Monitoring Guidance for Marine Litter in European Seas

Draft Report

CHAPTER 7

MICROLITTER

July 2013
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Draft Guidance Report:
TSG-ML was tasked to deliver guidance so that European Member States could initiate programmes for marine litter monitoring. As monitoring must be operational by 2014, first guidance was required by mid-2013. The draft Guidance report provides the basis for the marine litter programme however since new information continues to be compiled TSG-ML can review and update this guidance later in 2013.

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Disclaimer: This report has been prepared by a group of experts nominated by EU Member States and Stakeholders. It aims to provide guidance for the implementation of MSFD Descriptor 10 on Marine Litter. It does not constitute an official opinion of the European Commission, nor of the participating Institutions and EU Member States.
7. Microlitter

7.1. Introduction to Microlitter

Microlitter is considered in Section 4.4 of the MSFD descriptor 10 “Amount, distribution and composition of microparticles. The attribute will establish baseline quantities, properties and potential impacts of microparticles. Microplastic is likely to be the most significant part of this.”

In effect microparticles consist of similar materials to other types of litter; they are merely pieces of litter at the very small end of the size spectrum. Microparticles of a range of common material types including glass, metal, plastic and paper litter are undoubtedly present in the environment. The protocols outlined here focus on microplastics as descriptor 10 considers these to be the most significant component of the microlitter in the environment. This statement is partly based on the frequency of reports of microplastics (Hidalgo-Ruz et al., 2012), but relative proportions of material types will be influenced by the physical conditions of the habitat sampled, for example metal and glass microlitter is not likely to be found at the sea surface. The approaches described here are likely to capture other man-made particles. Where materials other than plastics contribute a major proportion of the microlitter in a particular location it is important that this is recorded and if necessary protocols are modified to ensure this litter is as completely sampled as possible.

When first described the term microplastic was used to refer to truly microscopic particles in the region of 20 µm diameter (Thompson et al., 2004). The definition has since been broadened to include all particles < 5 mm (Arthur et al., 2009). Microplastics are widely dispersed in the environment and are present in the water column, on beaches and on the seabed (Barnes et al., 2009; Browne et al., 2011; Gaessens et al., 2011; Collignon et al., 2012; Colton et al., 1974; Goldstein et al., 2012; Law et al., 2010). Hence microplastics are relevant to other protocols in descriptor 10, relating to the monitoring of larger items of debris; however they are treated separately here because their size necessitates specific methodology.

MSFD considers that in order to achieve GES that the quantities of microplastics in the environment should not result in harm. When defining methodological criteria it is essential to recognise that our understanding of the potential impacts of microplastic on organisms and the environment (i.e. the ‘harm’ that they might pose from the perspective of MSFD) is still not fully understood. A range of concerns have been outlined including: physical obstructions impairing feeding and digestion, particulates-type toxicity (analogous to airborne particulates) and the transfer of toxic substances to biota upon ingestion and physical damage to organs and tissues as a consequence of the physical presence particulates (Browne et al., 2008; Mato et al., 2001; Secretariat of the Convention on Biological Diversity and Scientific and Technical Advisory Panel GEF 2012; Teuten et al., 2007; Wright et al., 2013). The relative importance of these issues is likely to vary across the size spectrum of the plastic in relation to the size of the organism concerned. For example, items of the large end of the microplastic size distribution (1-5 mm) have been reported in the stomach of seabirds where they may compromise feeding and digestion (van Franeker et al., 2011). While in small invertebrates much smaller particles of plastic in the 10 µm size range have been shown to translocate from the intestine into the circulatory system (Browne et al., 2008) and there is considerable evidence for the translocation of even smaller nano particles. Work using larger particles (200-250 µm) has indicated the potential for the transport of persistent organic pollutants (Teuten et al., 2007).

While an upper size bound of 5mm has been widely (but not exclusively) adopted, current definitions do not explicitly state a lower size limit and lower size limits have seldom been reported for measured microplastic concentrations in the environment. The lower size limit is perhaps assumed to be the mesh size of the net or sieve through which the sample passed during the sampling, sample preparation or extraction steps. The size limits of microplastic particles that can be reported is also dependent on the method of detection, in many cases microscope-aided visual inspection. When identifying microparticles there are also size limits imposed by the analytical techniques employed (e.g. minimum sample intake requirements for detection and analysis). Hence an important part of establishing standard methods and protocols will first be to define the appropriate size range, and this aspect is considered in the present report.

Most studies have focused on sampling intertidal sediments and the sea surface / water column (Hidalgo-Ruz et al., 2012). However, despite the numerous studies one of the main limitations to our ability to make
spatial and temporal comparisons, especially at broad scales, is that a wide variety of approaches have been used to identify, quantify and report measured concentrations of microplastics (Hidalgo-Ruz et al., 2012). Work to date represents the critical pioneering steps towards understanding the distribution and fate of microplastics in the environment. After this initial period of discovery, microplastics research now finds itself at a stage of development where there is a lack of quality assurance/quality control (QA/QC) instruments available: e.g. no organisations yet offer proficiency training or testing, there have been no inter-laboratory studies, no certified reference materials are available, no standardized sampling and analysis protocols have been published, no accreditation certificates have been issued and some procedures in use have not yet been validated. Approaches for QA/QC will therefore be very useful for evaluating sources of variability and error and increasing confidence in the data collected.

Furthermore, microplastics comprise a very heterogeneous assemblage of pieces that vary in size, shape, colour, specific density, polymer type, and other characteristics. For meaningful comparisons and to answer the specific questions of the data users and to test hypotheses through monitoring, it is important to define specific methodological criteria to quantify such metrics as e.g. the abundance, distribution and composition of microplastics and to ensure sampling effort is sufficient to detect the effects of interest. Protocols to monitor microplastic in four compartments of the marine environment: 1) intertidal sediments, 2) Sea surface, 3) subtidal sediment and 4) biota are presented here however it must be recognised that at present our understanding of the sources, distribution and fate of microplastics in the environment are very limited as is our understanding of any associated harmful effects on the environment or wildlife. As a consequence it is not possible to present validated standard operating procedures for the compartments listed above. Instead we present recommendations for monitoring supported by a discussion of relevant considerations and limitations according to the scientific knowledge base at the time of writing. Most work to date has focused on intertidal sediments and sea surface sampling and so our recommendations for these compartments are more specific and detailed than for subtidal sediment and biota. The aim being to maximise consistency and comparability of the data collected by using the approaches outlined; and to contribute to on-going improvements in methodological aspects of sampling, analysis and experimental design for environmental microplastics.

Collection of data has an associated cost and so it will be critical to identify monitoring approaches (and associated meta data such as QA/QC data) that directly supports the aims of the monitoring programmes of, in this case, Descriptor 10, item 4.4 ‘microparticles (especially microplastics)’. In this respect it is important to note, as a general point, that mismatches in how monitoring data are collected and the hypothesis or question(s) being addressed by the data customarily act to limit the power of monitoring data and may weaken the conclusions that can be drawn from them. As we move forward toward GES the strategy of designing microplastic monitoring should therefore be to prevent the ‘data-rich but information-poor’ ailment that has affected various environmental monitoring data sets in the past. However, since our understanding about the distribution, principle types (e.g. shape, colour, polymer), relative importance of various sources and sinks, and any associated links to harm are currently limited it is important to recognise that the approaches outlined here should be re-evaluated and refined as new information emerges.

It is hoped that the recommendations for monitoring outlined here will help in the collection of new data to inform our understanding on trends in the abundance and distribution of microplastics; however in some instances the data collected may at this stage be more important for hypothesis generation rather than hypothesis testing. We strongly advocate the need for workshops to inter-calibrate methods and review data collected in order to refine specific monitoring and achieve the greatest level of efficiency.

7.2. Scope & key questions to be addressed

Technical Recommendations for the Implementation of MSFD Requirements were outlined in the 2011 report from the TSG-ML and concluded that:

‘There is a need to standardize sampling approaches in order to monitor the abundance of microplastic for MSFD. For samples from sea surface, water column, sediment and biota, this needs to consider both the sampling design in terms of number and size of replicates, spatial area and frequency of coverage as well as the methodological approach; type of net or core and method of identification used. Given this is an emerging area with numerous recent studies it is not reasonable to prescribe set methodologies at this time and the development of standard approaches and protocols should be seen as a goal over the next 4 years’
“By 2012 there should be identification and recommendation of protocols to provide consistent, reliable and relatively easily obtainable data on spatial and temporal trends in microplastic. Since patterns of distribution and the movement of particles between compartments, for example sea surface to seabed, is far from clear; it will be important to evaluate methods to sample shorelines, sea surface.”

Based on these recommendations this document presents a review of existing approaches considering sampling design, methods of sample collection and identification of microparticles and the extent of current usage which is important for comparative purposes. The main objective of the present document is to give guidance to Member States for monitoring of microplastics in marine habitats and consider appropriate monitoring design, sampling, analysis, reporting). Where possible, the basic criteria and approaches are recommended; such that future quantitative estimates are as comparable as possible. However, microparticles represent an emerging area of scientific research and as yet there are few robustly tested and validated approaches. Hence, in addition to providing recommendations that will be feasible and effective for Member States at the present time, this document also identifies areas where methods need developing. It is therefore essential that approaches are reviewed as our understanding and the literature on this topic evolve.

Sampling of microplastics will be considered for each of the following compartments: Beach, Water column and Sea surface, Subtidal sediment and Biota. Section 7.4 discusses the current status of sampling approaches for each of the four compartments considering the difficulties associated with applying these methods and any limitations. Section 7.5 then presents our recommendations for monitoring in each of the compartments. It also addresses aspects of quality assurance and quality control. However detailed power analyses to indicate levels of spatial and temporal replication required in order to be able to detect given levels of change (effect size) form back ground variability are yet to be undertaken. Such analyses are therefore an important priority in order to refine more efficient protocols in future. This Chapter makes recommendations on sampling (Section 7.5) based primarily on approaches that have been used to date. The Chapter considers monitoring approaches that address the full size spectrum of microparticles that can feasibly be sampled with recognised approaches i.e. millimetres, 100s of µm and 10s of µm. It seems inevitable that even smaller anthropogenic particles including nanoparticles are also present in the environment, however at present there is little that can be done to monitor particles of this size and they are considered beyond the scope of this review.

7.3. Key Questions of consideration

How to determine the abundance of microplastic in intertidal sediment?
How to determine the abundance of microplastic in subtidal sediment?
How to determine the abundance of microplastic at the sea surface?
How to determine the abundance of microplastic in biota?
How to introduce and maintain appropriate QA/QC measures in the field and the laboratory?

All of the above must be considered within the framework monitoring programs that are appropriate to the questions or hypotheses being tested.

7.4. General Sampling Methods

Sampling of microplastics in different main marine environments (sea surface, water column, sediment and biota) has been approached using a variety of methods: samples can be selective, bulk, or pre-treated to reduce their volume (Hidalgo-Ruz et al., 2012).

Selective sampling in the field consists of direct extraction from the environment of items that are recognizable by the naked eye, usually on the surface of sediments. For example particles in the size range 1–5 mm diameter are easily recognizable. However, when smaller microplastics are mixed with other debris or lack distinctive shapes there is a great risk of overlooking them. This form of sampling is therefore only valuable if the aim of the monitoring is to determine the abundance of specific items that are readily recognisable to the naked eye, such as resin pellets or if the aim is to quantify items of specific
sizes (e.g. those > 3mm). It cannot be used to quantitatively sample a variety of microplastic shapes and sizes. Bulk samples refer to collection of the entire volume of the sample (water or sediment) without reducing it during the sampling process. This enables the reporting of concentration units, (e.g. based on sample mass) and can facilitate more rapid sampling especially when microplastics cannot be easily identified visually in the field because for example because they are covered by sediment particles. Sediments and seawater can also be pre-treated to reduce the bulk of the sample. Here a known and recorded volume of the sample is processed preserving the portion of the sample that is of interest. For example, sediment can be sieved directly on the beach or particles can be separated according to density; while on board a vessel seawater samples can filtered or sieved.

Most studies use a combination of these steps after which a purification step is required to sort the micro litter from natural particulates. Visual characterisation is the most commonly used method for the identification of microplastics (using type, shape, degradation stage, and colour as criteria). Chemical and physical characteristics (e.g., specific density) can also be used. However, the most reliable method is to identify the chemical composition of microplastics is by infrared spectroscopy (Hidalgo-Ruz et al., 2012). This approach requires equipment that may be considered relatively costly compared to sampling of large items of debris (Euro 20 100k) however FT-IR is widely available in laboratories throughout Europe and can be used to identify particles down to around 20µm in size.

In all four compartments (sea surface, water column, sediment and biota) we recommend quantifying microplastics in the size range 20µm to 5mm. Microplastics should be categorised according to their physical characteristics including size, shape and colour (see Table 9). It is also important to obtain information on polymer type, since this can help identify potential sources and pathways, which is potential monitoring goal. Microplastics should be categorised according to size with a minimum level of resolution being to allocate the material found in to size bins of 100 µm (20-100 µm, 101-200 µm, 201-300µm etc). Ability to visually distinguish synthetic fragments from other natural and man-made particulates becomes increasingly difficult as the size of the piece under examination decreases, unless IR techniques are used (which is feasible >20µm). We advocate that all particles in the range 1-100 µm be subjected to further analysis to confirm identity (e.g. using FT-IR). For particles in the size range 0.1-5mm we recommend that a proportion (for example 10%) of the material in each size class, up to a maximum of 50 items per year or sampling occasion whichever is the least frequent) of the items considered to be microplastics be subjected to further analysis to confirm identity. This step is important in order to 1) ensure quality control of visual identification and 2) gain information on the relative abundance of different polymer types which can be used to help identify potential sources and pathways leading to the accumulation of microplastics.

**Sampling Frequency** - Detailed power analyses to indicate levels of spatial and temporal replication required in order to detect given levels of change (effect size) form background variability are yet to be undertaken for any habitat. This is an important priority in order to refine more efficient protocols in future. This document therefore makes recommendations on sampling based primarily on approaches that have been used to date. To achieve the greatest efficiency microparticles should be sampled alongside other routine sampling programmes. For example microparticles in beaches can be sampled at the same time as macro debris on beaches, or in parallel with any other routine intertidal monitoring for chemical contaminants, biota). Similarly sampling of subtidal habitats or the sea surface could also be incorporated into routine monitoring programmes. For biota it is not possible at this time to recommend specific organisms as indicator species of micro particles. Methods are provided indicating how biota such as birds, fish, and invertebrates can be sampled. For greatest efficiency we suggest microparticles be quantified as part of any routine sampling of macro litter within biota; for example in birds, as outlined in Chapter 6 on Biota.

7.4.1. Sampling intertidal sediments

A recent review identified over forty studies examining the abundance of microplastics in sedimentary environments, mostly on sandy beaches (Hidalgo-Ruz et al., 2012. The number of sites sampled in each study ranged from one to 300 beaches. Most studies examined between 5 and 18 beaches. The specific tidal zone sampled on a beach varied considerably among studies; some covered the entire extent of the beach, from the intertidal to the supralittoral zone, some distinguished several littoral zones, while others pooled samples across different zones. The majority of studies, however, focused on the most recent
flotsam deposited at the high tide line. As with other types of debris the accumulation of microplastics on shorelines is likely to vary according the depositional regime. This will most probably occur in a similar manner to the deposition of natural particulates, however attempts to relate microplastic abundance to differences in sediment type among shores have not shown significant correlations (Browne et al., 2011). To date most sampling in the intertidal has been on sandy shorelines. This is easier since separation of small pieces of microplastics (< 500 µm) from bulk sediment by density and filtration is more efficient in relatively coarse sediments since fine material such as silt and clay remains in suspension and can clog filters. More work is needed in order to understand factors influencing the distribution of microplastics along gradients of shear stress (wave exposure, tidal flow). However, since most work to date has been from relatively coarse sandy sediments our recommendation is that microplastics should be monitored on the top of the shore (strand line) and where available on sandy shores (0.1 – 0.0125 mm diameter). We suggest that separate samples be collected to monitor each of two sizes of debris (1-5mm and 20 µm – 1mm)

Sampling depths reported in previous studies ranged from 0 to 32 cm; most studies sampled a single depth layer within the top 5 cm of sediment. Given that beaches and subtidal coastal habitats are dynamic systems with continuous and seasonal erosion of sediment microplastics may become buried in sediment during periods of accretion; however more research is needed to establish the extent of this. Since most work to date has been from the surface of sediments our recommendation is that microplastics should be monitored on the top of the shore (strand line) and where available on sandy shores (0.1 – 0.0125 mm diameter). Samples should be collected from the surface 5cm of the sediment surface.

Most studies have sampled at the strand line, either: (i) sampling a linear extension along the strandline with a spoon and/or a trowel or (ii) sampling an area extension using quadrats. Sampling units were directly related to the sampling instrument used. Studies that sampled a specific areal extension (from 0.0079 to 5 m²) employed quadrats and corers. Other sampling units were weight (from 0.15 to 10 kg) and volume of sediment (from 0.1 to 8 L). Our suggestion (based on previous studies) is that a minimum of five replicate samples be collected from the strandline. Each replicate should be separated by at least 5m. Replicates can be distributed in a stratified random manner so as to be representative of an entire beach or a specific section of beach. This ultimately depends on the specific locations and questions of interest at a local scale. We suggest that power analyses be conducted to further guide the most appropriate level of replication.

**Microplastics 1 – 5mm** - This should be collected as an additional entirely independent sample at each location and, in order to minimise the risk of contamination form persons undertaking the sampling itself, should be obtained AFTER the sampling the smaller size fraction (< 1 mm, see below) The sediment can be sampled by collecting with a metal spoon or trowel the top 5cm of sand from the area contained within a metal 50 cm x 50 cm quadrat and passing through a 1 mm metal sieve and then be stored in metal (e.g. foil) or glass containers (i.e. not stored in plastic containers). Record the volume of sediment examined. Our recommendation ins that these be sampled using an extension of the protocol for meso debris (5-25mm) which uses a 5mm sieve to separate debris from beach sediment (see protocol for sampling beaches Section 3). This approach can be extended by including a further metal sieve of 1mm mesh to achieve volume reduction in the field. Preferably the sieves could be stacked together.

**Microplastics 20 µm – 1mm** - should be collected from the top 5cm of sand using a metal spoon (suggest 15ml). Because the weight of sediment can vary considerably according to water content we suggest standardising sampling by volume and collecting approximately 250ml of sediment Microplastics can subsequently be extracted in the laboratory by density separation (see later). Sediment should be stored in metal (e.g. foil) or glass containers (i.e. not stored in plastic containers). The sample can be collected by kneeling on the strand line and collecting a series of scoops at arms-length at intervals within an arc shaped area to the front.

### 7.4.2. Sampling seawater

Seawater samples have mostly taken by nets, the main advantage being that large volumes of water can be sampled quickly, retaining the material of interest. Most studies from surface waters have used Neuston nets and from the water column, zooplankton nets. Another instrument, that is deployed on a global scale and that has also been used for microplastic sampling is the continuous plankton recorder (CPR). The most relevant characteristics of the sampling nets are mesh size and the opening area of the net. Mesh
sizes used for microplastic sampling range from 0.053 to 3 mm, with a majority of the studies (rather than individuals samples collected) ranging from 0.30 to 0.39 mm. The net aperture for rectangular openings of Neuston nets (sea surface) ranged from 0.03 to 2.0 m². For circular-bongo nets (water column) the net aperture ranged from 0.79 to 1.58 m². The length of the net for sea surface samples has varied from 1.0 to 8.5 m, with most nets being 3.0 to 4.5 m long. Techniques using apparatus to collect Seawater and pass it through a filter on-board ship are being developed for example by CEFAS, UK they use the ships water inlet, collecting seawater from the side at specified depths, mostly ranging between 4m and 1m depth. The seawater is passed through sieves or nets in closed containers after which these can be removed and analysed for microplastics.

A key consideration in collecting seawater samples is the cost of ship time. Hence the potential to sample during existing cruises or from existing monitoring programmes such as the CPR is well worth considering. Manta and bongo nets have been used at the sea surface. It is important to deploy the trawl out of the wake zone as turbulence inside the wake zone does not allow for a representative sample to be collected. Use a spinnaker boom or a frame to deploy the trawl away from the side of the vessel. It is recommended to keep a close eye on the net while trawling to observe its performance and adjust speed and cable length if necessary. Avoid sampling at the peak of plankton blooms as this may clog the net.

Since most plastics are buoyant they are likely to accumulate at the sea surface. Surface sampling techniques can be used close inshore, but are restricted to calmer weather conditions, whereas CPR and other sub surface approaches can be used in rougher weather. High speed Manta trawls can be deployed in a range of sea states but CPR is the least sensitive to sea state and samples at an average depth of around 6m. Manta trawls can be used to sample large volumes of surface water, but are relatively insensitive to smaller size fractions (< 1mm) which can be difficult to separate or sort form the large surface area of the net. CPR has a very much smaller aperture (around 1.6 cm²) and hence samples smaller quantities of water per km but can be deployed much longer periods (distances) than the Manta without clogging as it has a continuous net spool which collects the sample. With the CPR the entire filter is sealed automatically and can easily be transferred to the laboratory for examination under the microscope. Preliminary data indicate CPR and Manta nets collect similar quantities of debris per unit volume of water sampled; however because of the larger aperture of nets such as Manta the quantity of debris collected per distance towed is substantially greater than CPR. During trawls it is important to maintain a steady linear course at a constant speed. A hi-speed manta trawl can be deployed up to 8 knots, build up the speed slowly towards maximum speed. Higher speeds reduce the ability to sieve seawater, creating a bow wake in front of the trawl.

At present it is not appropriate to recommend one approach over all others. Each approach has advantages and disadvantages and may be preferable according to local availability / sampling opportunities, the characteristics of the area to be sampled. Our recommendation is to obtain samples from sea water and to ensure the following details are recorded to accompany each sample: type of net, aperture, mesh size (preferably 333 μm mesh, 6m length for greatest inter-comparability among sampling programmes). It is not possible to specify standard haul duration as at some times of year, for example during a plankton bloom, nets may readily become clogged with natural material rendering them inefficient - a duration of 30 minutes is suggested and the duration of the trawl and the estimated water volume must be recorded. Samples from nets should be stored in glass jars taking care to rinse material as thoroughly as possible from the sides of the net using filtered sea water. Microparticles are determined as the total quantity of items captured by the net during the period it is deployed. Note this may well include some items that are smaller than the mesh of the net itself since with fine nets of this type approximately half the surface area of the net is the mesh material itself (the remainder being the gaps between the mesh) and this can directly trap small particles.

7.4.3. Sampling Subtidal Sediment

Material can be collected using any approach that recovers a sample of relatively undisturbed surface sediment from the sea bed (e.g. Van veen grab, multi corer, box core etc.). Once recovered onto the vessel a small sample of sediment ideally around 250 ml is recovered to best represent the location of the original 5 cm surface to sub surface of the seabed. Because the weight of sediment can vary considerably to water content we suggest standardising sampling by volume. Avoid sampling next to the edge of the apparatus to minimise risk of contamination form the equipment (e.g. paint flakes other contamination on the grab / core). The sample is transferred to a metal or glass container for subsequent density separation / FT-IR spectroscopy.
7.4.4. Sampling Biota for microplastics

A range of organisms including filter feeders, deposit feeders and detritivores have been shown to ingest microplastic in the laboratory (Browne et al., 2008; Thompson et al., 2004). There are a growing number of studies showing that organisms form natural habitats also contain microplastic in their gut. This has been shown for seals (from scats) (Eriksson & Burton, 2003), birds (van Franeker et al., 2011), fish (Lusher et al., 2012), crustaceans and echinoderms (Graham & Thompson, 2009). For some organisms a substantial proportion of the population is affected. For example data collected on the Northern Fulmar show that over 95% of individuals washed ashore dead contained plastic in their guts and much of this material was microplastic. While a study in the Clyde Sea, UK showed that contamination in the commercially important crustacean *Nephrops norvegicus*, was wide spread with 83% individuals containing plastic. A recent study in the English Channel showed that 10 species of fish and over all around one third of individuals (sample size n = 500) contained small quantities of microplastic (Lusher et al., 2012).

For biota it is not possible at this time to recommend specific organisms as indicator species of micro plastics. Protocols are provided indicating how biota such as birds, fish, and invertebrates can be sampled. For greatest efficiency we suggest microparticles be quantified as part of routine sampling of macro litter within biota; for example in Birds and Fish, as outlined in Section 6 on Biota.

If individuals are live then they must be humanely killed adhering to any prevailing ethical legislation. Small individuals can be stored whole. For larger individuals the gut can be dissected but otherwise left stored intact. Examination of the gut is facilitated with a dissecting microscope. The digestive tract is slit open using scissors and examined immediately. Depending on the size of the organism the gut can be examined in its entirety or samples of gut wall (e.g. 10cm x 10cm (or similar standard area) can be removed and viewed under a dissecting microscope. Any fragments of an unusual appearance are removed with forceps and placed on clean filter papers in petri dishes which are then sealed prior to further examination for example via spectroscopy (Picture 4).

![Picture 4: Figure 1- Microplastics from the gut of a fish collected in the English Channel. Scale bar represents 2mm (Lusher et al., 2012).](image)

7.4.5. Laboratory analyses of samples collected in the field

*Density Separation for extracting plastics from sediment* - The specific density of plastic particles can vary considerably depending on the type of polymer and the manufacturing process. Density values for plastics range from 0.8 to 1.4 g cm$^{-3}$. These values refer to virgin resins, without taking into account the effect on density of various additives that might be included during product manufacturing or the effects of biofouling on the surface of the plastic. Typical densities for sand or other sediments are 2.65 g cm$^{-3}$. This difference is exploited to separate the lighter plastic particles from the heavier sediment grains by mixing a sediment sample with a saturated solution of Sodium Chloride and shaking. After mixing, coarse sediment will rapidly settle to the bottom, while low density particles remain in suspension or float to the surface of the solution. Subsequently, the supernatant with the plastic particles can be extracted onto filter paper for further processing. Fine sediments such as silt and organic particulates such as fragments of algae and plants are likely to remain in suspension and will be separated together with any plastic present.

Of the 13 sediment studies examined by Hidalgo-Ruz et al. in 2012 ten included density separation using a saturate saline Sodium Chloride (NaCl) solution (1.2 g cm$^{-3}$). One limitation with this approach is that the density of some plastics (e.g. PVC) is greater than that of saturated NaCl and therefore separation of these denser polymers will be relatively poor. Other solutions of greater density have been applied for example, sodium polytungstate solution with a density of 1.4 g cm$^{-3}$ tap water, Sodium Iodide solution (NaI) and
Zinc Chloride (ZnCl₂). Plastics that float in fresh and seawater are polystyrene in foamed form, high and low density polyethylene, and polypropylene. Polystyrene in solid form also floats in a hypersaturated saline solution. The plastics that float in sodium polytungstate solution also include flexible and rigid polyvinyl chloride (PVCs), polyethylene terephthalate (PETs), and nylon. A range of separation devices have also been developed such as the Munich Plastic Sediment Separator (Imhof et al., 2012). There are merits to all of these approaches; however detailed cross calibration of extraction, efficiency, equipment cost, sampling time and health and safety are yet to be undertaken among methods. We therefore recommend extraction with Sodium Chloride as it has been most widely used, extraction apparatus is simple and widely available Sodium chloride is inexpensive and not hazardous.

With the Sodium Chloride separation a known volume (normally 50 ml) of sediment is added to a separating funnel using a metal spoon and 200 ml of saturated NaCl added. A stopper is added and the mixture agitated by hand for 2 minutes, and then allowed settling for 2 minutes. The supernatant is then transferred to suction filtration via a buckner funnel and passed through 10 µm retention glass fibre filter paper. Filter papers are removed and stored in sealed petri dishes prior to examination under a microscope. The NaCl separation procedure is repeated three times with each sediment sample to ensure a high proportion of buoyant debris is removed data form the three filter papers are added together. Subtidal sediments are typically finer than those from sandy beaches and so may be likely to clog filter papers and produce a relatively thick layer of fine natural particulates. This problem can be reduced by repeatedly filtering smaller volumes of sediment on and then pooling data form each separation. We recommend using a concentrated saline NaCl solution (1.2 g cm⁻³) to achieve bulk separation according to density. This is inexpensive, readily available, non-toxic has been most widely used to date and will achieve good separation for most polymers.

Filter papers can then be examined sealed within the petri dishes under a binocular microscope. The abundance of any pieces of unnatural appearance (due to colour, shape, dimensions) is recorded. Positions can be marked on the top of the petri dish lid to facilitate relocation / removal. It is advantageous for analysts to be familiar with the appearance of microplastics items (Picture 5 below) and also familiar with natural particulates such as sand / plankton. Trained plankton analysts can achieve around 70% accuracy for fragments down to 50-100 µm. For smaller (<100 µm) fragments FT-IR or Raman spectroscopy is essential. Even within the range 500 – 100 µm it is important to have a proportion of the items that are visually identified as plastic to be formally checked by FT-IR or Raman spectroscopy.

**Picture 5:** Examples of microplastic pieces collected from waters around Plymouth, UK (Courtesy of S. Sadri, Plymouth University).
**Separation from seawater (e.g. suspended material and seawater retained form plankton nets)** - Samples in seawater can be passed through a 500 µm sieve, and liquid passing through the sieve then filtered through 10 µm retention glass fibre filter paper using a Buckner funnel. Filter papers can then be examined under a dissecting microscope as for intertidal sediment. Sample on CPR silk filter screens can be examined directly under the dissection microscope.

<table>
<thead>
<tr>
<th>Size</th>
<th>Record size of each item. Minimum resolution is to allocate into bin sizes of 100 µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>Plastic fragments, pellets, filaments, plastic films, foamed plastic, granules, and styrofoam</td>
</tr>
<tr>
<td>Shape</td>
<td>For pellets: cylindrical, disks, flat, ovoid, spheruloids; For fragments: rounded, subrounded, subangular, angular; For general: irregular, elongated, degraded, rough, and broken edges</td>
</tr>
<tr>
<td>Colour</td>
<td>Transparent, crystalline, white, clear-white-cream, red, orange, blue, opaque, black, grey, brown, green, pink, tan, yellow</td>
</tr>
</tbody>
</table>

**CATEGORIES FOR MICROPARTICLES**

<table>
<thead>
<tr>
<th>Material</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plastic</td>
<td>Plastic fragments rounded</td>
</tr>
<tr>
<td></td>
<td>Plastic fragments subrounded</td>
</tr>
<tr>
<td></td>
<td>Plastic fragments subangular</td>
</tr>
<tr>
<td></td>
<td>Plastic fragments angular</td>
</tr>
<tr>
<td></td>
<td>Cylindrical pellets</td>
</tr>
<tr>
<td></td>
<td>Disks pellets</td>
</tr>
<tr>
<td></td>
<td>Flat pellets</td>
</tr>
<tr>
<td></td>
<td>Ovoid pellets</td>
</tr>
<tr>
<td></td>
<td>Spheruloids pellets</td>
</tr>
<tr>
<td></td>
<td>Filaments</td>
</tr>
<tr>
<td></td>
<td>Plastic films</td>
</tr>
<tr>
<td></td>
<td>Foamed plastic</td>
</tr>
<tr>
<td></td>
<td>Granules</td>
</tr>
<tr>
<td></td>
<td>Styrofoam</td>
</tr>
<tr>
<td>Other</td>
<td>Other (glass, metal, tar)</td>
</tr>
</tbody>
</table>

**Table 9:** Categories used to describe microplastics appearance

<table>
<thead>
<tr>
<th>Polymer type</th>
<th>Density (g cm⁻³)</th>
<th>No. of studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>polyethylene</td>
<td>0.917–0.965</td>
<td>33</td>
</tr>
<tr>
<td>polypropylene</td>
<td>0.9–0.91</td>
<td>27</td>
</tr>
<tr>
<td>polystyrene</td>
<td>1.04–1.1</td>
<td>17</td>
</tr>
<tr>
<td>polyamide (nylon)</td>
<td>1.02–1.05</td>
<td>7</td>
</tr>
<tr>
<td>polyester</td>
<td>1.24–2.3</td>
<td>4</td>
</tr>
<tr>
<td>acrylic</td>
<td>1.09–1.20</td>
<td>4</td>
</tr>
<tr>
<td>polyoximethylene</td>
<td>1.41–1.61</td>
<td>4</td>
</tr>
<tr>
<td>polyvinyl alcohol</td>
<td>1.19–1.31</td>
<td>3</td>
</tr>
<tr>
<td>polyvinylchloride</td>
<td>1.16–1.58</td>
<td>2</td>
</tr>
<tr>
<td>poly methylacrylate</td>
<td>1.17–1.20</td>
<td>2</td>
</tr>
<tr>
<td>polyethylene terephthalate</td>
<td>1.37–1.45</td>
<td>1</td>
</tr>
<tr>
<td>alkyd</td>
<td>1.24–2.10</td>
<td>1</td>
</tr>
<tr>
<td>polyurethane</td>
<td>1.2</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 10:** Number of Studies That Identified Polymer Type among the Sorted Microplastic Debris and Specific Densities of Different Polymer Types (n = 42 studies). From Hidalgo-Ruz et al. (2012).
Formal identification of particles using FT-IR or Raman Spectroscopy – this apparatus is relatively expensive and requires a trained operator. It is less critical for routine monitoring of larger fragments >500 µm. However it should be considered essential for fragments <100 µm and a proportion (5 – 10%) of all samples should be routinely checked to confirm the relative accuracy of any visual examination. This is achieved by comparing the spectra from the unknown sample collected from the environment against that of a known standard polymer in a database (Figure 1). It should be noted that this method is only definitive where a good match is obtained and this is not always possible. A suitable approach (used by one of us - RCT) would be to automatically accept any match >70% similarity, to individually examine matches between 60-70% similarity rejecting any samples which do not show clear evidence of peaks corresponding to known synthetic materials and to routinely reject (as being synthetic) any samples which produce spectra with a match <60%.

7.5. Recommended methods for sampling microplastics

7.5.1. Guidelines for sampling intertidal beach sediments

Goal: to determine number of microplastics per cm³ of strandline?

How data users can use this data: to compare the abundance between locations or times

Our recommendation is that microplastics should be monitored on the top of the shore (strand line) and where available on sandy shores (0.1 – 0.0125 mm sediment diameter). Samples should be collected from the surface 5cm of the sediment surface. This will maximise the potential for comparison between regions. Our recommendation is that five replicate samples be collected from the strandline at each site. Each replicate should be separated by at least 5m. Replicates can be distributed in a stratified random manner so as to be representative of an entire beach or a specific section of beach. This ultimately depends on the
specific locations and questions of interest at a local scale. Sampling should be conducted separately for each of two size categories.

**Microplastics 1 – 5mm** - These should be collected as an additional entirely independent sample at each location as sand should be obtained AFTER the sampling the smaller size fraction (≤1 mm see below) in order to minimise the risk of contamination from persons undertaking the sampling itself. The sediment can be sampled by collecting with a metal spoon or trowel the top 5 cm of sand from the area contained within a metal 50 cm x 50 cm quadrat and passing through a 1 mm metal sieve and then be stored in metal (e.g. foil) or glass containers (i.e. not plastic). Record the volume of sediment examined. Our recommendation is that these be sampled using an extension of the protocol for meso debris (5-25mm) which uses a 5 mm sieve to separate debris from sediment. This protocol can easily be extended by including a second metal sieve of 1 mm mesh to achieve volume reduction of the sediment sample in the field. Preferably these sieves could be stacked together.

**Microplastics 20 µm – 1 mm** - need to be collected as a bulk sample of sediment and subsequently extracted in the laboratory by density separation (see later). Sediment should be collected from the top 5 cm of sand using a metal spoon (suggest 15 ml) and then be stored in metal (e.g. foil) or glass containers (i.e. not plastic). Because the weight of sediment can vary considerably according to water content and type of sediment we suggest standardising sampling by volume. Approximately 250 ml of sediment should be collected of 50 ml will normally be sufficient for density separation. The weight used for the density separation should also be recorded so that the quantity of debris per gram can be determined approximately if required. The sample can be collected by kneeling on the strand line and collecting a series of scoops at arms-length at intervals within an arc shaped area to the front.

**Precautions to minimise contamination (field)** – Since the majority of microdebris is plastic care should be taken to avoid use of plastic. Metal scoops, trowels and quadrates should be used. These should be cleaned prior to sampling and wrapped in tinfoil or stout paper (not tissue as this may fray and introduce fibres). Samples should be collected and stored in stout paper bags / envelopes, metal or glass containers. People undertaking the sampling should minimise any synthetic clothing and avoid wearing garments that readily shed synthetic fibres (such as fleece). Position of the person sampling should be down-wind of the sampling area.

**Meta data** – To accompany each sample or set of replicates as appropriate it is worth noting any obvious local point sources of microdebris such as the proximity of relevant manufacturing industry or bulk handling facilities (e.g. for plastic pellets or powders) or local sources of small items of debris (e.g. sewage outfalls). Date of sampling, co-ordinates of location, sediment particle size. Also record relevant information from AQ/QC procedures such as the quantity of contamination recorded in blanks.

**Required reporting units** – items / ml of sediment, size of microparticles, in addition because our understanding of the sources, pathways and sinks for microplastics are currently limited, and because the main costs are in collection and processing it is considered very worthwhile to record additional observations including: relative abundance of main colours and shapes. If FT-IR or Raman is used then polymer type should also be recorded (Tables 9 and 10). Microplastics should be categorised according to size with a minimum level of resolution being to allocate the material found in to size bins of 100 µm (20-100 µm, 101-200 µm, 201-300 µm etc).

### 7.5.2. Recommendations for sampling surface waters

**Goal**: to determine number of microplastics per m³ of seawater?

**How data users can use this data**: to compare the abundance between locations or times

Deploy the net from the vessel out of the wake zone. The turbulence inside the wake zone does not allow for a representative surface sample to be collected. Use a spinnaker boom or A frame to deploy the trawl away from the side of the vessel. Keep close eyes on the net while trawling to observe its performance and adjust speed and cable lengths if necessary. Avoid periods of plankton blooms as this may clog the net and complicate further analysis.

Maintain a steady linear course at a constant speed. The hi-speed trawl can be deployed up to 8 knots, build up the speed slowly towards maximum speed. Higher speeds reduce the ability to sieve seawater, creating a bow wake in front of the trawl. The net can jerk forcefully as it surfs and ploughs through the waves, so watch the net while you trawl to observe its performance and adjust speed accordingly. Begin
with a half hour trawl. Use your judgment on duration based on your field observations and allowed trawling time e.g.: deploy the trawl when leaving a station and trawl up to the next station. Recover and secure trawl on the deck. Record STOP immediately and note down the values on the flow meter.

In order to process the sample for storage - rinse the net from the outside with a hose or bucket to concentrate the sample in the cod end. Never rinse the sample through the opening of the net.

a) You will need a large bowl, squirt bottles, sample container, spoon, tweezers, and a preservative (isopropyl alcohol or formalin).

b) Remove the cod end over a bucket, as a precaution to catch any spillage

c) Transfer sample into a large bowl.

d) Invert the cod end and wash it out from the outside using very little water, scrape left over sample into the large bowl using the spoon. Rinse the spoon into the bowl.

e) Pour entire sample into the sample container and add preservative. A sample may consist out of several containers.

Label the lid and outside of the sample container with the trawl number, date and time. Use waterproof marker for labels. Include a waterproof label in the sample. This label contains the same information as the external labels.

Sample Preparation:

a) Drain sample through 5 mm sieve into one large bowl.

b) Use fresh water wash bottle to rinse off plastic particles adhering to the inside of the sample jar.

c) Rinse sample inside sieve in order to separate plastics thoroughly.

d) Transfer each size class to a different large Petri dish.

e) Rinse equipment gently with the wash bottle so that no plastic particles are left behind.

f) If the process above does not result in adequate liquid in the Petri dishes for sorting, then add sufficient water to float all plastic bits – do not overfill

NOTE: If the sample is too large to perform the procedure above for the entire sample, then split carefully, sort separately, and combine the data later.

Separating sample into size classes >5mm and <5mm:

a) Place each Petri dish under a microscope.

b) Using forceps, remove all recognizable pieces of floating plastic.

c) Rinse off plastic bits with fresh water wash bottle to make sure smaller particles or plankton are not sticking to them.

d) Place rinsed bits of plastic in a separate labelled empty vial and set aside for later drying, typing, counting and weighing.

For size class <5mm, use a spoon to remove all remaining plastic. There may be more there, so start looking at centre of Petri dish and move out to the sides. Use a dissecting microscope to conduct a more thorough check of the sample. Once the plastic, plankton and organic debris are separated, the plastic is size classed and dried. The wet weight of the plankton and organic debris are measured and then dried.

Drying of separated plastic:

a) Set your drying oven at 20°C.

b) Sieve sample and spread onto Petri dishes or leave in sieves.

c) Place sample in oven or a secure dry location.

d) Dry samples at 20° for about 30 minutes. If the samples are still wet after 30 minutes, leave them in the oven and check regularly. If they are left in a dry location, then check every few hours.

When the sample comes out of the oven it is placed in a dissector to cool, then weighed.

Sorting plastic to determine type, count and weight:

a) With each size class dried in its own Petri dish or sieve, use forceps to sort sample into different types of plastic as categorized on the data sheet (see below).

b) Count number of plastics for each type for each size category.

c) Tare the scale with Petri dish and weigh sample on a gram scale.

d) Record weight and count on the data sheet
e) Transfer sorted and weighed plastic to labelled vials.

The plastic is removed from the sieves and each of the six size classes is sorted into shape type (fragment, pellet, line, film, and foam). The colour of each piece of plastic is also recorded (by size class) on a separate sheet. During this process each container is labelled and all data sheets are updated.

Precautions to minimise contamination (field) - Since the majority of microdebris is plastic care should be taken to avoid use of plastic during the protocol. Metal equipment should be used and should be cleaned prior to sampling and wrapped. Samples should be collected and stored in metal or glass containers. People undertaking the sampling should minimise any synthetic clothing and avoid wearing garments that are likely to shed synthetic fibres (such as fleece). Position for those undertaking sampling down-wind of the sampling apparatus during deployment and recovery. Prior to use equipment can be swabbed with damp filter papers which are sealed in petri dishes and checked for contamination.

Meta data – record: date, mesh size, aperture size, type of net, depth (preferably either at the sea surface or within surface 10m for greatest inter-comparability among sampling programmes) distance towed, location of tow (in / out of water) volume of water filtered (this is best obtained from a current meter as this will allow for tidal movement as well as ship speed). Also prevailing weather conditions and sea state, together with any relevant information on the volume of plankton or other particulates sampled, for example if there is concern that the net may have become clogged due to high concentration of plankton, this must be recorded.

Required reporting units – items / m of water, size, colour and shape etc. If FT-IR or Raman is used then polymer type should also be recorded (see descriptions in Tables 9 and 10). Microplastics should be categorised according to size with a minimum level of resolution being to allocate the material found into size bins of 100 µm (20-100 µm, 101-200 µm, 201-300 µm etc). See Figure 2 for example of recording sheet.

Figure 2: Example of standard recording sheet

7.6. Recommendations for sampling Subtidal Sediments

Goal: to determine number of microplastics per cm³ of sediment from the seabed?

How data users can use this data: to compare the abundance between locations or times

Material can be collected using any approach that recovers a sample of relatively undisturbed surface sediment from the sea bed (e.g. van veen grab, multi corer, box core etc.). Once recovered onto the vessel a small sample of sediment ideally around 250ml is recovered to best represent the location of the original 5cm surface to sub surface of the seabed. Because the weight of sediment can vary considerably to water content we suggest standardising sampling by volume. Avoid sampling next to the edge of the apparatus to minimise risk of contamination form the equipment (e.g. pain flakes other contamination on the grab / core). The sample is transferred to a metal or glass container for subsequent density separation / spectroscopy.

Meta data – Date, location, depth, sea state, type of equipment used, volume of sample collected, any relevant information e.g. complete quantitative sample, or some material lost during recovery etc. nature of sea bed sediment including particle size, organic matter, any available data on biota present.
Precautions to minimise contamination (field) - Since the majority of microlitter is plastic care should be taken to avoid use of plastic during the protocol. Metal equipment should be used and should be cleaned prior to sampling and wrapped. Samples should be collected and stored in metal or glass containers. People undertaking the sampling should minimise any synthetic clothing and avoid wearing garments that are likely to shed synthetic fibres (such as fleece). Position for those undertaking sampling down-wind of the sampling apparatus during deployment and recovery. Prior to use equipment can be swabbed with damp filter papers which are sealed in petri dishes and checked for contamination.

Required reporting units – items / ml sediment, size, colour and shape etc. If FT-IR or Raman is used then polymer type should also be recorded (see descriptions in Tables 9 and 10).

7.7. Suggestions for sampling microplastics in biota

*Goal:* to determine number of microplastics per individual or part thereof (e.g. gut)?

*How data users can use this data:* to compare abundance in individuals between locations or times

*Sampling* – At present it is not possible to recommend particular species or times of year that would be most appropriate to specifically monitor microplastics. For efficiency we suggest routine examination for microplastics in any organisms that are already being considered for macrolitter (e.g. Fulmars in northern Europe, see Biota Section 6). If individuals are live immediately prior to sampling then they must be humanely killed adhering to any prevailing ethical legislation. In many cases it may be possible to examine organisms that are dead at the time of collection for example fish or invertebrates from trawls or other sampling programmes, seabirds or turtles that have been washed ashore dead. Small individuals can be stored whole. For larger individuals the digestive tract can be dissected but otherwise left intact and stored intact.

Examination of the gut is facilitated with a dissecting microscope. The gut is slit open using scissors and examined immediately. Depending on the size of the organism the digestive tract can be examined in its entirety or samples of gut wall (e.g. 10cm x 10cm (or similar standard area) can be removed and viewed under a dissecting microscope. Any fragments of an unusual appearance are removed with forceps and placed on clean filter papers in petri dishes which are then sealed prior to further examination for example via spectroscopy.

*Meta data* – Please record: species and standard dimensions of length and weight (e.g. carapace length for crustaceans) together with gender, physical condition, alive, injured or dead at time of collection, reproductive state, quantity of food present in digestive tract, presence of parasites etc. Location collected, circumstances of capture, part of routine monitoring, from fisheries landings, individual brought to recovery facility (e.g. birds, seals).

Precautions to minimise contamination (field) - If organisms are collected alive in nets the possibility of plastic ingestion in the sampling net must be eliminated. Hence collecting fish from plankton nets where microplastic has been shown to accumulate is not a reliable approach. Where fish are caught in standard mesh nets the issue of contamination from the net is considerably reduced. However a confirmatory step should be included using FT-IR to confirm that fragments form the organisms do not match those of the polymer used in the nets.

Required reporting units – Items / g of intestine, size, colour and shape etc. If FT-IR or Raman is used then polymer type should also be recorded (see descriptions in Tables 9 and 10). Species of organisms and standard dimensions e.g. carapace length for crustaceans should be recorded and weight. Microplastics should be categorised according to size with a minimum level of resolution being to allocate the material found in to size bins of 100 µm (20-100 µm, 101-200 µm, 201- 300µm etc).
7.8. Recommendations for laboratory separation of microplastics from bulk samples

**Laboratory separation from intertidal sediment** - we recommend using a concentrated saline NaCl solution (1.2 g cm$^{-3}$) to achieve bulk separation according to density. This is inexpensive, readily available, non-toxic has been most widely used to date and will achieve good separation for most polymers. A known volume (normally 50 ml) of sediment is added to a separating funnel using a metal spoon and 200 ml of saturated NaCl added. A stopper is added and the mixture agitated by hand for 2 minutes, then allowed to settle for 2 minutes. The supernatant is then transferred to suction filtration via a Buckner funnel and passed through a 10µm retention filter paper. Filter papers are removed and stored in sealed petri dishes prior to examination under a microscope. The NaCl separation procedure is repeated three times with each sediment sample to ensure a high recovery (RCT unpublished data) of buoyant debris. Data from the three filter papers are added together.

**Laboratory separation from subtidal sediment** – This is conducted according to the protocol for intertidal sediment and using the same precautions to minimise / quantify procedural contamination. However subtidal sediments are typically finer than those from sandy beaches and so may be likely to clog filter papers and produce a relatively thick layer of fine natural particulates. This problem can be reduced by repeatedly filtering smaller volumes of sediment on and then pooling data form each separation.

**Formal identification of particles using FT-IR or Raman Spectroscopy** - This is not critical for identification of larger fragments (>500 µm). However it should be considered essential for fragments < 100 µm and a proportion (10%) of all samples should be routinely checked to confirm the relative accuracy of any visual examination. This is achieved by comparing the spectra from the unknown sample collected from the environment against that of a known standard polymer in a database. It should be noted that this method is only definitive where a good match is obtained and this is not always possible. A suitable approach would be to automatically accept any match >70% similarity, to individually examine matches between 60-70% similarity rejecting any samples which do not show clear evidence of peaks corresponding to known synthetic materials and to routinely reject (as being synthetic) any samples which produce spectra with a match < 60% . Microplastics should be categorised according to size with a minimum level of resolution being to allocate the material found in to size bins of 100 µm (20-100 µm, 101-200 µm, 201-300µm etc).

**Precautions to minimise contamination (laboratory)** – Extreme care must be taken to ensure the processing area is meticulously clean and in particular free from dust or particles. Cotton laboratory coat should be worn, minimise any synthetic clothing (e.g. synthetic fleece), do not process samples near to carpeted areas, minimise air circulation in the processing area (windows doors etc. that may carry air-borne particulates). Ensure samples are exposed to the air for the absolute minimum period required to transfer them between containers. At all other times containers remain covered. Ensure all containers and sampling equipment is scrupulously clean prior to use. Controls of clean NaCl should be run through apparatus and collected over filter papers as described above as a procedural control (blank) to check for contamination. Repeat cleaning until contamination in blanks is zero or negligible. As procedural controls to check ambient cleanliness place unused clean filter papers in petri dishes. Remove the lid and wrap it in clean foil, leave the petri-dish open for a fixed time period relevant to the time period for which samples might be exposed to the air during examination. Seal the petri-dish with the lid and count any fragments which have settled on the filter paper. Procedural contamination should < 10% of the average values determined form the samples themselves.

When examining biota in the laboratory it is important to record the time between the digestive tract first being cut open and the end of the examination. This can then be compared to levels of contamination collected on clean filter papers left exposed to the air for similar periods adjacent to the working area. Hence it is beneficial to work carefully and quickly once the digestive tract is opened. For larger specimens and in particular where there is a substantial quantity of food in the gut it may be necessary to wash the contents from the digestive tract using clean saline and collect in a petri dish and sealed from the air. Any fragments of unusual appearance should be removed and archived in sealed petri dishes prior to formal identification with FT-IR.
7.9. References


