Training Module for Marine Microplastics Monitoring

1 Introduction

1.1 Plastic litter as a global ocean concern

The light weight, high durability, resistance to chemicals, plasticity, and high buoyancy of foamed and resin products and the cost-effectiveness of plastics (Thompson et al., 2009) make them so-called "essential" materials in our daily life. A world without plastics, or synthetic organic polymers, seems unimaginable today, yet their large-scale production and use only dates back to ~1950. Plastics have outgrown most man-made materials. World plastic production of 1.7×10^6 ton in 1950 increased 189 times to 3.2×10^8 ton in 2015 (Plastics Europe, 2013, 2016). Moreover, the global production of polyethylene (PE) and polypropylene (PP) (the most common marine microplastics) grew at a rate of 8.7% per year from 1950 to 2012 (Andrady, 2017). By identifying and synthesizing dispersed data on production, use, and end-of-life management of polymer resins, synthetic fibers, and additives, researchers present the first global analysis of all mass-produced plastics ever manufactured. It is estimated that 8300 million metric tons (Mt) as of virgin plastics have been produced to date. As of 2015, approximately 6300 Mt of plastic waste had been generated, around 9% of which had been recycled, 12% was incinerated, and 79% was accumulated in landfills or the natural environment. If current production and waste management trends continue, roughly 12,000 Mt of plastic waste will be in landfills or in the natural environment by 2050(Geyer et al., 2017).

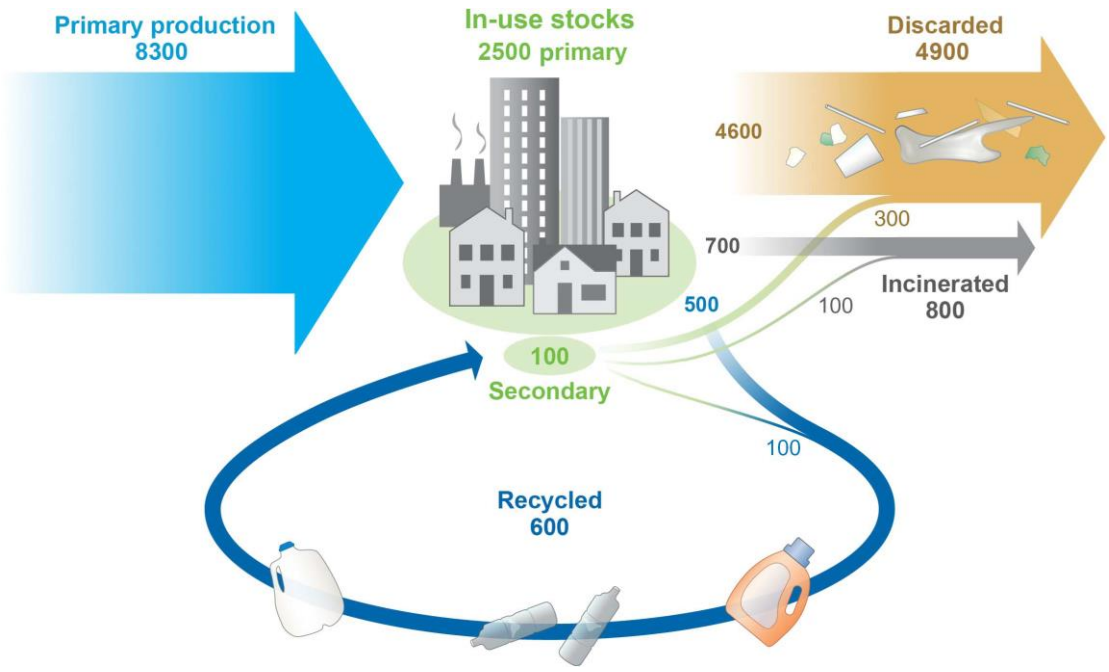

Figure 1.1 Global production, use, and fate of polymer resins, synthetic fibers, and additives (1950 to 2015; in million metric tons). (Geyer et al., 2017)

The vast majority of monomers used to make plastics, such as ethylene and propylene, are derived from fossil hydrocarbons. None of the commonly used plastics are biodegradable. As a result, they accumulate, rather than decompose, in landfills or the natural environment (Barnes et al., 2009). The only way to permanently eliminate plastic waste is by destructive thermal treatment, such as combustion or pyrolysis. Thus, near-permanent contamination of the natural environment with plastic waste is a growing concern. This is as a result of both land-based and sea-based human activities. Plastic debris has been found in all major ocean basins (Barnes et al., 2009). Contamination of freshwater systems and terrestrial habitats is also increasingly reported (Wagner et al., 2014; Rillig, 2012; Zubris & Richards, 2005), as is environmental contamination with synthetic fibers (Zubris & Richards, 2005, Dris et al., 2016). Plastic waste is now so ubiquitous in the environment that it has been suggested as a geological indicator of the proposed Anthropocene era (Zalasiewicz et al., 2016).

Since the 1950s, when large-scale production of plastics began, an increasing proportion of solid waste in the ocean has consisted of this material. It has been estimated that $4.8–12.7\times10^6$ ton of plastic waste entered the oceans from land-based sources in 192 coastal countries in 2010 (Jambeck et al., 2015), accounting for 1.8%–4.7% of the global plastic production in 2010. Plastic litter is most obvious on shorelines, where litter accumulates as a result of current, wave and wind action, river outflows and by direct littering at the coast. However, plastic litter occurs on the ocean surface, on the seabed and in association with biota, due to entanglement or ingestion (Figure 1.2).

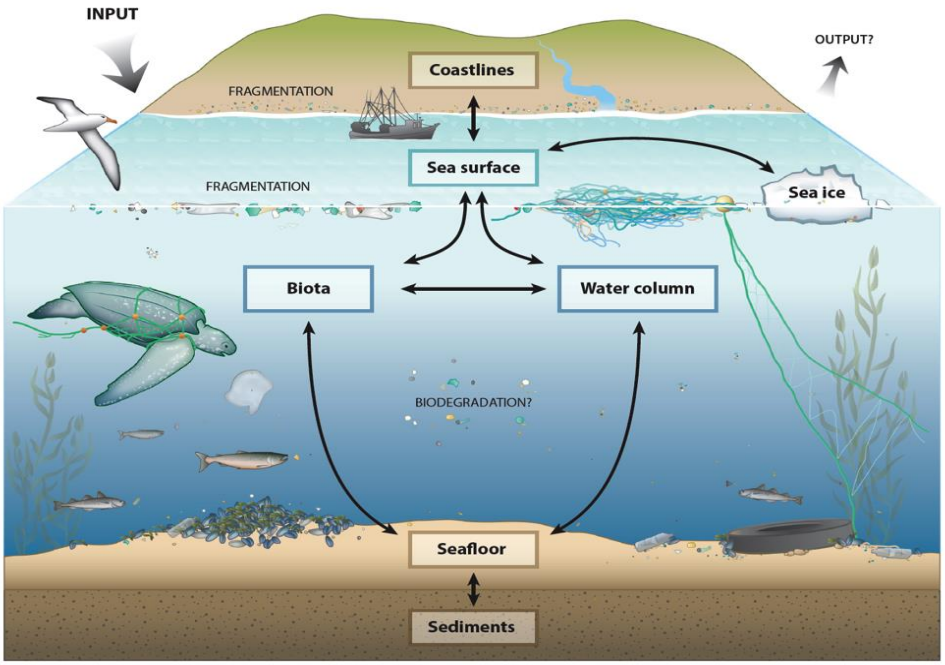

Figure 1.2 Microplastics do not reside permanently on the open water surface, but rather are subjected to a dynamic system of inputs and outputs between compartments. (Law et al., 2017)

Microplastics have been reported and found globally in multiple seas, including the Arctic Ocean and Antarctic Ocean. Once microplastics enter the sea, they persist and accumulate in water bodies and are transported around the world via winds and surface currents (Lusher et al., 2015). According to Co´zar et al. (2014), it has been estimated that approximately 7000–35,000 t of plastic, including microplastics, are floating and persistent in the open ocean. A similar study conducted by Eriksen et al. (2014) indicated that over 250,000 t and more than 5 trillion pieces of plastic have accumulated in the ocean, including in the Atlantic, North Pacific, South Pacific, and Indian Ocean gyres, and the amount of plastic debris continuously increases. The occurrence of microplastics in the oceans differs, with a high abundance of microplastics found near the regions with high levels of industrial activities or high population densities and in remote areas far from human habitation. The image of a plastic bag found at 10,898m in the famous Mariana Trench is a haunting one, suggesting that we have long passed the point where full or even partial recovery of extant plastic debris is feasible (despite some noble attempts in The Netherlands, the Adriatic Sea, and elsewhere) (Joanna & Peter, 2018).

The contamination of microplastics in the marine environment is regarded as a major risk for the health of marine organisms. Numerous studies have shown that many species suffer from plastic ingestion or entanglement (Gregory, 2009; Lusher, 2015; Auta et al., 2017). Marine organisms, including fish (Lusher et al., 2016), seabirds (Amelineau et al., 2016), sea turtles (Tourinho et al., 2010), invertebrates (Davidson and Dudas, 2016), and marine mammals (Besseling et al., 2015), are directly and indirectly vulnerable to microplastic ingestion. More importantly, microplastics can adsorb hydrophobic contaminants or heavy metals from the surrounding seawater and potentially act as a vector for these contaminants to enter the food web (Reisser et al., 2014). Therefore, it is essential to understand the distribution and the potential hot spots of microplastics.

Marine litter, including microplastics, is now a global challenge. In particular, pollution of the marine environment by microplastics has been recognized as a serious international issue over the past decade as microplastics are likely to affect marine ecosystems and are extremely difficult to recover. To promote policy planning based on a more concrete scientific knowledge while making a head start with preventive measures for plastic litter in the ocean, determining the current status of distribution and quantity of microplastics in the ocean is an urgent task.

1.2 Purpose and Objectives

In response to the growing interest worldwide in microplastics in the ocean, monitoring of microplastics (sampling and indoor analysis) has been carried out by many institutions around the world using various methods, and accordingly findings are gradually accumulating. Since 2004, when Thompson (Thompson et al., 2009) and colleagues pointed up marine microplastics as a new problem of high concern for our global ecosystems, over 2200 researchers published approximately 700 scientific articles on this topic. In this context, microplastics identification within a broad variety

of environmental compartments e.g. aquatic systems, sediments or organisms is an important aspect (Käppler et al. 2015). However, researchers criticise the lack of standardised analysis techniques and protocols which lead to insufficient result comparability, or even worse, uncertain conclusions (Gerrit et al., 2018).

It is expected that more monitoring will be conducted in the future, but as different sampling and analytical methods are used depending on the purpose of the survey of each country and research institution, there is now a global concern about lack of comparability of the accumulated data. There is also speculation that research conducted under limited resource availability, technical capacity and institutional arrangements, or monitoring by the latest equipment that are not yet globally common will be carried out. Inability to compare data obtained by different monitoring methods may pose an obstacle to research determining the global distribution and fate of microplastics in the ocean. Hence, it is recognized that standardization and harmonization of monitoring methods for marine litter, including microplastics, are important undertakings.

The principle purpose of this Module is to provide advice and practical guidance, for establishing programmes to monitor and assess the distribution and abundance of mircoplastics in the ocean. It is a product of the YSLME II Regional Working Group on Pollution. The main audience is intended to be national, inter-governmental and international organisations, NGO with responsibilities for managing the social, economic and ecological consequences of land- and sea-based human-activities on the marine environment. The decision to produce the Module reflects the lack of an internationally-agreed methodology to report on a key aspect of ocean (and freshwater) microplastics contamination, that is attracting increasing concern. Although the focus is on the marine environment, it is recognised that many of the methods of sampling and material characterisation will apply equally to freshwater systems.

The intention is to promote a more harmonised approach to the design of sampling programmes, the selection of appropriate indicators (i.e. type of sample), the collection of samples or observations, the characterisation of sampled material, dealing with uncertainties, data analysis and reporting the results. The Module coves <5mm of plastic litter encountered in the marine environment, on shorelines, floating on the sea surface, suspended in the water column, deposited on the seabed.

1.3 How to Use the Module - Structure

The Module document is divided in 3 sections:

- ✓ Chapter 1 – Introduction, addressing background, purpose, objectives and structure of the Module
- ✓ Chapter 2 – Definitions and Terminology: providing definitions of common terminology used in existing marine microplastics monitoring
- ✓ Chapter 3 –introduce the protocol of microplastic collection in seatersm and sample preparation procedure

- ✓ Chapter 4 –providing microplastic collection methods in sediments, beaches and subtidal sediments
- ✓ Chapter 5 –present a range of laboratory-based techniques of microplastics detection in biota
- ✓ Chapter 6 –introduce microplastics identification technologies
- ✓ Chapter 7 –present the necessary quality control and quality assurance

2. Definition and types of marine litter and microplastics

2.1 Definition of marine litter and microplastics

In 1995, UN Environment defined marine litter as any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment (UNEP, 1995). Marine litter consists of items that have been made or used by people and deliberately discarded into the sea or rivers or on beaches; brought indirectly to the sea with rivers, sewage, storm water or winds; accidentally lost, including material lost at sea in bad weather (fishing gear, cargo); or deliberately left by people on beaches and shores. Marine debris comprises of various material types, and can be classified into several distinct categories, including Plastics, metal, Glass, processed timber, paper and cardboard, rubber, clothing and textiles.

Once plastic enters the marine environment, it is difficult to degrade completely due to its biodegradation-resistant properties. Therefore, large plastic debris degrades into smaller fragments via different mechanisms such as weathering, photodegradation, and biodegradation and thus becomes small plastics fragments (Andrady, 2011). Definitions of Microplastics When reported in 2004 the term microplastics was used to describe fragments of plastic around 20 μm in diameter. However, while these early reports referred to truly microscopic particles they did not give a specific definition of microplastic. In 2008, the National Oceanographic and Atmospheric Agency (NOAA) of the US hosted the first International Microplastics Workshop in Washington and as part of this meeting formulated a broader working definition to include all particles less than 5 mm in diameter (Arthur et al. 2009). Particles of this size (i.e. <5 mm) have been very widely reported including publications that considerably pre-dated the use of the term “microplastics”.

However, some scientists have argued that microplastics should be defined as being < 1,000 μm (<1mm). The global assessment reports published by GESAMP (GESAMP 2015, 2016) mentioned the lack of an internationally-agreed size definition of microplastic particles. GESAMP recommends < 5mm diameter as the ‘common definition’ of the upper size boundary for microplastic particles for monitoring purposes.

Microplastics can be subdivided by usage and source as primary or secondary (Wright et al., 2013). Primary microplastics are commonly defined as manufactured raw plastic material (Browne et al. 2007, Arthur et al. 2009), such as microbeads in personal care products that enter waterways through wastewater, or loss of virgin

plastic pellets during production and transport; secondary microplastics result from the fragmentation of larger plastic materials.

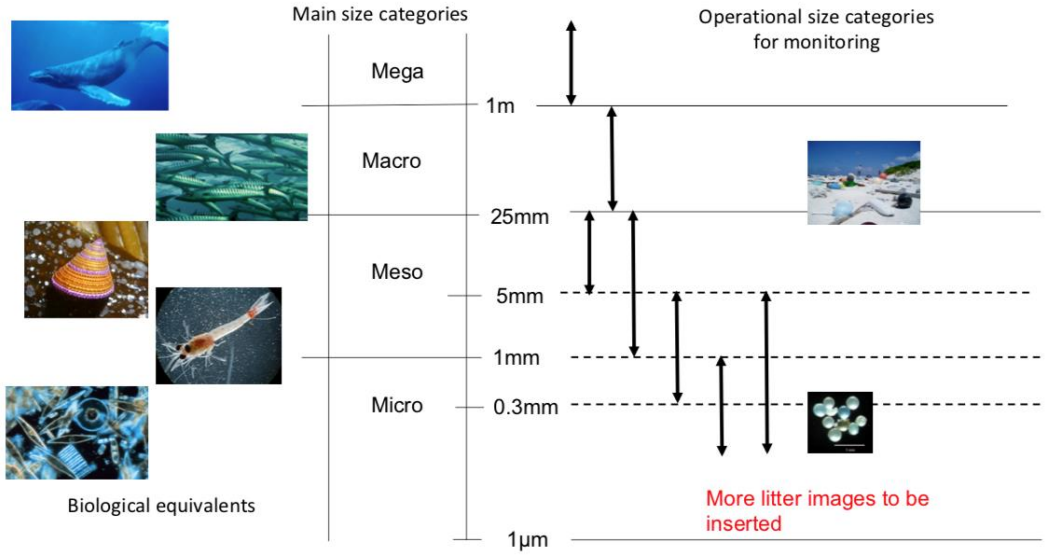

Figure 2.1 Schematic showing field descriptors, typical aquatic organisms in that size category, examples of marine litter and common size divisions（GESAMP, 2019）

2.2 Types and size of plastics

There are many hundreds of different types of polymer and mixtures of polymer in commercial production, but the market is dominated by: polyethylene (as both high-density, HDPE, and low-density, LDPE), polypropylene (PP), polyvinyl chloride (PVC), polyurethane (PUR), polystyrene (PS), and polyethylene terephthalate (PET). These six polymers make up about 80% of plastics production and are likely to form a large proportion of most marine litter (GESAMP, 2019). Table 2.1 provides examples of common products and their associated polymer resin, as well as their density as a virgin material and percentage of the global market.

Table 2.1 Common polymers and abbreviation codes

Polymer	聚合物	Abbreviation
Acrylonitrile-butadiene-styrene	丙烯腈 - 丁二烯 - 苯乙烯	ABS
Acrylate-styrene-acrylonitrile	丙烯酸酯 - 苯乙烯 - 丙烯腈	ASA
Butadiene rubber	丁二烯橡胶	BR
Cellulose acetate	醋酸纤维素	CA
Cellulose acetate-butyrate	醋酸纤维素 - 丁酸酯	CAB
Cellulose acetate propionate	醋酸纤维素丙酸酯	CAP
Cellulose	纤维素	CE

Polymer	聚合物	Abbreviation
Carboxymethyl cellulose	羧甲基纤维素	CMC
Cellulose nitrate	硝酸纤维素	CN
Cellulose propionate	丙酸纤维素	CP
Polychloroprene (neoprene)	聚氯丁二烯（氯丁橡胶）	CR
Chlorosulfonated polyethylene	氯磺化聚乙烯	CSM
Ethylene chlorotrifluoroethylene	乙烯三氟氯乙烯	ECTFE
Ethylene-propylene rubber	乙丙橡胶	EPR
Expanded polystyrene	膨胀聚苯乙烯	EPS
Ethylene vinyl acetate	乙烯醋酸乙烯酯	EVA
Ethylene vinyl alcohol	乙烯乙烯醇	EVOH
Fluorinated ethylene propylene	氟化乙烯丙烯	FEP
High-density polyethylene	高密度聚乙烯	HDPE
Hydroxyethyl methacrylate	甲基丙烯酸羟乙酯	HEMA
High-impact polystyrene	高抗冲聚苯乙烯	HIPS
Low-density polyethylene	低密度聚乙烯	LDPE
Linear low-density polyethylene	线性低密度聚乙烯	LLDPE
Methacrylate butadiene styrene	甲基丙烯酸酯丁二烯苯乙烯	MBS
Medium-density polyethylene	中密度聚乙烯	MDPE
Melamine formaldehyde	三聚氰胺甲醛	MF
Acrylonitrile butadiene rubber	丙烯腈丁二烯橡胶	NBR
Natural rubber	天然橡胶	NR
Polyamide (nylon)	聚酰胺（尼龙）	PA
Nylon 4,6	尼龙 4,6	PA 46
Nylon 6	尼龙 6	PA 6
Nylon 6,10	尼龙 6,10	PA 610
Nylon 6,6	尼龙 6,6	PA 66
Nylon 6,6/6,10 copolymer	尼龙 6,6 / 6,10 共聚物	PA 66/610
Nylon 11	尼龙 11	PA 11
Nylon 12	尼龙 12	PA 12
Polyarylamide	聚芳香酰胺	PAA
Polyamide imide	聚酰胺酰亚胺	PAI
Polyacrylonitrile	聚丙烯腈	PAN
Polybutylene	聚丁烯	PB
Polybutylene terephthalate	聚对苯二甲酸丁二醇酯	PBT
Polycarbonate	聚碳酸酯	PC
Polycaprolatone	Polycaprolatone	PCL
Polyethylene	聚乙烯	PE
Polyether block amide	聚醚嵌段酰胺	PEBA
Polyetheretherketone	聚醚醚酮	PEEK
Polyester elastomer	聚酯弹性体	PEEL

Polymer	聚合物	Abbreviation
Polyester imide	聚酯酰亚胺	PEI
Polyetherketone	聚醚	PEK
Polyether sulfone	聚醚砜	PES
Polyethylene terephthalate	聚对苯二甲酸	PET
Polyethylene terephthalate glycol-	聚对苯二甲酸乙二醇酯改性	PETG
Phenol formaldehyde	苯酚甲醛	PF
Perfluoroalkoxy alkane	全氟烷氧基烷烃	PFA
Polyhydroxybutyrate	聚羟基丁酸酯	PHB
Poly(3-hydroxybutyrate-co-3-	聚（3-羟基丁酸酯 - 共-3-羟基戊	PHBV
Polyhydroxyvalerate	聚羟基戊酸	PHV
Polyimide	聚酰亚胺	PI
Polyisocyanurate	聚异氰脲	PIR
Polylactic acid	聚乳酸	PLA
Poly(methyl methacrylate)	聚（甲基丙烯酸甲酯）	PMA
Polymethylpentene	聚甲基戊烯	PMP
Polyoxymethylene	聚甲醛	POM
Polypropylene	聚丙烯	PP
Poly(p-phenylene ether)	聚（对亚苯基醚）	PPE
Poly(p-phenylene oxide)	聚（对苯醚）	PPO
Polyphenylene sulphide	聚苯硫醚	PPS
Polyphenylene sulphide sulfone	聚亚苯基硫醚砜	PPSS
Polyphenylenesulfone	聚亚苯基砜	PPSU
Polypropylene terephthalate	聚对苯二甲酸丙二醇酯	PPT
Polystyrene	聚苯乙烯	PS
Polysulfone	聚砜	PSU
Polytetrafluoroethylene	聚四氟乙烯	PTFE
Polytrimethylene terephthalate	聚对苯二甲酸丙二醇酯	PTT
Polyurethane	聚氨酯	PUR
Polyvinyl acetate	聚乙酸乙烯酯	PVA
Polyvinyl butytral	聚乙烯醇缩丁醛	PVB
Polyvinyl chloride	聚氯乙烯	PVC
Chlorinated polyvinyl chloride	氯化聚氯乙烯	PVCC
Polyvinylidene chloride	聚偏二氯乙烯	PVDC

2.3 Shape of microplastics

Microplastics samples are usually sorted into different shapes according to observed morphology. GESAMP recommends five general categories of recommends, including fragment, foam, film, line and pellet (GESAMP, 2019). It is recommended the original data in these finer subdivisions with the recognition that subdivisions can be combined

for ease of harmonizing and comparing data. For example, separate the “Line” category into “Filaments” (from fishing) and “Fibres” (from textiles). In This report standard of GESAMP is recommended to be cited.

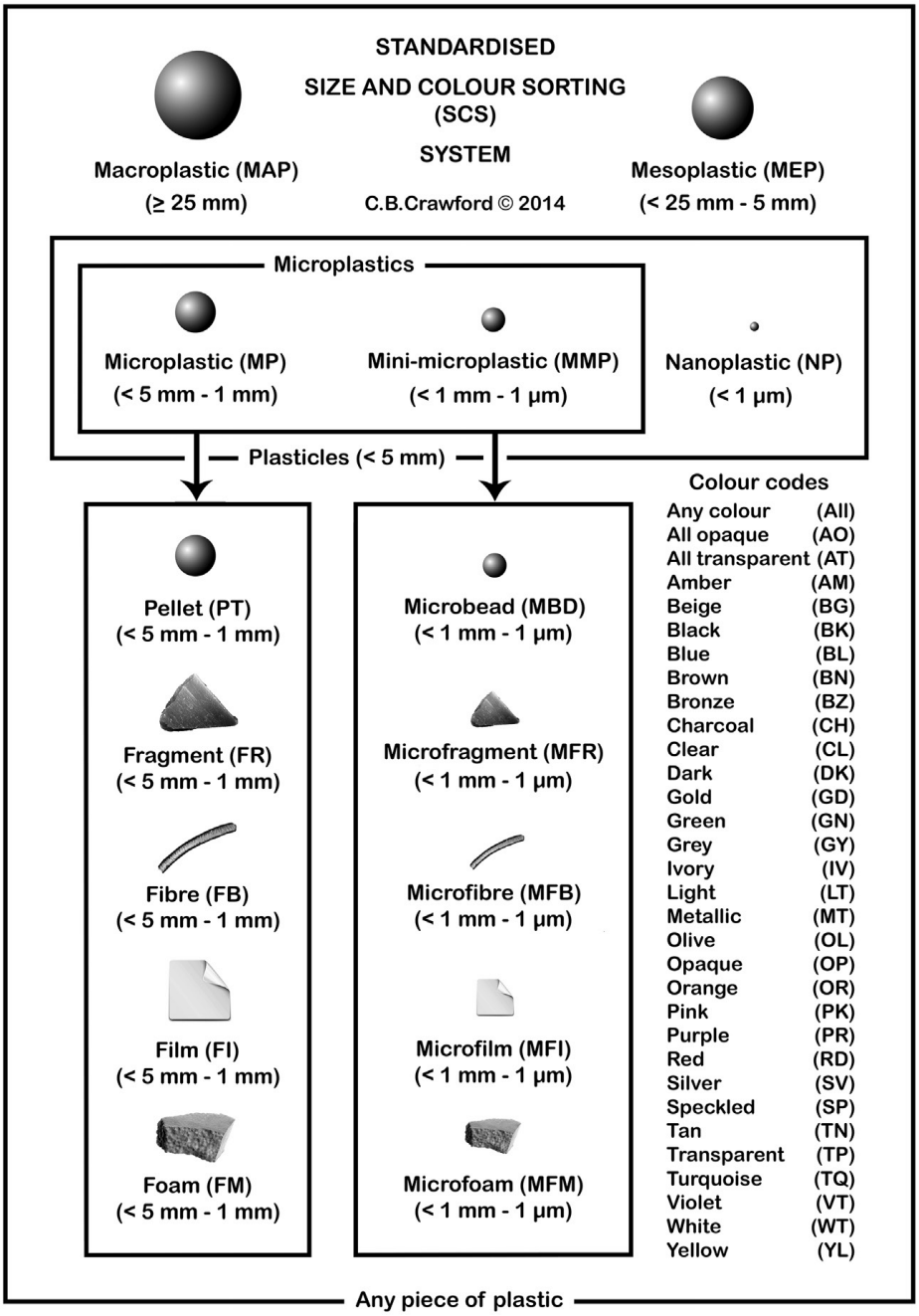

Figure 2.2 The standardised size and colour sorting (SCS) system.

3. Sampling and Analysis of Microplastics in seawaters

3.1 Microplastic-collection

3.1.1 Sampling of microplastics on the sea surface

The most common method for sampling microplastics in surface waters is to use

established methods for plankton sampling. This approach allows for the sampling of large volumes and surface areas of water relatively quickly in a volume-reduced method resulting in a relatively small, concentrated final sample. The mesh size used for sampling in previous studies ranged between 0.05 mm and 3 mm (Hidalgo-Ruz et al., 2012); a mesh size of approximately 330 μm is used most commonly and there is a tendency of becoming a standardized method of collection (Lusher et al., 2015).

There is no defined definition of what constitutes the surface water layer in microplastic sampling. Nevertheless, it has been described as the water surface layer less than 15 cm deep, which is where 95% of small plastic debris is concentrated (Carson HS et al.,2011). However, in most studies the depth of the surface layer is not specified and where it has been.

There are numerous standard operating procedures (SOPs) available from different monitoring agencies, such as the National Oceanic and Atmospheric Administration (Lippiatt et al., 2013, Masura et al., 2015). This module shows a general sampling procedure for water surface microplastics.

- A catamaran, manta or nueston trawl may be used to sample microplastics, with a body of 330 μm mesh size and of approximately 3-4 m in length, attach the flowmeter midway between the centre and the net rim.
- Clean the net and check for any contamination before use. Steps to avoid microplastic contamination should be undertaken, such as avoiding wearing synthetic garments, especially man made fabrics.
- Record the net aperture, the length and the mesh size. Check if the cod end of the net is closed. Record the date, time, location, weather conditions, vessel course and speed. Write down the initial GPS coordinates and initial time in the data sheet.
- Lower the net into the water to the correct depth. Wherever possible, a boom should be used to collect samples from the side of the boat (approx. 3 - 4 m distance from the boat) in order to prevent collecting water affected by turbulence inside the wake zone. the angle between the net and the ship's route is about 20°,
- Tow the net at a constant speed (2-3 knots) horizontally at the surface for a set period of time, such as 20 minutes per sample.
- Care should be taken to ensure that the net has not become clogged. If this has occurred, reduce the trawl time/speed.
- Once the tow is complete, retrieve the net and allow the water to drain away. Wash the debris collected by the net to the cod end of the net using a hose.

- Transfer the collected sample to an appropriate container for storage and preserve it where necessary, such as with 4% formaldehyde,135 70% ethanol or freezing.
- The net is then cleaned and checked for any contamination, after which it is ready for reuse.
- Using the data from the flow meters, the measurement of the net aperture, and the abundance of microplastics in the sample, calculate the results.
- The results can then be reported as the number of microplastics per m3 of water.

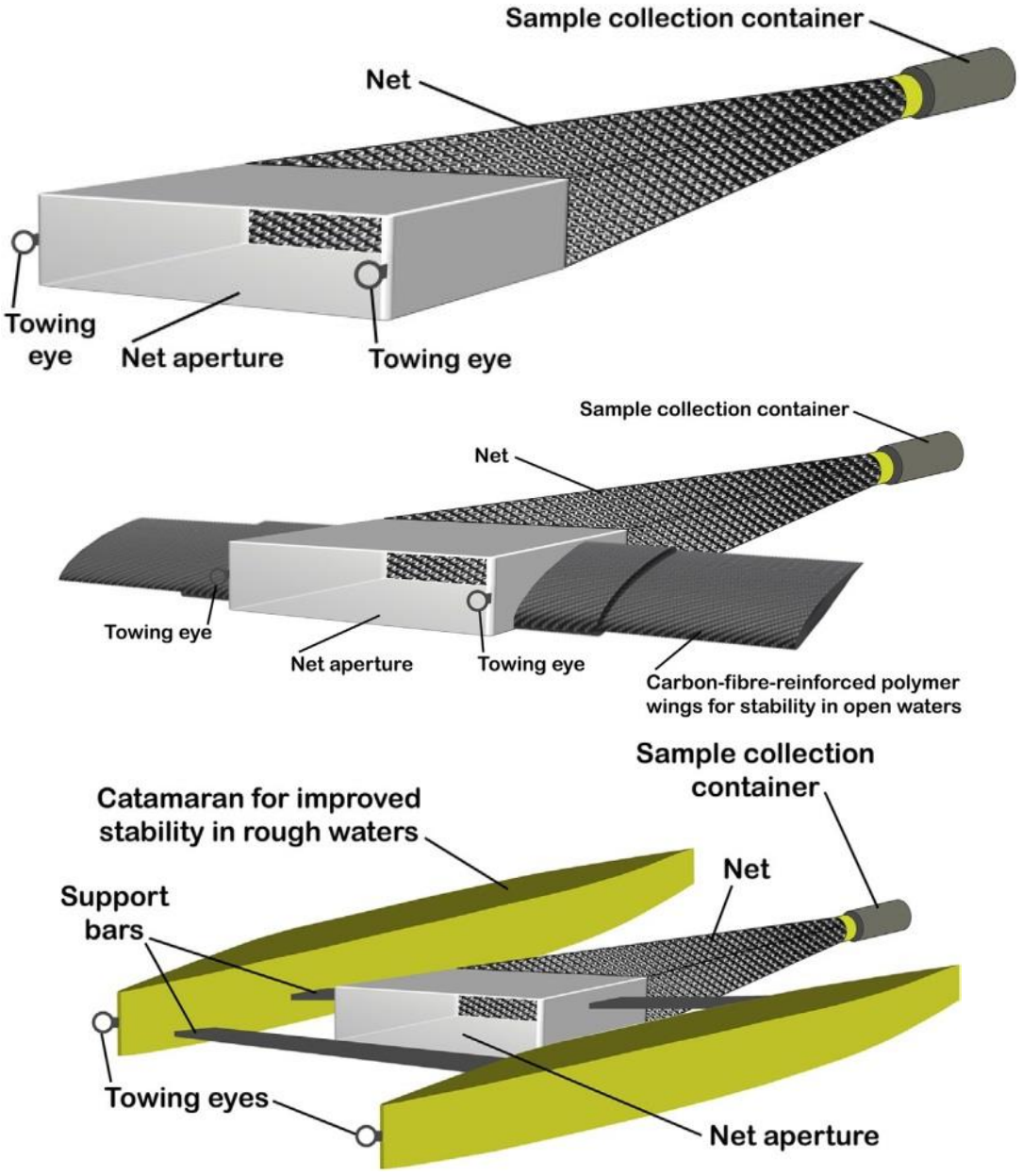

Figure3.1 (A) Neuston catamaran; (B) Manta trawl ; (C) Neuston trawl

When collecting samples in the environment it is important to take into consideration and to record the prevailing weather conditions, not only on the day of sampling but in the period leading up to sampling. On the day of sampling, it is necessary to note the wind direction as this may influence any potential contamination from the person carrying out the sampling, as well as from others nearby. Poor weather and sea conditions often cause the trawl to be difficult to balance on the surface water.

It is recommended that researchers collect a sample of vessel paint if possible to compare to anthropogenic debris that may not look like plastic, or sinks in sea water. In addition, it is often to get a small sample of the materials used for sampling, such as the net and rope, as these are often composed of plastic materials and can be used to exclude sampling induced contamination. Also, blanks should be collected, whereby a sample is collected from the equipment without it actually having touched the water.

3.1.2 Water column

Besides common net sampling, other techniques are occasionally used for assessing microplastic concentrations in the water column: bulk sampling with subsequent filtration (Ng and Obbard 2006; Dubaish and Liebezeit 2013), screening Continuous Plankton Recorder (CPR) samples (Thompson et al. 2004) or using direct in situ filtration (Norén and Naustvoll 2010). There are some advantages for pump filtration compared to plankton nets such as: flexible to use also smaller filter filters, exactly measured sample volume, applicable stationary for point measures. Limitations are however: Often too little sample volume for 300µm in practice to reach statistically reliable particle counts, do not sample sea surface layer so effectively floating particles will be missed (Karlsson et al., 2018).

3.2 Sample preparation

Since environmental samples usually contain organic matter, algae, wood products and other interfering impurities, it is generally impossible to directly analyze the microplastic samples. It is necessary to separate and extract the samples, e.g., density separation and biochemical separation (digestion). The pretreatment methods used for microplastics in different environmental media are different. It is recommended that the U.S. NOAA steps in the “Laboratory Methods for the Analysis of Microplastics” and EU pre-treat steps in the “Guidance on monitoring of marine litter in European seas” developed (European Commission, 2013).

In the laboratory, samples were poured through stacked 5.0-mm and 0.3-mm stainless steel mesh sieves. The plastics that were retained on the 5.0-mm stainless steel mesh sieves were separated using steel tweezers. Solids collected in the 0.3-mm sieves were transferred to a clean beaker using minimal rinsing with a low density polyethylene squirt bottle containing distilled water. To remove the organic material mixed in the sample, wet peroxide oxidation process procedure was conducted. Hydrogen peroxide and ferrous sulfate were analytical pure grade. 20 mL of aqueous 0.05 M Fe (II) solution (prepared by adding 7.5 g of $FeSO_4 \cdot 7H_2O$ to 500 mL of water and 3 mL of concentrated sulfuric acid) and 30% H_2O_2 solution were added to the beaker containing the 0.3-mm

size fraction of collected solids (Yonkos et al., 2014; Masura et al., 2015). After the reaction stopped, the beaker was heated on a 75°C hotplate for 30 min. If natural organic material was still visible, another 20 mL of 30% H_2O_2 was added. The beaker was placed at room temperature for at least one week. During the digestion process, the beaker was covered with tinfoil paper. Solids in the mixed solution were filtered through a glass fiber filterusing a vacuum system. Subsequently, the filter were placed in clean petri dishes which was covered with tinfoil paper and allowed to dry for further analysis.

4. Sampling and Analysis of Microplastics in Sediment

4.1 Sampling Microplastics in Sediment

4.1.1 Sediments

Analyzing sediment samples for the presence of microplastics began to appear in the scientific literature about 20 years ago and with greater frequency in the last 10 years. Sediment types investigated include deep sea (core) sand, beach sand, river sand, intertidal mangrove mud and municipal soil, as a result sampling methods vary greatly (Besley et al., 2017). Maximum depth collected varied from 2 cm to 5 cm to sediment cores of unknown depths (Miller et al. 2017). Microplastics in sediments or beaches are currently more frequently analyzed than microplastics in the water column. Sampling approaches depend on the sampling location i.e. sampling sediments directly on beaches or sampling subtidal sediments from a ship.

4.1.2 Beaches

Sampling beaches for microplastics is relatively easy and requires nothing more than a non-plastic sampling tool (tablespoon, trowel or small shovel), a frame or a corer to specify the sampling area and a container (if possible non-plastic) to store the sample. The quantity of samples reported in the literature varies between less than 500 g to up to 10 kg. While sampling on a beach poses no problem per se, the positioning of the sample location on the beach is still a matter of scientific debate as the distribution of microplastic particles is as dynamic as the beach itself (Hidalgo-Ruz et al. 2012). The high-tide line where flotsam accumulates is sampled mostly (Browne et al. 2010). Commonly applied sampling strategies include random sampling at several locations on the beach, on transects perpendicular or parallel to the water or in single squares. Often, several samples are pooled for an integrated estimate of the microplastic contamination of a beach. Every single sampling location for the pooled sample is then defined as described above. Another point of concern is the sampling depth. Sampling the top five centimetres is a common approach (as also suggested by the TSG-ML), but sampling to a depth of 0.3 m is also reported in the literature. If corers are used for sampling, different depth layers can be sampled so that microplastic concentrations can be related to sediment depth and eventually to the age of the corresponding sediment layer. The units of microplastic abundance reported depend on the sampling approach. Thus, abundance is normalized to sampling area, sediment weight or volume. Sampling

sediments for microplastics at beaches might appear trivial. However, currently no standard protocol exists for sampling microplastics with respect to location, sampling technique and sample quantity, and thus the comparability of the data produced is limited. Accordingly, there is an urgent need for the development of standardized sampling approaches. Because of the patchy distribution of microplastics at beaches a standardized, spatially integrating sampling design appears reasonable and would facilitate the generation of comparable data. A first step towards the standardization of sampling microplastics at beaches in the EU has been made by the TSG-ML (Hanke et al. 2013). They recommend to monitor microplastics at sandy beaches at the strandline with a minimum of five replicate samples separated by at least five metres and to distinguish two size categories: large microplastics (1–5 mm) and small microplastics (20 µm–1 mm). Small microplastics should be sampled from the top five centimetres with a metal spoon by combining several scoops at arm length in an arc-shaped area at the strand line to collect ca. 250 g of sediment; large microplastics should be sampled from the top five centimetres and several kilograms of sediment sample can be reduced by sieving over a 1-mm sieve directly at the beach (Löder & Gerdts, 2015).

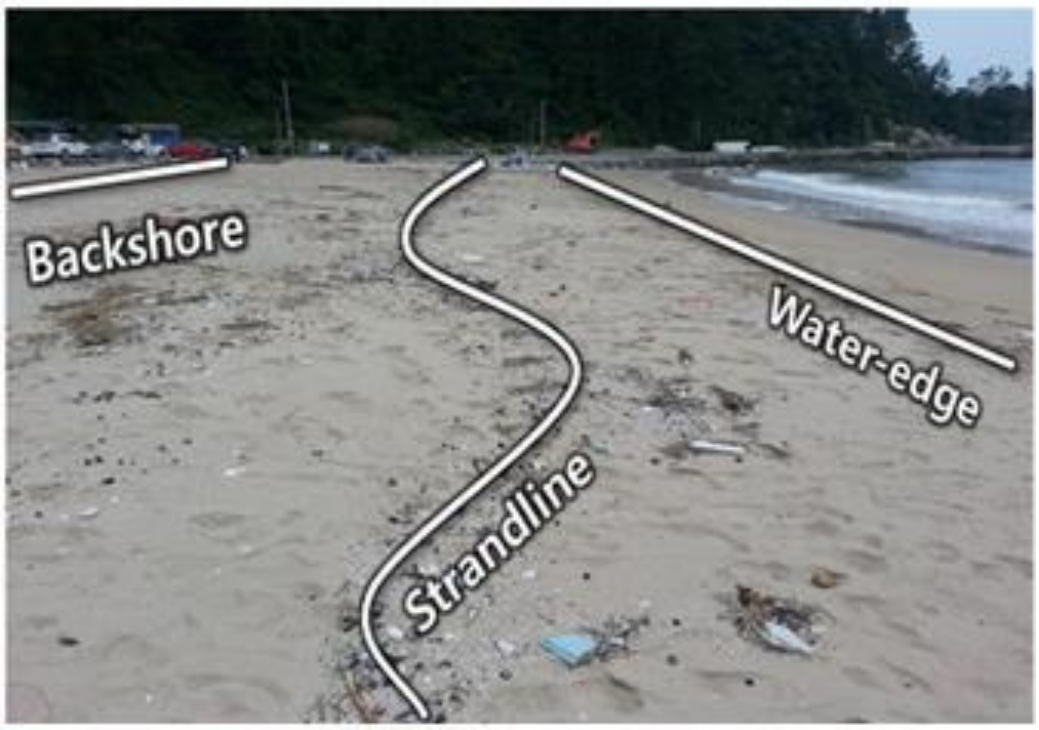

Figure 4.1 The referent lines for beach sediment sampling (IOC-WESPAC, 2018)

4.1.3 Subtidal Sediments

Subtidal sediments can be sampled from vessels with grabs, e.g. Van Veen or Ekman grab or corers of different design, e.g. a multiple corer. Grabs tend to disturb the sediment and are suited for surface (e.g. top five centimetres) or bulk sampling, whereas undisturbed core samples enable the simultaneous sampling of surface and depth layers but yield smaller sample volumes. The size of the instrument applied as well as the time needed for its retrieval depends strongly on the water depth at the sampling location.

The use of corers enables sampling to a water depth of more than 5,000 m (Van Cauwenberghe et al. 2013). Sediment samples are usually stored frozen or dried and kept in the dark until further analysis (Löder & Gerdts, 2015).

4.2 Separation of microplastics in sediment

Density flotation methods using either sodium chloride (NaCl, most commonly of 140 g L−1) or sodium iodide (NaI) were widely used, regardless of the sediment type or depth of sampling. Most studies suspended the sediments in hypersaline NaCl solution after which they were allowed to settle (10 min to overnight), while others (Claessens et al., 2011; Miller & Motti, 2017) conducted multiple (exhaustive) settlements to ensure all plastics were recovered. Horton et al. (2017) implemented a 3-step procedure involving visual inspection of whole sample, density flotation in $ZnCl_2$, followed by further visual inspection of unfloated sample. This procedure revealed the inefficiency of visual sorting through sediment samples (37% recovery of total plastics), yet the effectiveness of a $ZnCl_2$ density separation (75% recovery). While these recovery rates were not established from spiked samples, the difference demonstrates the importance of density flotation separation when processing sediment samples. Maes et al. (2017) similarly suggests a ZnCl2 density separation, saying a solution with density of 1.37 g mL−1 will allow for the flotation of PA, PS, PVC, PET, PE, and PP. In addition, Maes et al. (2017) proposed an alternative method allowing for the identification of plastic particles from sediments by staining samples with a Nile Red (NR) acetone solution. While this method proved effective at allowing for slightly faster visual inspection and promises (with further validation) general particle categorization, it is unknown whether this additional step (~60 min) would speed up analysis of samples. In addition, any subsequent FTIR analyses of NR-stained plastic particles is reliant on the use of "very small amounts" i.e. final concentration of 1, 10 or 100 µ g mL−1 suspension, and requires adaptation of the FTIR imaging optics (Maes et al., 2017). Masura et al. (2015) suggested using a commercial separator lithium metatungstate solution as an alternative due to its greater density (1.62 g cm−3) compared to NaCl. This allows for denser particles (i.e. PVC, PET) to be recovered more readily (Quinn et al., 2017).

Claessens et al. (2013) used elutriation, whereby an air stream lifts lower density particles to the surface, followed by decanting and sieving. They suggest the implementation of thorough cleaning, as well as procedural blanks when using an elutriation method for field samples, since there is the potential for contamination during extraction. Wessel et al. (2016) used a custom-made automated density flotation separator with> 35 PSU filtered water, which achieved an average recovery rate of 97.25% (± 2.5) in only 26 min. Crichton et al. (2017) proposed an innovative and cost-effective flotation methodology exploiting the oleophilic properties of microplastics by using retail grade canola oil yielding average recovery rates of 96.1%, and proving a more time efficient method than NaI or $CaCl_2$ methods, although this method will impact on any subsequent chemical analysis, particularly FTIR. More recently, Fuller and Gautam (2016) investigated pressurized fluid extraction using methanol (CH_3OH) and dichloromethane as a means of chemically extracting the microplastics. This

extraction procedure dissolved the plastics, producing plastic residues, thereby destroying the morphology of microplastic particles making physical characterization impossible. Only three studies reported using an alkaline, acid or oxidative digestion on sediments (Fuller and Gautam, 2016; Masura et al., 2015; Quinn et al., 2017). Claessens et al. (2013)

Similar to the seawater samples, only a small number of sediment studies conducted recovery checks to establish robustness of their methods. Claessens et al. (2013) spiked uncontaminated sediment samples with known microplastics and achieved a recovery efficiency range of 68.8%–97.5% dependent on sediment and polymer type. In another experiment using elutriation, clean sediments were spiked with known PVC or PE, and fibers collected from environmental samples, with a 100% and 98% separation efficiency, respectively. Claessens et al. (2013) achieved similar recovery rates to Claessens et al., 2011 study, reporting a 69–98% recovery with control beach samples (unknown plastic polymer types). Quinn et al. (2017) observed higher recovery rates with increasing solution density, from a 55%–90% range in saturated NaCl (1.17 g cm^{-3}), to 91% in saturated NaI (1.57 g cm^{-3}) and 99% in saturated 25% $ZnBr_2$ (zinc bromide, 1.71 g cm^{-3}). Nor and Obbard (2014) obtained recovery rates for spherical PE beads from spiked mangrove sediment samples of 55–72% after grinding samples with a mortar and pestle, followed by two density flotation separations using NaCl. Implementing a grinding step is not recommended for environmental samples as it can physically damage and break apart plastic particles, especially if already weathered (Pers. Observation). Fuller and Gautam (2016) spiked composted municipal waste sediments with known plastic polymers and, after grinding, separated the microplastics using a pressurized fluid (dichloromethane) extraction protocol, producing a microplastic residue and average recoveries of> 80%. FTIR analysis of the microplastics was also performed before (beads) and after (residue) spiking. Although the appearance of the plastic beads was altered due to the solvent extraction process, the FTIR spectra revealed no significant chemical changes to the plastic residue. However, the application of this technique is limited by the fact that the residue may contain mixtures of plastics requiring sophisticated spectral deconvolution.

Based on our review of the recovery rates from density flotation techniques applied to sediments, the use of $ZnBr_2$ is recommended (Quinn et al., 2017), however, this method has not been validated for all polymer types. To ensure all plastic particles (fragments and fibers) are recovered from sediment samples, an elutriation method, similar to that reported by Claessens et al. (2013), is also recommended. As for seawater samples, there is a need to establish a reliable, standardized and efficient approach for the separation and characterization of microplastics from sediments, with an emphasis on determining recovery rates.

5. Detection methods of microplastics in biota

While visual detection and separation is a mandatory step for the removal of debris and naturally occurring organic fragments, in many cases it may be impractical to completely rely on visual detection and separation to remove microplastics from

biological material. For this reason, techniques have been developed that allow faster separation of plastic materials from organic material by digesting away the organic material to leave behind the plastic material for quantification. This is typically accomplished with the use of acids, bases and enzymes. Ultimately, this approach is particularly appropriate for smaller organisms where the entire body or visceral mass may be digested, as well as with fish stomachs, and ensures that all of the plastic materials present are collected. Nonetheless, care has to be exercised when utilising this approach since although the reagent may successfully digest away the biological tissue, it may also have a chemical impact upon the microplastics themselves, especially with small items such as fibres（Rocha-Santos T. et al., 2015）.

5. 1 Acid digestion

One of the most extensively studied methods is based upon the principle of wet digestion of biological tissues using acids. Indeed, the most successful is the acid destruction method and is recommended by the International Council for the Exploration of the Sea (ICES, 2015) as part of a preliminary protocol which was introduced for the monitoring of plastics in fish stomachs and shellfish. The acid destruction method (also known as the acid mix method) uses a mixture of 65% nitric acid (HNO3) and 68% perchloric acid ($HClO_4$) in a 4:1 ratio (HNO_3:$HClO_4$ 4:1 v:v) and completely digests the tissues and removes other organic material, leaving behind only silica and plastic. This method has the added advantage that it also removes rayon fibres, which are common fibres composed of regenerated cellulose and are not considered to be microplastics, and which have been known to skew results. However, the technique is still under development and variations on the concentrations of the acids may be required since there have been some reports of detrimental effects on nylon fibres, which are known to be sensitive to acids and alkalis.

Other methods which have been developed involved the use of nitric acid, hydrogen peroxide and have demonstrated effective rates of tissue digestion (particularly in mussels), with high recovery yields of polystyrene microbeads at 94–98%, but highly variable results for nylon fibres at 0–98% recovery (Claessens M, et al., 2013). Furthermore, considerable variation was found based upon the size and type of the microplastics extracted.334 A review428 of the presence of microplastics in organisms from natural habitats reported that crustaceans, fish, molluscs (mostly Mytilus edulis mussels) and polychaetes (lugworms) were assessed using visual detection and separation, as well as with tissue dissociation methods utilising potassium hydroxide (KOH), hydrogen peroxide (H_2O_2) and various acid destruction methods. Treatment with H_2O_2 was undertaken in various studies (Foekema EM et al., 2103; Mathalon A, 2014; Wesch C et al., 2014) but was demonstrated in other studies to result in incomplete tissue dissociation and a significant loss of microplastics of specific sizes from the sample.

5.2 Alkaline digestion

Relative to any acid digestion, studies have found an alkaline hydrolysis utilizing a

strong base (which denature proteins and hydrolyze chemical bonds) more efficient and generally less damaging to inherent plastics, especially with regard to fish and invertebrates (Claessens et al. 2013, Cole et al. 2014, Lusher et al. 2017). The impact of this method on polymeric material depends on the plastic, with some conflicting reports for certain polymers. Polyethylene, polypropylene, and polyamides are all reported to be resistant, while polycarbonate and polyesters seem to be degraded (Lusher et al., 2017), which limits the applicability of these reagents. The optimized alkaline digestion protocol recommends 40 mL of 10 M KOH per 0.2 g dry weight of sample maintained at 60°C for 24 h.

5.3 Enzymatic digestion

To avoid the prospect of dissociation of the microplastics themselves, the use of enzymes, such as proteinase, lipase, cellulase and chitinase, have been recommended107 for use in tissue dissociation as an alternative to acids and alkalis. Indeed, a study66 which investigated the ingestion of microplastics in zooplankton, the enzyme proteinase-K was used in a sample clean-up step to remove large amounts of biogenic material (97% by weight) which was successfully filtered from water samples without destroying any microplastics present. Cole et al. (2014) optimized the original enzymatic protocol. A homogenizing solution (400 mM Tris-HCl buffer, 60 mM EDTA 105 mM NaCl, 1%SDS) is added to a dried sample. The mixture is incubated for 15 min at 50°C prior to the addition of 500 g/mL of Proteinese-K per 0.2 g dry weight of the sample. This mixture is then further incubated at 50°C for 2 h. Sodium perchlorate (5 M) is added and samples shaken at room temperature for at least 20 min. the solution is then physically homogenized a second time using a finer (21G) needle prior to further incubation at 60°C for 20 min. After ultrasonication on ice (to prevent excess heat) using a sonication probe, samples are vacuum filtered, with filters being rinsed with Milli-Q water, removed, covered and dried at 60°C. Filters are then visually examined with the use of an optical microscope, for plastic particles.

6. Microplastic identification

6.1 Visual Identification

Following sample collection and separation, the final stage in the assessment of microplastics in the environment is the positive identification of those items suspected to be composed of plastic. Noren F. (2007) suggested the following strict criteria to identify microplastics, which work best for microplastics in the size range 0.5-5 mm.

- The particle or fibre in question has no observable organic or cellular structures.
- In the case of fibres, the diameter should be consistent along the length with no evidence of tapering or bending in three-dimensional space. If the fibre is not straight, biological origin is suspected.

- In the case of red coloured fibres, additional scrutinisation with high-magnification microscopic examination, fluorescence microscopy and staining of chloroplasts is required to preclude algal sprouts.
- Particles should be clear and unvaryingly coloured.
- In the case of transparent, opaque or white particles, further high-magnification microscopic examination, as well as fluorescence microscopy, should be undertaken to preclude the possibility of biological origin.

It is strongly recommended to subsequently analyze sorted particles by techniques that facilitate a proper identification of plastics (Hidalgo-Ruz et al. 2012; Dekiff et al. 2014) because the quality of the data produced by visual sorting depends strongly on (1) the counting person, (2) the quality and magnification of the microscope and (3) the sample matrix (e.g. plankton, sediment, gut content). Another fundamental drawback of visual sorting is the size limitation, i.e. particles below a certain size cannot be discriminated visually from other material or be sorted because they are unmanageable because of their minuteness. Furthermore, visual sorting is extremely time-consuming. In summary, even an experienced person cannot discriminate all potential microplastic particles unambiguously from sand grains, chitin fragments, diatom frustule fragments, etc. Thus the error rate of visual sorting reported in the literature ranges from 20 % (Eriksen et al. 2013) to 70 % (Hidalgo-Ruz et al. 2012) and increases with decreasing particle size.

6.2 Fourier-transform infrared (FTIR) spectroscopy

Fourier-transform infrared (FTIR) spectroscopy is the most popular and widely used technique for the positive identification of the type of plastic that microplastics in environmental samples are composed of. The reason for the popularity of the technique is due to its straightforwardness and reliability, the predominant reason is that FTIR is highly accurate in identifying the type of plastic present by producing highly specific infrared (IR) spectra which contain distinct band patterns, thereby allowing differentiation between plastic materials and natural materials. The technique relies upon the actuality that most molecules absorb light in the infrared region of the electromagnetic spectrum.

Large particles can be easily analyzed by an FTIR surface technique—“attenuated total reflectance”(ATR) FTIR spectroscopy— at high accuracy in less than one minute. A step forward with respect to the characterization of small-sized particles is the application of FTIR microscopy. Although micro-FTIR mapping has been successfully applied for microplastics identification, however this technique is still extremely time-consuming when targeting the whole sample filter surface at a high spatial resolution because it uses only a single detector element **(Harrison et al., 2012).** Focal plane array (FPA)-based FTIR imaging, allows for detailed and unbiased high throughput analysis of total microplastics on a sample filter **(Levin & Bhargava**

2005). This technique enables the simultaneous recording of several thousand spectra within an area with a single measurement and thus the generation of chemical images. By combining FPA fields, whole sample filters can be analyzed via FTIR imaging. It's important to note that samples must be dried prior to measurement via IR spectroscopy as water strongly absorbs IR radiation.

6.3 Raman spectroscopy

Raman spectroscopy can also be coupled to microscopy (Raman microspectroscopy) to identify microplastics as small as 1 μm **(Cole et al., 2014)**. It is a straightforward, efficient and reliable technique which requires minimal sample preparation and has been successfully used for the identification of microplastics that have been separated from environmental samples.

Like FTIR spectroscopy, Raman spectroscopy is a non-destructive technique that does not affect the sample. Thus, further analysis can be undertaken following identification of the microplastic, such as the extraction of any sorbed persistent organic pollutants (POPs) for identification and quantification via gas chromatography–mass spectrometry (GC–MS). However, unlike the transmission and reflectance methods utilised in infrared spectroscopy, Raman is a scattering technique. This is advantageous over infrared spectroscopy in that thicker and strongly absorbing microplastics can be analysed. Furthermore, in comparison to FTIR, microplastics of a very small size can be analysed by Raman spectroscopy and a wider range of infrared wavelengths can be utilised for analysis of the sample. Nevertheless, Raman tends to be the second choice in polymer identification after FTIR. This is mainly a result of issues with sample fluorescence（Lenz R et al., 2015）. Most plastic materials are rarely the pure polymer and are typically of impure composition as a result of the incorporation of a wide variety of additives and colouring pigments during manufacture.

6.4 Pyrolysis–gas chromatography–mass spectrometry

Pyrolysis–gas chromatography–mass spectrometry (Pyr-GC–MS) is a technique which thermally decomposes the large high-molecular weight molecules of a sample via heat mediated cleavage in the presence of an inert atmosphere. The composition of these moieties is subsequently determined by mass spectrometry (MS) and provides characteristic information as to the structural composition of the samples large high-molecular weight molecules, thereby allowing the sample composition to be identified (Nuelle M. et al.,2104) As a destructive technique which thermally decomposes the sample, further analysis of the microplastics is precluded. Consequently, this may be a limiting factor in some cases. Nevertheless, the great advantage of Pyr-GC–MS is that the technique utilises direct introduction of the sample with minimal pre-treatment. The technique can be utilised for the identification of microplastics in environmental samples, as well as simultaneously identifying any plastic additives present.142 However, since samples must be manually placed in the instrument and analysed individually, the analysis of large quantities of microplastics is limited, as well as the

range of sizes of items which can be effectively handled (Löder M et al., 2015).

7. Contamination mitigation

Due to their ubiquitous nature, the contamination of a solid or liquid environmental sample with microplastics that were not originally part of that sample is one of the major issues involving the examination of samples for microplastics and consequently it has been raised by several researchers. Indeed, the processes involved in the collection, separation and identification of samples for microplastics often result in the inadvertent introduction of microplastics that would not otherwise be found in the sample. Mini-microplastics (particularly microfibers) can be introduced from the ambient air, but also via the use of sampling or laboratory equipment, improper storage of samples or even from the clothing of the researchers themselves. In many cases this contamination can compromise the analysis.

The following contents list the microplastic analysis quality control steps (Quinn B., 2017)

Step 1: Preparation

- A clean white cotton laboratory coat and nitrile gloves should be worn at all times.
- Only clothing composed of natural fibers should be worn, synthetic garments should be avoided, even if worn underneath a laboratory coat.
- Air movement in the laboratory should be minimised by closing all windows and doors.

Step 2: Cleaning

Ensure that the laboratory is kept clean and free from dust. Avoid working below overhead fixtures which may have accumulated settling dust.

- Clean all equipment with 70% ethanol and then rinse 3 times with distilled water.
- After cleaning, cover all equipment with aluminium foil.
- Wipe all work surfaces with 70% ethanol 3 times prior to the commencement of work.
- Examine all petri dishes, filter papers and forceps with a dissection microscope before use.

Step 3: Solid particulate surface monitoring

- This process is carried out before and after all analyses of microplastics in samples.
- While ensuring that gloves are worn at all times, the adhesive side of fresh 5 cm2

sections of transparent high tack adhesive tape is pressed 3 times onto the work surface and then lifted. Any solid particulates present should adhere to the adhesive on the tape.

- Each 5 cm2 section of adhesive tape is then adhered to a clean piece of cellulose acetate film.
- The date and time that each 5 cm2 section of adhesive tape was used is written next to it on the cellulose acetate film with a permanent marker pen.
- The 5 cm^2 sections of adhesive tape on the cellulose acetate film are then examined under a microscope for the presence of mini -microplastics, such as microfibers and microfragments.
- Further analysis, and positive identification, of any mini-microplastics found adhered to the 5 cm2 sections of adhesive tape can be undertaken by infrared spectroscopy and subsequently excluded from the sample of interest.

Step 4: Solid airborne particulate monitoring

- Before work commences, clean pieces of dampened filter paper are placed in 9 cm standard glass petri dishes, ensuring that the filter paper covers the entire internal area of the petri dish.
- The petri dishes are then placed around the work surface, where they remain for the duration of the laboratory work.
- Upon completion of the work, the filter paper is then examined for the presence of mini- microplastics using a microscope, or a glass lid is placed on the petri dish and labelled with the date and time for subsequent microscopic analysis at a later date.
- Further analysis, and positive identification, of any mini-microplastics found on the filter paper can be undertaken by infrared spectroscopy and subsequently excluded from the sample of interest.

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