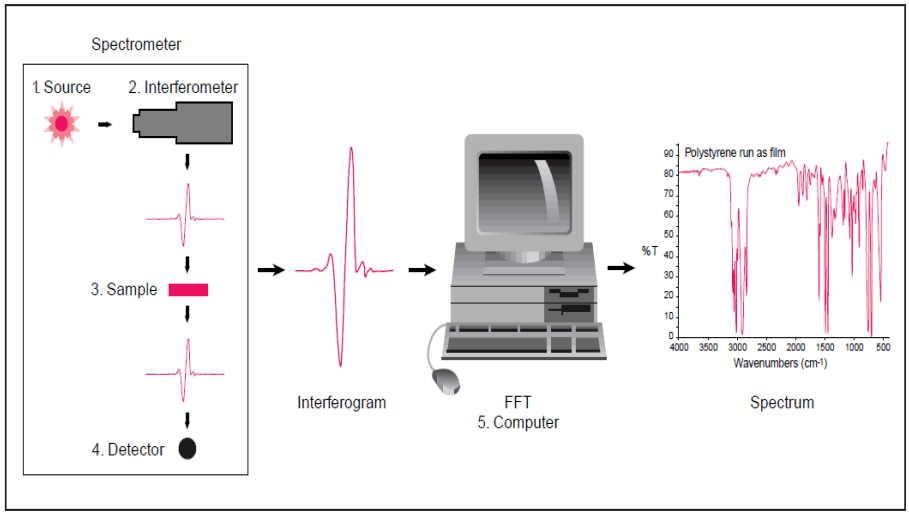


Figure 12) Diagram showing the sample analysis process of FT-IR spectrometry (Source: Thermo Nicolet Corporation)

Fragments were identified using a Bruker IFS66 Fourier transform infrared (FT-IR) spectrometer with a MCT detector operating in the 4000-600 cm^{-1} wave number range and attached to a Bruker Hyperion 1000 microscope. A Specac DC2 Diamond compression cell (2 mm in diameters) was used to prepare the samples. Each sample was transferred from the petri dish on to the diamond cell and compressed between the two plates into a thin uniform thickness enough to allow for adequate transmission of IR beam through the sample to the detector and resulting in a better quality spectrum. For measurement, processing and evaluation of the spectra Burker's Opus 6.5 spectroscopy software was used to best match spectra of the unknown debris following a protocol similar to that used by Thompson *et al.* (2004) as follows:

1. Sample spectra were corrected for background noise.
2. An initial search was conducted against the reference spectra in a database of common polymers to find the best match.

3. The top 10 matches with the highest hit quality score were selected and a Euclidean distance analysis performed (see Appendix 3 for details)

4. Matches with quality index ≥0.7 were accepted. Any border line matches with quality index <0.7 but ≥ 0.6 were individually examined and interpreted based on the closeness of their absorption frequencies to those of chemical bonds in the known polymers. Matches with quality index < 0.6 were rejected.

Spectra of the latex gloves used during the CPR silk sample and the protective plastic sheet in which they are wrapped were added to the spectra library and was included in the search in order to eliminate any contamination from these unlikely, but possible sources.

2.3.6 Data Analysis

2.3.6.1 Normalisation of data

The presence / absence data reported by the analysts (Figure 7) was normalized to produce a frequency of occurrence map (Figure 9) using the data management and spatial analysis tools in ArcGIS (ESRI 2011, ArcGIS Desktop: Version 10). This was achieved by first producing a sampling grid (5˚x5˚) for the whole of the North Atlantic Ocean using the *Fishnet* data management tool and then the *Spatial Joint* analysis tool to join the data points to the gridded sampling area.

Using the *Dissolve* data management tool the girded point data was then normalized for sampling effort to calculate the frequency of occurrence of microplastics for each of the grid cells as follow:

$$\textit{Frequency of occurrence} = \frac{Number\ of\ samples\ with\ microplastics}{Total\ number\ of\ samples\ processed}$$

2.3.6.2 Standardisation of results

The concentration of microplastics was standardised by dividing the total number of pieces per CPR silk sample by the volume of water that had passed through each silk, approximately 3m³, thus giving the standardised value for the average number of pieces per m³ of seawater.

$$Concentration = \frac{Total\ number\ of\ microplastic\ pieces\ per\ sample}{Volume\ of\ seawater\ filtered\ per\ sample\ (3m^3)}$$

2.3.6.3 Statistical analysis

All statistical analysis were conducted using SPSS (IBM 2011, SPSS Desktop: Version 20) software package. Data was first assessed for normality using the Shapiro-Wilk test and was shown not to be normally distributed hence the non-parametric Mann-Whitney U test was used to test for difference in the abundance data between the nearshore and offshore samples.

2.4 Results

2.4.1 Distribution and abundance of microplastic debris in CPR samples

In all areas of study the mean concentration of microplastics along the selected CPR routes was higher in the nearshore samples compared to those from the offshore waters with samples from UK's nearshore waters having generally higher concentrations compared to those from the European waters (Figure 13). The difference in abundance of microplastic was significant in samples from 2 of the 3 routes: the M route in northern North Sea (Mann Whitney U=76.50, n=35, P=0.045) and the V route in northeast Atlantic Ocean (Mann Whitney U=160.50, n=60, P=0.048) (Figure 14).

The overall mean concentration between routes was highest in samples of southern North Sea (HE-Route-: 0.29/m^3; n=35) followed closely by the northeast Atlantic Ocean (V-Route: 0.21/m^3; n=60) and lowest in the northern North Sea (M-Route: 0.18/m^3; n=35) (Figure 15).

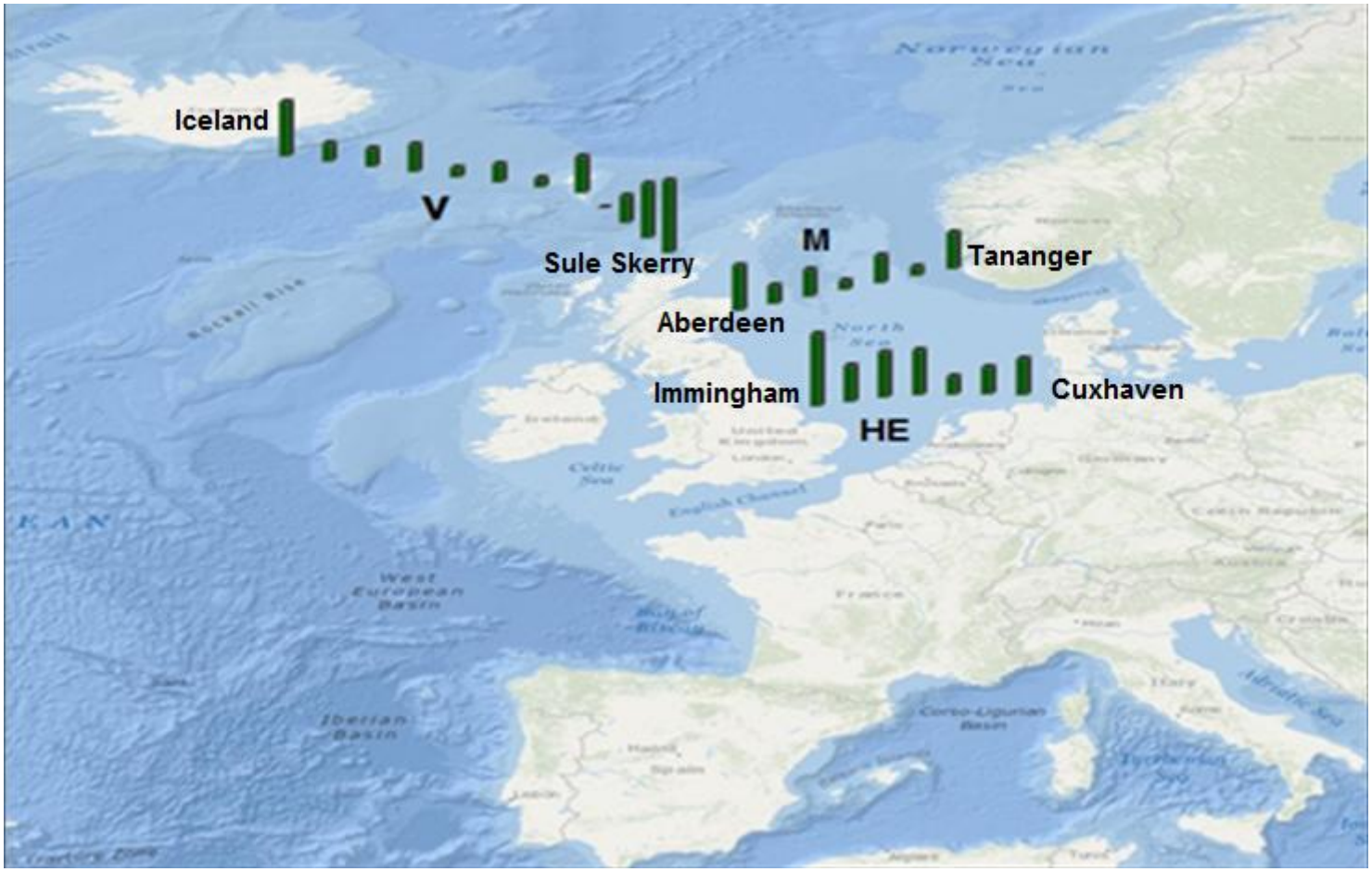


Figure 13) Mean abundance of microplastics along CPR routes across the southern North Sea (HE), northern North Sea (M) and the northeast Atlantic Ocean (V). Each bar represents the mean concentration of microplastic/m³.

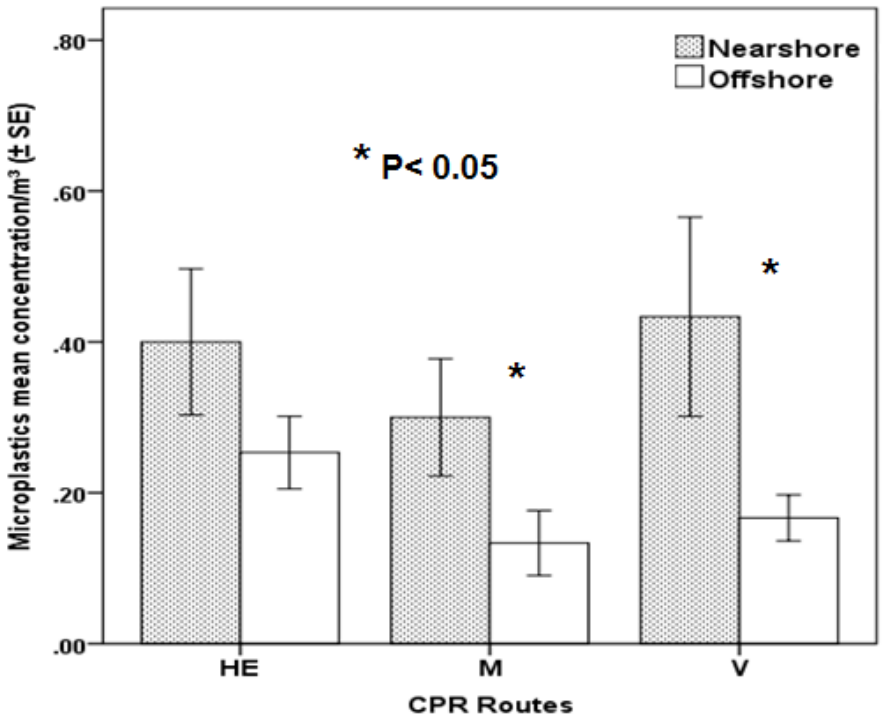


Figure 14) Abundance of microplastic debris from the samples along CPR routes in southern North Sea (HE), northern North Sea (M) and northeast Atlantic Ocean (V). Values expressed as means/m³ ± SE.

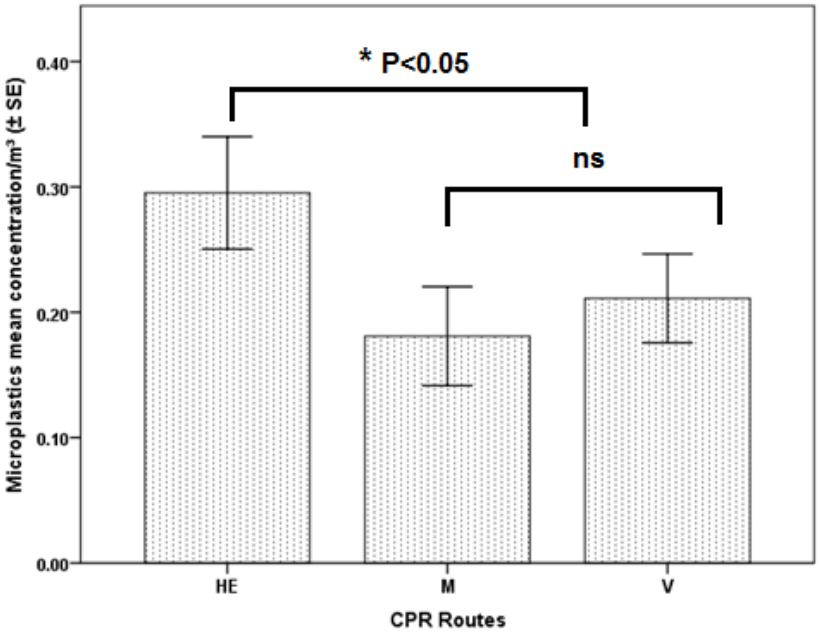


Figure 15) Overall abundance of microplastic debris was significantly higher in samples from routes in southern North Sea (HE, n=35) compared to those from northern North Sea (M, n=35) and northeast Atlantic Ocean (V, n=60). Values expressed as means/m³ ± SE.

2.4.2 Composition of microplastic debris in CPR samples

A total of 89 pieces of suspected microplastic debris from 130 CPR silk samples were identified by the FT-IR spectrometry (Figure 16). These included 38% plastics and a substantial (62%) quantity of the semi-synthetic polymer 'rayon'. The most common types of plastics were Polyester (20%), Polyethylene terephthalate (10%), Nylon (3%), Polyvinyl Chloride (2%), Polypropylene (2%) and Acrylic 1% (Figure 17). Most pieces were in the form of filaments such as polyester fibres and lines similar to those used in the fishing industry and the occurrences of fragments were rare (Figure 18).

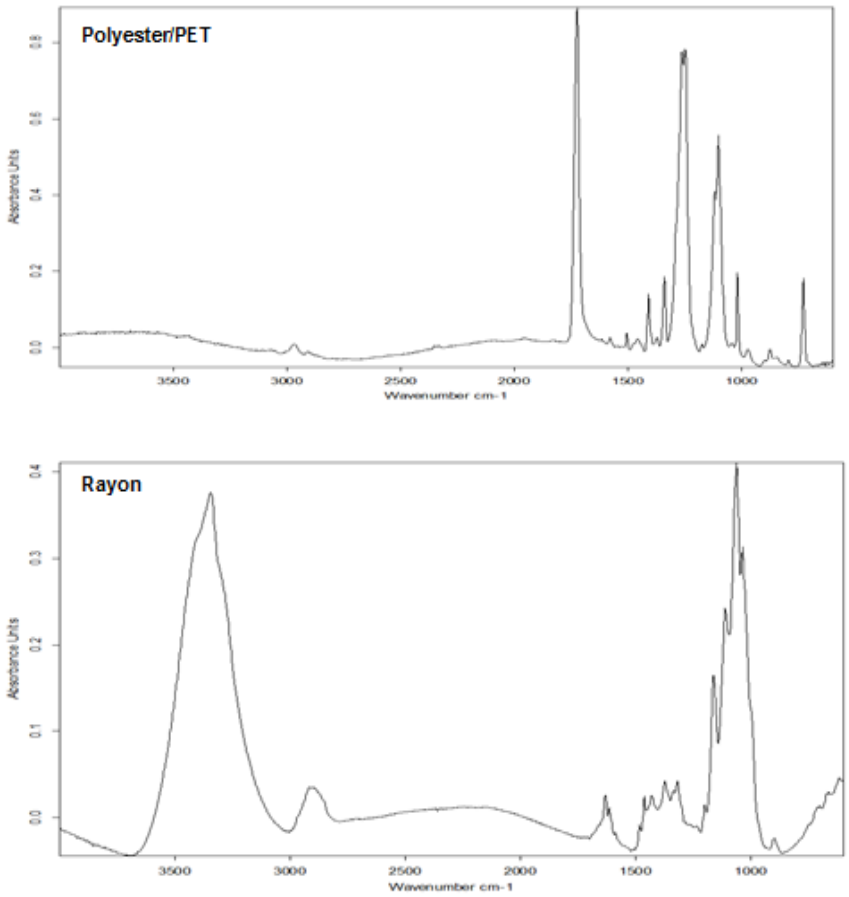


Figure 16) Examples of Fourier Transform Infrared spectra of microplastic debris found in CPR samples from North Atlantic Ocean

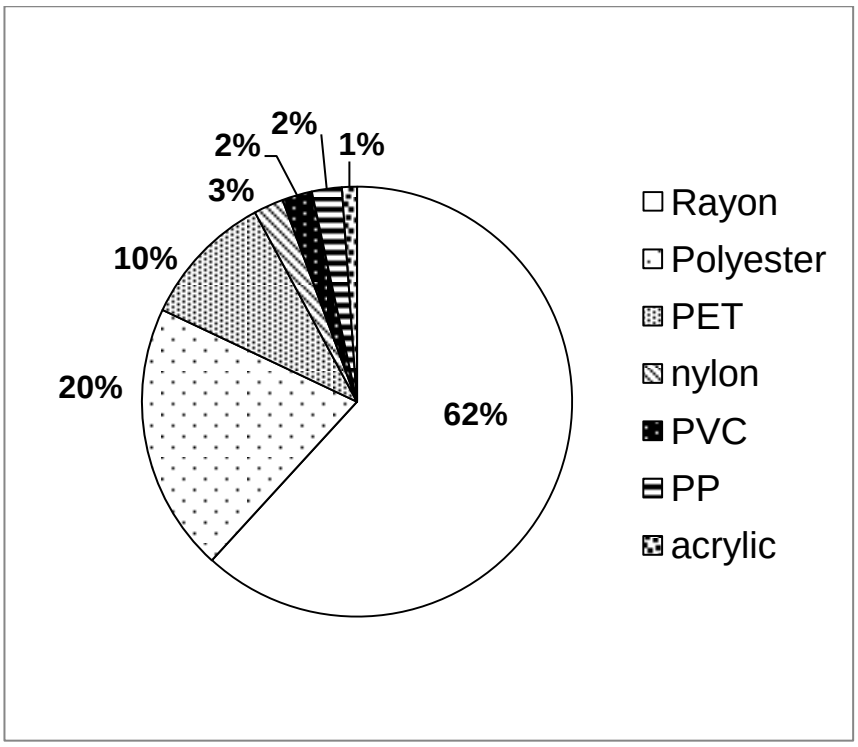


Figure 17) Composition (%) of microplastic debris found in the CPR samples

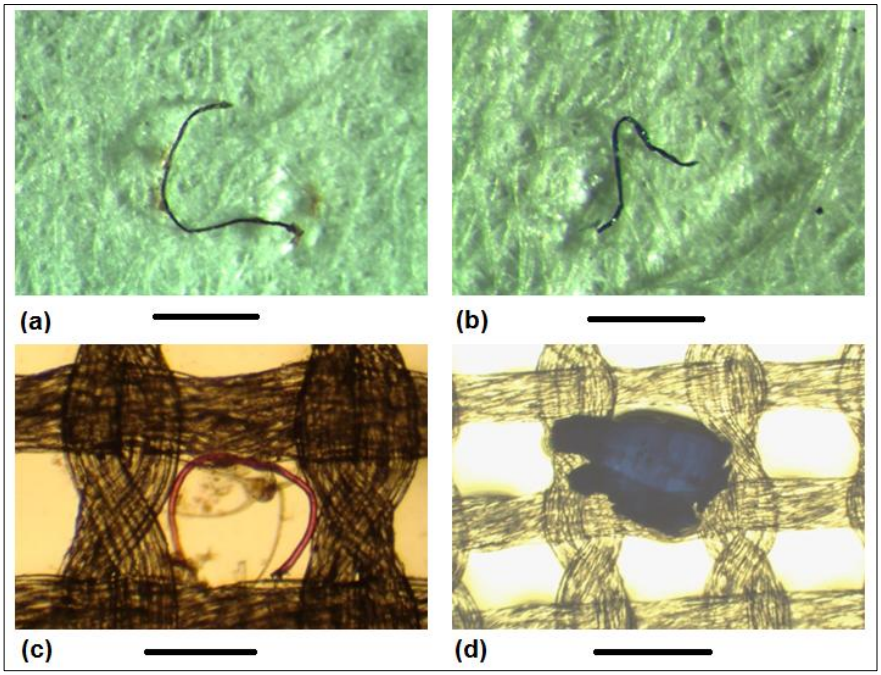


Figure 18) Examples of different forms of microplastic debris found in CPR samples from the North Atlantic Ocean. Polyester fibres (a,b), nylon (c) and Polyethylene terephthalate fragments (d). Scale bar represents 270 µm.

2.4.2.1 Rayon fibres

The ubiquity of rayon fibres in samples demanded further investigation. This was examined by considering both natural and anthropogenic materials as possible sources of these fibres. Spectra were obtained from a range of potential sources of natural cellulosic fibres such as algae, seagrass and salt marsh plants as well as manmade products including sanitary towels, cigarette filters and oil absorbing synthetic material that are commonly used by the industry in the marine waters after accidental spillages. The respective FT-IR spectrum of these fibres was added to the existing FT-IR library and compared these to that of rayon fibres. Figure 19 shows electron-micrographs of some of the rayon fibres found in CPR samples and those of natural and synthetic fibres.

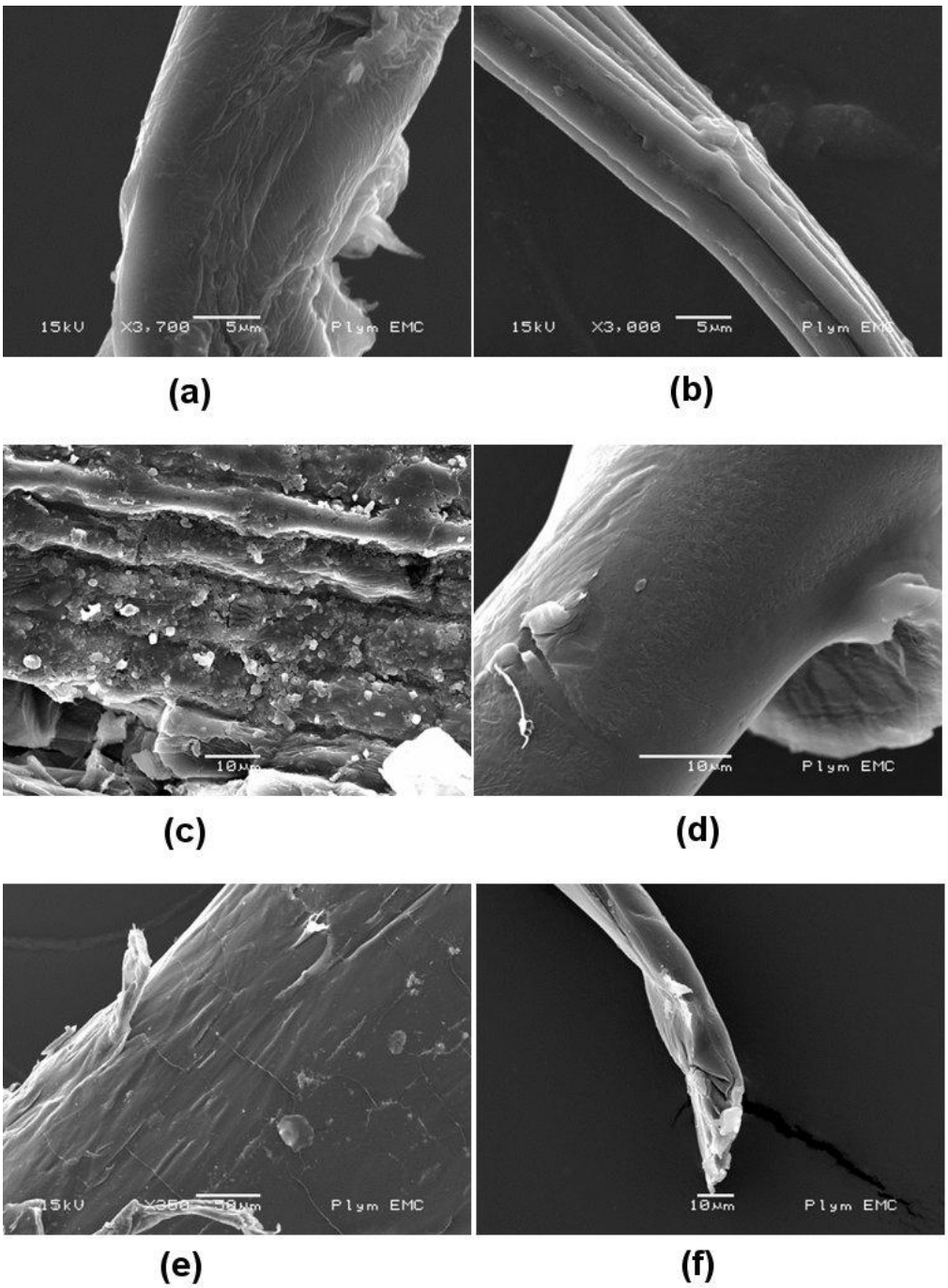
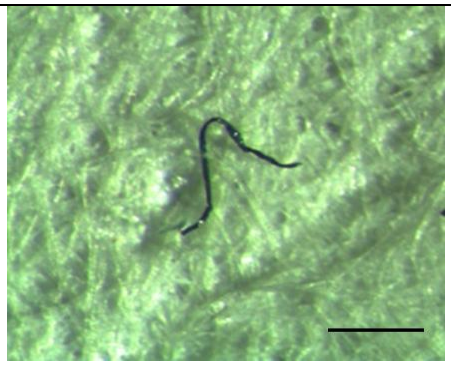
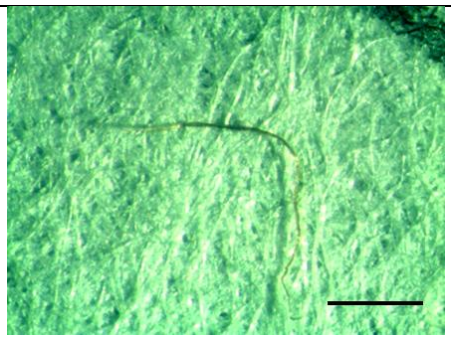
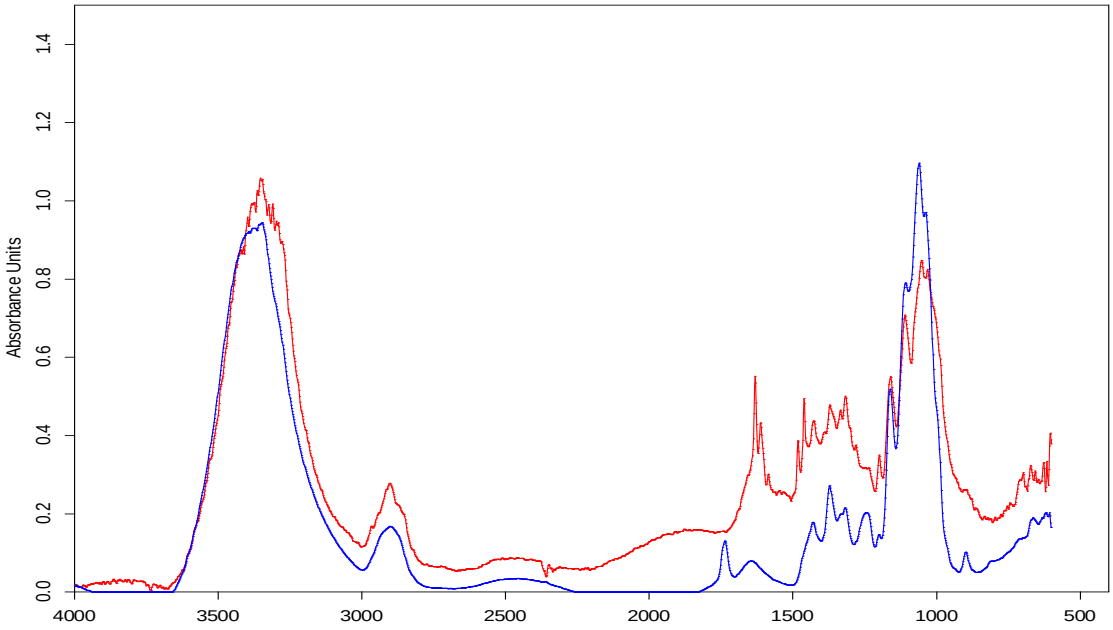


Figure 19) Electron-micrograph of rayon fibres found in CPR samples (a, b), Natural fibre: seagrass leaf (c). Synthetic fibres: sanitary towel (d), Polypropylene fishing line (e) and Polyester (f)

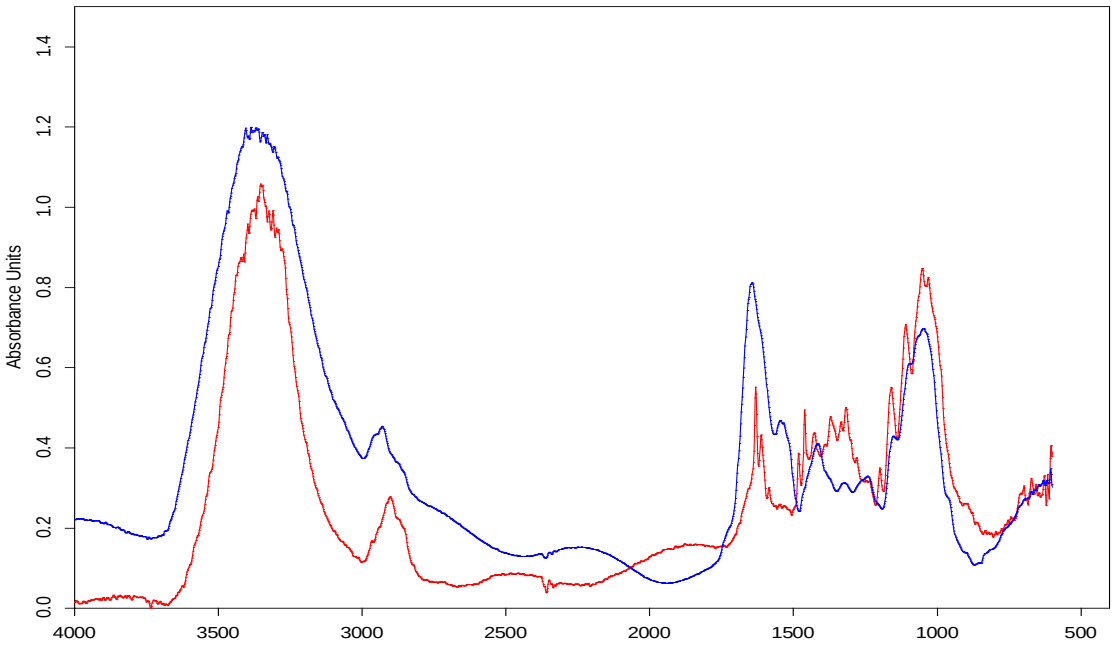
Characteristics and photos of some of the fibres from CPR samples compared to the natural and synthetic fibres are presented in Table 1. As expected most of the cellulosic fibres produced similar spectrum with spectrum of seagrass blade (*Zostera marina*) matching the spectra of rayon fibres closely (80% confidence); however the slightly better (84% confidence) match was that of the sanitary towel fibres (Figure 20).

Table 2) Characteristics of rayon fibres found in CPR samples compared to those of natural (seagrass) and synthetic (sanitary towel) fibres

Source	Image	Form	Colour	size
Rayon (CPR sample)		Fibre	black	~167 µm
Sanitary towel		Fibre	Pale brown	~1.5 mm
Seagrass (*Zostera Marina)*		Fibre	Dark green	~ 2.5 mm



(a)



(b)

Figure 20) FT-IR spectra of rayon in red compared to that of (a) sanitary towel fibre with ~84% similarity and (b) seagrass leaf with ~80% similarity.

2.5 Discussion

2.5.1 Spatial distribution of microplastics in the North Sea and Northeast Atlantic Ocean

This study has provided new data on the amount and composition of microplastics and shown higher concentrations of microplastic debris in the CPR samples from coastal waters of the northeast Atlantic Ocean and North Sea compared to those of oceanic waters from further offshore.

The higher concentration of plastic debris in the nearshore waters is consistent with findings of other studies who also found greater abundance of floating debris in the nearshore coastal waters (Dixon and Dixon, 1983; Theil *et al.*, 2003; Desforges *et al.*, 2014;). It is not easy to determine whether the origins of microplastic debris in the nearshore samples are all from the nearby coastal zones or transported by currents. However these studies all emphasise the importance of hydrographic features such as coastal and estuarine fronts, upwelling systems and eddies. Frontal zones in particular are a common feature in the North Sea and are known to have an accumulating effect on floating objects by restricting their horizontal dispersion (OSPAR, 2000) and the higher concentrations of microplastic debris observed in the nearshore samples of this study may be attributed to these frontal systems.

The frequency of occurrence map in (Figure 9) indicated areas of high concentration mainly in coastal waters but also in the offshore waters close to the North Atlantic Ocean subtropical gyre where high concentrations of plastic and microplastic debris have been reported (Lavender *et al.*, 2010).

It is likely that some of this debris are pushed deeper as a result of wind induced mixing of water column similar to those observed by Kukulka *et al.*

(2012) and also become negatively buoyant due to fouling by marine organisms where they may be captured by CPR.

CPR samples are from subsurface waters (~10 m depth) hence this study provides new information on spatial distribution of microplastics in marine subsurface waters, however, more studies with broader spatial and temporal coverage are required to more comprehensively characterise any patterns of abundance and its relation to the hydrographical features.

2.5.2 Amount and composition of synthetic pieces

Rayon and polyester fibres were the most abundant types of fragment found this study. These fibres are also amongst the most commonly used synthetic material in clothing which could easily detach from clothing during the laundering and have been shown to find their way into the marine waters through the sludge produced by the waste water treatment plants (Zubris & Richards, 2005; Browne et al., 2011). Rayon has absorbent properties and is commonly used in hygiene products and nappies. Although the occurrences of these items have decreased in beach litter since 2010; they remain among common litter items (MCS, 2012). Rayon is also known to break up easily (Park *et al.*, 2004) and hence dispersed more widely which may explain its ubiquitous presence in our samples. Lusher *et al.* (2012) also found the rayon fibres as the most common type of microplastic debris ingested by 10 species of fish from the English Channel.

The prevalence of fibres commonly used in clothing (i.e. rayon and polyester) in comparison to other types and forms elevated the concerns about the potential risk of contamination of plankton silk samples during the preparation and analysis at SAHFOS and required further examination. This is

addressed in Chapter 3 where the validity of the analysts' reports of microplastics and the question of procedural contamination are more closely examined.

Chapter 3. Validation and analysis of microplastic debris from the Continuous Plankton Recorder (CPR) samples

3.1 Abstract

Plastic is the most abundant type of marine debris and as it fragments into smaller pieces it becomes even more widely dispersed and harder to detect. Monitoring the abundance of plastic litter will be an important factor in assessing the Good Ecological Status (GES) in marine waters as required by the European Marine Strategy Framework Directive 2008/56/EC (MSFD). Monitoring is important in evaluating the effectiveness of any measures implemented to reduce the inputs of marine debris. However, because of considerable temporal and spatial variability in their distribution large sample sizes and reliable processing procedures are required to accurately detect any changes in abundance.

The Continuous plankton recorder (CPR) survey by the Sir Alister Hardy Foundation for Ocean Science (SHAFOS) is the largest plankton recording program of its kind in the world. The aim of this chapter was to establish the extent to which the consistent CPR methodology and widespread archived samples could be used to provide a reliable index of microplastic in surface waters. The most conclusive method of confirming the identity of unknown fragments that could potentially be plastic is to use Fourier Transform Infrared (FT-IR) spectroscopy but this method is time-consuming and the equipment relatively expensive.

The results of the validation and analysis of microplastic fragments found in the CPR samples as reported by the SAHFOS' analysts are presented. These showed a good level of accuracy (66%) in the ability of the SAHFOS' analysts to detect microplastic fragments visually during their normal processing of plankton samples (i.e. without use of FT-IR). However, it also indicated the susceptibility of CPR samples to contamination prior to sea deployment and

also during examination after deployment. This suggested that more rigorous protocols should be developed and adopted and quality assurance measures should be implemented before CPR samples could be used as a reliable microplastic monitoring tool.

3.2 Introduction

The occurrence of plastic debris in the marine environment was first reported in the coastal regions in 1970s (Carpenter *et al.*, 1972; Morris and Hamilton, 1974; Gregory, 1978) and has since been the focus of many studies (Laist, 1987; Derraik, 2002; Moore, 2008; Thompson *et al.*, 2009; Law *et al.*, 2010; Frías *et al.,* 2011, Sul *et al.*, 2011). Plastic is the largest component of marine debris accounting for up to 80% of the debris that contaminates and degrades marine habitats at a global scale (STAP, 2011). We know less about the distribution of smaller (< 5mm in diameter) fragments known as 'microplastic'.

The occurrences of microplastics in the form of pellets and polystyrene spherules found in the plankton net hauls from the north-western Atlantic were first reported in 1970s (Colton *et al.*, 1974) and have since been reported worldwide on beaches (Gregory, 1978; Gregory, 1983; Ng & Obbard, 2006; Browne *et al.*, 2010; Costa *et al.*, 2010; Hirai *et al.* 2011;), sediments (McDermid & McMullen, 2004; Reddy *et al.*, 2006; Rios, Moore & Jones, 2007; Browne *et al.*, 2011; Claessens *et al.*, 2011; Vianello *et al.*, 2013) and water column (Moore *et al.*, 2001; Thompson *et al.*, 2004; Law *et al.*, 2010; Collignon *et al.*, 2012). This debris could enter the marine environment either via direct discharge from the land based sources or through the gradual fragmentation of larger items of plastic debris into smaller pieces by the photo-oxidation and mechanical processes in the environment also known as 'weathering'.

The European Marine Strategy Framework Directive 2008/56/EC (MSFD) recognise Marine Litter as one of the indicators for the Environmental State of the European Seas. The Commission decision on criteria and methodological

standards on good environmental status (GES) of marine waters (Commission Decision 2010/477/EU), lists specific indicators for assessment of Marine Litter. Indicator 10.1.3 considers marine micro-particles, in particular microplastics and calls for more data on the amount, distribution and, where possible, composition of this debris (MSFD, 2011).

However, current knowledge of the sources and fate of microplastic debris in marine waters is limited and there is no evidence that this small debris is transported in the same manner as larger items. For example, particles of relatively buoyant polymers such as polyester have been found on the seabed alongside denser polyvinyl-alcohol which would have been expected to sink (Thompson *et al.*, 2004). Furthermore recent data shows that traditional net based surface measurements may significantly underestimate abundance through the effect of wind mixing on the vertical distribution of buoyant debris (Kulkulka *et al.*, 2012). In order to assess the extent of microplastic contamination, and its impact worldwide, there is a need for comparable data to be collected across different marine habitats using standard methodologies (Hidalgo-Ruz *et al.*, 2012).

The efficacy of any measures implemented to reduce the abundance of plastic debris need to be assessed by an effective monitoring programme capable of addressing the inherent spatial and temporal heterogeneity in distribution of plastic debris. At-sea this task is further complicated as it is harder to access than shoreline habitats and requires large sample sizes for reliable statistical analysis (Ryan *et al.*, 2009; Hanke *et al.*, 2013).

The Continuous plankton recorder (CPR) survey is the longest plankton recording programme of its kind in the world and is currently under

consideration as a possible platform for monitoring the abundance of microplastic debris in near surface marine waters (TSG, 2012). The presence of microplastics in CPR samples as reported by Thompson *et al.* (2004) led SAHFOS to include the inspection of samples for 'suspected' microplastic pieces as part of their standard visual analysis protocol which includes up to 500 planktonic taxa. This inspection is based on a visual examination and reporting of silks which appear to contain particles resembling plastics and indicates the presence or absence of these particles. However, for small particles it can be difficult to distinguish plastics from natural debris based on visual examination alone.

The most conclusive method to confirm the identity of unknown fragments that are potentially plastic is to use FT-IR spectroscopy but this method is time-consuming and the equipment is expensive. In this study, given the clear trends shown in Chapter 2, I aimed to validate the accuracy of the analysts reports which were obtained through visual examination by re-examining a subset of the samples that they had marked to contain microplastic pieces and then conclusively identified this using FT-IR spectrometry. I also investigated the susceptibility of CPR samples to 'procedural contamination' prior to sea deployment and also during examination after deployment. The objectives being:

1) To establish if SAHFOS's analyst detection of microplastic fragments by eye under the microscope during their normal plankton analysis could be used to give a reliable index of contamination without need for formal FT-IR analysis. If so this would facilitate more rapid monitoring and permit the use of archived data.

2) To establish whether the contamination was introduced into CPR samples during routine preparation and analysis.

3.2.1 Hypothesis tested

H01: The presence of microplastic in CPR samples as reported by visual examinations of SAHFOS' analysts is accurate and agrees with the results of the more detailed FT-IR analysis.

3.3 Materials and method

3.3.1 Sample processing protocol

Each sample was examined under a binocular dissecting microscope at 5x magnification and a circular field of view of approximately 2mm. This was the magnification that had been used by the analysts for the initial identification. Both 'covering' and 'filtering' silks were examined in a systematic manner using a longitudinal top to bottom traverse method starting from top left hand corner.

During visual examination the same criteria as used by the analysts was adapted. Initial discrimination of plastic fibres or fragments was mainly based on basic physical features such as colour and form. Exceptionally bright hues of colours that are not usually present in planktonic organisms or natural particulates were selected for further examination. Fibres and fragments suspected of being plastic were manipulated using forceps and a fine needle mounted on an inoculation loop handle to better distinguish them from the naturally occurring material such as plants and soft gelatinous animal parts. These unknown, but potentially plastic, pieces were then transferred on to filter papers, labelled and kept covered in petri dishes prior to identification using a Bruker IFS66 Fourier transform infrared (FT-IR) spectrometer. Sample spectra were corrected for background noise, and then compared to the reference

spectra in a database of common polymers (*Bruker Optics ATR-Polymer Library*) to match spectra of the unknown debris to those of known polymers

3.3.2 Accuracy of Analysts in detecting microplastics

Out of the total of 399 CPR samples marked as plastic contaminated by SAHFOS analysts since 2004, 54 were randomly selected from the year 2009 (Figure 21) as this was the most recently completed set and also exhibited the highest number of samples containing plastic. To further assess the reliability of analysts' reports an additional 40 samples from the same sampling area and time which were not marked as "contaminated" was randomly selected and examined for comparison.

To get a true value for the amount of plastic on each sample after deployment, but before analysis, 15 previously unexamined samples were examined. These samples closely resembled the analysed samples spatio-temporally.

3.4 Statistical Analysis

To determine the extent of agreement between quantities of microplastic confirmed by the FT-IR technique to those of the analysts visual accounts, the Fisher test function in RStudio software package, version 0.98 was used to calculate the P-value of the Fisher's Exact test for the count data

To test if there was a significant difference between the results from even samples (those not examined by the analysts) and those that were analysed by the analysts and used for the pilot study the Mann Whitney U non-parametric test was used.

3.5 Results

3.5.1 Accuracy of Analysts' reports

In total 94 silks were examined. Thirty-five of the 54 samples that were marked as "contaminated" and 6 out of the 40 that were marked as "uncontaminated" contained synthetic fragments, giving an overall accuracy of ~66% between the FT-IR results and those reported by the analysts. The Fisher test function in R was used to calculate the P-value of the Fisher's Exact test for the count data (Fisher's Exact Test, P = 0.03474).

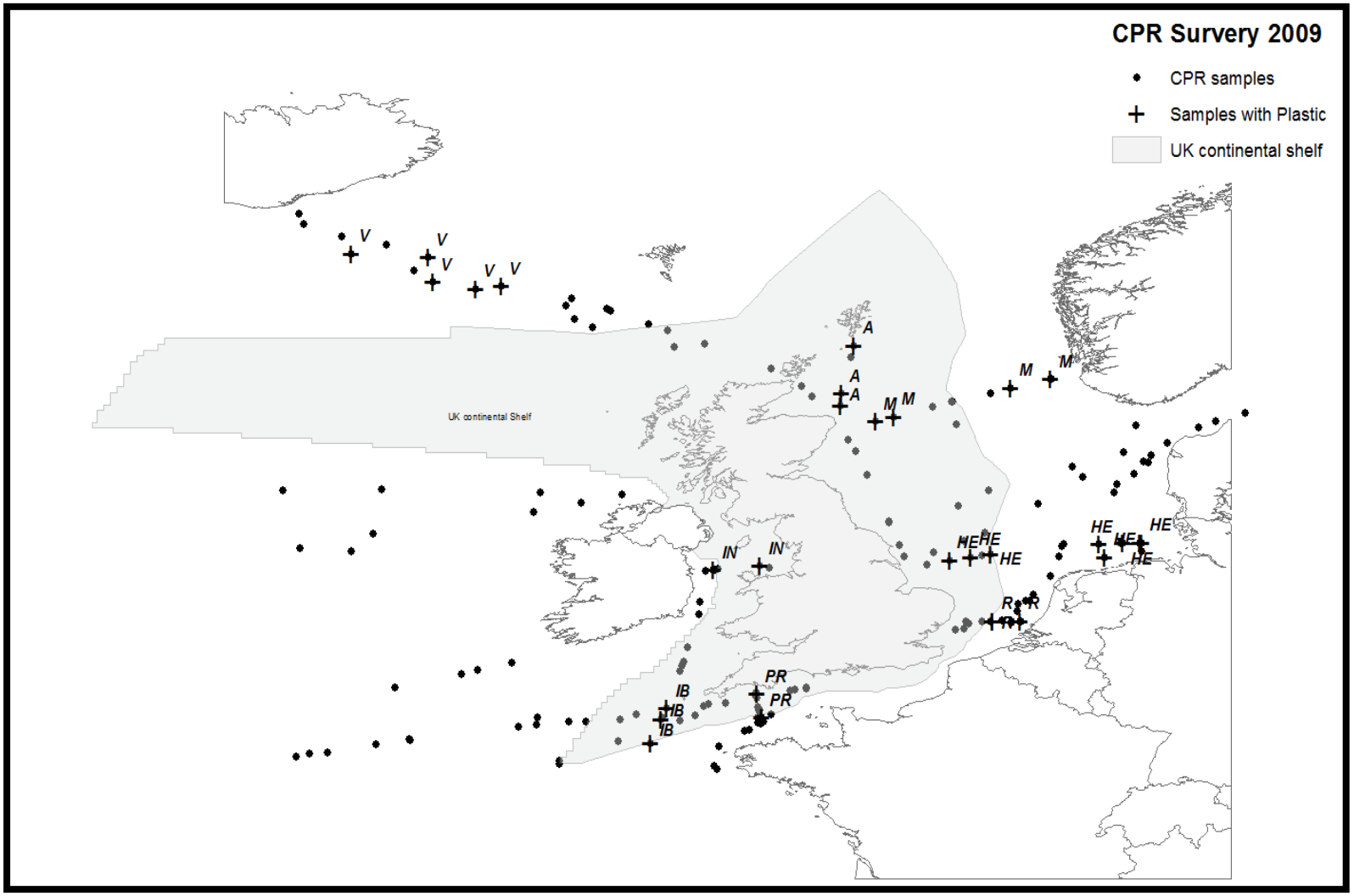


Figure 21 Map of CPR samples examined and confirmed for microplastics by FT-IR.

3.5.2 Amount and composition of microplastics

Most pieces were in the form of fibres and included a substantial (43%) amount of the semi-synthetic cellulosic material, rayon. The most common plastic types were Polyethylene Terephthalate (PET/Polyester) comprising 35% of the samples followed by Nylon (11%), Acrylic (7%) and Polyethylene 4% (Figure 22). Mean concentration of microplastics was 0.26 /m^3 seawater and varied in between routes from 0.13/m^3 to 0.53/m^3 (Figure 23). Analysis of the samples not processed by the analysts found on average 1.66 pieces of plastic on each silk which equals to mean concentration of 0.55/m^3 (each CPR silk sample filters ~ 3 m^3 of seawater).

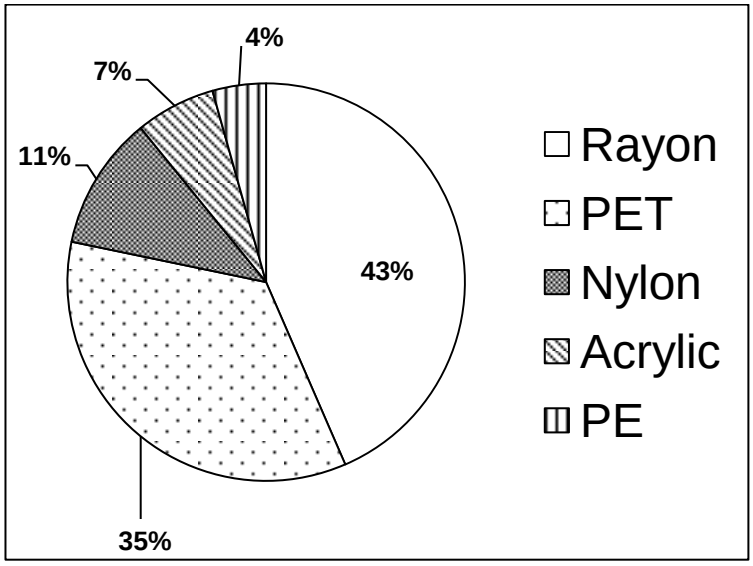


Figure 22) The relative proportions of different polymers found in CPR samples from the North Sea and North Atlantic Ocean

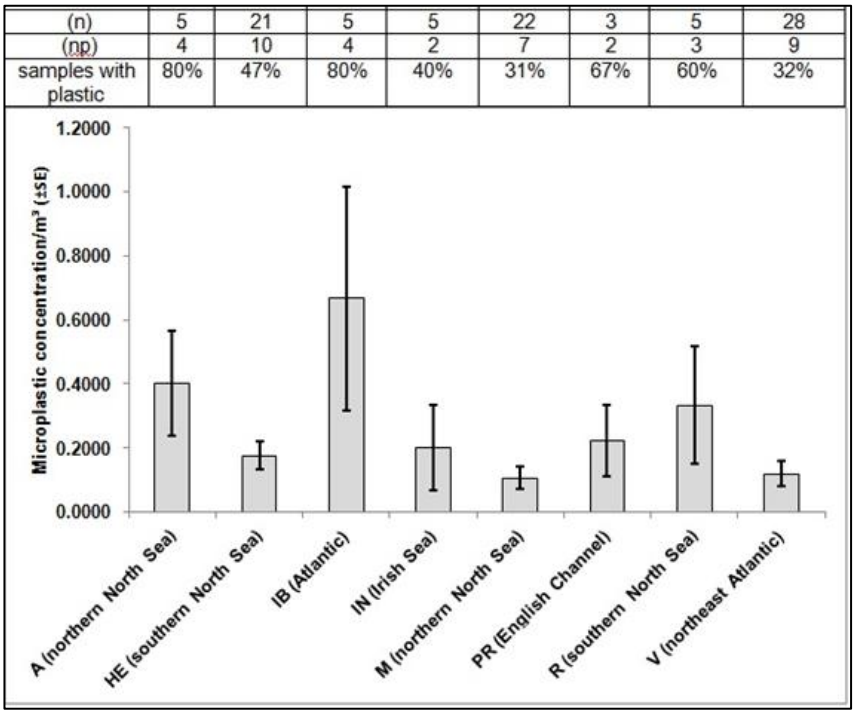


Figure 23) Mean concentrations of microplastics/m³ (± SE) for each CPR route. Number of silk samples examined (n), number of silk samples with plastic (np).

3.6 Discussion

3.6.1 Accuracy of the Analysts reports

In this study we have confirmed the presence of microplastic debris in marine surface waters around the UK and also have provided evidence for reliability (overall accuracy of ~66%) of SAHFOS's analysts to visually detect this debris during their standard analysis of plankton samples. This, along with the long-time archive of CPR samples and the broad spatial coverage of the survey, could be a cost effective standard method to investigate spatiotemporal trends of the floating microplastics in oceans. However, the CPR needs an operational speed of at least 20 knots and towed at about 10 m depth so is not suitable for sampling from slow vessels or in shallow coastal waters and estuaries; it has a relatively small aperture and so is also likely to significantly under-sample the more buoyant types of microplastics at the sea surface.

3.6.2 Amount and composition of microplastics

These preliminary findings indicate low concentrations of microplastic in UK waters but this data are too limited to establish spatial trends. A more comprehensive analysis of the dataset and mapping the distribution of more samples is needed in order to determine regional trends.

Further studies such as comparisons with other commonly used sampling devices (e.g. manta and bongo nets) will also provide more information on the amount and characteristic of microplastics in the surface waters and help to better understand the vertical distribution and residency time of various floating plastic debris in the marine waters under different environmental and biological conditions.

The comparison between the quantities of microplastic found on the even samples (those not analysed by the analysts) and those that were examined by the analysts showed that there were greater quantities of microplastics on the even samples (average 1.66/silk than on the odd samples that were examined by analysts ~ 0.55/silk). One possible consideration was that perhaps some of the microplastics captured at sea being lost during the analysis in labs, most likely during the eye-count analysis where the contents of silk is washed into a petri dish for closer examination and then put back on the silk. If not done thoroughly it is possible that some microplastic particles could have remained in the petri dish and become lost and resulted in lower counts of microplastic in the samples that were previously examined by the analysts and used for this study.

Chapter 4. Comparison of the quantity and composition of buoyant microplastic debris collected by two different methods (CPR vs. Manta)

4.1 Abstract

‘Microplastic’ debris (pieces < 5mm) is contaminating marine habitats via both direct sources such as those used in personal care products and indirectly through mechanical and photo-oxidative fragmentation of larger plastic debris into smaller pieces. In comparison to the larger items of plastic debris our understanding of distribution and full impact of microplastic debris is relatively limited. The European Marine Strategy Framework Directive 2008/56/EC (MSFD) regards microplastics as an emerging issue of concern and calls for more data on the quantity, distribution and composition of this debris.

However, this task is complicated due to the spatial and temporal heterogeneity of this debris and lack of sufficient comparable data from different marine habitats. Most studies of buoyant microplastic debris have been conducted using equipment designed to sample plankton. Two commonly used devices are the Continuous Plankton Recorder (CPR) and the Manta net. The aim of this chapter was to compare the amount and composition of the debris collected by these two different approaches.

The results showed significantly higher abundance of microplastic in samples collected by the manta net per unit of distance (0.58 pieces/km vs. 0.02 pieces/km). However, when the results were standardised per cubic volume of water there was no significant difference between the two approaches (0.14 pieces/m^3 vs. 0.13 pieces/m^3). There was also greater variety (type, form and size) of plastic debris in the manta net samples than that collected by the CPR. The most common type of plastic collected by the manta net was Low Density Polyethylene (LDPE) whereas Polyester fibres were the most common type in CPR samples. These results provide additional data on

the amount, composition and distribution of buoyant plastic debris in water column using different sampling devices.

4.2 Introduction

Plastic is the most abundant type of marine debris and its detrimental socioeconomic and ecological impacts is well documented (Hall, 2000; GEF, 2012; UNEP, 2011). Successful management of marine debris problem requires a comprehensive approach that is within the context and in relation to regional, national and international legislations and includes all the marine compartments such as shorelines, sea surface and seabed. On-going monitoring of abundance and composition of plastic debris is essential for better understanding of its sources, pathways and fate and also for assessing the efficacy of the implemented measures to reduce its abundance. Current monitoring programmes in Europe have been predominantly conducted by the volunteers through Non-Governmental Organisations (NGOs) and have been a valuable source of information.

However these programmes have mainly focused on the larger items of debris from beaches and used varying methodologies (Hidalgo-Ruz *et al.* 2012) which too often makes the results incomparable and also further complicated by large spatial and temporal heterogeneity of plastic debris (Cheshire *et al.*, 2009; Ryan *et al.*, 2010; Hanke *et al.*, 2013).

Many plastics are positively buoyant and once adrift they can spend a long time floating around but studies shown that even the most buoyant plastics will be slowly fouled by micro-organisms and eventually sink deeper in the water column (Lobelle and Cunliffe, 2011). Most studies have only considered debris from the surface waters but some have shown that wind induced turbulence during the stormy conditions plays a significant role in vertical transport of floating debris from the surface layer. The affected water depth is typically a few

meters and the residence time of the floating objects will depend on its density and the sea state. Size and shape of the objects are also play a role and one study indicates that smaller particles might rapidly be mixed under water, but take longer time to re-surface (Prokurowski *et al.*, 2011).

Floating marine litter objects come in a great variety of shapes and sizes and nearly 80% of them are estimated to have been made of synthetic polymers and have anthropogenic origin. They are introduced to the oceans from various sources such as riverine input, shoreline runoff and from sources at sea such as direct disposal or loss from ships and installations. It is important to understand the dynamics of floating litter as they represent the pathway between different marine compartments and therefore necessary to development of monitoring strategies.

Indicator 10.1.3 of the Commission Decision (2010/477/EU) specifically highlights need for information on trends in the amount of microliter in the water column including analysis of its composition and spatial distribution. For plastic waste these are small (<5mm) pieces of plastics commonly referred to as microplastics. They come from both primary sources such as those used as exfoliates in cosmetic products from spillage of preproduction plastic pellets and powders but they are also formed from breakdown of larger plastic material and are known as secondary sources of microplastics (Arthur *et al.* 2009; Fendal & Sewell, 2009; Thompson *et al.* 2009; Andrady 2011).

Current methodologies for monitoring larger items of litter are based on observational techniques from fixed platforms, ships and airplanes. These are highly dependent on weather conditions and do not collect and identify the litter items (Ribic *et al.*, 1992). The suitability of existing approaches for the

implementation of MSFD Descriptor 10 for marine litter are currently being assessed based on the size range of the objects to be monitored (Piha *et al.*, 2011). For micro-litter and in particular microplastics as described in Descriptor 10.3 of MSFD, the widely accepted size category of <5mm puts it within the range of mesh sizes typically used in the trawl nets for sampling planktonic organism including: manta, bongo and plankton nets. However, these are small, and believed to be more widely dispersed, therefore much harder to monitor. In order to acquire a better understanding of the potential risks of this debris to marine organisms at different trophic levels it is important to monitor its abundance and distribution at several depths in water column and to standardise and compare the results from different methodologies.

Floating micro and macro plastic debris in the marine environment have commonly been sampled using manta plankton nets (Brown & Cheng, 1981; Lattin *et al.*, 2004; Law *et al.*, 2010). In addition, examination of CPR samples has shown the capability of this device in capturing plastic debris in subsurface waters (Thompson *et al.*, 2004). However, CPR and plankton nets vary in many ways such as speed, volume, mesh size and operational depth and no studies, as yet, have compared these methods in terms of amount and type of microplastics captured.

The aim of this chapter was to quantify and describe the types of microplastic debris collected by the CPR and manta net as two commonly used and possible methods for monitoring buoyant microplastics in marine waters. The objective of this experiment was to provide baseline information on characteristics of the microplastic debris captured by each device. These data would help in better understanding of the vertical position and movement of

microplastics in water column as well as giving an insight into the strength and limitations of each method for the monitoring microplastics in marine waters.

Due to the nature of the investigation particular care was taken to avoid introduction of any additional contamination from synthetic clothing worn during these investigations. A cotton laboratory coat and latex gloves were worn at all times. However, small pieces of synthetic material from the laboratory and storage area could accidently be introduced on to the sampling equipment and potentially contaminate the samples. This could happen before, during and after the deployment of the CPR. In order to assess the level of contamination from these sources and to compare the potential for contamination between sampling approaches I also performed checks during different stages of sample processing both before and after deployment of sampling devices to the sea and found the presence of polyester and rayon fibres in the sampling equipment used in both methods.

4.2.1 Hypotheses tested

H01: The amount and characteristics of microplastics captured by CPR and manta net do not differ significantly.

H02: The CPR samples are free from contamination by ‘external/non -marine’ sources of microplastic (procedural contamination).

4.3 Methods

4.3.1 Study area and sampling design

RV Quest of Plymouth Marine Laboratory (PML) and *Sepia* of Marine Biological Association (MBA) were used to tow the CPR and a Manta net along a similar path in Plymouth's coastal waters during the 3 days of sampling in August and September 2012. On each day the tows started at the same time and passed through two different bodies of water: first through the shallower (~25m) nearshore waters and the other further away from coast in deeper (~50m) offshore waters. There were 6 replicates of manta samples on each day and 2 of the CPR. However, due to the operational and time limitations, the full coverage of the CPR's sampling area by manta was not possible and, therefore, partial comparison of the overlapping segments of the path closer to the shore was made (Figure 24).

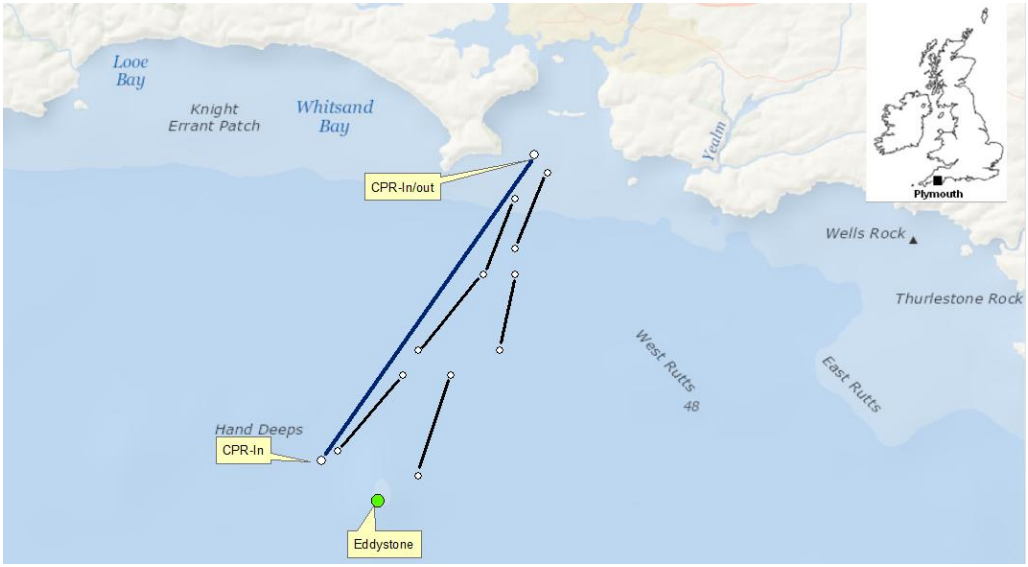


Figure 24) Map of UK showing location of Plymouth (insert top right corner). Plymouth coastal waters and traverses (black line: manta; blue line: CPR) used to collect debris.

Surface samples were collected using a manta trawl with a rectangular opening of 0.5 x 0.15 m² lined with a 3m long 300 µm net fitted with a 30 x 10 cm² screw-fit collecting bag. Each trawl sampled on average the top 7.5 cm of the sea surface at an average speed of 4 knots for 30 minutes covering an approximate total linear distance of 24 km. The net was towed from a boom installed on the side of the boat away from the wake to minimise the disturbance of the debris by the bow wave and propulsion system.

Subsurface samples were collected using a CPR device with a square opening of 1.27x1.27 cm² lined with a 270 µm silk net. The same CPR unit was in all three days of sampling and the silks were prepared and cut into sample blocks following similar protocols as the ones used by SAHFOS for sampling plankton (detailed in Chapter 2, Section 2.3.1). Samples were taken at an average depth of 10m and speed of 9.6 knots covering an approximate linear distance of 31 km (Figure 25).

Figure 25) Photographs of the Continuous Plankton Recorder (left) and Manta net (right).

4.3.2 Procedural contamination checks (Manta net)

Small pieces of synthetic material from the laboratory and storage area could accidently be introduced on to the sampling equipment and potentially contaminate the samples. This could happen before, during and after the deployment of the. In order to assess the level of contamination from these sources the following checks were conducted:

Procedural check before the deployment

The Manta net and its cod end mesh were washed out thoroughly with high pressure clean water and then stored in an outdoor area. After one week the manta net was washed again this time with its cod end mounted, to capture any contaminants that might have been introduced on to the net during the storage time. The content of the cod end was then washed into a stainless steel bucket and finally transferred into a clean glass jar which was taken back to the laboratory for further analysis. This experiment was repeated 3 times with one week between each.

Procedural check after the deployment

A similar experiment to that of manta net was carried out on the sieve with the smallest (27 µm) mesh size that was used for filtering the contents of the samples back in the laboratory. First the sieve was washed with high pressure clean water and stored away as it was done during the investigation. After a week the sieve was washed again but this time into a clean glass jar the content of which was captured onto the finely pored filtering papers using a filtration system that is commonly used for separating the suspended material from liquids (Figure 26). This experiment was repeated 3 times with one week between each.

Figure 26) Filtration system used for separation of suspended material (Source: SciLabWare Ltd)

Furthermore, to assess the level of potential contamination from the microscopy laboratory at Plymouth University, a petri dish loaded with a clean filter paper (55mm in diameter ~ 24 cm^2) was placed exposed to open air at different locations close to the microscopes where are commonly used for processing of the samples. This was done once during each working day of the week between 10:00 and 13:00 hours (5 replicates in total). During this time the lid of the petri dish were kept wrapped in clean tissue paper and was not exposed to the air.

4.3.3 Procedural contamination checks (CPR)

Small synthetic particles, in particular fibrous forms that are commonly used in textiles can become airborne and could contaminate samples. During a meeting with the analysts and technicians at SAHFOS the possible sources of contamination on to the silk samples were discussed and were identified to be:

- Before the CPR deployment at the workshop during the:
    1. Preparation of silk role for loading into the CPR device
    2. Loading of the prepared silk role into the CPR device
- After the CPR deployment:
    1. Unloading and cutting of the silk role into sample blocks.
    2. During the analysis of silk blocks in the microscopy lab.

To quantify contamination introduced during these stages the following checks were conducted.

4.3.3.1 Procedural checks during the silk preparation

To determine the level of contamination introduced during the silk preparation process prior to the loading of silk roll into the CPR device, 15 silk rolls were randomly selected and examined under a similar staged-microscope as those used by the SAHFOS's plankton analysts. To make the data as representative as possible the silk rolls were selected from those that were cut at different years (i.e. 2008, 2009 and 2010) and also from different size categories (e.g. 25, 40, 70, 80 and 110 division). On each silk roll an area equivalent to that of the plankton silk samples (~ 150 cm^2) typically examined by the analysts was checked. Silks were examined at 3 different locations (beginning, middle and end of silk) along the silk's length and in a systematic way using a longitudinal top to bottom traverse method.

4.3.3.2 Procedural checks during the loading/unloading and cutting

A short length (~50 cm) of the silk roll typically used for the CPR routes (in this case the PR route from Plymouth to Roscoff) was visually examined under the microscope in a systematic way using a longitudinal top to bottom traverse method to check that the silk as supplied to SAHFOS was free from

any particles. The silk was then put through the normal loading and unloading procedures (apart from the CPR unit being deployed at sea) and subsequently cut into silk samples same as those typically examined by SAHFOS analysts and were re-examined for any contamination.

4.3.3.3 Procedural checks during sample analysis in microscopy laboratory

A stretch of silk roll typically used for the CPR routes was prepared, loaded into the CPR device, unloaded from the CPR device and cut into silk samples block of same size as those typically examined by SAHFOS analysts using the normal procedures. These samples were thoroughly examined under the microscope and cleared from any contamination and were subsequently distributed between the analysts for mock analysis using the standard 'traverse' analysis technique for same length of time that is normally used for plankton samples (~ 20-30 minutes). Samples were analysed at 3 different locations in the laboratory using different microscopes and different analysts. 18 silk samples were equally divided between the morning and afternoon sessions and examined by the analysts during 3 days (e.g. 6 samples per day, 3 mornings and 3 afternoons).

During the above checks all the unknown pieces found on the silks or settled onto the filter papers were transferred and stored in petri dishes prior to identification by the Fourier transform infrared (FT-IR) spectrometry following the same protocol as previous experiments (See Chapter 2, Section 2.3.5).

4.3.4 Data Analysis

A mechanical flowmeter was initially used to measure the amount of water filtered through the manta net but due to the repetitive entanglement of its propeller by the floating vegetation its use became a hindrance and was eventually abandoned. However, since the speed and length of tows were known the volume of water passing through the net was calculated using the following formula:

$$V = L * W * H$$

Where L is the length of the linear distance covered by each tow and was calculated based on the average boat speed of 4.3 nautical miles per hour for a 30 minutes tow equating to 3780m. W width of the aperture (0.5 m) and H the height of the aperture with a 1/3 of the aperture submerged on average (0.15/3=0.05 m), hence V the volume of water filtered through the net calculated to 97m³ for each tow.

The amount of water filtered through the CPR was similarly calculated based on the total length of the tow (~31284m) and the aperture size of 1.27cm² and equated to ~ 5m³ (31284x 0.0127 x 0.0127=5.04).

4.4 Results

4.4.1 Quantity and composition of microplastic (Manta net)

In total 70 pieces of suspected plastic debris were found in the manta net samples. FT-IR analysis conducted on 50% of these pieces and conclusively identified 30 pieces (85%) as plastics. Figure 27 shows the relative proportions of different polymer types. The most common synthetic polymer types were

Polyethylene (50%), Polypropylene (27%) and Polystyrene (17%). Microplastic (pieces <5mm) comprised 60% of samples and ranged from 0.6 mm to 4.87mm in size with the 1-3mm size class being the most abundant. Figure 28 shows the relative proportions of different microplastic forms. The most common forms of microplastic were fragment (84%) and pellets (10%) followed by lines, sheet and fiber with similar proportions (~2%). The most abundant form of macroplastic (>5mm) were lines (79%) followed by fragment (14%) and sheet (7%). Figure 29 shows photographs of different types and forms of plastic debris found in the manta samples.

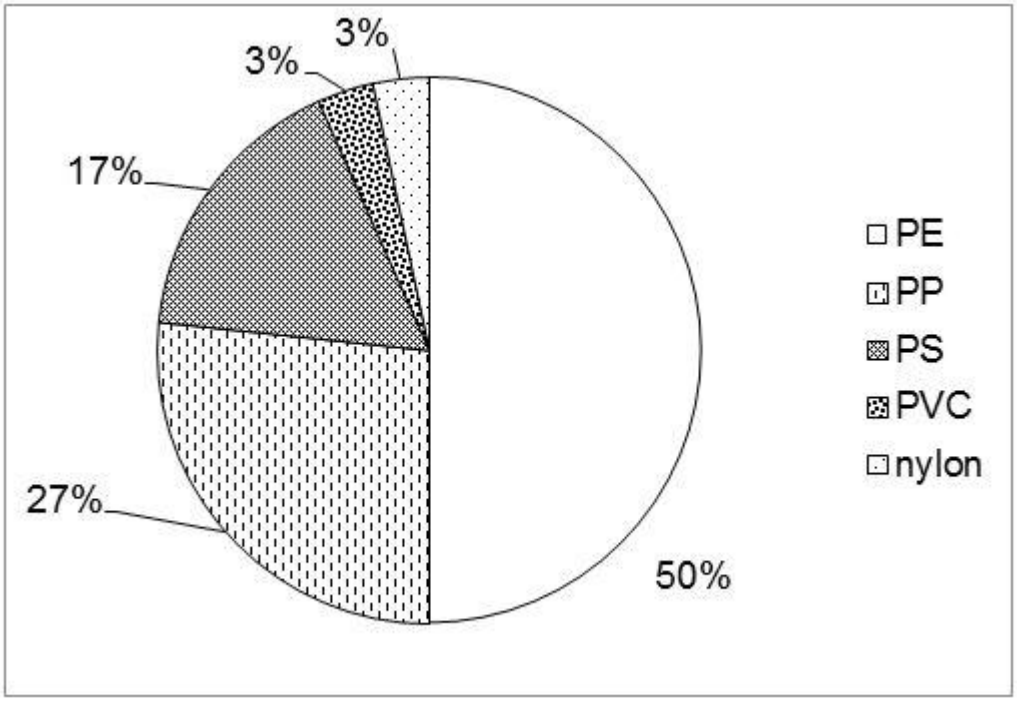


Figure 27) The relative proportions of different polymers found in manta net samples.

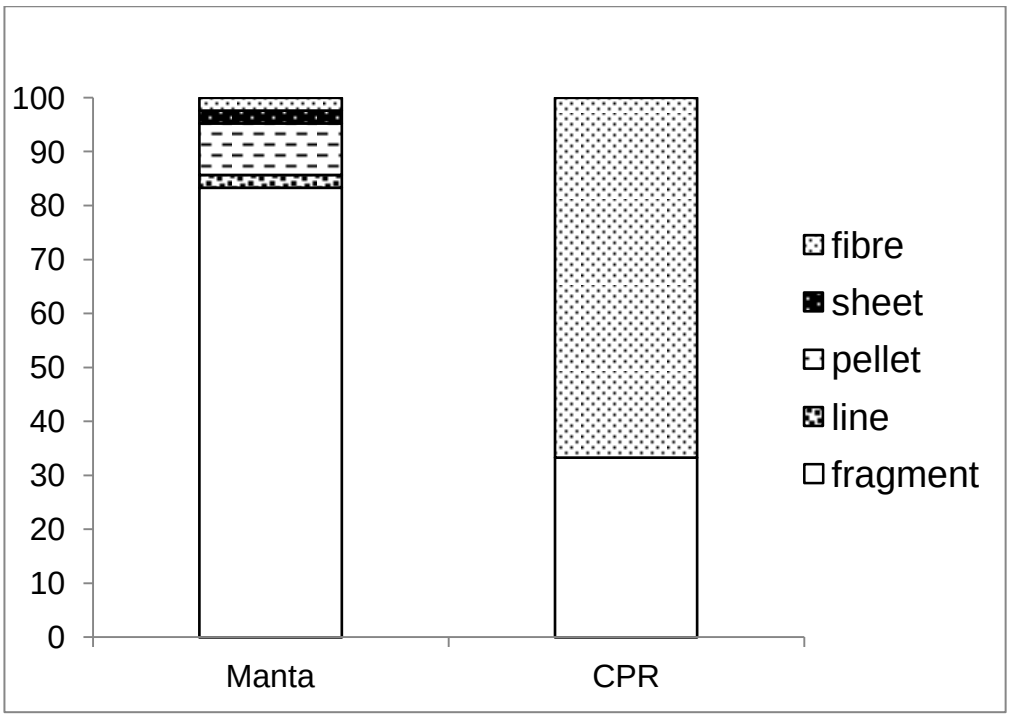


Figure 28) Percentage of plastic pieces found in Manta and CPR samples from the whole sampling period by form.

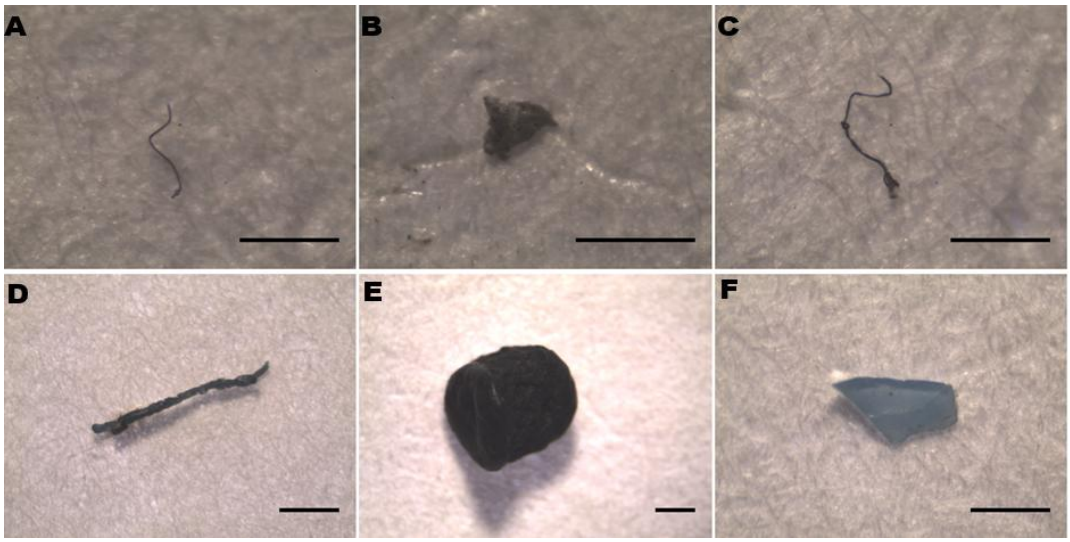


Figure 29) Photographs of plastic debris identified using FT-IR.

Top row CPR: (A) Rayon, (B) Polypropylene, (C) Polyester,

Bottom row Manta: (D) Nylon, (E) Polypropylene, (F) Polyethylene.

Scale bars represent 1 mm.

4.4.2 Quantity and composition of microplastic (CPR)

In total 7 pieces of suspected microplastic were found in CPR samples of which using the FT-IR analysis, 3 pieces were conclusively confirmed as synthetic polymers: one Polypropylene fragment (~ 0.49 mm), one Polyester fiber (0.82 mm) and one Rayon fiber (1.4 mm). The average number of pieces across 3 days of sampling was one.

The average number of microplastic pieces was 1 giving a concentration of 0.2 per cubic of water over total of 5 m³ filtered water compared to that of 0.14 for the Manta with average of 14 pieces over 97 m³ filtered water (Figure 30).

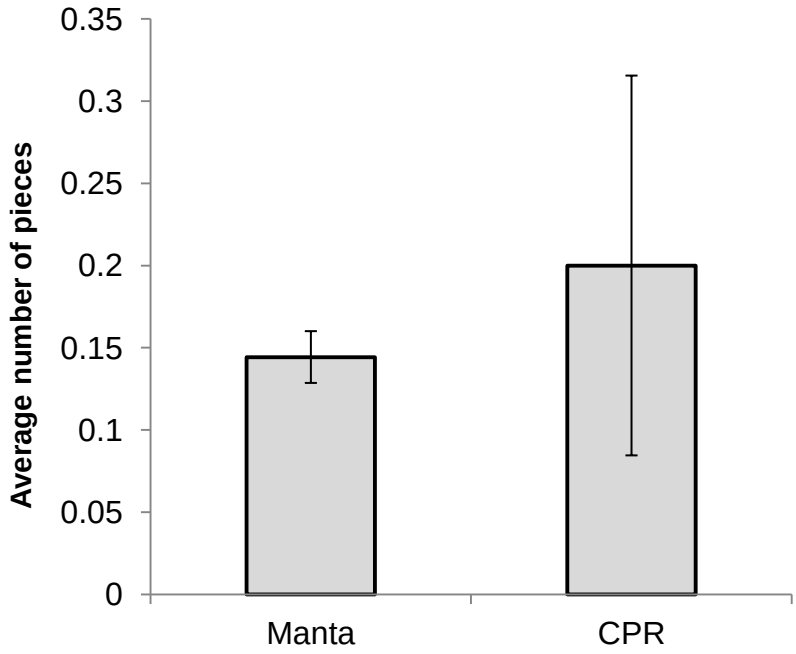


Figure 30) Average number of plastic pieces/m³ (±SE, n=3) found in the CPR and Manta samples

4.4.3 Procedural contamination (Manta net)

On average the numbers of contaminant particles found were: 7.3 pieces on the manta net mesh, 1 piece on the sieve's mesh and 1piece on the petri dish from the microscopy lab. Rayon fibres were the most common type of contamination and were observed in samples from all 3 items of equipment (Manta net, sieve and petri dish). Other contaminant fibres were polyester and acrylic fibres as well as polypropylene and PVC fragments. PVC was found only on sieve samples (0.3 pieces on average) and Polypropylene only on Manta samples (0.18 pieces on average). Petri dish samples contained only rayon and polyester fibres on average at 0.6 and 0.4 pieces respectively. Figure 31 shows proportion and total number of the contaminants found on each equipment.

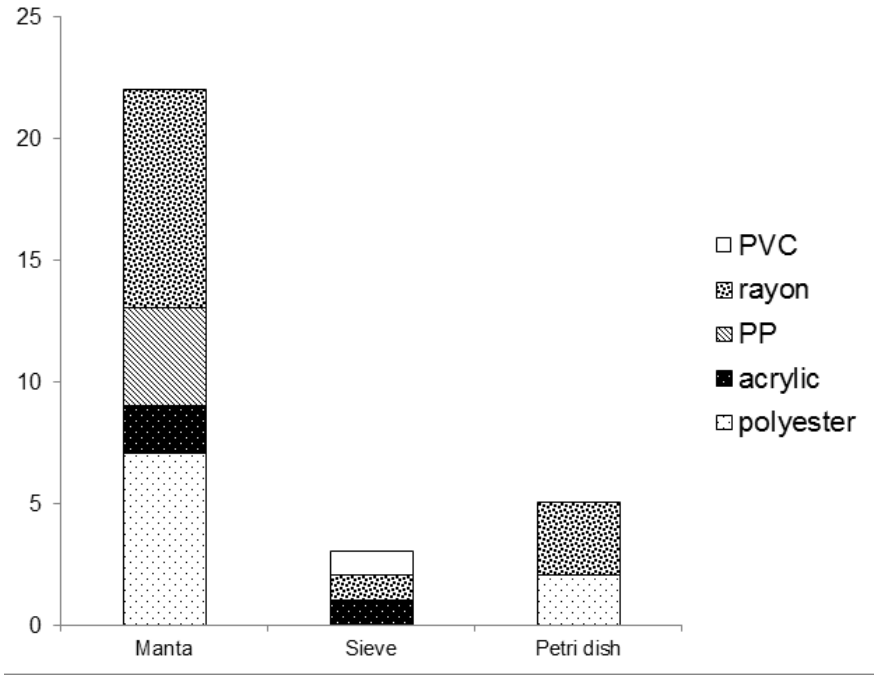


Figure 31) Proportion and total number of the contaminants found on each of the sampling equipment.

4.4.4 Procedural contamination (CPR)

The highest level of contamination was observed during the silk preparation process prior to the deployment to sea at an average of 3.6 pieces per silk. Levels of contamination from processes after the deployment were lower: 1.66 pieces per silk for the laboratory and 0.2 for the “silk loading and cutting” process (Figure 32). Using the FT-IR technique the contaminants were analysed and formally identified as rayon or polyester fibres. Figure 33 shows photos of some of the contaminant fibres found on the silk. The fibres were mostly dark blue and typically 2 to 6 mm in size but fibres as small as 0.5 mm were also observed.

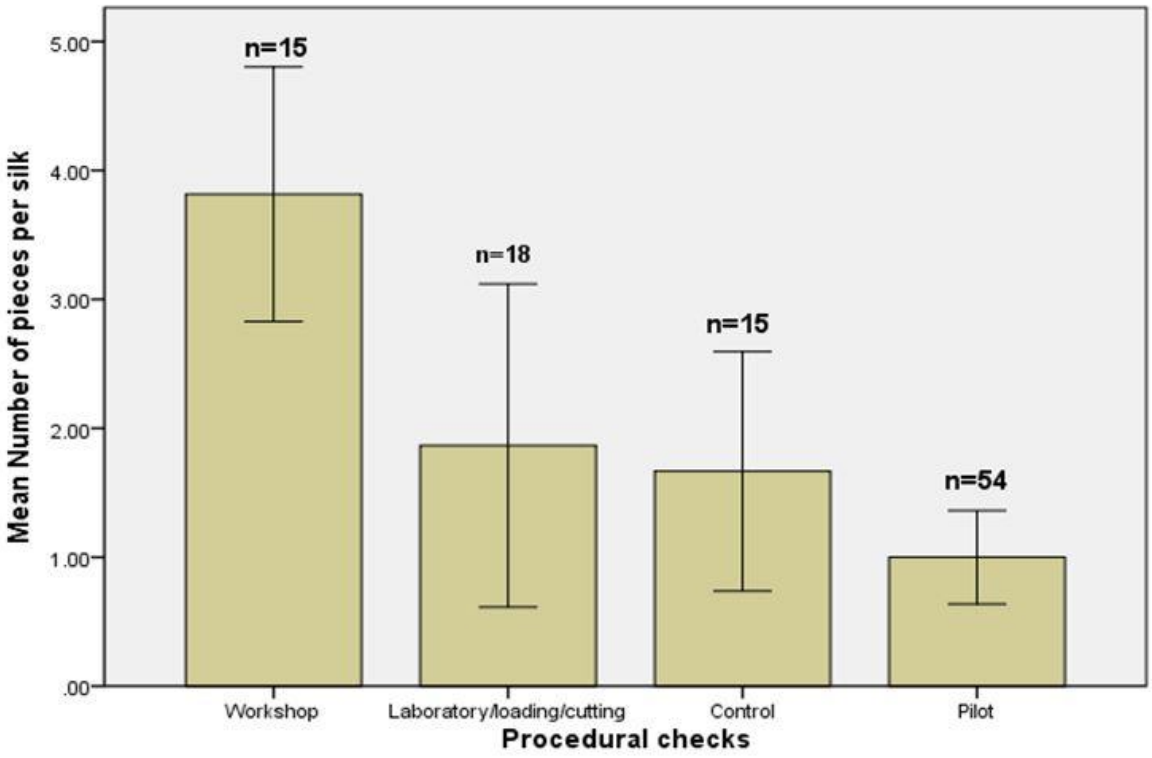


Figure 32) Mean number of synthetic pieces (± SE) found on CPR samples after silk preparation but before examination by the analysts (Workshop) and after examination by the analyst (Laboratory/loading/cutting) compared to microplastics found in CPR samples from sea without laboratory analysis (Control) and with laboratory analysis (Pilot). Number of silk samples examined (n).

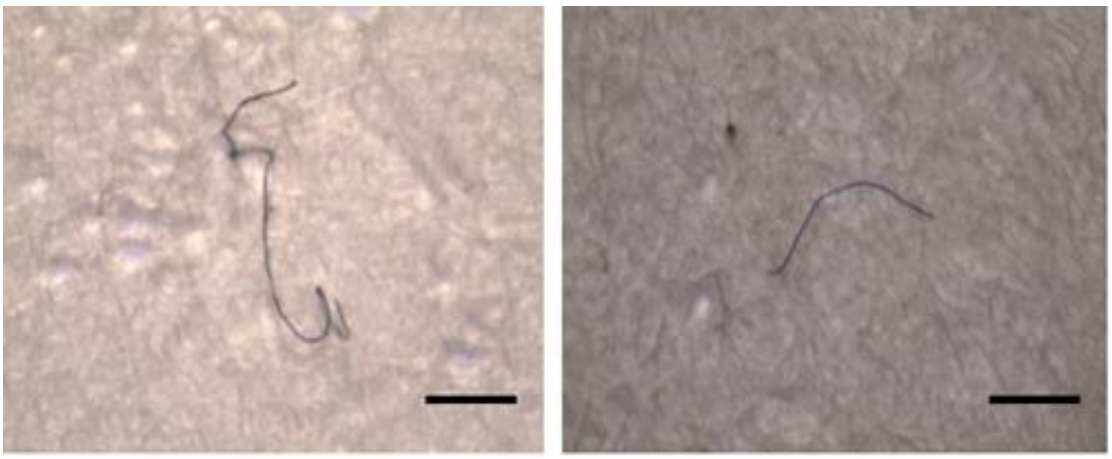


Figure 33) Photographs of some contaminant fibres found on CPR silks. Scale bar represents 1mm.

4.5 Discussion

4.5.1 Quantity and composition of microplastic debris

In this study I have shown that the composition (type, size and form) of microplastic debris in surface waters as captured by the manta net are clearly more diverse than those sampled in subsurface waters by the CPR. The Manta net captured significantly more plastic when assessed by the number of items but not when standardised by the volume of water (Figures 31). This was expected as the Manta filters more water than CPR but it may also be an indication of a shorter suspension time in the middle column possibly due to the increased mixing regimes at this depth pushing the debris to lower depths.

A study by Lattin *et al.* (2004) who compared the abundance of plastic debris at different depths in the water column (surface, middle and bottom) before and after storm events using different plankton nets (manta, bongo and epibenthic sled) found the middle column (10m) samples less abundant than surface samples and the near bottom samples most abundant in both

nearshore and offshore waters. However in the nearshore waters the middle column samples taken after a storm event showed higher abundance than the ones before storm highlighting the effect of wind on vertical positioning of debris within the surface boundary layer similar to those reported in a more recent study by Kukulka *et al.* (2012). The higher concentrations of microplastic debris in nearshore subsurface waters is also consistent with findings of recent study by Desforges *et al.* (2014) which found the concentration of microplastic debris to be 4–27 times greater at sites nearshore than sites offshore in NE Pacific Ocean.

4.5.2 Procedural contamination (CPR)

The fact that most of the contamination was found during the silk preparation stage and prior to the deployment of the CPR at sea was not surprising since it is during this stage that the silk is most manipulated and exposed and therefore highly likely to pick the contaminant fibres from the surrounding environment such as clothing of the technicians and also the surface of the table on which they are prepared. In this stage both the filter and cover silk are unrolled on a table in the workshop in order to be marked into sections then edges are glued and finally aligned on top of each other before being rolled back.

The next step involves mounting of the prepared silk roll into the CPR device. In this phase the silk remains mostly rolled with minimal contact with the working table but it is still exposed to air and likely to be contaminated by the fibres detached from the technician’s clothing and the mechanisms within the CPR device. However both the time period and exposed area of the silk during this stage are much less than the preparation stage.

After return from sea the spooled CPR silk is unloaded from the device and cut into silk blocks in the cutting room. The cutting room is the most isolated environment in SAHFOS's laboratories covered by a protected screen and a ventilated hood to keep the air clear from the toxic formalin vapour. Therefore it is least likely that silks get contaminated during this stage. Once the silk is cut into sample blocks they are ready for analysis in the laboratory. On average it will take 20-30 minutes for silk sample to be analysed during a traverse screening, however it is not unlikely for silks to be exposed longer due to other tasks such as need for close examination of specimen under a more powerful microscope or consultation with other analysts and/or identification catalogues.

The analysis stage after the preparation is the second, most likely, period during which samples could get contaminated by external fibres. These scenarios are consistent with results of our experiment in which the highest contamination levels were observed during the preparation stage (3.6 pieces/silk) followed by the laboratory/loading/cutting stage at 1.86 pieces/silk (Figure 32).

4.5.3 Procedural contamination (Manta)

Similar to the CPR the highest number of contaminants was found on the net and prior to the deployment to sea an average of 7.3 pieces which is much higher than average of 3.6 pieces in CPR but this is expected as manta net has a much larger surface area hence more likely to pick higher levels of contamination. However, level of contamination was slightly lower at the Plymouth University microscopy laboratory compared to the SAHFOS's lab (1 vs 1.66 pieces per silk).

This study has shown the susceptibility of both the CPR and manta net to contamination prior to sea deployment and also during the laboratory examination after deployment. Therefore, more rigorous protocols and quality assurance measures should be implemented if these methods are to be used as a reliable microplastic monitoring tool. Chapter 6 will address some of these issues and recommends measures that could help in lowering the contamination levels.

Chapter 5. On the quantity and composition of floating plastic debris entering and leaving the Tamar Estuary, Southwest England

5.1 Abstract

The majority of plastic debris found in the marine environment has land based sources and rivers are considered an important medium for the transfer of this debris. However, there are few published studies on the amount, composition or trends of riverine plastic debris. Here we report on the quantity and composition of floating plastic debris collected from surface waters of the Tamar Estuary. This represents the first study of riverine transport of plastic debris in to European waters during different tidal regimes.

A key question in this study was to establish whether the estuary was a net source or a net sink for plastic debris. This was evaluated during both spring and neap tides. Plastics were found in a variety of forms (fragments, line, fibres, and sheet) and sizes (0.2mm-112mm). Microplastics (<5 mm) comprised 82% of the debris. FT-IR analysis indicated the most abundant types of plastic to be Polyethylene (40%), Polystyrene (25%) and Polypropylene (19%). The overall mean concentration of plastic items was (0.028/m^3) and there was a significant difference in size frequency distribution between the spring and neap tides with more fragments of larger size observed during spring tides. While it is clear that debris has accumulated on shorelines within the estuary, during our study this river did not identify as a net source or sink.

5.2 Introduction

Plastic production has increased rapidly in recent years from 5 million tons in 1950s to 288 million tons in 2012 (Plastics Europe, 2013). With its unique properties and multifaceted applications plastic has become an indispensable part of modern life. Plastic are inexpensive, durable and versatile, however these attributes have also led to extensive use in disposable items that are used once and then discarded. This combined, with inadequate recycling, waste management and littering behaviour, have made the accumulation of plastic waste a global environmental problem (Thompson *et al.*, 2009).

The occurrence of plastic debris in the marine environment was first reported in the coastal regions in 1970s (Carpenter *et al.*, 1972; Morris and Hamilton, 1974; Gregory, 1978;). Since then it has been the focus of numerous studies and has been shown to be widely distributed (Thompson *et al.*, 2004; Barnes *et al.*, 2009; Browne *et al.*, 2011; Moore, 2008; Sul *et al.*, 2011) as well as ingested by a wide range of organisms. However, we know relatively little about the associated environmental impacts (Laist, 1987; Derraik, 2002; Thompson *et al.*, 2009; Lusher *et al.*, 2012).

Plastic is the largest component accounting for 50-80% of debris that contaminates marine habitats at a global scale (Barnes *et al.*, 2009). The majority of this litter has land-based sources such as: landfill sites, tourism, storm water outflows. Rivers are considered to be an important medium for transfer of different types of debris to the sea; however there are few published data on the riverine and estuarine plastic debris. A study of the intertidal banks of the River Taff, UK by Williams and Simmons, (1996) showed plastic as the

most abundant type of debris with sewer overflows and fly tipping as main sources.

The analysis of the solid wastes in one isolated beach in Brazil also identified plastic, with urban origins, as being the most abundant type of debris and highlighted the importance of the riverine contribution (Araújo& Costa, 2007; Ivar & Costa, 2013). There is one published study on shorelines in the Tamar Estuary, UK showing that habitats that are downwind could act as potential sinks for debris (Browne *et al.*, 2010), and thus indicating the potential importance of water movement and wind patterns within the estuary in the transport of debris. A recent study in the tidal River Thames (Morritt *et al.*, 2014) also reports on the movement of substantial quantities of debris close to the river bed. However because sampling was not stratified according to tidal cycles it is not possible to determine the direction of transport.

It is important to monitor the quantity and distribution of marine litter in order to fully assess its socio-economic and ecological impacts and to monitor the success of measures targeted at reducing litter. The European Marine Strategy Framework Directive 2008/56/EC (MSFD, 2011) recognises Marine Litter as one of the indicators for the Environmental State of the European Seas and calls for more data on the amount, distribution and, where possible, composition of this debris (Piha *et al.*, 2011). A key part of any strategy to reduce marine litter is to understand and then potentially regulate its sources.

Current knowledge of the sources and fate of plastic debris, particularly for microplastics is limited and it is not clear whether this small debris is transported in the same manner as larger items. Floating debris could be pushed into estuaries during the rising tide from nearby coastal waters and/or

travel out into the coastal waters during the falling tide. But estuarine water circulation is complex and influenced by many environmental parameters such as riverine inflow, tides, wind and evaporation. The nature of estuarine circulation affects the residence time of its water body and consequently the concentration of its solutes and suspended solids some with human-induced sources such as litter that may affect the health of estuaries (Balls, 1994). A recent study (Bakir *et al.*, 2014) has suggested a correlation between the desorption rates of the Persistent Organic Pollutants (POPs) from the contaminated microplastics and their retention time in estuaries and marine waters and highlights the importance of both natural and anthropogenic processes such as flushing and dredging in pulse release of these substances.

This chapter presents the first comparison of the quantity and composition of buoyant plastic debris in European estuarine waters according to daily and lunar tidal cycles. The specific aims were: a) to describe the abundance and composition of floating litter in estuarine surface water; b) to compare the abundance, size frequency and composition of debris between the different stages of the daily tidal cycles (flood vs. ebb) and different lunar phases (spring and neap).

5.3 Material and methods

5.3.1 Study area

The River Tamar has a catchment area of ca. 1700 km² and flows south from North Cornwall for about 78 km (Evans *et al.*, 1993). The estuary is tidal for about 31 km from Gunnislake Weir to its mouth at Plymouth Sound (Figure

34) and is considered macrotidal with semi-diurnal tidal flows ranging from 6.5 m at Devonport during the springs and 1.5 m at neaps in the upper reaches (Miller, 1999). Land use in the upper reaches of the catchment is mainly agricultural with relatively clean waters (Mighanetara, *et al.*, 2009). There are no major landfill sites along the estuary but a number of anthropogenic impacts such as run off form historic mining sites and discharges from sewage works are visible towards the mouth of the estuary around naval dockyard closer to the more populated areas of the city of Plymouth (Miller, 1999; Langston *et al.*, 2003) As such the estuary could be considered typical of many other estuaries within Northern Europe.

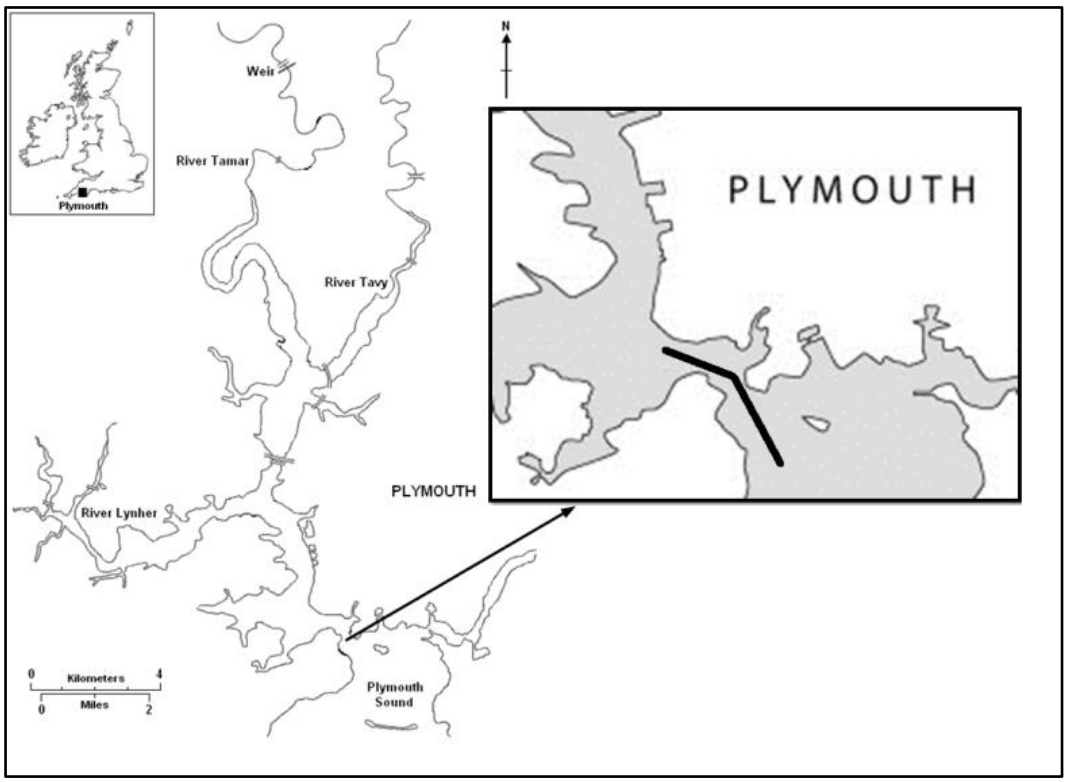


Figure 34) Map of UK showing location of Plymouth (insert top left corner). Tamar catchment and the traverse used to collect debris

(black line in insert top right).

Figure 35) Manta net on board *RV Aquatay* (left) and deployed in the Tamar Estuary (right). The overall width of manta including its floating wings was ~ 150cm.

5.3.2 Sampling and processing protocol

Samples were collected from surface waters during May and July 2012 near to the mouth of the river during both spring and neap tides using a 0.50m by 0.15m manta net (300 µm mesh) aboard the Plymouth University's RV *Aquatay* (Figure 35). Spring and neap tides were both sampled on three replicate dates and on each occasion with three replicate samples taken during both the flood and the ebb tides. The net was towed against the tidal flow at a speed of 4 knots for 30 minutes during the maximum flow period. Samples were transferred into glass jars and taken to the laboratory where their content was filtered through a set of sieves with varying mesh sizes (3mm, 1mm and 270 µm). Any unknown but potentially plastic pieces were transferred on to Petri dishes and categorised under 4 different size groups (>5mm, 3-5mm,1-3mm and <1mm) and forms (Fragments, Sheet, Fibre and Pellet). Fourier transform infrared (FT-IR) spectrometry analysis was conducted on 50% of the pieces

from each size groups in order to indicate the relative proportions of different polymers.

Fragments were identified using a Bruker IFS66 Fourier transform infrared (FT-IR) spectrometer with a MCT detector operating in the 4000-600 cm-1 wave number range and attached to a Bruker Hyperion 1000 microscope. A Specac DC2 Diamond compression cell (2 mm in diameters) was used to prepare the samples. Each sample was transferred from the petri dish on to the diamond cell and compressed between the two plates into a thin uniform thickness enough to allow for adequate transmission of IR beam through the sample to the detector and resulting in a better quality spectrum. For the measurement, processing and evaluation of the spectra Burker's Opus 6.5 spectroscopy software was used to best match spectra of the unknown debris following a protocol similar to that used by Thompson *et al.* (2004).

Due to the nature of investigation special measures were taken to reduce the likelihood of introduction of any contaminations into the samples. Before sampling all equipment including the manta net were thoroughly washed with clean fresh water and a cleaned steel bucket and funnel were used to transfer the content of each sample into glass jars. In the laboratory, during the processing and analysis of the samples, a cotton coat and latex gloves were worn to reduce the contamination of samples by synthetic fibres from the clothing material.

5.4 Statistical Analysis

The non-parametric Kolmogorov-Smirnov (2 sample K-S test) was used to test the differences in size frequency distribution pattern between the tidal states. To test for any difference in the abundance of debris across the tidal

states (neap vs. spring and flood vs. ebb) the non-parametric Mann-Whitney U test was used.

5.5 Results

In total 204 pieces of suspected plastic were found and of these 84% were confirmed to be plastic in the following relative proportions: Polyethylene (40%), Polystyrene (25%) and Polypropylene (19%) (Figure 36). Polyvinyl Chloride, Polyester and Nylon were amongst the other types of plastics present. Microplastic (<5mm) comprised 82% of this debris and were found in different forms in all samples (Figure 37). The 1-3mm size category was the most abundant and contained all the polymer types. The proportion of polyethylene was similar across all the size categories. PVC was present only in 1-3mm and 3-5mm and nylon only in <1mm and 1-3mm size groups (Figures 38 and 39).

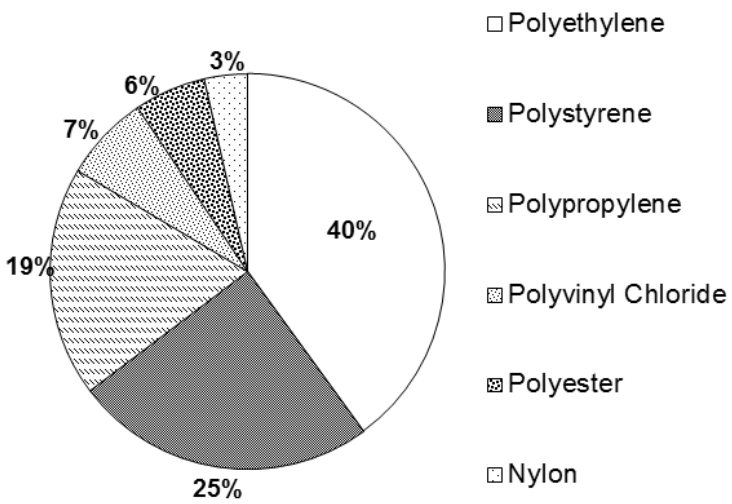


Figure 36) The relative proportions of different polymers found.

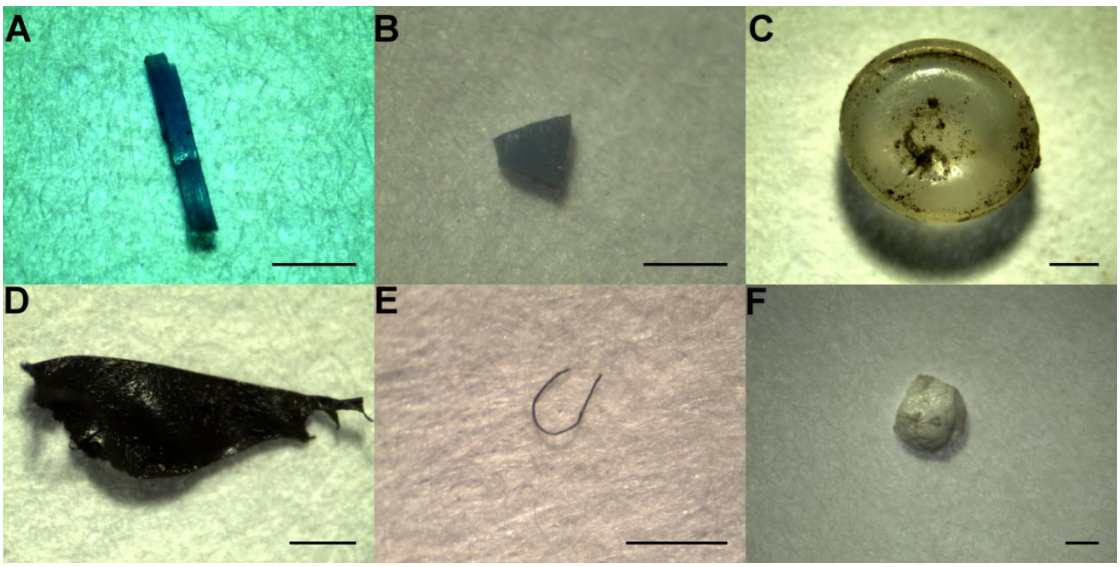


Figure 37) Photographs of some of the plastic debris found in the River Tamar and identified using FT-IR as: (A) Polypropylene, (B, C and D) Polyethylene, (E) Nylon, (F) Polystyrene. Scale bars represent 1 mm.

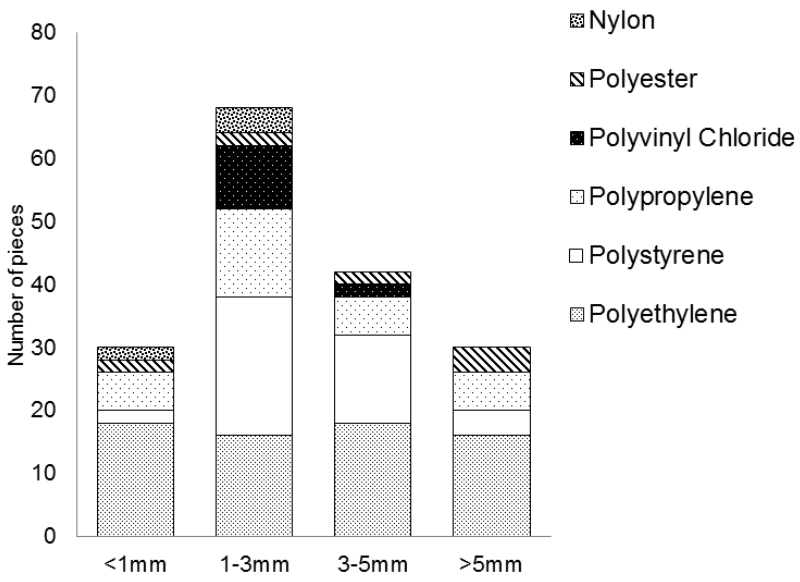


Figure 38) Total number of plastic pieces found in the River Tamar from the whole sampling period by polymer type and size.

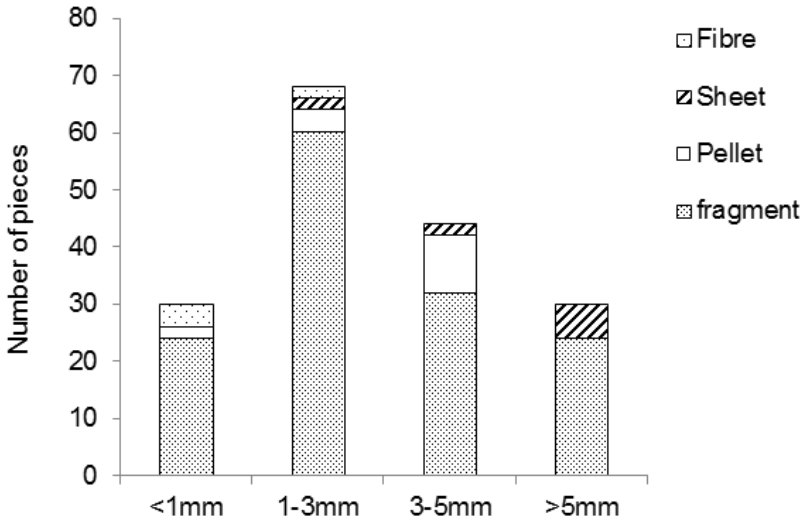


Figure 39) Total number of plastic pieces found in the River Tamar from the whole sampling period by form and size.

The frequency distribution of size classes across tidal states was significantly different between the spring and neap tides (Kolmogorov-Smirnov $Z = 1.832$, $p = 0.002$, $n=36$) with a shift towards a smaller central tendency during the neap/ebb tide (Figure 40). Mean abundance between tidal phases varied but there was no significant differences between the tidal phases or cycles (Mann Whitney $U=283$, $n=18$, $P=0.118$; $U=287$, $n=18$, $P=0.152$). The overall mean concentration of plastic was 0.028/m³ (Figure 41)

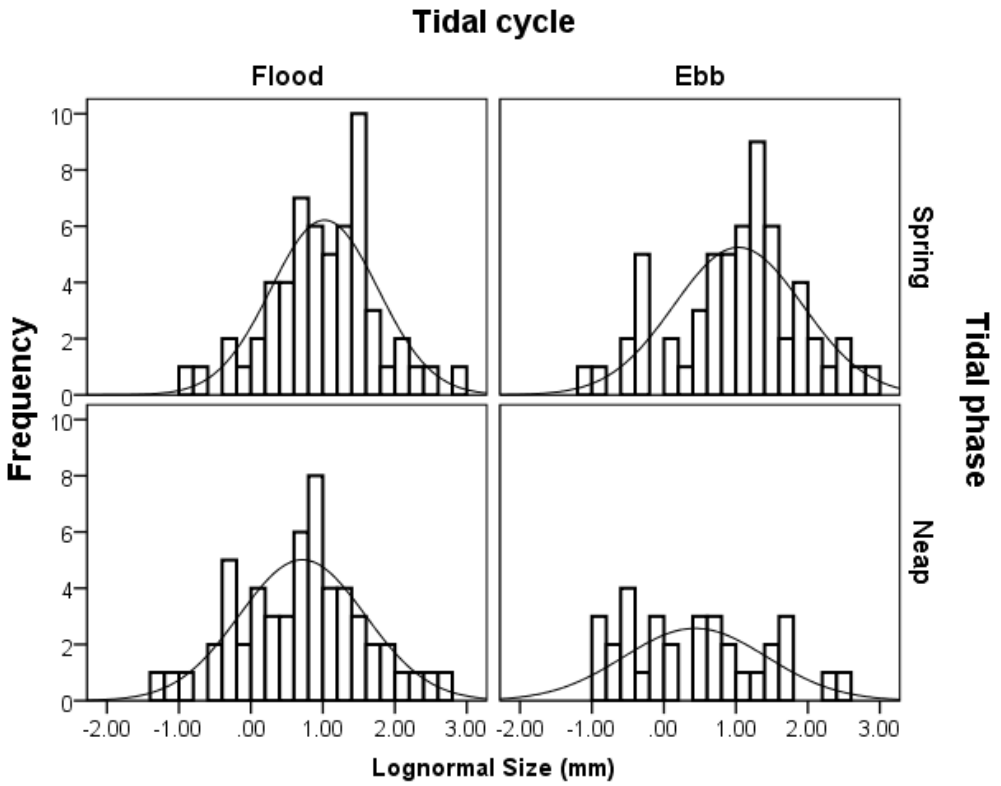


Figure 40) Lognormal of frequency distribution across different size categories and tidal cycles, showing a shift during neap/ebb tides.

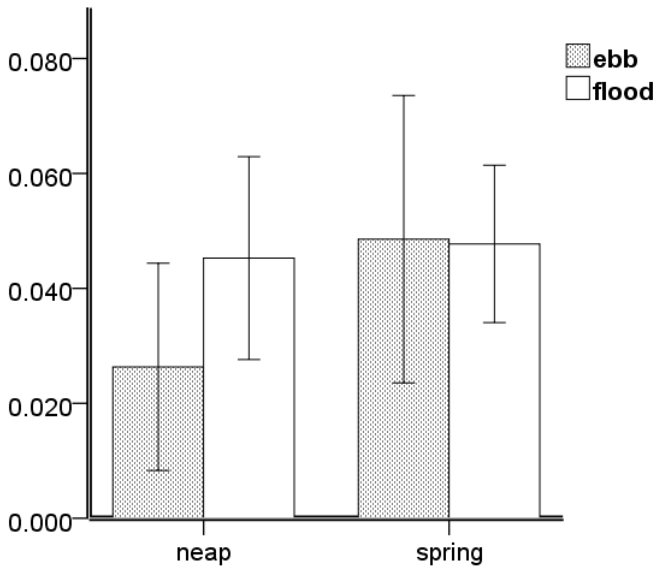


Figure 41) Abundance of plastic/m³ ($\bar{x}$ ± SE) found in River Tamar by tidal cycles (Flood/Ebb) and according to the tidal phases (Spring/Neap).

5.6 Discussions

Composition and quantity of plastic

As with other studies (Browne *et al.*, 2010; Costa *et al.*, 2010), microplastics (<5 mm) accounted for the majority of the debris in terms of numerical abundance, which generally is an indication of weathering (photo oxidation) and physical (abrasion by waves) defragmentation. However, in this study the 1-3mm size class was the most abundant size class whereas in the study by Browne *et al.* (2010) it was the <1 mm class. The skew towards smaller debris seen in the study of Browne *et al.* (2010) is probably due to the difference in sampling and extraction methodology where the use of glass filter papers would have given them a much lower cut-off value compared to the 300 µm mesh of manta used in this study.

It is not easy to determine the origins of plastic debris in the marine environment, in particular, the buoyant plastic, but the types of polymer used for different applications provide a potential indication. In this study similar to those of the larger items found on adjacent shorelines by Browne *et al.* (2010) buoyant polymers: polyethylene, polypropylene and polystyrene were the most abundant types of debris (Figure 38). These polymers are predominantly used in the packaging industry which could indicate an urban origin of this debris. However, polymers such as polyester, nylon and PVC are denser than seawater and their presence in our samples indicates that the transport of debris is influenced by factors other than density alone. One likely explanation could be the introduction of some of these fibres via the sewage outlets on to shorelines (Browne *et al.*, 2011) and/or their re-suspension in water column as a result of turbulent mixing induced by wind and tidal currents.

Influence of tides on the abundance and size distribution of floating plastic debris

In this study we found a shift towards the smaller debris and although not significant also some indication of lower abundance during the neap/ebb tide. It is possible for some particles that leave the estuary on the ebb tide to re-enter the estuary during the flood tide and vice versa. This is especially likely near the mouth of estuaries where complex circulation patterns can occur (Wolanski, 2007). Perhaps the similar overall abundance in the spring tide samples (Figure 41) in this study could be partially attributed to this phenomenon. The shift towards the smaller pieces and lower abundance of debris during neap tide could be due to the elevated clogging of the net if phytoplankton concentrations were greater during neap tides compared to spring tide as observed by Cloern (1991). The cumulative effect of clogging could result in a bias towards the smaller particles and also decrease in the overall sampling rate, thereby artificially reducing the overall amount of microplastics that are eventually captured. However, visual inspection of the recovered nets did not reveal substantial clogging and our short sampling durations were chosen to help minimise clogging. Clearly, in future sampling, the duration of tow needs to be considered in relation to potential clogging so as to maximise the capture of debris.

The wind direction and force could also have an important role in the spatial distribution of floating debris as indicated by Browne *et al.* (2010) where shores downwind accumulated a greater abundance of plastic debris. There are some indications in our data of higher abundance of debris in samples collected during North-westerly wind regime than the predominant South-westerly (Table1); however more work would be needed to confirm this.

Table 3) Wind directions and speed data. Samples with highest number of plastics are shown in bold.

Trawl Id	Sampling Day	Date	Tidal Phase	Tidal Cycle	Wind Speed/knots	Wind Direction	Plastics count
MFOD2S2	2	14/05/2012	Neap	ebb	7-10	**NW**	**20**
MFOD4S2	4	29/05/2012	Neap	ebb	7-10	S	6
MFOD5S2	5	13/07/2012	Neap	ebb	7-10	W	6
MFID4S2	4	29/05/2012	Neap	flood	7-10	**NW**	**29**
MFID2S2	2	14/05/2012	Neap	flood	11-15	W	18
MFID5S2	5	13/07/2012	Neap	flood	11-15	SW	8
MFOD1S2	1	08/05/2012	Spring	ebb	4-6	**W**	**31**
MFOD3S2	3	24/05/2012	Spring	ebb	4-6	S	7
MFOD6S2	6	20/07/2012	Spring	ebb	4-6	NW	21
MFID1S2	1	08/05/2012	Spring	flood	4-6	**W**	**21**
MFID3S2	3	24/05/2012	Spring	flood	7-10	S	19
MFID6S2	6	20/07/2012	Spring	flood	4-6	SW	18

The stratification and mixing processes between the saline and fresh water are important factors affecting both horizontal and vertical positions of buoyant items such as plastic debris. Wind direction and force have been shown to play an important role in distribution and positioning of floating objects (Browne *et al.*, 2010; Thiel *et al.*, 2011; Kukulka *et al.*, 2012) future studies should therefore examine more extensively the temporal effects of different wind regimes. Tidal currents are typically strongest between high and low tides and could considerably influence the residence time and transport of floating objects within an estuary.

When designing sampling or monitoring programmes, factors such as timing, location and length of trawls in relation to the strength of tides should be

carefully considered in order to ensure that the most representative body of water is being sampled. If effects of a particular land-based feature (e.g. landfill, sewage output, etc.) are of interest then the spatial coverage of the trawls relative to these features becomes a priority compared to trawl times or distance . However, if like our study the effect of tides is the main interest then the timing of trawls must be standardised in preference to spatial coverage. For instance, in our study (trawl time: 30 minutes; Boat speed: 4 knots) all samples were taken during periods of maximum-flow-in and maximum-flow-out (approximately 2.5 hours after the high and low tides). This allowed for relative consistency with regards to the tidal flow but resulted in less linear distance towed along the estuary during the spring tides.

Ultimately data should be standardised for the volume of water through the net using either a flowmeter or calculations based on the distance travelled by the boat at a constant speed. In order to gain comprehensive estimates of the volume of litter that is transported via estuaries it will be necessary to use a combined approach to ensure various types and sizes of debris are captured and that different parts of the water body are sampled including mid-water and the river bed. For example substantial quantities of litter have recently been reported in a study using traps fixed near to the seabed in the tidal Thames (Morritt *et al*., 2014).

Chapter 6. General Discussion: Investigation of microplastic debris in marine surface waters

6.1 Summary of the research findings

The research validates existing data and also acquires new data using different sampling and processing methodologies. It also identifies some of the methodological challenges that are to be considered and recommends measures for addressing some of these issues .

Archived CPR samples were first used to evaluate microplastics abundance in sub-surface waters of the northeast Atlantic Ocean (Thompson *et al.*, 2004). The analysis and mapping of microplastic debris using samples from the Continuous Plankton Recorder (CPR) reported by the SAHFOS' analysts as presented in Chapter 2 showed a wide distribution of microplastic debris in the North Atlantic Ocean and confirmed the presence of synthetic and semi-synthetic polymers in several European waters (including North Sea, Irish Sea, English Channel and the Northeast Atlantic Ocean) with the abundance generally being higher in the nearshore coastal waters than in offshore oceanic samples; potentially indicating the importance of the urban centres similar to findings by Browne *et al.* (2011) and the modulating effect of oceanographic features such as frontal zones on distribution of plastic debris in coastal waters (e.g. Hinojosa *et al.* 2011, and references therein).

The validation and analysis of microplastic fragments reported by the SAHFOS' analysts in the CPR samples as presented in Chapter 3 showed an acceptable level of confidence in the accuracy of the SAHFOS' analysts to visually detect microplastic fragments during their normal processing of plankton therefore allowing for a tentative consideration of CPR methodology as an index of microplastic in subsurface marine waters.

Selecting a suitable sampling method and depth is crucial for evaluating floating microplastic pollution (Lattin *et al.*, 2004; Kukulka *et al.*, 2012; Song *et al.*, 2014). Field trials comparing data from manta trawl net and CPR as presented in Chapter 4 showed a significantly higher abundance of microplastic in samples collected by the manta net per unit of distance (0.58 pieces/km vs. 0.02 pieces/km) however, there was no significant difference between the two approaches when the results were standardised for cubic volume of water (0.14 pieces/m^3 vs. 0.13 pieces/m^3). There was also greater variety in type, form and size of plastic debris in the manta net samples than that collected by the CPR suggesting a wider spatial distribution of microplastic debris in marine surface waters.

Further field trials as presented in Chapter 5 evaluated for the composition and abundance of microplastic debris in estuarine waters of the River Tamar during different tidal regimes showed that microplastic debris compromised 82% of the total debris and the most abundant types of plastic were: Polyethylene (40%), Polystyrene (25%) and Polypropylene (19%) with a significant difference in size frequency distribution between the spring and neap tides with more fragments of larger size observed during spring tides.

These results provide additional data on the amount, composition and distribution of buoyant plastic debris in marine waters using different sampling devices and show further evidence of the spatial heterogeneity of this debris and therefore also highlighting the need for more comparable data from different marine habitats using standardised methodologies.

6.2 Importance of Monitoring Programme

On-going monitoring programme to assess the abundance and composition of plastic debris are essential for better understanding of its sources, pathways and fate as well as for assessing the efficacy of the implemented measures to reduce its abundance. Current monitoring programmes in Europe have been predominantly conducted by the volunteers through Non-Governmental Organisations (NGOs) and have been a valuable source of information. However these programmes have mainly focused on the larger items of debris from beaches and used varying methodologies which too often makes the results incomparable (Cheshire *et al.*, 2009; Ryan *et al.*, 2010; Hidalgo-Ruz *et al.*, 2012; MCS, 2013). Public awareness and engagement is an integral part of any remedial solution to tackle the marine debris problem and monitoring programmes can also provide important information for raising public awareness (Cheshire *et al.*, 2009).

Most marine litter monitoring programmes have focused on beaches at a local scale, however, regional and local surveys do not provide a suitable baseline since the selection of sampling sites is subjective and, therefore, not representative of the area in general. Marine debris is a global problem and increasingly in need of a harmonised scientific approach with tailored protocols that are specific to the questions being asked (Ryan *et al.*, 2010; Hanke *et al.*, 2013) and sensitive to changes in the amount of 'new' plastic litter entering the marine waters (Thompson, 2007).

6.3 Suitability of CPR and Manta trawl for investigation of microplastics in the marine surface waters

Monitoring floating debris in particular is a logistically complex, expensive and time consuming operation. Net-based methods used on board of research vessels have been the most common sampling approach as they give researchers a greater control over choosing the time and area of sampling. However, net-based methods are suitable for calmer coastal waters since in oceanic waters their operational capabilities are significantly reduced under the adverse weather conditions. Also they can only cover relatively small areas and for large scale offshore sampling due to their limited maximum operational speed (~ 6 Nm) would prove impractical and costly as large numbers of net tows are required to sufficiently estimate the average density of the floating litter (Ryan *et al.*, 2010). In order to understand the dynamic and spatio-temporally heterogeneous processes that drive the composition and distribution of the floating debris at global scale a more extensive sampling in both space and time is required.

In contrast to conventional net based sampling programmes with limited spatial and temporal coverage the CPR survey has over 75 years of archived samples and a network of over 50 routes sampling nearly 10,000 nautical miles of water every month. In addition its sampling and analysis methods have remained largely unchanged since 1939 (Richardson *et al.*, 2006) and therefore provides a standardised framework for spatiotemporal studies. Another advantage of CPR samples is the ease of their geographical traceability in that for each sampling unit the exact location, time and date is known therefore allowing for convenient and efficient investigation of temporal and spatial patterns.

However, the CPR only samples sub-surface waters and despite the mixing of water by the ship's wake, it is believed that some plastic items will remain at the surface, only sinking lower if their overall density changes as a result of fouling. Though not substantiated there may also be some limitations on the type and amount of microplastic debris collected by CPR. This could be a product of the speed at which the CPR unit is towed, its rather small aperture size as well as the inherent patchiness of this debris in the marine waters. Although validation of the plankton analysts on microplastic from the examined samples shows good agreement with the formal identification (FT-IR) results the closer examination of the original records had revealed high variability in the analysts' report of microplastics both in time and also between the individual analysts therefore requiring some data reduction before final analysis (see Section 2.3.3).

Nonetheless, with the exception of laboratory based contamination which clearly needs corrective measures in order to be reduced (see Section 6.4) based on both published and unpublished results so far the advantages of CPR survey seem to outweigh its limitations, in particular for broad scale and remote offshore locations where the operational cost would be much more affordable compared to surface nets.

6.4 Measures to reduce the procedural contamination

The problem of 'procedural contamination' in general is a well-recognised issue in other areas of research with standardised protocols in place to minimise and control its effect. Our understanding of microplastic debris is grown as reflected in the increasing number of studies, at the same time so is

our appreciation of the limitations and subtlety of some of the procedural and methodological challenges that must be considered. For researchers of microplastic debris the problem of microplastic contaminants from sources other than the sampling area is even more challenging due to the widely presence and usage of plastic products in all aspects of today's modern society.

We need to build a comprehensive and harmonised body of knowledge not only on the raw compositional and spatiotemporal but also methodological to help us devise standardised protocols to be followed by the microplastic research community such as the recommendations by the Marine Strategy Framework Directive Task Group 10 published by EU (Galgani *et al.* 2010). Some of these measures may be generic and applicable more widely and some may be more specific and depend on the underlying question and the adapted sampling methodology. Appendix A lists some of the generic and specific measures that are believed to reduce the likelihood of 'microplastic contamination' from external sources.

6.5 Future research recommendations

6.5.1 Spatiotemporal patterns of floating microplastic

Research presented in this thesis has confirmed the presence of microplastic debris in marine subsurface waters as reported in CPR plankton samples and also provided evidence of its higher abundance in coastal, compared to, oceanic waters. However to prepare for effective monitoring of floating plastic debris at sea more samples over larger spatial and temporal scales is needed in order to ensure patterns can be observed despite the large

heterogeneity in distribution of plastic litter at sea. This is a very challenging task both in terms of size and also the dynamicity of the sampling environment and would require the sampling programmes to be prioritised based on a goal-oriented approach that is tailored to answer specific questions regarding the floating plastic litter.

For instance if the main question is the sources of plastic litter then sampling of the inshore waters should be the priority for which based on practical field data Manta trawl would be the most suitable sampling method. However, if the question is focused on distribution and spatiotemporal patterns of the floating microplastic debris in wider oceans then CPR would be a more appropriate sampling method. Regardless of what sampling tools are used targets must be clearly defined and linked to specific legislations and mitigation measures. For example, if a new code of practice has been introduced to reduce the accidental loss of raw pellets during transport in an urban catchment, then a goal-oriented monitoring approach would allow for use of appropriate sampling methods with adequate spatial and temporal scales to assess the effectiveness of such measures.

6.5.2 Identification, processing and recording of microplastic debris in CPR

Since only fragments that differed in appearance from plankton were reported and identified, the amount of microplastic recorded in this research is likely to have underestimated the amount of microplastic in the natural environment. Further research is needed to investigate the applicability of novel identification methods such as portable FT-IR spectroscopy techniques (Sorak

et al., 2012; Šuštar *et al.*, 2014) so that a more complete and efficient assessment can be performed.

Although the CPR's archived samples and its relatively unchanged methodology offer a promising monitoring platform, further work is needed to establish standardised plastic detection and 'clean' sample processing protocols in order to minimise the variability in the analysts' reports of microplastic and also reduce the amount of 'procedural contamination' as highlighted in this thesis. Currently the SAHFOS analysts only report the presence and absence of microplastic debris during their routine examination of plankton in CPR samples. Further properties such as quantity, colour, size and form must also be recorded in order to allow for a more complete analysis of the amount and composition of the debris. Also ongoing training and practical workshops would ensure a common approach and interpretation of the microplastic composition in between the analysts.

6.5.3 Vertical distribution of floating microplastic debris

The persistence of floating plastic debris in water column under different environmental conditions is poorly understood. Knowing the degradation and persistence properties of different plastic types under different conditions would be useful for predicting its pathways and sinks and also for devising targeted monitoring approach using most suitable sampling methods that are most appropriate for the body of water and/or plastic type of interest. This information would also be helpful in assessing the likelihood of exposure and impact of a particular type of plastic on marine organisms at different trophic levels more accurately.

The data presented in Chapter 4 of this thesis provides some preliminary data on the amount and composition of microplastic debris as collected by manta trawl from surface waters and CPR from subsurface waters but only over a very short spatial and temporal scale. Further field and laboratory experiments using different types of buoyant plastics that is commonly found as litter across a range of marine habitats and over larger spatiotemporal scales is needed to better understand the effects of environmental variables such as UV radiation, temperature, salinity and wind on vertical distribution of floating plastic debris in water column.

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Appendices

Appendix 1:

Resin types and codes of the recyclable plastics

Recycling Code	Resin Type	Common uses
	Polyethylene terephthalate	Fizzy drink bottles and oven-ready meal trays
	High-density polyethylene	Bottles for milk and washing-up liquids
	Polyvinyl chloride	Food trays, cling film, bottles for squash, mineral water and shampoo.
	Low/ Linear low density polyethylene	Meat and poultry wrapping. Carrier bags and bin liners.
	Polypropylene	Margarine tubs, microwaveable meal trays.
	Polystyrene	Yoghurt pots, foam meat or fish trays, vending cups.
	Any other plastics that do not fall into any of the above categories	An example is melamine, which is often used in plastic plates and cups

Appendix 2:

Recommended measures to reduce contamination of field samples from external microplastic sources.

Generic Measures

- Use of non-synthetic clothing such as cotton laboratory coats and gloves during the sample processing
- Use of non-plastic laboratory equipment such as glass petri dishes, metal tweezers, etc. when possible.
- Thorough cleaning of the sampling device and equipment before and at the end of sampling day.
- Ensuring that the exposure of the dishes used for storage of the specimens is kept to the minimum necessary.
- Storage of the sampling equipment in a clean and preferably enclosed area in between the sampling days/sessions.

Specific Measures - CPR

Before deployment to sea:

- Ensure that the silk roles are stored in an enclosed storage area away from any sources of contamination after being delivered.
- Discard of the first section (~ 1m) of each role of silk before use.
- Make sure that the surface area where the silk role is to be opened up for marking is clean (use special surface cleaner which reduces the dust/particle attraction)

- Ensure that when the silk is too long to fit on the table the end of it doesn’t fall on the floor. Use a cardboard box at the end of the table for the silk to fall on.
- Ensure that the marked silk roles are stored in a clean enclosed area while waiting to be loaded onto CPR device.
- Ensure that CPR device is as clean as possible from synthetic particles before loading of the silk role (use high pressure air to blow away any particles).

After deployment from sea:

- Once the silk role is unloaded from the CPR unit make sure it is stored in an enclosed are.
- Ensure the surface use for cutting and silk preparation is clean
- Make sure the surface and area around the microscopes are free from dust and particles.
- Minimise the exposure of the silk samples to the open air by covering them with a glass lid when it is not being processed.

Appendix 3:

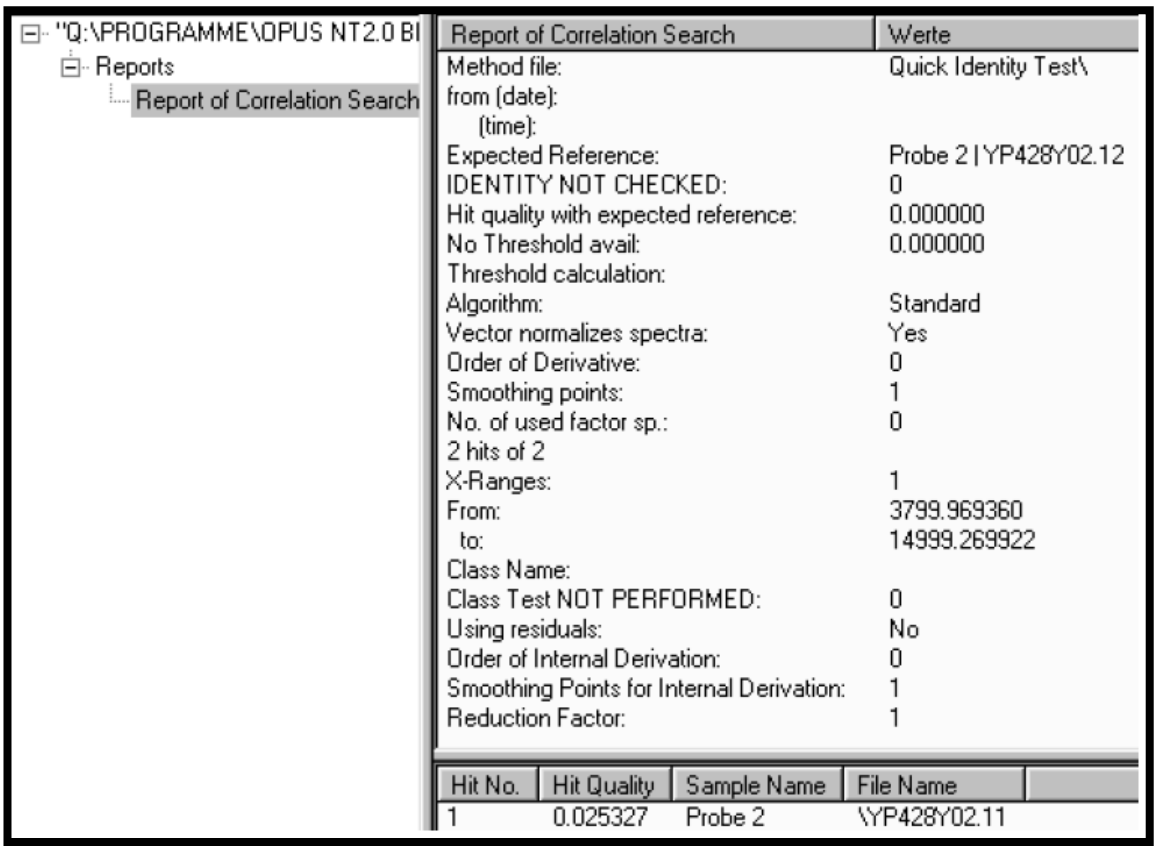
Normalisation of spectral data

Normalisation is used to compensate for the differences in sample quantity used for acquiring the database spectrum and the unknown spectrum. There are two commonly used methods to normalize spectral data:

1. The dot product normalization, which essentially normalizes the spectrum base on the total area under the curve
2. The scaling normalization, which normalizes the spectrum based on the height of the strongest peak.

The Euclidean search normalizes spectra by the dot product method. This is done by dividing each data point for both the reference and the unknown by the square root of the dot product of its spectrum.

The spectroscopic software, Opus by Bruker provides for a 'Quick Identity Search' test which allows to judge the similarity of two spectra. The test determines the Euclidean distance between the test and reference spectrum. Selecting the 'Vector Normalisation' as the data preprocessing method (as used in this study) will produce a report shown spectral distances compared to that of reference spectrum in a value range between 0 and 2 displayed as *Hit Quality* in the results section as seen here:



Quick Identity Test Report (source: Bruker/OPUS Reference Manual)

The Euclidean Hit Quality index value is calculated by summing the square of the difference between each data pair. Unlike other methods such as the scaling where normalisation is done using only maximum and minimum data points, the Euclidean algorithm uses all of the data points of the spectrum. However, as the Euclidean distance algorithm aims at areas under the curve it is possible for certain bands in spectrum with broad area under curve such as O—H to being weighted heavily whereas with small areas and sharp peaks such as C—N ignored or underestimated.

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On the quantity and composition of floating plastic debris entering and leaving the Tamar Estuary, Southwest England

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ABSTRACT

The majority of plastic debris found in the marine environment has land based sources and rivers are considered an important medium for transfer of this debris. Here we report on the quantity and composition of floating plastic debris collected from surface waters of the Tamar Estuary. This represents the first study of riverine transport of floating plastic debris into European waters during different tidal regimes. Plastics were found in a variety of forms and sizes and microplastics (<5 mm) comprised 82% of the debris. The most abundant types of plastic were Polyethylene (40%), Polystyrene (25%) and Polypropylene (19%). There was a significant difference in size frequency distribution between the spring and neap tides with more fragments of larger size observed during spring tides. While it is clear that debris has accumulated on shorelines within the estuary, during our study this river did not identify as a net source or sink.

1. Introduction

Plastic production has increased rapidly in recent years from 5 million tons in 1950s to 280 million tons in 2011 (Plastics Europe, 2011). With its unique properties and multifaceted applications plastic has become an indispensable part of modern life. Plastic are inexpensive, durable and versatile, however these attributes have also led to extensive use in disposable items that are used once and then discarded. This combined with inadequate recycling; waste management and littering behaviour have made the accumulation of plastic waste a global environmental problem (Thompson et al., 2009). The occurrence of plastic debris in the marine environment was first reported in the coastal regions in 1970s (Carpenter and Smith, 1972; Gregory, 1978; Morris and Hamilton, 1974). Since then it has been the focus of numerous studies and has been shown to be widely distributed (Barnes et al., 2009; Browne et al., 2011; Moore, 2008; Sul et al., 2011; Thompson et al., 2004) as well as ingested by a wide range of organisms. However, we know relatively little about the associated environmental impacts (Derraik, 2002; Laist, 1987; Lusher et al., 2012; Page et al., 2004; Thompson et al., 2009).

Plastic is the largest component accounting for 50–80% of debris that contaminates marine habitats at a global scale (Barnes et al., 2009). The majority of this litter has land-based sources such as: landfill sites, tourism, storm water outflows. Rivers are considered to be an important medium for transfer of different types of debris to the sea; however there are few published data on the riverine and estuarine plastic debris. A study of intertidal banks of River Taff, UK by Williams and Simmons (1996) showed plastic as the most abundant type of debris with sewer overflows and fly tipping as main sources. The analysis of the solid wastes in one isolated beach in Brazil also identified plastic with urban origins as being the most abundant type of debris and highlighted the importance of the riverine contribution (Araújo and Costa, 2007; Ivar and Costa, 2013). There is one published study on shorelines in the Tamar Estuary, UK showing that habitats that are downwind could act as potential sinks for debris (Browne et al., 2010), and thus indicating the potential importance of water movement and wind patterns within the estuary in the transport of debris. A recent study in the tidal Thames (Morritt et al., 2014) also reports on the movement of substantial quantities of debris close to the river bed. However because sampling was not stratified according to tidal cycles it is not possible to determine the direction of transport.

It is important to monitor the quantity and distribution of marine litter in order to fully assess its socio-economic and ecological impacts and to monitor the success of measures targeted at reducing litter. The European Marine Strategy Framework Directive 2008/56/EC (MSFD, 2011) recognises marine litter as one of the indicators for the Environmental State of the European Seas and calls for more data on the amount, distribution and, where possible, composition of this debris (MSFD, 2011). A key part of any strategy to reduce marine litter is to understand and then potentially regulate its sources.

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Current knowledge of the sources and fate of plastic debris, particularly for microplastics is limited and it is not clear whether this small debris is transported in the same manner as larger items. Floating debris could be pushed into estuaries during the rising tide from nearby coastal waters and/or travel out into the coastal waters during the falling tide. But estuarine water circulation is complex and influenced by many environmental parameters such as riverine inflow, tides, wind and evaporation. The nature of estuarine circulation affects the residence time of its water body and consequently the concentration of its solutes and suspended solids some with human-induced sources such as litter that may affect the health of estuaries (Balls, 1994). A recent study (Bakir et al., 2014) has suggested a correlation between the desorption rates of the Persistent Organic Pollutants (POPs) from the contaminated microplastics and their retention time in estuaries and marine waters and highlights the importance of both natural and anthropogenic processes such as flushing and dredging in pulse release of these substances.

This paper presents the first comparison of the quantity and composition of buoyant plastic debris in European estuarine waters according to daily and lunar tidal cycles. The specific aims were: (a) to describe the abundance and composition of floating litter in estuarine surface water; (b) to compare the abundance, size frequency and composition of debris between the different stages of the daily tidal cycles (Flood vs. Ebb) and different lunar phases (Spring and Neap).

2. Methods and materials

The River Tamar has a catchment area of ca. 1700 km^2 and flows south from North Cornwall for about 78 km (Evans et al., 1993). The estuary is tidal for about 31 km from Gunnislake Weir to its mouth at Plymouth Sound (Fig. 1) and is considered macrotidal with semi diurnal tidal flows ranging from 6.5 m at Devonport during the springs and 1.5 m at neaps in the upper reaches (Miller, 1999). Land use in the upper reaches of the catchment is mainly agricultural with relatively clean waters (Mighanetara et al., 2009). There are no major landfill sites along the estuary but a number of anthropogenic impacts such as run off form historic mining sites and discharges from sewage works are visible towards the mouth of the estuary around naval dockyard closer to the more populated areas of the city of Plymouth (Miller, 1999; Langston et al., 2003) As such the estuary could be considered typical of many other estuaries within Northern Europe.

Samples were collected from surface waters during May and July 2012 near to the mouth of the river during both spring and neap tides using a 0.50 m by 0.15 m manta net (300 μm mesh) aboard the Plymouth University's RV *Aquatay* (Fig. 2). Spring and neap tides were both sampled on three replicate dates and on each occasion with three replicate samples taken during both the flood and the ebb. The net was towed against the tidal flow at a speed of 4 knots for 30 min during the maximum flow period. Samples were transferred into glass jars and taken to the laboratory where their content was filtered through a set of sieves with varying mesh sizes (3 mm, 1 mm and 270 μm). Any unknown but potentially plastic pieces were transferred onto Petri dishes and categorised under 4 different size groups (>5 mm, 3–5 mm,1–3 mm and < 1 mm) and forms (Fragments, Sheet, Fibre and Pellet). Fourier transform infrared (FT-IR) spectrometry analysis was conducted on 50% of the pieces from each size groups in order to indicate the relative proportions of different polymers.

Fragments were identified using a Bruker IFS66 Fourier transform infrared (FT-IR) spectrometer with a MCT detector operating in the 4000–600 cm^{-1} wave number range and attached to a Bruker Hyperion 1000 microscope. A Specac DC2 Diamond compression cell (2 mm in diameters) was used to prepare the samples. Each sample was transferred from the petri dish onto the diamond cell and compressed between the two plates into a thin uniform thickness enough to allow for adequate transmission of IR beam through the sample to the detector and resulting in a better quality spectrum. For the measurement, processing and evaluation of the spectra Burker's Opus 6.5 spectroscopy software was used to best match spectra of the unknown debris following a protocol similar to that used by Thompson et al. (2004).

The non-parametric Kolmogorov–Smirnov (2 sample K–S test) was used to test the differences in size frequency distribution pattern between the tidal states. To test for any difference in the

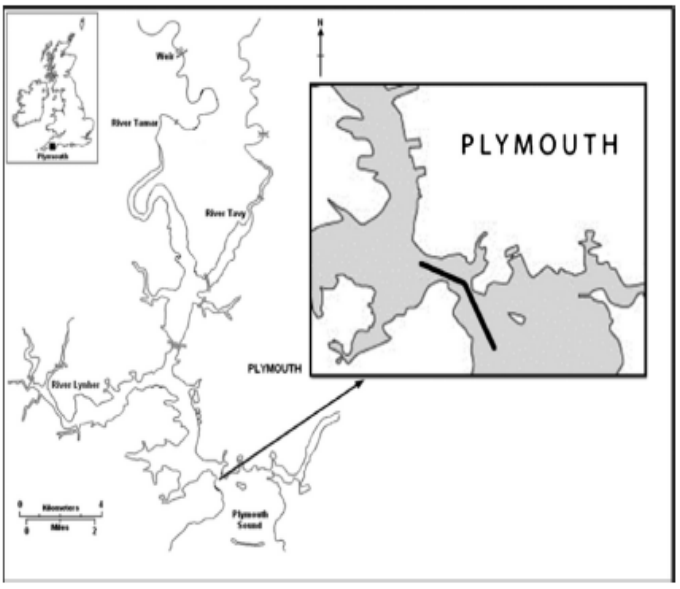

Fig. 1. Map of UK showing location of Plymouth (insert top left corner). Tamar catchment and the traverse used to collect debris (black line in insert top right).

Fig. 2. Manta net on board RV Aquatay (left) and deployed in the Tamar Estuary (right). The overall width of manta including its floating wings was ~150 cm.

abundance of debris across the tidal states (Neap vs. Spring and Flood vs. Ebb) the non-parametric Mann–Whitney *U* test was used.

3. Results

In total 204 pieces of suspected plastic were found and of these 84% were confirmed to be plastic in the following relative proportions: Polyethylene (40%), Polystyrene (25%) and Polypropylene (19%). Polyvinyl Chloride, Polyester and Nylon were amongst the other types of plastics present. Microplastic (<5 mm) comprised 82% of this debris and were found in different forms in all samples (Figs. 3 and 4). The 1–3 mm size category was the most abundant and contained all the polymer types. The proportion of Polyethylene was similar across all the size categories. PVC was present only in 1–3 mm and 3–5 mm and nylon only in <1 mm and 1–3 mm size groups (Figs. 5 and 6).

The frequency distribution of size classes across tidal states was significantly different between the spring and neap tides (Kolmogorov–Smirnov $Z = 1.832$, $p = 0.002$, $n = 36$) with a shift towards a smaller central tendency during the neap/ebb tide (Fig. 7). Mean abundance between tidal phases varied but there was no significant differences between the tidal phases or cycles (Mann Whitney $U = 283$, $n = 18$, $P = 0.118$; $U = 287$, $n = 18$, $P = 0.152$). The overall mean concentration of plastic was 0.028/m^3 (Fig. 8)

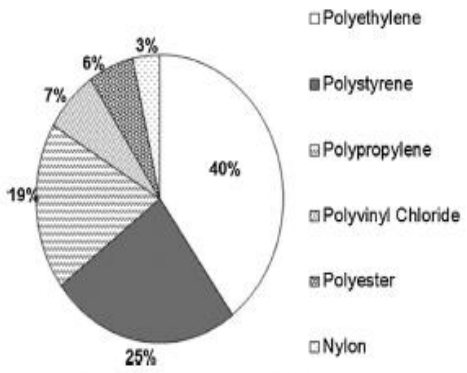

Fig. 3. The relative proportions of different polymers.

4. Discussions

4.1. Composition and quantity of plastic

As with other studies (Browne et al., 2010; Costa et al., 2010), microplastics (<5 mm) accounted for the majority of the debris in terms of numerical abundance, which generally is an indication of weathering (photo oxidation) and physical (abrasion by waves) defragmentation. However, in this study the 1–3 mm size class was the most abundant size class whereas in the study by Browne et al. (2010) it was the <1 mm class. The skew towards smaller debris seen in the study of Browne et al. (2010) is probably due to the

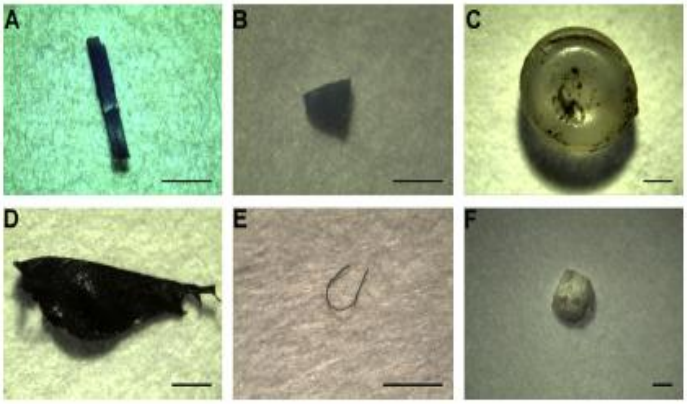

Fig. 4. Photographs of plastic debris identified using FT-IR as (A) Polypropylene, (B–D) Polyethylene, (E) Nylon, (F) Polystyrene. Scale bars represent 1 mm.

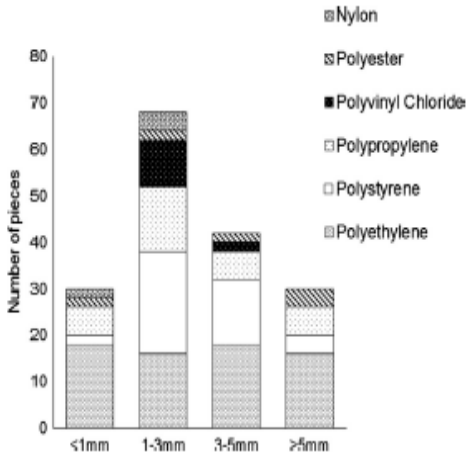

Fig. 5. Total number of plastic pieces from the whole sampling period by polymer type and size.

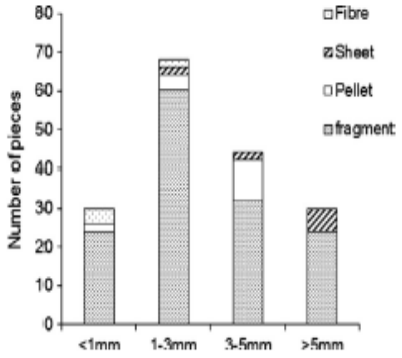

Fig. 6. Total number of plastic pieces from the whole sampling period by form and size.

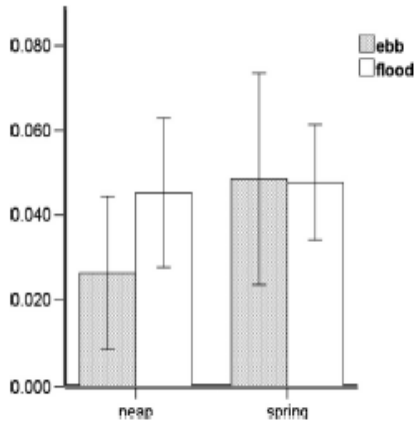

Fig. 8. Abundance ($\bar{x} \pm se$) of plastic m^3 of water by tidal cycles (Flood/Ebb) according to the tidal phases (Spring/Neap).

difference in sampling and extraction methodology where the use of glass filter papers would have given them a much lower cut-off value compared to the 300 μm mesh of manta used in this study.

To determine the origins of plastic debris in the marine environment, and in particular the origins of buoyant plastic is not easy but the types of polymer used for different applications provide a potential indication. In this study similar to those of the larger items found on adjacent shorelines by Browne et al. (2010) buoyant polymers: Polyethylene, polypropylene and polystyrene were the most abundant types of debris (Fig. 3). These polymers are predominantly used in the packaging industry which could indicate an urban origin of this debris. However, polymers such as polyester, nylon and PVC are denser than seawater and their presence in our samples indicates that the transport of debris is influenced by factors other than density alone. One likely explanation could be the introduction of some of these fibres via the sewage outlets onto shorelines (Browne et al., 2011) and/or their re-suspension in water column as a result of turbulent mixing induced by wind and tidal currents.

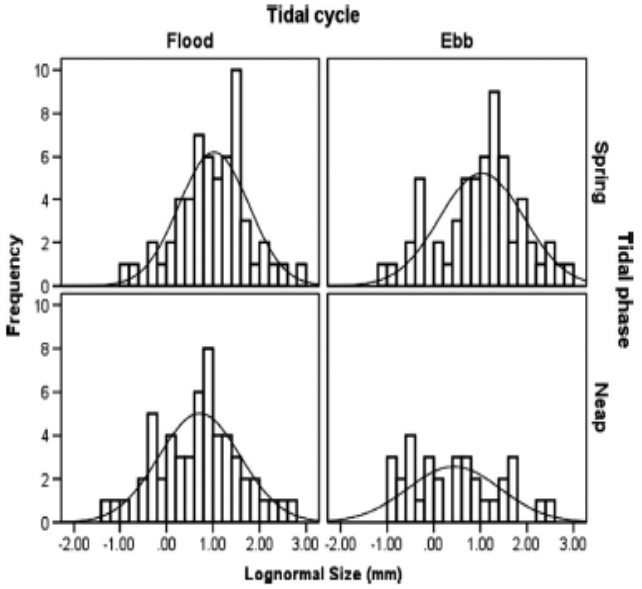

Fig. 7. Lognormal frequency distribution across different size categories and tidal cycles, showing a shift during neap/ebb tides.

Table 1
Wind directions and speed data. Samples with highest number of plastics are shown in bold.

Trawl id	Sampling day	Date	Tidal phase	tidal cycle	Wind speed/knots	Wind direction	Plastics count
MFOD2S2	2	14/05/2012	Neap	Ebb	7–10	**NW**	**20**
MFOD4S2	4	29/05/2012	Neap	Ebb	7–10	S	6
MFOD5S2	5	13/07/2012	Neap	Ebb	7–10	W	6
MFID4S2	4	29/05/2012	Neap	Flood	7–10	**NW**	**29**
MFID2S2	2	14/05/2012	Neap	Flood	11–15	W	18
MFID5S2	5	13/07/2012	Neap	Flood	11–15	SW	8
MFOD1S2	1	08/05/2012	Spring	Ebb	4–6	**W**	**31**
MFOD3S2	3	24/05/2012	Spring	Ebb	4–6	S	7
MFOD6S2	6	20/07/2012	Spring	Ebb	4–6	NW	21
MFID1S2	1	08/05/2012	Spring	Flood	4–6	**W**	**21**
MFID3S2	3	24/05/2012	Spring	Flood	7–10	S	19
MFID6S2	6	20/07/2012	Spring	Flood	4–6	SW	18

4.2. Influence of tides on the abundance and size distribution of floating plastic debris

In this study we found a shift towards the smaller debris and although not significant also some indication of lower abundance during the neap/ebb tide. It is possible for some particles that leave the estuary on the ebb tide to re-enter the estuary during the flood tide and vice versa. This is especially likely near the mouth of estuaries where complex circulation patterns can occur (Wolanski, 2007). Perhaps the similar overall abundance in the spring tide samples (Fig. 8) in this study could be partially attributed to this phenomenon. The shift towards the smaller pieces and lower abundance of debris during neap tide could be due to the elevated clogging of the net if phytoplankton concentrations were greater during neap tides compared to spring tide as observed by Cloern (1991). The cumulative effect of clogging could result in a bias towards the smaller particles and also decrease in the overall sampling rate, thereby artificially reducing the overall amount of microplastics that are eventually captured. However, visual inspection of the recovered nets did not reveal substantial clogging and our short sampling durations were chosen to help minimise clogging. Clearly in future sampling the duration of tow needs to be considered in relation to potential clogging so as to maximise the capture of debris.

The wind direction and force could also have an important role in the spatial distribution of floating debris as indicated by Browne et al. (2010) where shores downwind accumulated a greater abundance of plastic debris. There are some indications in our data of higher abundance of debris in samples collected during North-westerly wind regime than the predominant South-westerly (Table 1); however more work would be needed to confirm this.

The stratification and mixing processes between the saline and fresh water are important factors affecting both horizontal and vertical positions of buoyant items such as plastic debris. Wind direction and force have been shown to play an important role in distribution and positioning of floating objects (Browne et al., 2010; Kukulka et al., 2012; Thiel et al., 2011) future studies should therefore examine more extensively the temporal effects of different wind regimes. Tidal currents are typically strongest between high and low tides and could considerably influence the residence time and transport of floating objects within an estuary.

When designing sampling or monitoring programmes, factors such as timing, location and length of trawls in relation to the strength of tides should be carefully considered in order to ensure that the most representative body of water is being sampled. If effects of a particular land-based feature (e.g. landfill, sewage output, etc.) are of interest then the spatial coverage of the trawls relative to these features becomes a priority compared to trawl times or distance. However, if like our study the effect of tides is the main interest then the timing of trawls must be standardised in preference to spatial coverage. For instance, in our study (trawl time: 30 min; Boat speed: 4 knots) all samples were taken during periods of maximum-flow-in and maximum-flow-out (approximately 2.5 h after the high and low tides). This allowed for relative consistency with regards to the tidal flow but resulted in less linear distance towed along the estuary during the spring tides. Ultimately data should be standardised for the volume of water through the net using either a flowmeter or calculations based on the distance travelled by the boat at a constant speed. In order to gain comprehensive estimates of the volume of litter that is transported via estuaries it will be necessary to use a combined approach to ensure various types and sizes of debris are captured and that different parts of the water body are sampled including mid-water and the river bed. For example substantial quantities of litter have recently been reported in a study using traps fixed near to the seabed in the tidal Thames (Morritt et al., 2014).

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