Best Practices for the Extraction and Enumeration of Microplastics in Various Marine Environmental Matrices

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BEST PRACTICES FOR THE EXTRACTION AND ENUMERATION OF MICROPLASTICS IN VARIOUS MARINE ENVIRONMENTAL MATRICES

by

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ABSTRACT

Dimitrijevic, J., Kelly, N.E., Moore, A.M., Breeze, H., and Ross, P.S. 2019. Best practices for the extraction and enumeration of microplastics in various marine environmental matrices. Can. Tech. Rep. Fish. Aquat. Sci. 3334: vi + 79 p.

There is growing need and interest in quantifying and characterizing microplastics in Canada's coastal and open ocean environments. However, microplastic research is a developing field, where sampling, extraction, and reporting techniques are evolving rapidly. This report aims to provide necessary information for researchers to start working in the field quickly with rigorous standards, by conducting a thorough examination of the most prominent methods currently available. A comprehensive review of 46 papers is provided with an overview of sampling and extraction techniques and the advantages and limitations for each. Papers were selected based on prominence in the literature and variation in sample collection, polymer identification, and reporting practices. Focus is given on controlling for background and procedural contamination, investigating microplastic abundances to size fractions <50 µm, and accurate reporting for sediment, seawater, zooplankton, and shellfish. Methods to extract microplastics from each matrix are discussed with a brief introduction on the state of research for each as of March 31, 2019. While this document focuses on microplastic particles between 1–5000 µm in size, a brief discussion on nanoplastics, which are particles smaller than this range, is also provided. Various options for microplastic identification and enumeration are evaluated, including the critical step of post-extraction chemical identification, which is needed to confirm the identity of microplastic particles from all types of samples. This guide should help researchers characterize and quantify microplastics in the marine environment, which will help develop programs that provide accurate, high resolution data useful for management of this anthropogenic stressor.

RÉSUMÉ

Dimitrijevic, J., Kelly, N.E., Moore, A.M., Breeze, H., and Ross, P.S. 2019. Best practices for the extraction and enumeration of microplastics in various marine environmental matrices. Can. Tech. Rep. Fish. Aquat. Sci. 3334: vi + 79 p.

La quantification et la caractérisation des microplastiques présents dans les zones côtières et les océans du Canada sont des sujets récents et d'intérêt. Ceci étant dit, la recherche associée aux microplastiques est en développement et dont les techniques d'échantillonnage, d'extraction et d'analyse évoluent rapidement. Ce rapport met de l'avant l'information nécessaire afin de permettre aux chercheur(e)s de débuter un échantillonnage rapidement tout en ayant des standards robustes, en présentant une synthèse des méthodes les utilisées ainsi que les plus récentes. Une revue littéraire de 46 articles primaires est présentée incluant un survol des techniques d'échantillonnage, d'extraction, ainsi que les avantages et limites de chacune. Les articles considérés furent sélectionnés basés sur leur visibilité comme référence, la variété des techniques utilisées, l'identification des polymères et les méthodes de diffusion des résultats. L'accent fut entre autres sur le contrôle des valeurs ambiantes (de base), de la possibilité de contamination lors des manipulations, l'évaluation de l'abondance des microplastics <50 µm, la présentation adéquate de résultats provenant de sédiment, zooplancton et bivalves. La méthode d'extraction des microplastiques de chacun de ces substrats est discutée incluant une évaluation de l'état global des publications sur le sujet en date du 31 mars 2019. Bien que ce document se concentre sur les microplastiques incluant les particules de taille entre 1–5000 µm, une brève discussion sur les nanoplastiques de taille encore plus réduite est aussi présentée. Diverses méthodes d'identification et de quantification des microplastiques sont évaluées, incluant l'étape critique de l'identification chimique post-extraction, qui est nécessaire afin de confirmer l'identité des microplastiques provenant des différents types d'échantillons. Ce guide devrait aider les chercheur(e)s à caractériser et quantifier les microplastiques présents dans l'environnement marin, et ainsi développer des programmes pouvant permettre la production de résultats robustes, de haute précision et utile pour la gestion de stresseurs anthropique en milieux aquatiques.

1.0 BACKGROUND

Fisheries and Oceans Canada has renewed its focus on researching the individual and cumulative effects of ecosystem stressors by establishing the national Ecosystem Stressors Program in 2016. There is growing need and interest within and outside of DFO to characterize and quantify microplastics in coastal and open ocean environments. Plastic pollution is ubiquitous in the modern natural world (Geyer et al. 2017); however, very little is known about the distribution, abundance, origin, and potential impacts of microplastics in Canada (Anderson et al. 2016). Thus, it is important to develop baseline abundances, identify major sources and types of microplastics, and understand the impacts microplastics are having on biota and human health both individually and cumulatively with other stressors (e.g., nutrient loading, climate change, and contaminants).

Microplastic research is considered an emerging science (Jahnke et al. 2017) and globally microplastic sampling and extraction techniques are not standardized, making comparison of results across time and space difficult (Catarino et al. 2017; Crichton et al. 2017; Mai et al. 2018). As research requires an initial significant investment in reagents and equipment, monitoring and research programs need to be undertaken with great consideration and care to appropriate methodologies to ensure accurate, high-resolution data and useful results that can be compared within and across DFO regions as well as with data from external researchers.

This report aims to provide necessary information for researchers to start working in the microplastics field quickly with rigorous standards. Guidance was developed by the Ocean Wise Conservation Association's Environmental Microplastics Lab scientists (authors J.D. and P.R.) as requested by, and in consultation with, DFO Maritimes Science and Aquatic Ecosystems (authors N.K., A.M., and H.B.). A comprehensive review of 46 papers is provided with an overview of sampling and extraction techniques and the advantages and limitations of each. Papers were selected based on prominence in the literature and variation in sample collection, polymer identification, and reporting practices. Focus is placed on controlling for background and procedural contamination, investigating microplastic abundances to size fractions <50 µm, and accurate reporting for sediment, seawater, zooplankton, and shellfish. The current (as of March 31, 2019) state of research is provided in brief for each matrix, and extraction methods from each matrix are discussed with recommendations for modification and improvement with consideration to expense, time for analysis, and data resolution obtained. A brief discussion on nanoplastics and available technology to identify polymer chemistry is also provided.

This report provides a foundation for researchers interested in pursuing microplastics research; however, recent literature should always be consulted prior to conducting experiments to incorporate the most recent knowledge into final decisions on experimental design and analysis.

2.0 MICROPLASTIC CHARACTERIZATION

Reporting of plastic debris in the marine environment began in the 1970s (Jambeck et al. 2015; Mai et al. 2018), and the term 'micro-plastic' was coined in 2004 (Thompson et al. 2004) when the Plymouth research group began investigating small plastic pieces along the coast of England. The term microplastic is now globally accepted as plastic polymers <5 mm in diameter (Arthur et al. 2009). An initial lower limit of 333 µm was proposed based on mesh size of neuston nets most commonly used to sample seawater (Arthur et al. 2009); however, the lower limit is now

shifting to 1 µm (Shim et al. 2017). It is also generally accepted that the number of microplastics smaller than 333 µm increases exponentially as particle size becomes smaller (Andrady 2017). It is important to identify the smallest possible size fractions of microplastics as bioavailability to small organisms increases when particle size decreases (Shim et al. 2017). Particles < 1µm are generally considered nanoplastics, although there is no current consensus (Gigault et al. 2018; but see Hartmann et al. 2019) (see also Section 7.0).

Sources of microplastics to the marine environment include mismanaged waste, waste water effluent, storm water outflows, maritime fishing activities (i.e. gear loss), and atmospheric fallout (Browne et al. 2011; Jambeck et al. 2015; Dris et al. 2016; Martin et al. 2017). Density is an important consideration in microplastics research as it influences which sampling methods and extraction techniques will be effective in separating microplastics from their matrix. Plastic polymers most commonly found in the ocean (Fig. 1; see also Fig. 3 in Silva et al. 2018) have different densities depending on the polymer (Table 1), which is a predictor of where microplastics may settle in the marine environment (Van Cauwenberghe et al. 2015a). However, for smaller-sized particles (< 250 µm) at low Reynolds numbers, sinking rates may be influenced more by size and shape than polymer type (Kaiser et al. 2019). Biofouling and weathering as well as incorporation into plankton aggregates (Long et al. 2015) affect particle buoyancy and alter settling patterns in the water column (Van Cauwenberghe et al. 2015b). Additives to virgin polymers can also affect density (Andrady 2011).

3.0 CONTAMINATION

Due to their ubiquity in the environment, microplastics sampling and analysis are prone to contamination from multiple sources in both field and laboratory settings; for example, from sample exposure to air, from solutions, clothing worn by researchers, and transfer from equipment (Fig. 1e; Lusher et al. 2017b). A study along the Seine River (France) showed 2–355 particles·m^{-2}·day of atmospheric fallout, wherein 29% of the microfibres were synthetic (e.g., PET, polyamide, polyurethane) or a mix of natural and synthetic materials (e.g., cotton and polyamide; Dris et al. 2016); the highest rates of fallout occurred in urban centres. In South Korea, Song et al. (2014) noted a high abundance of paint particles in water samples taken from the surface microlayer (SML), with the authors noting that though unlikely, contamination from paint peeling off the research vessel was possible. Adequate contamination control measures, including clothing worn by researchers, material and maintenance of equipment, creation of a polymer library, and inclusion of positive and negative controls, are required in the field and lab to ensure reliable and comparable results. Overall, it is recommended that procedural contamination should account for <10% of the average values of a study's samples (Galgani et al. 2013).

In both field and laboroatory environments, it is necessary to use ultrapure and/or filtered water for rinsing equipment and filtering reagents and/or fixatives to reduce contamination. For rinsing equipment, ultrapure water (e.g., from a MilliQ system; www.emdmillipore.com/) is recommended. If a water purification system is not available, filtered water (using tap water or seawater) can be created in the laboratory via vacuum filtration using a sub-micron (<1 µm) filter. If preservatives or reagents are to be used, filtration of the solution prior to use is required to reduce potential contamination (De Witte et al. 2014). Vacuum filtering using a filter paper that is compatible with the solution is critical to ensure the filter is not impaired by exposure to certain chemicals, and ensures that the filter media does not shed fibres or particles into the

sample. For both purposes, glass microfibre filters (e.g., Whatman® GMF/GF filters) are recommended, as they are manufactured from 100% borosilicate glass, are binder-free, biologically inert, resistant to most chemicals, can withstand temperatures up to 500°C, and are cost-effective. Further information on the chemical compatibility of filter media can be found at: https://cdn.gelifesciences.com/dmm3bwsv3/AssetStream.aspx?mediaformatid=10061&destinationid=10016&assetid=16470.

3.1 FIELD CONTROL MEASURES

Generally, wearing clothing made from plastic materials (Browne et al. 2011) or wool, which is prone to shedding, should be avoided and colour and type of clothing worn by the field team should be recorded. Latex or nitrile gloves and non-plastic equipment should be used as much as possible (Mai et al. 2018). Observations on other potential contamination sources should be recorded. If plastic material is used for sampling (e.g., plastic netting), a piece should be retained and analyzed to obtain the chemical signature for inclusion in a polymer library (Lusher et al. 2017b). Contamination microplastics can then be identified and removed from the analysis.

Ideally, field apparatus should be rinsed with ultrapure or filtered water and sealed in the laboratory for later use (Section 3.0) (Lusher et al. 2017b); however, filtered tap water or seawater can also be taken to the field to rinse equipment in situ as necessary. Exposure time (i.e. the time a sample is exposed to air after being removed from the marine environment) should be minimized to reduce airborne contamination (Lusher et al. 2015a). To properly account for exposure time, background blanks are recommended (see Section 2.4). Procedural blanks (Section 3.3) can also be collected in the field if the sampling method allows.

3.2 LABORATORY CONTROL MEASURES

A clean room is the best location to operate a microplastics lab, although such a facility does not completely eliminate the risk of airborne contamination (Woodall et al. 2015; Catarino et al. 2017; Comnea-Stancu et al. 2017). A clean room is defined as a laboratory space that controls airborne particulate matter down to 0.1–5 µm. The room should be non-ventilated or have a negative flow, limited access (e.g., doors and windows closed), and foot traffic should be minimized (Lusher et al. 2017b). Adding filters to vents (Courtene-Jones et al. 2017) and muslin cloth over doorways (Woodall et al. 2015) can further reduce the import of microfibres into the lab space. A laminar flow hood (designed for PCR work and cell or algal culture) provides many of these requirements, is much cheaper and easier to implement than establishing a clean room, and reduces contamination by 96.5% (compared to only 50% reduction under a fume hood alone; Wesch et al. 2017). We recommend that sample processing involving hazardous chemicals should be conducted in a fume hood (Lusher et al. 2017b); processing not involving hazardous chemicals should be conducted under a laminar flow hood whenever possible (Foekema et al. 2013; van Cauwenberghe and Janssen 2014; Catarino et al. 2017; Lusher et al. 2017b).

Lab technicians should wear nitrile gloves (Gewert et al. 2017), headscarves (Comnea-Stancu et al. 2017), and (low-shedding) cotton lab coats or Tyvek® coveralls over their clothing. It is also recommended that synthetic clothing not be worn underneath the lab coat (Galgani et al. 2013). Researchers should also take small samples of fibres from all materials (e.g. putative fibres from clothing, curtains, carpets, ceiling tiles, cleaning sponges, etc.) within the vicinity of any laboratory work. These reference samples can be stored in small glass vials and archived for

subsequent reference when encountering odd particles within a sample, to facilitate the idenfication of background airborne contamination. Further, bright or unusually coloured uniforms are recommended as fibres are easier to identify as contamination. Once a suit is purchased, the material should be analyzed to obtain the chemical signature for inclusion in a polymer library. Contamination fibres can then be identified and removed from the analysis.

All working surfaces (particularly on and around the microscope) should be wiped with 70% ethanol and paper towels or a brightly coloured sponge (for ease of contamination identification) directly before lab work begins (Lusher et al. 2013, 2015b; Barrows et al. 2017; Prata et al. 2019). The use of glass and metal equipment is preferable to plastic consumables, although if no alternative exists, plastic consumables should be used directly from packaging (Prata et al. 2019). All equipment used during microplastic extraction should be rinsed with filtered (see Section 3.0) or ultrapure water three times prior to use (Li et al. 2015; Lusher et al. 2015b; Rochman et al. 2015; Wagner et al. 2017; Wang et al. 2017). Heating glassware and other equipment (e.g., glass fibre filters) to 500°C for 4 hours has also shown to be effective in reducing cross-contamination (Dris et al. 2018). Reagents should be filtered (see Section 3.0) prior to use (Maes et al. 2017a). Lab equipment without lids should be covered with tinfoil to reduce contamination risk from atmospheric fallout (Nuelle et al. 2014). During visual analysis, a plastic covering placed over top of a stereomicroscope can minimize fibres settling onto samples (Torre et al. 2016). Procedural blanks (Section 3.3) should be used during laboratory procedures to correct for false positives in samples. Both negative (procedural (Section 3.3) and background (Section 3.4) blanks) and positive controls (Section 3.5) should always be conducted (Hermsen et al. 2018).

3.3 PROCEDURAL BLANKS

A procedural blank [i.e. negative blank (Phuong et al. 2018) or blank (Sun et al. 2017)] is the most critical and informative method for controlling for contamination because it provides error rates on the number of microplastics contaminating samples (De Witte et al. 2014). A procedural blank is a “clean” sample (e.g., a sample without the medium being measured for microplastics; Hermsen et al. 2018) that is processed in parallel to a batch of samples in the laboratory. The procedural blank undergoes all steps involved in extraction using the same equipment and reagents for the same duration. Procedural blanks should be replicated a minimum of 3 times per every batch of samples processed. After the extraction, the procedural blank is visually examined using a microscope in the same manner as samples. Suspected microplastics are recorded and a subset is sent for chemical polymer identification [minimum 10% (Lusher et al. 2017b)]. A conservative approach can be adopted whereby any particle identified in a sample that resembles a contaminant be eliminated from the analysis (Martin et al. 2017). Lusher et al. (2014) state that background levels of microplastics should be under 10% of the total reported values. The values obtained are used to develop a correction factor to account for false positives present in the sample and these procedural blanks are subsequently removed from analysis (Lusher et al. 2017b). In the field, collection methods can be repeated in the absence of a sample and stored for analysis in the lab (Galgani et al. 2013; Mai et al. 2018). However, this can be a complex process depending on the method and may not be possible for all sampling situations.

3.4 BACKGROUND BLANKS

A background blank is a clean filter paper (or petri dish) that remains close to a sample or batch during sampling or extraction, and provides information on airborne contamination (Crichton et

al. 2017). During sampling, clean filter papers should be put in rinsed petri dishes and placed in areas near the sampling station but safe from high winds that may blow the filter paper away. The petri dish should remain open when sample collection is occurring and be placed in an area that has similar environmental conditions as the sampling area. Laboratory background blanks involve placing a wetted filter paper within a petri dish and removing the lid each time the sample or batch is exposed to air (Woodall et al. 2015; Davidson and Dudas 2016). If the extraction procedure occurs in multiple locations, the background blank follows the sample around until all processing is complete. Like a procedural blank, the resultant filter paper is examined under the stereomicroscope for airborne contamination and is enumerated. Any microplastic particles found can then be incorporated into the correction factor.

3.5 POSITIVE CONTROLS

Extraction methods should always be trialled using spiked blank samples (spiking clean environmental samples (i.e. sediment, water) with known concentrations of plastic particles), in order to test the efficiency of microplastic recovery (Miller et al. 2017; Silva et al. 2018). This can be followed by a blind extraction test, where the researcher extracting the microplastics does not know what was added into the spiked sample (Davison and Asch 2011). For these tests, it is recommended that the spiked plastics comprise similar shapes, size ranges, and polymer types as that found in environmental samples, as these factors influence extraction efficiency (Wang and Wang 2018). In general, these trials have been hampered by the lack of certified reference materials with known concentrations, which is important for method validation, measurement uncertainty estimations, internal quality control, external proficiency tests, and inter-laboratory studies (Silva et al. 2018).

To date, few publications report performing this level of quality control. For example, in a recent survey of quantification methods of microplastics in sediments, only 7 out of 43 studies conducted laboratory control sample or validation trials (Hanvey et al. 2017). In terms of protocol, typically a mixture of select polymer types (polystyrene, polyethylene) and shapes (spheres or spherical beads) of known number, have been introduced to blank samples (e.g., filtered water) or a biological matrix (e.g., sediment, biological tissues) and then this spiked sample is subjected to the same extraction method under study (Claessens et al. 2013; Davidson and Dudas 2016; Hermsen et al. 2017). The numbers of retrieved microplastics are then compared to the amount initially added to calculate the microplastic detection efficiency of the extraction method. However, the plastics used for spiked samples typically comprise larger size ranges (i.e. > 1 mm) than those found in environmental samples (likely due to logistical limitations in manually creating and enumerating microplastic particles for this purpose), making it difficult for researchers to assess the efficacy of their protocol in extracting smaller plastics. Additionally, efficiencies may be overestimated if 100% of the environmental sample (i.e. sediments) is not removed from the sieves before weighing.

In a recent development, Ziajahromi et al. (2017) reported a validation protocol to create spiked water samples using environmentally representative mixtures of various polymer types (i.e., polyester beads, polyester fibres, polystyrene particles) sourced from common household and laboratory plastics (see also Cole et al. 2014; Nuelle et al. 2014). Instead of enumerating individual particles, the method sorted particles using a series of stacked sieves, which categorized microplastics by weight across various size classes (60–125 µm, 125–250 µm, 250–

500 µm, and >500 µm). After extracting the spiked samples, the recovered particles were weighed, and the efficiency was calculated as (Ziajahromi et al. 2017):

$$Efficiency\ (\%) = \left(\frac{mass\ of\ recovered\ particles}{mass\ of\ added\ particles}\right) \times 100\% \quad (1)$$

This method could be easily adapted to incorporate a wider range of sizes and types of plastics, although a sensitive weigh scale is required. As with all extraction procedures, background blanks should be run concurrently with spiked samples to account for airborne contamination during extraction.

Some reagents (i.e. HNO_3, H_2O_2, $HClO_4$) used in extraction protocols can damage certain polymer types, particularly when used at high temperatures or concentrations (see Section 4.3.3), obscuring samples or underestimating microplastic concentrations (Lusher et al. 2017a). Thus, it is important to conduct application tests to determine the effects of any applied chemical digestive agents on plastics prior to using them for sample digestion (Wang and Wang 2018).

4.0 SAMPLE COLLECTION, PRESERVATION, AND EXTRACTION

The collection of representative samples using appropriate sampling tools in a carefully designed sampling strategy is a critical step for the accurate assessment of microplastics (Crawford and Quinn 2017a). Yet, such standardization is currently lacking in global research (Silva et al. 2018). The methods used to collect microplastic samples in the field affect the abudance, size, and shape of the microplastics obtained, which can greatly influence the outcome of the study. The three main methods used are: (1) selective sampling, where particles visible to the naked eye are directly collected from the environment; (2) volume-reduced sampling, where the volume of a bulk sample is reduced until only specifc items of interest remain; and (3) bulk sampling, where the entire sample is taken without reducing its volume (Crawford and Quinn 2017a). Sampling methods will always develop and adapt over time, so it is crucial that sampling details are recorded to increase reproducibility of the study, identify potential contamination from the sampling gear or during sampling, enable the replication of the sampling, and provide insight into comparability with other studies (Hermsen et al. 2018; Silva et al. 2018). For sampling microplastics in seawater, Hermsen et al. (2018) suggest including information on the exact sampling gear used (i.e. net type, material, and its mesh sizes), recording sampling location, depth, date, sea state, and time of day sampled. For sampling microplastics in sediments, depth, weight or volume, and density and water content of sediments sampled should be recorded (Hanvey et al. 2017).

Environmental samples must be properly preserved and stored to reduce degradation and contamination. When possible, samples should be stored in glass jars with metal lids. Best practices include rinsing all storage containers three times with filtered water (see Section 3.0) prior to use to avoid contamination (Cole et al. 2014; Lusher et al. 2015b; Gago et al. 2018). Taking filtered water to the field (see Section 3.0) is also recommended. This allows field equipment to be rinsed prior to use in situ. If it is not possible to bring large volumes of water to the field, filtered water can be created on site by pouring seawater through a 63-µm sieve into a rinsed metal bucket. Although this water can be used, it may contain microplastics <63µm and is not recommended if the lower limit of quantification is less than this size fraction.

Various methods have been described for storage (Table 2), though the majority of studies do not mention the use of a preservation method (Miller et al. 2017; Hermsen et al. 2018). If

identification and/or characterization of the biological material within a sample is not needed, refrigeration or freezing is ideal as there is no risk of sample loss or an impact on microplastics, and it is a cost-effective and simple storage method (Miller et al. 2017). If using glass and/or metal containers, it is important to leave an expansion gap when freezing samples. Preservation techniques are primarily implemented to obtain information on the biological material present within a sample. However, fixatives can impact polymer integrity. Lusher et al. (2017b) notes that polyamide (nylon; PA) is only partially resistant to 10% formaldehyde solution based on theoretical resistance tables and 100% ethanol is known to damage polystyrene (PS). In contrast, Courtene-Jones et al. (2017) noted that the preference for sample preservation by freezing over a preservative lacked evidence. There was no significant difference observed visually (discolouration, cracks, new cavities, increases to brittleness, and/or changes in length) for spikes of weathered beach fibres, polyethylene terephthalate (PET), high-density polyethylene (HDPE), polyvinyl chloride (PVC), polypropylene (PP), polystyrene (PS) and polyamide (PA) in frozen mussel tissue (-20°C) versus fixed (4% formaldehyde buffered to pH 7.5 using borax for 3 days and then transferred to 70% ethanol for 7 days). There was also no difference on the number of microplastics quantified per gram of wet weight tissue for individual mussels (Courtene-Jones et al. 2017). However, fixatives may interfere with subsequent enzymatic digestion of biological tissues (i.e. zooplankton; see Section 4.3).

We conclude that storage method should be based on research objectives and the type of downstream analysis required (i.e. chemical digestion of biological tissues, FTIR or GC-MS analysis, examination of microplastic-associated microbial communities, or adsorbed chemicals). For long-term monitoring studies, fixatives (pre-filtered in the laboratory to avoid contamination; see Section 3.0) may be advantageous as available freezer space can impose an upper limit on the number of samples able to be stored. If specific microplastic polymers are being targeted, specific preservatives may be used to reduce degradation potential. While the use of formaldehyde, formalin, and ethanol were found to have no effects on microplastics (see Section 4.0), the use of other fixatives for preserving biota should be tested prior to use (Courtene-Jones et al. 2017; Hermsen et al. 2018). Further, strict contamination procedures (see Section 3.0) should always be adhered to alongside the use of procedural and background blanks to reduce and quantify airborne contamination during any application testing.

The general analytical practice for quantifying microplastics in environmental samples consists of four steps: extraction, isolation (or separation), identification, and classification (Shim et al. 2017). However, there are currently a multitude of methods used in the wider literature to accomplish these steps, as microplastics can be extracted from any marine environmental or biological matrix with context-specific considerations. In addition, the extraction and visual identification of microplastics is difficult as the combination of size, shape, and colour are infinite (Hale 2017). The ideal analytical approach should: (1) efficiently remove organic and inorganic material from environmental samples while not affecting the plastic particles; (2) collect the purified samples on filters with a small pore size (<1 μm) appropriate for microscopic measurement (see Section 5.0); (3) establish criteria for visual identification; (4) include measures to reduce cross-contamination; and (5) be cost and labour effective (Löder et al. 2017; Prata et al. 2019). While keeping these suggestions in mind, the remainder of this section provides an overview of sampling and extraction methods for sediment, seawater, zooplankton, and shellfish; however, it is recommended that the content of each section be further reviewed in the recent literature, as method standardization is still a prevalent data gap for the global analysis

of microplastics in the marine environment. The most reliable and accurate (i.e. optimal) method that matches the long-term research objectives should be the primary consideration when choosing a laboratory analysis method for microplastics. If funding restricts the protocols subsequently chosen, the assumptions and limitations of the methods should be clearly stated in any publication.

4.1 SEDIMENT

Seafloor sediments may act as a sink for microplastics in the marine environment (Woodall et al. 2014) as microplastics denser than seawater (e.g., PVC, PET, acrylic, PE; see Table 1) are assumed to eventually sink and settle onto the seafloor (Hidalgo-Ruz et al. 2012; GESAMP 2016). Particles less dense than seawater can also sink after biofouling, being "repackaged" as faeces, or being entrapped within aggregrates or marine snow (Andrady 2011; Woodall et al. 2014; Long et al. 2015; Zalasiewicz et al. 2016).

Seven papers examining microplastic concentrations in sediments were reviewed. The papers discussed were chosen to represent the variety of sampling and extraction methods available in addition to their prominence as cited methods. Table 3 provides an overview of sampling techniques and the advantages and limitations for six of the seven studies. Table 4 provides extraction techniques, with a discussion on contamination control, relative cost, and if the method is recommended for use or modification. Note that methodology is not yet standardized, which presents a research gap to be updated as literature becomes available (Crichton et al. 2017; Hanvey et al. 2017).

4.1.1 Collection

Sediment sampling for microplastics has occurred using sediment cores (Martin et al. 2017), box corers (Mai et al. 2018), or by simply using a metal spoon to scoop surface sediments into a glass jar (Hanvey et al. 2017). Pagter et al. (2018) compared three commonly applied benthic sampling tools to assess their efficiency in sampling microplastics. No significant difference was found in the concentrations of microplastics collected using a Van Veen grab, a box corer, or a gravity corer, suggesting multiple methods are suitable and proficient at determining the abundance of microplastics in sediments (Pagter et al. 2018). We found only one study that used a sediment trap to collect microplastics in the marine environment (Käppler et al. 2016). Sediment traps collect particles falling out of suspension from the overlying water column, and would significantly underestimate low-density polymers that typically remain in suspension (e.g., polyethylene microbeads; see Ballent et al. 2016), but may be useful for targeted studies examining sinking rates of particular microplastics. Overall, sampling methodology should be based on the research question and the most robust methodology available. Long-term monitoring studies looking to determine relative microplastic abundances over time benefit from using a box corer or grab that provides large samples that are homogenized and assessed as a single unit (Maes et al. 2017b).

Multi-layer sediment cores allow for a depositional analysis of microplastics over time (Martin et al. 2017; Mai et al. 2018) and are recommended for high-resolution studies on a small scale (i.e. the time spent analyzing a single sample takes more time, but detailed information is provided on microplastics by sediment layer). Analyzing core layers also eliminates the weight versus volume decision for reporting values. Abundances are simply reported for each layer, allowing

for inter-study comparisons globally and a picture of deposition history (Martin et al. 2017). Studies examining coastal deposition of microplastics in urban areas can use transects at low tide and hand collect sediments to a particular depth (Crichton et al. 2017; Gago et al. 2018). Most studies sample within the top 5 cm of the surface layer (Gago et al. 2018), which is suggested to be the "modern layer" of sediments that will contain most particles (Martin et al. 2017). Carson et al. (2011) report that 95% of microplastics are found within the top 15 cm of sediments, with 50% found within the upper 5 cm. In contrast, Fisner et al. (2017) recommend sampling should be conducted to a depth of 1 m (for at least a subset of samples) in order to fully capture the depth distribution of microplastics.

4.1.2 Preservation

Once the sample is collected, refrigeration (for short-term storage) or freezing (for longer-term storage) is recommended. Samples should be collected into pre-rinsed glass jars (with metal lids or lids lined with aluminum foil). If refrigerating samples, amber glass jars with metal lids are preferred for storage as they block sunlight to deter biofouling and algal growth or decomposition and do not pose a contamination risk. If freezing samples, a gap should be left in the jar to adjust for expansion of the sample as it freezes and prevent breakage of the glass container.

4.1.3 Extraction

Extracting microplastics from sediment is intrinsically difficult due to matrix complexity. Considering the vast spectrum of polymer densities likely present within marine sediments, it is important to focus efforts on retaining dense particles that may be in the form of fibres (e.g., acrylic and PE) or fragments (PVC and PET). The most common approach for extracting microplastics from sediments involves density separation using hypersaline solutions, but other methods are also reviewed here that require inexpensive equipment and have the potential to capture microplastics at all densities (density-independent method and fractional sieving).

A common cost-effective density separation method uses table salt (Fries et al. 2013; Nuelle et al. 2014). However, sodium chloride (NaCl) has a reported density of 1.2 $g \cdot cm^{-3}$ which is unable to capture denser plastics such as PVC (density = 1.16–1.58 $g \cdot cm^{-3}$), polyester fibres (density = 1.24–2.3 $g \cdot cm^{-3}$), PET (density = 1.37–1.45 $g \cdot cm^{-3}$), PVA (density = 1.19–1.31 $g \cdot cm^{-3}$) and polytetrafluoroethylene (PTFE) (Hidalgo-Ruz et al. 2012; Prata et al. 2019). Although more expensive and dangerous to work with, alternative solutions can be created using sodium iodide (NaI; density = 1.8 $g \cdot cm^{-3}$), zinc bromide (ZnBr; density = 1.7 $g \cdot cm^{-3}$), zinc chloride ($ZnCl_2$; density = 1.7 $g \cdot cm^{-3}$), calcium chloride ($CaCl_2$; density = 1.3–1.46 $g \cdot cm^{-3}$), or sodium tungstate dihydrate ($Na_2WO_4 \cdot 2H_2O$; density = 1.4 $g \cdot cm^{-3}$) (Crichton et al. 2017; Hanvey et al. 2017; Pagter et al. 2018). Coppock et al. (2017) created an inexpensive, reproducible, and easily portable separation appartatus using $ZnCl_2$ as a flotation medium, reporting a mean recovery efficiency of 95.8% (range 70–100%). However, the most common alternative to NaCl in the papers reviewed was NaI. In a review of density separation methods, Prata et al. (2019) recommend the use of NaI over other alternatives, as long as it is not used with a cellulose filter (as exposure to NaI turns them black and complicates visual identiifaction of microplastics). Although NaI is more costly than table salt, Kedzierski et al. (2017) developed a method to recycle NaI up to 10 times without any density alterations, resulting in significant cost reductions.

Density flotation has been combined with the process of elutriation, whereby a sediment sample is perturbed by a constant flow of water to separate the less dense particles from the heavier ones. Claessens et al. (2013) built an elutriation column to separate microplastics from sand samples followed by a density flotation step using a high density NaI solution. This method reported an extraction efficiency for PVC particles of 100% and 98% for microfibres (Claessens et al. 2013). Other designs have since been adapted. Kedzierski et al. (2016) expanded the original design of Claessens et al. (2013) to operate as a closed circuit and reduce overconsumption of water during the elutriation process, while controlling the water temperature. Hengstmann et al. (2018) used a glass elutriation column (as opposed to the PVC column built by Claessens et al.), which avoids plastic contamination during elutriation, provides a view of the separation process, and is more resistant to chemicals (although increases the risk of the apparatus breaking). Because denser particles are more difficult to extract, these studies reported a 100% recovery rate for buoyant particles (PE and PP), but lower rates for heavier particles (e.g., PET, PVC, polyamide; 72–100%) (Kedzierski et al. 2016; Hengstmann et al. 2018).

Crichton et al. (2017) use a density-independent method utilizing the oleophilic properties of microplastics to capture particles of all densities. Canola oil is used to coat microplastics present in a sample and isolate them using a 1 µm polycarbonate filter. We recommend this method for use or modification, but it is labour intensive and may not be practical for large-scale monitoring studies.

Martin et al. (2017) used fractional sieving to separate a single slice of a sediment core into manageable sizes using 500-µm, 400-µm, and 250-µm sieves. Although this technique is recommended for modification, there are multiple improvements that should be considered. Because sediment is not removed from the sample prior to visual analysis, small microplastic fragments that look like sediment are easily missed. Also, the smallest size fraction is quite large (250 µm) and it is recommended that it be lowered to 63 µm. Finally, when working with fine-meshed sieves, airborne fibres may attach themselves to the mesh and sample fibres invisible to the naked eye may be missed if they become intertwined in the mesh. To take this into consideration, the method should be trialled using a blind spike test (i.e. spikes are added by someone other than the researcher extracting the microplastics).

Overall, we recommend the elutration column method in combination with density separation created by Claessens et al. (2013) for modification (see also Kedzierski et al. (2016), Hengstmann et al. (2017), and Pageter et al. (2018) for various modifications to the size of the column and materials used), as the column apparatus can be easily replicated in the lab, has high recovery rates, and requires only a small volume of NaI.

To remove biogenic matter (which aids in the visual identification of microplastics on filter papers), three papers utilized a 30% hydrogen peroxide (H_2O_2) oxidation period to remove remaining biogenic matter present in samples (Section 4.4.3 describes this in further detail). Nuelle et al. (2014) suggest that this step should not be applied routinely in the extraction protocol, but determined on a case-by-base basis. Although H_2O_2 is generally recommended as the best solution to digest samples with high organic matter content (Hanvey et al. 2017), a 35% solution has been shown to decrease PE and PP fragments by roughly 16% (Nuelle et al. 2014). Further, Nuelle et al. (2014) caution that an extreme exothermic reaction can occur between NaI and H_2O_2, resulting in strong gas development, and suggest additional filtration and rinsing of

the sample through a new filter if a digestion step is to be used after density separation with NaI. Additionally, during the reaction period, H_2O_2 will often leave a foamy residue on the inside of the glassware, creating an opportunity for small microplastic particles to adhere and be missed during quantification. Instead, a 10% potassium hydroxide (KOH) soak is recommended (Dehaut et al. 2016).

4.2 SEAWATER

Seawater is the most commonly sampled environmental matrix by oceanographers and biologists. As a result, seawater has been the best sampled reservoir of buoyant microplastic particles on a global scale, demonstrating a worldwide distribution of plastic on the surface of the open ocean, associated mostly with convergent currents and accumulating in ocean gyres (Law et al. 2010; Cózar et al. 2014; van Sebille et al. 2015). Ten papers were reviewed in detail to highlight different sampling and extraction techniques of microplastics in seawater. Table 5 provides an overview of sampling techniques and the advantages and limitations for each. Table 6 provides extraction techniques, with a discussion on contamination control, relative cost, and if the method is recommended for modification. As with the case for sediments, methodologies concerning seawater samples have not yet been standardized (Miller et al. 2017).

4.2.1 Collection

Methods exist to collect seawater samples at the surface, sub-surface, and surface micro layer (SML); however, all seawater collection methods are affected by sea state. Low density microplastics are easily mixed by oceanic turbulence during rough weather (Isobe et al. 2017; Maes et al. 2017b). Weather conditions should be recorded during sampling, including temperature, salinity, wind speed, wind direction, Beaufort sea state or wave height, vessel speed, and/or current strength/direction (Lusher et al. 2015b; Isobe et al. 2017; Maes et al. 2017b). Onboard instruments are preferred for measuring vessel speed as they provide better estimates of distance travelled. Ideally, sea state would be consistent throughout the survey to allow for study comparisons (Maes et al. 2017b).

Surface trawls by manta or neuston net are a common survey method for surface seawater collection. Reported mesh size of nets ranged from 300 µm to 333 µm and tows occurred within the top 10 cm of the sea surface. Vessel speed ranged from ~1.2–8.2 knots for between 10 and 30 minutes. Trawl surveys are considered to cover a moderate survey area compared to SML and subsurface sampling. It is noted that choppy conditions can reduce the area surveyed if the net comes partially out of the water in waves (Maes et al. 2017b). Nets should be fitted with a flow meter to get real-time data on water volume passing through (Lusher et al. 2017a), particularly for nets with <64 µm size (Mack et al. 2012). However, pressure build-up within the net, due to tow velocity, mouth size and shape, and net and mesh size, can also affect mechanical counters (Evans and Sell 1985). At a minimum, vessel speed and total time of tow (or tow length), and net dimensions should be recorded in order to calculate a conservative estimate of microplastic concentrations per volume sampled.

The biggest drawback of net tows are the large mesh sizes used (i.e. >300 µm); studies are likely missing a large proportion of small microplastics present at the sea surface. A study comparing mesh size (see also Section 4.3.1) found that manta trawls using a 160 µm mesh better reflected true microplastic abundances compared to a 505 µm mesh (Sun et al. 2017). In a survey of the

literature, Covernton et al. (2019) reported that using a 300–350 µm mesh can underestimate total microplastic concentrations by 1 to 4 orders of magnitude compared to smaller mesh sizes (e.g., <100 µm), despite the smaller volume of water collected. As a large fraction of microplastics found in fish and invertebrates are <300 µm, the use of large mesh sizes underestimates the concentrations of a class of biologically relevant microplastics, limiting the accurate quantification of ecological risk to pelagic organisms (Covernton et al. 2019). Further, large mesh sizes likely underrepresent the number of microfibres present within a sample as certain fibre orientations will allow them to pass through nets, which becomes likelier the larger the mesh size (Desforges et al. 2014; Covernton et al. 2019). Considering fibres often constitute >50% of the microplastic types observed, it is important these particles are captured when sampling.

Another technique to sample subsurface seawater uses the continuous water intake system fitted with a series of mesh filters on larger research vessels (Desforges et al. 2014; Lusher et al. 2014, 2015b). This technique is recommended for large-scale monitoring studies looking to determine relative microplastic abundances over a large distance. Some retrofitting is required to ensure samples are not contaminated as they travel through the pipes. For these reasons, this method is considered moderately expensive but is cost effective after the initial investment is made.

A unique study conducted in South Korea used surface tension to sample within the top 400 µm of the SML (Song et al. 2014). A metal sieve was gently tapped 100 times to collect particles floating at the sea surface. This method was able to collect heavy particles that are theorized to have recently entered the marine environment and were trapped in the top layer by surface tension. This inexpensive method is also noted for collecting small microplastics (i.e. <300 µm) with minimal biogenic material as a byproduct. The authors note that SML sampling is biased towards small particulates and can exclude particles >1 mm in size. Song et al. (2014, 2015a) also suggest that SML and manta trawl sampling be combined to provide a comprehensive understanding of microplastics present at the sea surface.

Bulk collection methods (also refered to as “grab” or “discrete” samples) involves sampling subsurface (~50 cm–1 m) water in 1 L glass bottles, typically collected by hand from either the back of a vessel (Barrows et al. 2017; Green et al. 2018) or from shore (Covernton et al. 2019). The bottle grab method captures micro- and nano-scale plastics and yields up to 4 orders of magnitude greater concentrations of microplastics per litre, compared to using common zooplankton sampling methods (bongo, manta, and neuston nets) (Barrows et al. 2017; Green et al. 2018; Covernton et al. 2019). One limitation is that this method only samples small volumes of water, which may result in high variability among samples, although sufficient replication of small-volume discrete samples can be used to increase sample representativeness. On the other hand, this method of bulk sampling can significantly reduce cross-contamintaion, as clean bottles are submerged and capped underwater, which can then be filtered in controlled conditions in the laboratory.

Overall, a combination of sampling methods is needed for an accurate understanding of the concentration of both large and small microplastics in seawater. We recommend that in selecting a seawater sampling method, researchers carefully consider their study objectives and the size range of microplastics they wish to target. Best practices indicate: (1) large-meshed zooplankton nets should be paired with concurrently sampled bottle grabs (Barrows et al. 2017; Green et al. 2018); (2) to sample large volumes over wide geographic ranges, pressurized pumps or

continuous intake systems should be modified with an adequate series of filters (Desforges et al. 2014; Song et al. 2014; Covernton et al. 2019); (3) sample sizes should be carefully considered *a priori* when employing discrete/bottle grab sampling methods; and (4) sampling methods should be designed to filter <10 µm in order to reliably capture microfibres (Covernton et al. 2019).

4.2.2 Preservation

Seawater samples should be collected into pre-rinsed glass jars (with metal lids or plastic lids lined with aluminum foil) and refrigerated or frozen (leaving an air gap for expansion if freezing samples). Many of the studies discussed preserved their samples using various fixatives; however, this is not necessary if the primary focus of the study is to recover microplastics and not characterize the biological material (Miller et al. 2017). Fixatives may be an option if refrigerator or freezer space is unavailable (see Section 3.0). If refrigerating samples, amber glass jars with metal lids are preferred for storage as they block sunlight to deter biofouling and algal growth and do not pose a contamination risk.

4.2.3 Extraction

Vacuum filtration is preferred to sieving when concentrating samples for visual identification. Vacuum filtration requires much less time to concentrate samples than sieving and does not involve hand rinsing to remove material from a fine mesh, which reduces contamination and the opportunity for sample loss. The method proposed by Wagner et al. (2017) is recommended as it isolates microplastics to <1µm (Table 6). Considering the colloidal nature of microfibres, however, it would be of interest to determine the accuracy of reporting microplastic values from a fraction of the filtrate, as it is possible microfibres intertwine and mix. Rochman et al. (2019) suggest counting masses of similar looking fibres as "bundles" if the fibres are intertwined and cannot be pulled apart, have the same appearance and occur in masses of >20. This method, does not, however, account for microfibre "bundles" that consist of heterogeneous polymer types. If this occurs, there is the potential that large numbers of particles are missed in pipetting and may not be accurately reflected in the total count.

Desforges et al. (2014) used an acid digestion prior to dying any remaining material with Nile red, which allowed particles to be identified by fluorescence with minimal background noise from remnant biological material. Nile red can lead to false positives, whereby non-plastic particles (e.g., small fragments of mineralized chitin and other proteinaceous materials) become stained (Maes et al. 2017a), and should be trialled with various organic materials to determine a potential error rate prior to analysis. Although the efficacy of this staining process is a recommended technique to test further, the use of a strong acid to eliminate biogenic material is not recommended (see discussion on strong acid digestions in Section 4.3.3). Instead an enzymatic digestion should be trialled. The use of potassium hydroxide (KOH) should also be considered (Covernton et al. 2019), as it is effective in removing non-cellulosic organic biological materials and has little effect on most plastic polymers (Dehaut et al. 2016). However, Wagner et al. (2017) reported that preliminary extractions with KOH interfered with downstream spectroscopic based analysis (e.g., SEM, FTIR).

The critical aspect of microplastic extraction methods from seawater relates to the separation of microplastics from the biological biomass (i.e. plankton) (Miller et al. 2017; see also discussion in Section 4.3). After surveying the literature, we have observed that some studies (e.g., Cole et

al. 2014; Desforges et al. 2014; Song et al. 2014) estimating microplastic abundance in the ocean perform a chemical and/or enzymatic digestion step prior to vacuum filtration to remove biological material and ease visual sorting of microplastics, particularly for net samples that have dense concentrations of biota. However, this digestion step results in the collection of microplastics ingested by the plankton in addition to the seawater matrix (i.e. water + biota vs. water only). To isolate microplastics from the seawater fraction collected by a neuston net, Barrows et al. (2017) performed a two-step filtration process which involved using a density flotation step with a hyper-saline (NaCl) solution to separate plastics from biological material prior to vacuum filtration. The use of the hyper-saline solution, combined with the small pore size of the filter, captured more microplastics than had been previously reported in other neuston tow studies (Barrows et al. 2017).

4.3 ZOOPLANKTON

As of June 2016, ingestion of microplastics has been recorded in over 220 species of marine organisms across all trophic levels (Lusher et al. 2017b). In a recent review, 39 zooplankton species from 28 taxonomic orders were reported to have ingested microplastics (Botterell et al. 2019). Microplastics have the potential to negatively affect marine organisms through blockage, false satiation, translocation to cells and by acting as vectors for the transport of harmful chemicals that are either present through additives or adsorbed onto the particle in the marine environment (Barnes et al. 2009; Cole et al. 2013; Rochman et al. 2015; Van Cauwenberghe et al. 2015a). Given their small size and range of feeding methods, interactions between zooplankton and microplastics are probable (Cole et al. 2013). Several physical and biological factors, such as particle size, shape, age, and abundance, appear to influence the bioavailability of microplastics to zooplankton (Botterell et al. 2019). It is also argued that zooplankton are more vulnerable to microplastic interactions than other marine species, due to their indiscriminate feeding as suspension feeding filter feeders (Desforges et al. 2015; Dawson et al. 2018; Foley et al. 2018), with the potential for ramifications throughout the food web. Additionally, researchers have recently suggested that microplastic ingestion by zooplankton may act as a potential avenue for the formation of nanoplastics (plastic particles <1µm). PE beads fed to Antarctic krill were reduced in size by 78% and were significantly altered in shape post ingestion (Dawson et al. 2018). This finding articulates the need to better understand interactions between zooplankton and microplastics, especially as zooplankton tie primary producers to larger trophic levels in pelagic ecosystems (Cole et al. 2013, 2015; Sun et al. 2017).

In 2016, the DFO Atlantic Zone Monitoring Program (AZMP) conducted a pilot study to examine microplastics and estimate their potential risks to zooplankton populations in the offshore waters of the Scotian Shelf (spring and fall) and Newfoundland Shelf (summer) in the Atlantic Ocean (C. Johnson, DFO Maritimes, pers. comm). A series of manta net tows (202 µm mesh) were conducted, sampling approximately 100–340 m^3 of seawater. Various digestion methods (both chemical and enzymatic) were trialled to find the optimal methodology for microplastics extraction from neuston samples; each had their own advantages and limitations. A standard microplastic characterization protocol was recommended based on key characteristics relevant for zooplankton, since ingestion of microplastics by zooplankton are suspected to be related to the external particle surface colour and texture. Preliminary results report that fragments were detected in 4 of 13 tows, with extrapolated maximum values of ~40 000 pieces·km^{-2}. Fibres were detected in all tows, ranging from 18 000–180 000 pieces·km^{-2}.

Although higher concentrations in the slope water suggest an open ocean source, the composition of microplastics was uncertain and additional work is required before any conclusions can be drawn. These results will be available in a forthcoming DFO Canadian Technical Report of Fisheries and Aquatic Sciences (C. Johnson, DFO Maritimes, pers. comm).

Table 7 provides an overview of sampling techniques and the advantages and limitations for each. Table 8 provides a description of the sampling and extraction protocols for three studies examining microplastic abundances in zooplankton. We believe these to be the only published reports in the primary literature determining microplastic abundances in wild zooplankton available in the scientific literature as of March 2019. Expert advice was sought where necessary to provide more well-rounded recommendations, given that representative sampling and extraction of microplastics in zooplankton presents a current research gap.

4.3.1 Collection

Zooplankton are sampled by horizontal net tows along the bottom, surface, and sub-surface using nets with a mesh size ranging from 50 µm to 505 µm. There is general agreement among authors that smaller mesh sizes better reflect microplastic abundances and zooplankton encounter rates. Mesh sizes <300 µm are recommended for use (Cole et al. 2014; Desforges et al. 2015; Sun et al. 2017). Few studies have examined microplastic ingestion in field collections of zooplankton, but the same biases that affect sampling for microplastics in seawater would also apply to sampling zooplankton (see Section 4.2.1). In this case, the choice of net mesh size is particularly important in order to capture the overlap between the target zooplankton and the microplastic particles that span their preferred food size range. For a more thorough discussion of the effect of net mesh size on filtration efficiencies and accurate quantification of zooplankton abundance and size, readers are referred to Mack et al. (2012), Salvanes et al. (2018), and Wiebe et al. (2017).

4.3.2 Preservation

Zooplankton are often fixed in preservative (10% buffered formalin or 70% ethanol) as this maintains cellular structure and enables species identification in the lab. If proper contamination procedures are used with low concentration fixatives, polymer integrity should not be affected (Courtene-Jones et al. 2017). However, Lusher et al. (2016) warn some plastics can be affected and therefore, underestimates may be possible. Following extraction and drying, particles should be stored in a dark location until analysis (Hidalgo-Ruz et al. 2012). If preservatives are used, filtration of the fixative solution is required (De Witte et al. 2014). Vacuum filtering the preservative solution using a 1µm filter paper that is resistant to the chemicals present in the solution (see Section 3.0) inside a laminar flow hood can minimize contamination potential. Note that some digestion methods used to breakdown the proteins of organisms will not work once the sample is fixed (e.g., enzymatic digestion using Corolase), so freezing zooplankton samples is recommended if taxonomic identification of zooplankton is not needed (L. Howell, Ocean Wise Conservation Association, pers. comm.; Cole et al. 2014). Sun et al. (2017) report that after preservation in 5% formaldehyde solution zooplankton were split into two equal parts (one for abundance analysis, one for microplastics anlaysis). We suggest, given appropriate contamination protocols are conducted, that zooplankton samples could be split prior to preservation, so that one part could be frozen for microplastic analysis, while the other part is fixed for identification and abundance analyses.

4.3.3 Extraction

Two of the three studies that examined microplastics in zooplankton samples separated the zooplankton from the sampled seawater matrix. Both Desforges et al. (2015) and Sun et al. (2017) isolated zooplankton from the volume-reduced sample, examined each individual for attached/adhered particles, and rinsed each thoroughly with deionized water (Sun et al. 2017) prior to performing chemical digestion methods. In contrast, Cole et al. (2014) only removed macrozooplankton and large debris from a sieved sample, subsequently processing all material retained on 200 μm mesh screens.

Two protocols used strong acid digestion (HNO_3) (Desforges et al. 2015; Sun et al. 2017) while one (Cole et al. 2014) developed a complicated technique using the enzyme Proteinase-K to breakdown biological tissue. Enzymatic digestion is preferred over the use of a strong acid, which can dissolve, degrade, or discolour pH-sensitive microplastic particles and fibres (as reviewed in Lusher et al. 2017b). Further, Claessens et al. (2013) reported a 0% recovery rate for small nylon fibres that were added during trials using nitric acid (HNO_3), while Dehaut et al. (2016) trialled a similar method and determined PA degraded and some plastics yellowed. In contrast, the enzymatic method described by Cole et al. (2014) is expensive, labour intensive, and involves many steps that require physical interaction with the sample. Contamination risk is always increased when protocols involve stirring, homogenization, or moving samples between containers. Physically homogenizing a sample likely breaks brittle plastics into smaller pieces resulting in an overestimation of the total count. Proteinase-K is costly to acquire in Canada and does not achieve 100% efficiency. Further, Cole et al. (2014) reported undigested material remained embedded within a thin film of a clear, glutinous biological material post-digestion, and recommended that chitinase be added to the protocol, further increasing costs. Lastly, the volume processed is relatively small and requires mathematical extrapolation to understand microplastic encounter rates.

All three studies (Cole et al. 2014, Desforges et al. 2015, Sun et al. 2017) ran procedural blanks alongside sample extraction. Specifications to the number of blanks were vague; however, contamination results were reported. Cole et al. (2014) highlighted the importance of running blanks as it was determined NaOH was corroding tinfoil lids into their samples and physical homogenization of the samples was introducing plastic shavings. Procedures were altered and contamination was eliminated from the final results. This highlights the importance of running blanks throughout the lifespan of a project to understand and minimize contamination risk. Background blanks were not discussed and are also recommended moving forward as they inform on airborne contamination within the lab.

We suggest that for the extraction of microplastics in zooplankton, methods described in Section 4.4.3 be modified with the aim of minimizing sample handling time and sample processing costs.

4.4 SHELLFISH

As some microplastics have similar sizes and dimensions to plankton and other suspended particles, they are available to, and can be retained by, suspension-feeding bivalve molluscs (Wright et al. 2013). Both wild and farmed populations of mussels, clams, and oysters have been shown to directly ingest microplastics, where they are found on the gills or in the digestive tract (Mathalon and Hill 2014; Van Cauwenberghe et al. 2015a; Sussarellu et al. 2016), and if small enough, may also be taken up into cells (Von Moos et al. 2012). As a result, the presence of

marine microplastics in shellfish could pose a threat to food safety. While van Cauwenberghe and Janssen (2014) estimated the annual human dietary exposure for European shellfish consumers to be 11 000 microplastic particles per year, Catarino et al. (2018) measured much lower numbers in shellfish, suggesting annual per capita consumption rates of 123–4 620 particles·year^{-1}. Although the consequences of transfer of microplastics to humans through the food chain is currently unknown, there is growing concern over the capacity for microplastics to transfer adsorbed contaminants (e.g., plastic additives and persistent organic pollutants) once ingested (e.g., Teuten et al. 2009) or to act as bioaccumulative vectors for diseases, bacteria, or viruses in cultivated shellfish destined for human comsuption. There is currently no regulatory framework concerning the presence of microplastics in seafood for human consumption (EFSA CONTAM Panel 2016).

Thirteen papers were reviewed to examine microplastic extraction techniques in shellfish. It is noted that papers digesting fish tissues were included as these methods are highly cited and easily adapted for shellfish, although the applicability of a specific method may depend upon the exact tissue chosen for microplastic extraction (e.g., a fish filet may be easier to process than the shellfish digestive gland). Table 9 summarizes the methodology, results, and discussion of each paper critiqued. Attention was focused on digestion efficiency, effect of the digestion method on microplastic integrity, potential for contamination and/or sample loss, and the use of procedural or background blanks. A qualitative assessment of relative cost is provided based on total extraction time and the relative cost of reagents in Canada. An overview of collection methods is not provided as shellfish collection for wild samples simply occurs by hand.

4.4.1 Collection

Upon collection, shellfish should have their shell exterior thoroughly rinsed with filtered water to eliminate potential contamination. If microplastic abundance is being quantified within the entire body cavity, including the digestive tract, the animal should be handled minimally (Lusher et al. 2017b) and flash frozen as quickly as possible to minimize sample loss through ejection of pseudofaeces or elimination of faeces (Lusher et al. 2017a). Additionally, bivalves should be secured shut with an elastic rubber band after collection to prevent gaping during freezing. If microplastic abundance is being quantified within the tissues only, exluding the digestive tract, depuration in filtered seawater is recommended. This allows the animal to clear the gut and reduce the amount of sediment or microplastics that may be present in the digestive tract (Mathalon and Hill 2014; van Cauwenberghe and Janssen 2014). Although the studies examined have only incubated their shellfish for 24 hours (Claessens et al. 2013; De Witte et al. 2014), depuration rate will be a function of species, temperature, water flow, and initial microplastic load. It is also recommended that depuration would need to take place in a closed system where introduced water is filtered to at least 1 μm, which may be extremely difficult conditions to recreate in a laboratory. There is no current consensus on the requirements for depuration of nanoplastic and microplastic particles in shellfish (Ribeiro et al. 2019).

4.4.2 Preservation

Samples can be frozen whole in tinfoil or freezer bags; samples from the plastic bags should be run under the FTIR and the spectra stored in a polymer library for background contamination removal (Lusher et al. 2017b).

4.4.3 Extraction

Visual sorting without degrading biological tissues (Lusher et al. 2013) is no longer recommended and considered an outdated process, although it may be considered under specific circumstances (i.e. the provision of naïve microplastics in a controlled environment with background and procedural blanks). Although it is extremely cost effective, high error rates are associated with this technique as biological contents are examined under the microscope for large durations of time, exposing tissues to airborne contamination. Furthermore, for shellfish, it is possible that microplastics present in the folds of the gill surface or within the digestive tract may be missed.

Until recently, acid digestion techniques were common for biological tissues. Short digestion periods combined with high temperatures resulted in high efficiency tissue degradation. It is now established, however, that strong acids affect pH sensitive plastics (Lusher et al. 2017b) as discussed in Section 4.3.3.

Multiple authors have used oxidation to break down biogenic material. Hydrogen peroxide (H_2O_2) is most commonly used as it is relatively inexpensive to purchase and has a long shelf life. The studies reviewed digested a mix of shellfish (Mathalon and Hill 2014; Li et al. 2015; Waite et al. 2018) and biogenic matter (Nuelle et al. 2014) over a period of 1–3 days between 50°C and 65°C. Li et al. (2015) digested numerous shellfish species using 30% H_2O_2 over a 2–3 day period. However, temperatures reached 65°C, which is considered slightly too high and resulted in loss of some microplastics due to melting during processing (see Munno et al. 2018). Li et al. (2015) reported microfibres as the most common plastic type identified, including nylon. Authors do report foaming, however, leading to potential sample loss if plastics adhere to the sides of the glassware. A flaky residue also remained on the filter paper (Mathalon and Hill 2014), which could result in false negatives. For these reasons oxidation methods are not recommended for shellfish tissue processing.

Multiple studies used strong bases to hydrolyze chemical bonds and denature proteins. Most notable is the use of KOH to dissolve tissues at room temperature (Foekema et al. 2013) or when incubated at 60°C (Rochman et al. 2015). In contrast, Karami et al. (2017) demonstrated that the maximum inclubation temperature should be 40°C, while extending the digestion period to 48–72 hours, to account for the slower digestion rate. These methods use a simple digestion process with few procedural steps in which contents are minimally exposed to air. The resultant solution is then filtered within the micrometre range and plastics are identified to type via FTIR or Raman spectroscopy. Additionally, the use of 10% KOH at these temperatures (~20–60°C) minimally affects polymer integrity (as reviewed in Dehaut et al. 2016 and Lusher et al. 2017b). KOH is relatively cost effective and is an easily obtained chemical (Lusher et al. 2017a).

Considering the short digestion period, Dehaut et al. (2016) recommended applying the protocol put forth by Rochman et al. (2015). It is noted that incubation of KOH should occur in the fume hood. If this set up is not possible (i.e. an incubator is unavailable and/or unable to fit in the fume hood due to size restrictions), then the Foekema et al. (2013) method can be adopted. The latter involves a 2- to 3-week KOH digestion period at room temperature, whereby individual flasks are placed inside a fume hood during the reaction period (instead of an incubator). This does, however, introduce an upper limit to the number of flasks that can be digested at one time based on available bench space.

Highly regarded in the literature, enzymatic digestions that target various tissue types at low temperatures are ideal because they do not attack plastic polymers and they avoid the use of caustic chemicals that could damage some types of microplastics. For example, protease catalyzes the decomposition of protein chains into easily dissolved peptides, cellulase targets phytoplankton cell walls, and chitinase breaks down the glycosidic bonds within chitin-containing materials. The majority of enzymes are often expensive, however, and require relatively large volumes compared to the sample volume processed. Löder et al. (2017) developed a universal enzymatic purification protocol for the extraction of microplastics from all types of environmental matrices, through the application of a series of technical grade enzymes in combination with H_2O_2 oxidation, washing with sodium dodecyl sulfate (SDS), a density separation with $ZnCl_2$, and filtration through an aluminum oxide filter. The protocol was safe to use with micro-FTIR analysis (Löder et al. 2017), but contained a large number of methodological steps to be conducted over the span of ~2 weeks. Catarino et al. (2017) attempted to establish a standardized technique for blue mussel digestion using a cost-effective industrial grade enzyme (Corolase 7089). Corolase 7089 (AB Enzymes) is an industrial protease that can hydrolyze a broad range of substrates at a neutral pH. It is safe to handle and a liquid, which reduces the potential for airborne contamination when preparing a solution. In addition, only two steps are involved in the Catarino protocol, which further reduces contamination risk. The sample is digested overnight on a magnetic stir plate and filtered using a 0.8 µm cellulose nitrate filter. Considering the short digestion period, minimal steps, and the cost-effective reagent, it is recommended that this protocol could be adopted (see Dimitrijevic 2018 for adopted methodology and results).

We are aware of two studies that examined the impacts of microplastic ingestion on the ecophysiology of shellfish, with observed negative effects on fecundity, offspring development, and cellular and neurotoxic effects from microplastic-associated chemical pollutants (Avio et al. 2015a; Sussarellu et al. 2016). Exposure of shellfish to microplastics occurred in controlled laboratory settings and required the implementation of specific methodologies (i.e. histological techniques, transcriptomic and proteomic analyses) that are beyond the scope of this document to analyze critically. It is noted, however, that initial studies examining the deleterious effects of microplastic ingestion on shellfish health often use extremely high microplastics concentrations of a single polymer type and are not currently environmentally relevant. Readers are referred to the review by Ribeiro et al. (2019) for further information.

5.0 IDENTIFICATION AND QUANTIFICATION

Extracted microplastic particles are typically captured and then enumerated on a filter paper. Unlike the filter media that capture particles within the filter matrix ("depth filters"), which are recommended for purifying water, reagents, and fixatives, we recommend membrane filters to extract and enumerate microplastics. Membrane filters are classed as "screen or surface filters," which capture particulates on the smooth filter surface, enabling their easy visualization under a microscope. Also, the retention levels for these types of filters extend to very small pore sizes (i.e. down to 0.02 µm), allowing for the efficient collection of sub-micron particulates on the surface of the filter. While the chemical compatibility of membrane filters should always be examined prior to use (see Section 3.0), hydrophilic (i.e. possess an affinity for water and can be wetted with virtually any liquid) polycarbonate membrane filters (e.g., Whatman® Cyclopore®

polycarbonate membrane filters) are an effective non-plastic option that are recommended for use with most microplastic extraction protocols reviewed herein.

5.1 LIGHT MICROSCOPY

Microplastics are generally quantified visually using a stereomicroscope or a compound microscope. To reduce the risk of airborne contamination and to ensure quantified particles are from the sample being investigated, visual identification should use a closed container (i.e. petri dish or petri slide) under a covered microscope (GESAMP 2016). A grid or coloured lines placed behind the petri dish will ensure filter papers are examined systematically. Random squares or rows of the grid can be quantified and values extrapolated to maximize efficiencies. Whereas the smallest size fraction of microplastic particles collected from environmental samples is dictated by the filter size used during the extraction step, microscopic confirmation has been recommended for particles <1 mm (Hidalgo-Ruz et al. 2012) or <500 µm (Löder and Gerdts 2015). It is difficult to visually detect plastics <100 µm even under a microscope (Lenz et al. 2015).

The greatest challenge during visual identification is deciphering whether a particle may be biogenic, anthropogenic, or plastic. According to Hidalgo-Ruz et al. (2012), GESAMP (2015), Lusher et al. (2015b), and the Marine & Environmental Research Institute (2015), particles are recorded as plastic if the following characteristics are observed:

- Absence of cellular or organic structures
- Constant thickness of fragments and fibres
- Homogeneous colour and brightness
- Unnatural colour and/or shininess
- Equal roundness throughout the entire length of fibre
- 3-dimensional bending of fibres

Hidalgo-Ruz et al. (2012) note that these characteristics work best for microplastics in the 0.5–5 mm size range.

The hot needle test is a common tool used to observe the physical response of a particle after being poked with a hot needle. If the particle shatters or breaks, it is considered natural; if the particle melts or bends, it is considered plastic (De Witte et al. 2014; Karlsson et al. 2017). Note that this method is destructive and is not recommended as the sole method for plastic determination as the melting point of polymers varies (Lusher et al. 2017a).

When a microplastic is identified, photographs and measurements can be taken using a camera that is fitted to the microscope. Images can then be analyzed further using computer software such as ImageJ. Characteristics such as size, shape, and colour are recorded. Particles are usually binned into the following categories based on shape (Hidalgo-Ruz et al. 2012; Marine & Environmental Research Institute 2015; Lusher et al. 2017b; but see Rochman et al. 2019):

- **Beads** – nurdles and or/microbeads
- **Films** – flat, exhibit a 2-dimensional shape
- **Foamed** – air pockets present
- **Fibres** – thin and round
- **Fragments** – 3-dimensional with irregular shape

Crawford and Quinn (2017b) recommend sorting visually identified microplastics using a standardized size and colour sorting (SCS) system, which categorizes particles based upon their size and appearance in a specific sequence of steps. Plastics are first separated based on their size (measured along longest dimension or classed into ranges), then morphology (i.e. pellet, fragment, fibre, film, or foam), colour, polymer type, and finally quantity. This information is all recorded using appropriate category abbreviations so that the exact information for each piece of plastic is represented in the SCS code (Crawford and Quinn 2017b). Recently, Hartmann et al. (2019) developed a framework for defining and categorizing "plastic debris," which considers seven criteria: chemical composition, solid state, solubility, size, shape and structure, colour, and optionally the origin of the microplastic particle. Each criterion can be precisely defined and quantified, thus removing ambiguity and increasing the generation of comparable data among studies of plastic pollution in the environment.

Using visual identification alone, rates of false positives and negatives range from 20% to 70%, particularly for small microplastics, likely as a result of observer and/or technical error (Lusher et al. 2017b; Shim et al. 2017). Lavers et al. (2016) noted that the probability of visually detecting various types of plastics in beach sediments ranged from 60–100%, and varied by observer, observer experience, and amount of biological material present that resembled plastics. For example, microplastic fibres are often overestimated while fragments are often underestimated (Song et al. 2015a); blue fragements have the highest detection rates, and white fragments the lowest (Lavers et al. 2016); and due to their inconspicuous colour and size, white microbeads and microplastics <300 μm are likely undertestimated in most studies (Nel et al. 2019). While training and experience is likely to lower the error rates of visual identification (Lusher et al. 2017a, 2017b), there remains a high risk of misclassification error due to human bias. It is therefore recommended that chemical polymer identification be used to confirm results (see Section 5.3), which will reduce the risk of false positives or negatives from misidentification (Lusher et al. 2017a; Maes et al. 2017a).

5.2 OTHER IDENTIFICATION METHODS

The use of dyes is a low-cost method that may aid the visual identification of microplastics. Staining particles with a fluorescent dye has been used to identify plastic polymers (Norén 2007; Desforges et al. 2014; Maes et al. 2017a; Tamminga 2017). The dye sticks to hydrophobic particles causing them to fluoresce brightly under specific light conditions (Andrady 2011). The use of fluorescent dyes to quantify microplastics in environmental samples is relatively unexplored; this method has been primarily used to study uptake of microplastics in controlled experiments. Recently, Maes et al. (2017a) developed an automated fluorescence scanning rig whereby the microscope is set up to scan a filter paper and identify plastic particles without the need for visual identification. Used in combination with a density separation technique, Maes et al. (2017a) used a cost-effective stock solution (Nile red at 1 $mg \cdot mL^{-1}$) to dye the separated sample. An orange filter with blue light is then used to identify plastic polymers, which shine brightly compared to natural particles, which do not. The trial reported high recovery rates (96.6%) using spiked samples of microplastics with known sizes and shapes. This method is less time-consuming and less costly than using other chemical polymer techniques (Tamminga 2017; see also Section 5.3) and holds much promise in substituting the subjective visual sorting of microplastics with a semi-automatic procedure (Erni-Cassola et al. 2017). Although the weathering of plastics does not seem to affect staining (Shim et al. 2017), false positives can

arise from proteinaceous biogenic material (e.g., crab claw fragments, marine algae), which can absorb the dye, and false negatives can arise due to weak fluorescent signals from some types of polymers (PC, PUR, PET, PVC; Erni-Cassola et al. 2017), as well as from polyester fibres, which can be difficult to stain (JD, pers. obs.; Tamminga 2017). This method should therefore be trialled using a range of natural polymers following a blind identification test using spikes.

An alternate method involves the use of Rose-Bengal solution (4,5,6,7-tetrachloro-2′,4′,5′,7′-tetraiodofluorescein) to stain non-plastic particles in a sample (Liebezeit and Liebezeit 2014; Ziajahromi et al. 2017). Rose-Bengal stains natural and non-plastic particles, such as cotton and other natural fibres, easing visual identification by allowing the separation of pink-coloured natural particles from non-coloured plastics. Ziajahromi et al. (2017) assessed the suitability of this method for polyester fibres and PE microbeads in spiked samples, which were unstained after a 5 minute treatment with Rose-Bengal solution at room temparture, and confirmed the staining method had no effect on the subsequent anlaysis with FTIR. In contrast, Liebezeit and Liebezeit (2014) found that some non-plastic particles (i.e. sand grains) did not stain with Rose-Bengal. This method should also be trialled using a range of natural and synthetic polymers, as a potential low cost and time-saving method to aid in the visual separation of natural and non-plastic particles. However, counting fluorescent and non-fluorescent particles concurrently may prove difficult as it eliminates sorting based on additional particle characteristics (i.e. all non-fluorescent particles appear as black/grey under a microscope; JD, pers. obs.).

Recently, Woods et al. (2018) used an imaging flow cytometer (VS series FlowCam) to identify and enumerate low concentrations of microplastic fibres and algal cells during laboratory ingestion and uptake experiments with blue mussels. Their methodology was optimized to compensate for the intrinsic properties of the microfibres used in their experiments, and a 98% accuracy was obtained through comparison to manual microscope counts. Bergmann et al. (2017) applied a FlowCam to visualize and quantify amounts and sizes of microplastics sampled from deep-sea Arctic sediments; however, a large amount of coal particles present in the samples ultimately impeded accurate quantification using this method. Benefits of using the FlowCam technology include the ability to image, count, and calculate the concentration of particles 300 nm to 5 mm. However, limitations for its use in microplastics analysis include the availability of this specialized equipment, the potential saturation of the equipment if concentrations of particles are large, and its inability to characterize polymer types (Prata et al. 2019). While this technology may be useful under controlled experimental situations (e.g., Sgier et al. 2016; Woods et al. 2018), its application to the enumeration of environmental samples remains unclear.

5.3 CHEMICAL POLYMER IDENTIFICATION

Chemical polymer identification is used to validate the results of visual identification and to determine the composition of polymer type within a sample, which can inform on their origins. The four main methods currently in use are Fourier-transform infrared spectroscopy (FTIR), Raman spectroscopy (Raman), scanning electron microscopy (SEM), and pyrolysis–gas chromatography–mass spectrometry (Pyr-GC-MS) (Prata et al. 2019). For visual examples of the output of these chemical identification methods for various types of microplastic particles, readers are referred to Figures 4 and 6–12 in Silva et al. (2018). A minimum of 10% of identified particles should undergo this second verification between 100 µm–5 mm (Lusher et al. 2017b), up to a maximum of 50 items per year or sampling occasion, whichever is less (Gago et al.

2016). It is further suggested that all particles <100 µm be analyzed to polymer type (Lusher et al. 2017b), as well as any plastics that could have contaminated the samples. Chemical polymer identification equipment is large and expensive and access to machinery and/or funding usually dictates which method is used. Table 10 compares chemical polymer identification methods, including the lower size limit for particle detection, relative contamination potential and cost. Table 11 examines the pros and cons for each, from various studies.

FTIR is a relatively low cost (after the initial investment in the cost of the machine), non-destructive spectroscopic technique (Shim et al. 2017) that can identify polymers of various sizes, shapes, and colours. To date, FTIR has been the most common procedure used (Gago et al. 2018; Prata et al. 2019). Generally, a particle is isolated from the environmental matrix and examined using the FTIR microscope. An infrared beam is used to excite the molecules of a sample particle and develop a spectrum. For single particle analysis, FTIR instruments can be used in two different measurement modes that have different advantages and limitations: reflectance and transmittance (Huppertsberg and Knepper 2018). Attenuated total reflectance (ATR) FTIR requires full contact between the particle and the crystal (GESAMP 2016) to get a good spectrum, which can sometimes be difficult to achieve, as both particle and crystal surfaces can be prone to contamination and/or sample loss (i.e. while attempting to make contact between the particle and crystal). Alternately, transmission spectra can be recorded directly from IR transparent filters (silica or aluminum oxide), which is beneficial for measuring particles <500 µm (Huppertsberg and Knepper 2018). Polymer confirmation must have a match of at least 60% (Lusher et al. 2013; Avio et al. 2015b). A match is determined by running the spectra against a library of known polymers, which needs to be purchased on a subscription basis (e.g., Bio-Rad KnowItAll library is roughly \$10,000 CAD·yr^{-1} for 250,000 IR spectra of commercial products, A. Posacka, Ocean Wise Conservation Association, pers. comm). Once a subset of particles has been identified, particle counts are updated based on the results (Gago et al. 2018). A notable challenge for polymer identification via FTIR occurs when trying to distinguish between polymers that are made of cellulose (natural origin) from those of rayon (anthropogenic origin) (Comnea-Stancu et al. 2017). Compared to other methods, FTIR has the largest lower size limit (10 µm); it is difficult to yield a usable spectrum from particles smaller than this limit, as the wavelength of the laser beam is too large compared to the particle size (GESAMP 2015). Focal plane array reflectance FTIR micro-spectroscopy (FPA-FTIR) can be used to record several thousand spectra simultaneously across a grid of detectors, which can facilitate a high throughput of samples, but is more cost-intensive and aggregated or irregularly shaped particles may produce refractive errors (Shim et al. 2017; Huppertsberg and Knepper 2018; Serranti et al. 2018).

Raman spectroscopy is a non-destructive technique that uses a laser to create a polymer-specific spectrum. Polymer analysis can be done at multiple locations on the particle's surface, and the spectrum obtained is used to compare the crystalline structure of polymers to a library (GESAMP 2016). No sample preparation and no contact is required to run the sample (Shim et al. 2017). Raman spectroscopy uses a smaller laser than FTIR and has a correspondingly lower limit for particle detection (1µm) (Shim et al. 2017). However, a laser for Raman spectroscopy is roughly five times more expensive than an FTIR laser (Mai et al. 2018). Raman spectroscopy is prone to spectral distortion induced by fluorescence, and thus requires relatively clean samples, which can be problematic for particles extracted from environmental matrices (due to particle surface oxidation, biofouling, or UV degradation), and heavily dyed polymers can be difficult to

identify (e.g., primarily fibres) (GESAMP 2016; Shim et al. 2017). Reference databases are typically composed of virgin plastic spectra provided by the manufacturer, making the precise identification of degraded particles more difficult. Käppler et al. (2016) compared the number, size, type, spectra quality, measurement time and handling of detectable microplastics between Raman and FTIR transmission imaging, demonstrating that while measurement time of Raman was considerably higher, FTIR imaging underestimated microplastics <20 µm in size by ~35%. They recommended that microplastic size fractions in the 50–500 µm range be analyzed by FTIR, while Raman should be employed for the 1–50 µm particle size range (Käppler et al. 2016). Cabernard et al. (2018) reached similar conclusions, demonstrating that μ-Raman imaging analysis of microplastics extracted from surface water samples quantified twice as many particles ≤500 µm in size than ATR-FTIR. However, they recommended further research to enable a more time-efficient routine application of Raman for reliable counting of particles down to 1 µm. A recent review by Araujo et al. (2018) showcases solutions that may contribute to faster and better identification of microplastics using Raman spectroscopy, while highlighting potential non-conventional techniques that may permit more advanced applications.

SEM is a non-destructive technique that creates high-resolution images by running a beam of electrons through the particle (Rocha-Santos and Duarte 2015). This technique can be used to characterize polymers and determine microbial surface communities. If combined with mass spectrometry, SEM can also provide insight onto polymer additives by looking at thermal degradation properties (Lusher et al. 2017b). This method enables visual analysis of weathering (e.g., visible cracking) for particles at the nanometre scale (Ter Halle et al. 2017). The elemental composition of particles can be determined when coupled to energy dispersive spectroscopy (SEM-EDS), thus discerning carbon-dominant plastics from inorganic particles. However, SEM-EDS is expensive and labour-intensive, limiting the number of samples that can be analysed in a given timeframe (Shim et al. 2017). Overall, SEM techniques are valuable for select purposes, such as to distinguish diatoms from microplastic particles (Li et al. 2016) or as an auxiliary technique to calibrate spectroscopic ones (i.e. FTIR, Raman) (Ding et al. 2019).

A thermo-analytical method, Pyr-GC-MS is a destructive technique that heats particles at high temperatures to obtain gases and determine the structural information of macromolecules (Fries et al. 2013). Quantification of polymer materials is obtained through the comparison of sample pyrograms with reference pyrograms of known polymers (Shim et al. 2017). No pre-treatment of samples is required with this technique, and only a small quantity of sample need be analysed in any one measurement (down to 0.5 mg). Although many particles can be run simultaneously, results only provide data on the mass of specific polymers present, not individual counts, sizes, or morphology (Mai et al. 2018). Because the goal in determining polymer type is to inform visual counts, Pyr-GC-MS would require individual particle analysis, similar in efficiency to other methods. However, compared to spectroscopic techniques, this method provides rapid measurements, which can be useful for routine analyses (Silva et al. 2018). A more recent development is the combination of thermos-extraction and desorption with mass spectrometry (TED-GC-MS), which allows rapid analysis of the 5 most common polymer types (PE, PP, PS, polyamide 6, and PET) (Dümichen et al. 2017). This method is advantageous, as it can handle relatively large sample amounts (up to 100 mg), and does not require any pre-treatment of particles; that is, separation of microplastics from environmental samples by flotation, filtration, sieving, and/or chemical or enzymatic digestion techniques are not required (Strungaru et al. 2019). One caveat to note for the TED-GC-MS technique is that the decomposition products of

plastic polymers need to be different from those of the environmental matrix for accurate determination. Further suitable calibrations for each polymer of interest remain to be conducted, as well as a more thorough investigation on detection limits of microplastics in environmental samples, prior to more widespread use of this method (Dümichen et al. 2017).

Ideally, new methods would move from individual particle identification to complex sample analysis composed of both natural and anthropogenic material, as well as incorporating automation to provide faster and more complete analysis of samples. Shim et al. (2017) proposed the addition of a fluorescent filter onto the FTIR microscope to combine visual and chemical identification in real time. The authors theorize this would reduce manual microplastic identification and decrease the risk of lost particles when transferring samples from the dissecting scope to the FTIR microscope. Similar to Maes et al. (2017a), Nile red is suggested as a fluorescent dye, but this protocol is not recommended until it can be confirmed that false positives from natural particles are eliminated. Primpke et al. (2017, 2018) combined FPA-FTIR with image analysis to create an automated routine that compares particle spectra against a reference database for chemical identification. This method significantly reduced the time and human bias to a minimum, and successfully identified small microplastic particles (<30 μm) that are often missed during manual (optical) analysis. However, the analytical time demand remains high with this method (Primpke et al. 2017) and a specialized reference database design is required to distinguish among different polymers in specific spectral ranges (Primpke et al. 2018). HyperSpectral Imaging (HSI) is a rapid, non-invasive, non-destructive method that uses optical sensing technology to capture hyperspectral images of microplastics in the short-wave infrared range (1000–2500 nm) to compare sample spectra with polymer reference spectra. Using classification models (partial least-squares discriminant analysis), Serranti et al. (2018) were able to correctly classify PP and PE present in 97.68% and 99.68% of PP and PE image pixels, respectively, while PS was properly classified in 81.30% of image pixels. In addition to characterizing polymer type, this method also showed promise in accurately and simlutaneously determining the abundance, size, and shape of microplastic particles sampled as floating plastic debris from across the globe, thus opening a promising way for improving plastic pollution monitoring (Serranti et al. 2018).

Ultimately, the preference of one method over another is dependent on the availability of instruments, project budget, and the research objectives. If a combination of polymer identification and additive information is desired, SEM or Pyr-GC-MS may prove more useful. FTIR and Raman spectroscopy are (usually) non-destructive, so samples can be rerun if necessary. However, for all methods, further research is needed to understand the impact that degradation processes, hitchhikers, and biofouling have on particle identification based on changes to their resultant spectra (Comnea-Stancu et al. 2017). A comparative study of FTIR, Raman, and SEM found similar results when identifying microplastics isolated from fish gut matrices (Wagner et al. 2017). All three methods exhibited similar labour times and overall cost and were concluded to be a complementary set of techniques with the potential to characterize polymer type. Murrell et al. (2018) used ATR-FTIR in combination with SEM and optical profilometry (OP) to determine the composition and physical surface characteristics of microplastics from personal care products and wastewater effluent, demonstrating that many particles were significantly distorted from the spherical shapes of the virgin standards. Käppler et al. (2018) critically compared μ-ATR-FTIR and Pyr-GC-MS methods for the analysis of microplastics from environmental samples, concluding that both methods are effective and

produce complementary results. Moving forward, a combination of chemical identification methods should be employed to maximize the accurate characterization of polymer types across a broad size range and improve the overall reliability, comparability, and quality of data collected.

The analysis of toxic substances associated with microplastics requires different analytical protocols compared to environmental samples and is beyond the scope of this document. Readers are referred to Avio et al. (2015a), Hong et al. (2017), Silva et al. (2018), and Huppertsberg and Knepper (2018) for more information.

6.0 REPORTING

Microplastic abundance reporting varies by environmental matrix and sampling method, and this lack of standardized reporting makes global comparisons difficult (Lusher et al. 2017b; Gago et al. 2018; Mai et al. 2018). Table 12 provides a synthesis of the reporting techniques for several papers in each of sediment, seawater, zooplankton, and shellfish. Descriptions of particle shape, colour, and polymer type are listed as well as metrics reported.

Microplastic abundance tends to be reported under shape and colour categories into which observed particles can be classified. Results are then reported as the percentage for each shape and colour that is found either by area, weight, or species. This can be combined with average length and width and the percentage of polymers identified by chemical polymer identification if applicable. Microplastic particle weight is not reported, as 100% digestion is not yet possible, so plastic particle weight cannot be distinguished from residual organic matter (Song et al. 2014, 2015a).

Generally, sediment values are reported by either weight in kilograms or grams (Claessens et al. 2013; Besley et al. 2017; Maes et al. 2017b; Gago et al. 2018; Mai et al. 2018), or by volume in millilitres (Woodall et al. 2014; Crichton et al. 2017), which is an important consideration for inter-study comparisons. Hanvey et al. (2017) recommend reporting using SI units for sediment mass (g) and dry weight values, rather than volume, as a path forward for standardization.

Results from seawater are generally extrapolated to the number of microplastics per cubic metre (Desforges et al. 2014; Lusher et al. 2015b) or square kilometre (Maes et al. 2017b; Mai et al. 2018). Considering the small volume of water Song et al. (2014, 2015b) collected at the SML, microplastic abundances were provided per litre of water, however, this is not common.

Like seawater, microplastic abundances for zooplankton are reported per cubic metre (Cole et al. 2014; Desforges et al. 2015; Sun et al. 2017), although Desforges et al. (2015) also reported the plastic encounter rate (no. plankton per plastic particle) and a plankton density-corrected plastic ingestion (no. ingested particles$\cdot m^{-3}$ seawater). Unique to this area of research is the ability to develop an encounter rate, based on the number of individuals counted, compared to the number of microplastics identified for the same unit of water. This is achieved by splitting a sample in two, with one half analyzed for species and the other for microplastics (Sun et al. 2017). In contrast, Desforges et al. (2015) assessed microplastic ingestion directly from individual zooplankton, then estimated the risk to juvenile and adult salmon through consumption of microplastic-containing zooplankton.

Reporting for shellfish generally includes two metrics: microplastic abundances per individual (by species) and per gram of tissue (Li et al. 2015; Davidson and Dudas 2016; Waite et al. 2018).

The latter is defined as either a wet weight or dry weight, which is dependent on the digestion method used. For example, wet tissue is required for the enzymatic digestion developed by Catarino et al. (2017) which does not allow for a dry weight estimate.

Correction factors can be generated to adjust for contamination using procedural blanks. Exact descriptions of techniques are limited and should be determined on a study by study basis. De Witte et al. (2014) used blank values to develop a limit of detection (LOD) value whereby the average number of particles + 3 SD was obtained for each microplastic particle by colour and type. Particles that were observed frequently were subsequently eliminated from the analysis.

7.0 NANOPLASTICS

Microplastic researchers are recognizing the need to also investigate the presence and effects of nanoplastics within the natural environment. Effects of nanoplastics on marine organisms and ecosystems are not currently understood (see review by Mattsson et al. 2018), but preliminary studies have shown translocation to tissues (Dawson et al. 2018) and lipid membrane permeation, which can lead to altered cell function (Rossi et al. 2014). Bioaccumulation of nanoplastic-associated organic contaminants (i.e. polycyclic aromatic hydrocarbons) may also occur in exposed organisms (Jiang et al. 2019).

Currently, there is no clear definition of what constitutes a nanoplastic (da Costa et al. 2016; Ter Halle et al. 2017; Gigault et al. 2018). Proposed upper limits include 100 nm (Alimi et al. 2018) and <1µm (da Costa et al. 2016; Ter Halle et al. 2017). The lower limit is either undescribed or set at 1nm (Ter Halle et al. 2017). Gigault et al. (2018) argue that nanoplastics are inherently different from nanoparticles that are industrially engineered to be a specific size or shape. Gigault et al. (2018) propose that the definition of a nanoplastic should only include particulates resulting from the degradation of industrial or weathered plastics. They also propose that the size range of a nanoplastic be from 1 nm to 999 nm as this aligns with the rationale behind the descriptions of "macro," "meso," and "micro" plastics, which is that plastic particulates fall within a specific size range based on shape. Hartmann et al. (2019) express a similar opinion, defining nanoplastics from 1 to <1000 nm.

Due to their small size, nanoplastics can interact with microorganisms and macromolecules in the water column (Alimi et al. 2018). Nanoplastics exhibit colloidal behaviour and are asymmetrical in shape (Gigault et al. 2018). Aggregation occurs when two particles collide resulting in a hetero- (comprised of a mix) or homo- (comprised of the same) complex that is inclusive of clays and other organic materials (da Costa et al. 2016; Alimi et al. 2018; Gigault et al. 2018). Aggregation formation is dependent on the physical and chemical environment, and the behaviour of aggregates is difficult to predict. Nanoplastic particles do not fit the density-driven model for predicting deposition as behaviour is predominantly random (referred to as Brownian Motion) in a solution (Gigault et al. 2018). For example, PVC at the nanoplastic scale will readily disperse in the water column rather than sink (Gigault et al. 2018). A study examining the behaviour of nanoplastics found that PS particles were stable at 350 $mmol \cdot L^{-1}$ NaCl but readily aggregated at 500 $mmol \cdot L^{-1}$ NaCl, which was an irreversible event (Gigault et al. 2018).

Researching nanoplastics presents new challenges, particularly when trying to isolate and quantify concentrations in environmental samples. Only one study to date has attempted to do so in seawater. Ter Halle et al. (2017) set out to quantify nanoplastics present in 1 L of seawater

using a combination of steps. Using a chemometric approach and principal component analysis, the authors could only conclude on the proportion of PVC, PS, and PET in a 1 L sample; it was not possible to quantify or characterize the individual particulates or aggregates present. Ter Halle et al. (2017) conclude that the extraction of nanoplastics be improved by increasing the total sample volume to 5 L as 1 L did not obtain enough sample to adequately describe the quantity of nanoplastics present.

Nanoplastics are largely below the detection limit available using current monitoring technology (da Costa et al. 2016). Development efforts need to focus on automated or semi-automated methods that provide both physical and chemical sample analysis (Shim et al. 2017). Researchers in South Korea have proposed using the scanning function of combined atomic force microscopy with infrared spectroscopy or Raman spectroscopy. Visually locating nanoparticles is time consuming and thus unlikely to provide high throughput based on current standard practices (Shim et al. 2017). Shim et al. (2017) propose further investigation into particle staining technologies, with appropriate validation to prevent false positives, that can be subsequently viewed using fluorescence as this would reduce visual identification time and error rates. Correia and Loeschner (2018) tested the suitability of asymmetric flow field–flow fractionation (AF4) coupled to multi-angle light scattering (MALS) for detecting nanoplastics in a homogenized fish sample spiked with 100 nm polystyrene nanoparticles (PSNPs). While their light scattering (LS) signal of the PSNPs was easily detected with a LOD of 52 $\mu g \cdot g^{-1}$ fish, the method was unable to detect PE particles due to their elevated LS background (Correia and Loeschner 2018). Although promising methodologies are currently being tested, nanoplastics research remains in its infancy at present.

8.0 CONCLUSIONS AND RECOMMENDATIONS

A comprehensive overview of the best practices required for accurate and representative reporting of microplastics is provided for sediment, seawater, zooplankton, and shellfish. The following are general recommendations:

- Research and reporting objectives should be used to help determine sampling and extraction techniques.
- Rigorous contamination prevention protocols should be adopted in the field and in the lab.
    - Ample use of proper personal protective equipment
    - Conducting procedural and background blanks to control for contamination
    - Using glass and metal equipment preferentially
    - During sample collection or handling, avoiding the use of synthetic textiles, and wearing non-shedding coveralls (Tyvek® suits) and/or 100% cotton lab coats and head scarves
    - Take small samples of fibres from materials that are in the vicinity of any laboratory work (e.g. putative fibres from clothing, carperts, ceiling tiles, cleaning sponges, etc.) and store in small vials for the subsequent identification of airborne contamination in samples
    - Rinsing all sampling and laboratory equipment with ultrapure water 3 times prior to use
    - Filtering all working solutions

    - Working in a clean air environment (e.g., in a clean room, under a laminar flow hood, and/or using HEPA air filters) as much as possible in both field and laboratory settings.
    - Covering samples during filtration, digestion, and visual identification and/or performing these steps in a laminar flow hood
- Sampling methods should aim to collect particles to the smallest size fraction, within reason and when possible (i.e. given the limitations when working in field-based settings vs. in a controlled laboratory environment).
- Although depending somewhat on the analytical method chosen, refrigeration or freezing should be the first choice for sample storage, followed by preservation in low concentration fixatives.
- All procedures should be trialled with a positive control (i.e. blind spike test) and recovery rates determined and reported.
- For water and sediment extraction, techniques should minimize handling time and transfers of samples between containers. Efforts should be made to retain particles of all densities to the smallest possible size fraction.
- For extraction from biological samples (i.e. zooplankton and shellfish), procedures using enzymatic or 10% KOH digestion are preferred over the use of strong acids or oxidizing agents in order to maintain polymer integrity.
- It is critical that enumeration using light microscopy is combined with post-extraction chemical identification to confirm the identity of microplastic particles in all marine environmental samples.
- A minimum 10% of suspected microplastics should be identified to polymer type by examining particle chemistry using either FTIR, Raman, SEM, Pyr-GC-MS at minimum, and ideally using a combination of two or more techniques.
- Sample reporting should aim to be representative and facilitate inter-study comparisons when possible.This can be achieved by reporting concentrations in terms of count by both weight and volume for sediment, by volume for seawater, and by individual, gram of organ, and/or whole body weight for biota.

As of March 2019, a wide variety of analytical methods for the study of microplastics in aquatic environments has been published in the peer-reviewed literature. With this report, we have conducted a thorough discussion of the advantages and limitations of the most prominent and promising methods currently available and aimed to provide a path forward for researchers starting out in the field of microplastics research. Towards this goal, we have created a flow-chart that outlines 5 key stages that we recommend should form the basis of a standardized methodological protocol for the overall collection, extraction, and identification of microplastics from marine environmental matrices (Fig. 2). Some methodological choices will be dictated by the focus of any particular study (e.g., rapid environmental assessments vs. quantifying impacts to biota), as well as out of necessity (i.e. due to restraints on budgets, time, personnel to conduct labour, and/or access to laboratory equipment), and will always be at the discretion of the researcher. Thus, standardization of methodologies is not necessarily possible across research studies, unless restricted to certain cases, such as environmental monitoring programs where the same sampling equipment can target the same particle size ranges from the same environmental matrix. However, under all circumstances, researchers should strive for the best methodology

available to them, always employ rigorous contamination protocols, and report results quantitatively.

Microplastic research is an emerging science. While sampling, extraction, and reporting techniques are evolving quickly in the literature, many obstacles still remain that prevent comparisons of microplastic abundances between studies. Moving forward, it is important to refine techniques that capture microplastics down to size fractions <50 µm, to capture the majority of the bio-available particles that pose the greatest health risks, and to provide a comprehensive understanding of microplastic contamination in aquatic environments. Combining microplastic abundances with oceanographic data over time will permit the visualization of microplastic distribution at multiple spatial or temporal scales and allow for the prediction of "hotspots" of microplastics in marine environments.

Chemical polymer identification of microplastics is expensive and requires specialized machinery; thus, it may not be a readily available option for all researchers, particularly those conducting small-scale experiments. However, polymer identification is an essential piece of information needed to accurately and quantitatively characterize microplastic contamination in marine biota and the environment. Given these limitations, engaging in collaborations among laboratories, and pooling resources among researchers in particular would facilitate the sharing of data and results and is likely the best avenue to make future advances in the field of marine environmental pollution.

Nanoplastic quantification and enumeration methods remain a prominent data gap, and improvements in technologies to isolate and quantify nanoplastics in the marine environment are needed. The further development of particle staining technologies combined with fluorescence microscopy presents a promising solution.

At present, microplastics research requires a large investment in time and resources, particularly during the extraction and identification phases, in order to be rigorous, to allow for comparisons among studies, and to generate the best data for answering key questions about distribution, transport, and biological effects of microplastics. By working towards a common goal of accurately reflecting true micro- and nanoplastic abundances with minimal bias, researchers will be able to provide a global assessment on plastic contamination in the marine environment.

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10.0 TABLES

Table 1. Common plastic polymers and associated densities (adapted from Andrady 2011; Crichton et al. 2017; Hanvey et al. 2017). Rows in grey represent plastics that are denser than seawater (density = 1.03 $g \cdot cm^{-3}$), which are likely to sink.

Plastic Polymer	Abbreviation	Density ($g \cdot cm^{-3}$)	Common Commercial Products	Settling Location
Expanded polystyrene	EPS, styrofoam	0.015–0.03	Floats, foam cups	Floating at sea surface
Polypropylene	PP	0.85–0.83	Rope, bottle caps, netting, carpet	Floating at sea surface
Low-density polyethylene	LDPE	0.91–0.925	Plastic bags, six-pack rings, bottles, straws	Floating at sea surface
High-density polyethylene	HDPE	0.959–0.965	Milk and juice jugs, milk crates	Floating at sea surface
Polystyrene	PS	1.05	Plastic utensils, food containers	Mesopelagic
Polyamide	PA, nylon	1.02–1.15	Netting, traps, textiles	Mesopelagic/Sediments
Thermoplastic Polyester	PES	1.3–1.4	Beverage bottles, textiles (carpet and clothing)	Mesopelagic/Sediments
Polyvinyl chloride	PVC	1.3–1.45	Plumbing pipes, plastic film, bottles, cups, garden hoses	Sediments

Table 2. Storage methods for microplastic samples for sediment (SED), seawater (SW), zooplankton (ZOOP), and shellfish (SF). Efficiency rating is a qualitative assessment based on storage method, impact on microplastic integrity, and the potential for contamination, as well as a relative cost assessment based on fixative cost and preparation time, assuming unlimited storage space.

Method	Matrix	Efficiency	Degradation Potential	Contamination Potential	Relative Cost	Notes	Reference
Temperature methods							
Frozen at -20°C	SED	High	No	No	Low	Refrigerated to minimize biofouling.	Crichton et al. (2017)
4°C on vessel; -20°C in lab	SED	Med	No	No	Low	Potential for biofouling during refridgeration	Maes et al. (2017b)
Room temperature	SED	Med	No	No	Low	Potential for biofouling	Nuelle et al. (2014)
4°C in darkness	SW	High	No	No	Low	Minimized algal growth prior to analysis	Gewert et al. (2017)
-20°C in unused freezer bags	SF	High	No	Yes	Low	Potential for bag to contaminate sample. Need to rinse shell prior to dissection	Phuong et al. (2018)
Depurate 24 hr; frozen -20°C in tinfoil	SF	High	No	No	Med	Removes microplastics from gut, requires depuration facility	De Witte et al. (2014)
Frozen at -20°C	SF	High	No	No	Low	Samples can be frozen in tinfoil or unused plastic bags.	Catarino et al. (2017)
Cooled 1°C, -20°C with elastic bands	SF	Med	No	No	Low	Samples were measured and rinsed and then frozen.	Davidson and Dudas (2016)

Method	Matrix	Efficiency	Degradation Potential	Contamination Potential	Relative Cost	Notes	Reference
Preservation methods							
10% formalin	SW	Med	Yes	Yes (if solution not filtered)	Med	Lids covered with tinfoil. Does not discuss formalin being filtered.	Maes et al. (2017b)
5–10% HCl; stored at 4°C in lab	SW	Med	Yes	Yes (if solution not filtered)	Med	HCl solution requires filtering.	Desforges et al. (2014)
5% formaldehyde	ZOOP	Med	Yes	Yes (if solution not filtered)	Med	Formaldehyde solution requires filtering to avoid contamination.	Sun et al. (2017)
Other methods							
Deionized water in 20 mL vials	ZOOP	High	No	Yes	Med	Requires deionized water prior to survey or on vessel.	Desforges et al. (2015)
Mesh filters oven-dried at 60°C for >24 hr	ZOOP	Low	No	Yes (if not covered during filtration)	Med	Dessicated samples ground with mortar and pestle prior to enzymatic digestion.	Cole et al. (2014)
½ depurated for 3 days, ½ digested	SF	No	No	No	Med	Requires immediate processing of samples directly after collection, which limits distance sampling can occur from collection location.	Van Cauwenberghe and Janssen (2014)

Table 3. Collection methods for sediments with advantages and limitations. Collection methodology is project specific. Information can be used to make informed decisions that best suit study objectives. MP = microplastic.

Collection Method	Advantages	Limitations	Reference
- 3 kg sediment randomly at 3 cm depth using stainless steel spoon - stored room temperature	- processed large volumes of sediment - area analyzed larger than a core sample would provide	- unable to determine depositional information (i.e. accumulation over time) - variation with sampled area and depth	Nuelle et al. (2014)
- ROV push core in quiver mechanism - top water layer pipetted - cores sliced at 2/5 cm - slices in preserving solution or wrapped in aluminum foil and stored at -80°C	- able to analyze depositon of microplastics through time - easy to use - allows replication	- relatively small area sampled - require specialized equipment - boat with winch required - sampling may disturb sediment surface	Woodall et al. (2015)
- transect placed vertically between water line and high tide line - samples collected at low, mid and high tide - collected 250 mL sediment below top 5 cm - stored -20°C within 4 hr of collection	- higher resolution provided as able to look at differences in abundances at differing tidal heights - samples unlikely to become biofouled post collection as frozen within 4 hr of collection - rapid sampling - allows for replication	- examining 'relatively new' sediments - unable to determine deposition through time	Crichton et al. (2017)
- randomly collected top 1 cm sediment layer within 10 m^2 area using metal spoon	- relatively large area examined (due to random sampling technique)	- relatively small sample processed - looking at very recent deposition of microplastics	Karlsson et al. (2017)
- shallow samples collected with 'scoop' - deep locations collected using van Veen grab - collected top 5 cm layer - homogenized - cooled 4°C on vessel - frozen at -20°C in lab	- collected samples at multiple depths (intertidal and subtidal) - samples unlikely to biofoul after sampling due to immediate cooling/freezing	- unable to look at MP deposition through time - use of van Veen may disturb sediment surface	Maes et al. (2017b)

Collection Method	Advantages	Limitations	Reference
- 2 core liners placed into reineck box corer (66 mm diameter) - water-sediment layer siphoned and filtered GF/F - core sliced at 0.5 cm intervals with metal blade (0 cm–4.5 cm)	- able to look at MP deposition through time - due to multiple coring locations, study examined MP distribution (intra-study comparison) - examined MPs in water layer giving insight into exposure at time of grab	- equipment relatively expensive compared to intertidal/subtidal collection techniques - boat with winch required	Martin et al. (2017)

Table 4. Extraction methods for sediments with advantages and limitations for each and whether the method is recommended for adaptation (Adapt). Cost = relative cost is estimated as high, moderate, or low based on materials required and anticipated labour*; Cont. = relative potential for contamination estimated as high, moderate, or low; MP = microplastic.

Extraction Method	Design Advantages	Design Limitations	Cont.	Cost	Adapt?	Reference
Elutriation & NaI Density Separation - PVC column fitted with 1 mm sieve at top and 35 µm mesh at bottom filled with 500 mL sediment - upward flow created and aeration separates lighter particles from sand - NaI extraction for particles retained 35 µm - top layer filtered (5 µm filter) - density separation repeated 2 to 3 more times	- relatively large sample size - captured particles <10 µm - 100% recovery rate of PVC and 98% recovery for fibres - elutriation step minimizes volume NaI required by 97% - NaI density greater than NaCl - can use FTIR/Raman after separation - NaI low chemical hazard	- NaI costs 7 times more than NaCl (recognized by authors) - required to create elutriation tube and trial complex steps	Mod	Mod	Yes	Claessens et al. (2013)
Air-Induced Overflow & NaI Density Separation - sediment dried at 60°C - 1 kg sieved 1 mm mesh and added to NaCl solution - air-induced overflow used fluidization and NaCl solution to separate light particles into supernatant (see paper for details) - dried 60°C for 12 hr - filter residue transferred onto glass beaker - NaI density filtration - supernatant filtered (0.45 µm) - 35% H_2O_2 soak; 7 days (if needed)	- filtered down to 1 µm range - processed large volumes of sediment - ran 2 procedural blanks	- complicated processing involving high number of instruments and sample transfer - contamination deemed high and attributed to large number of occurrences sample exposed to air - potential for sample loss on sides of glassware (see their Fig. 2)	High	High	No	Nuelle et al. (2014)

Extraction Method	Design Advantages	Design Limitations	Cont.	Cost	Adapt?	Reference
Centrifuge - each slice centrifuged (4 000 rpm for 5 min) - supernatant poured through 32 µm sieve - repeated 8 times - material washed into cleaned pot with Millipore water	- ran multiple protocols to reduce airborne contamination - does not require costly chemicals	- require specialized equipment with possibility of breakage - multiple transfers of sample increasing contamination risk	Mod	Low	No	Woodall et al. (2015)
Density Independent Oil Extraction - sample dried 50°C - 50 g weighed out - 5 mL canola oil & 100 mL filtered water added to Erlenmeyer flask - oil layer decanted into separatory funnel 2 times - rinse twice with filtered water - settle 2–20 min - sediment/water removed - oil layer filtered with (1 µm polycarbonate) - detergent rinse 2 times and filter (onto same filter paper) - soak filter paper 10 min with ethanol (repeated twice)	- density independent separation cost effective - particles readily identified via FTIR after ethanol soak - procedural blanks run in parallel to samples - background blanks in laminar flow hood and fume hood	- numerous steps are labour intensive (processing estimated 2 hr·sample^{-1}) - max 6–8 samples run daily - potential for sample loss in waste (weathered plastics not tested)	Low	Low	Yes	Crichton et al. (2017)

Extraction Method	Design Advantages	Design Limitations	Cont.	Cost	Adapt?	Reference
<u>NaCl Density Separation</u> - sample homogenized; 20 g (w.w.) into separation flask - NaCl solution added - stir 10 min (magnetic stir) - add 1 drop olive oil - solution precipitated >5 hr - peristaltic pump push supernatant upward (see their Fig.1 for flask set up) - supernatant held in separate arm of the flask - vacuum filtered (0.7 µm filter) - 20 min soak with 30 mL 30% H_2O_2 to reduce organic matter - 1 drop dish soap rinsed glassware and filtered onto a second 0.7 µm filter	- method did not affect polymer identification via Raman spectroscopy - 1 procedural blank (assumed) per 3 samples, corrected for contamination - NaCl inexpensive, easily accessible, and a low chemical hazard	- relatively small sample processed - achieved 82% recovery rate spiked particles - NaCl solution excludes dense particles (e.g., PVC and PET) - labour intensive with relatively high number of steps for <90% recovery rate	Low	Mod	No	Karlsson et al. (2017)
<u>NaCl Density Separation</u> - 25 g sediment added to flask and NaCl solution (1.2 $kg \cdot L^{-1}$) - stirred 2 min - settle 1 hr - filter suspension 0.7 µm glass filter paper - rinse 30% H_2O_2 to remove residual organic matter	- captured particles down to 1 µm - ran two blanks alongside sample and 2 duplicates - corrected for contamination - followed standardized protocol for inter-study comparisons	- density = 1.2 $g \cdot cm^{-3}$; likely lost particles of greater density - small sample size processed followed by large extrapolation (25 g sample to $MP \cdot kg^{-1}$) - unable to stratify sediment	Low	Low	No	Maes et al. (2017b)
<u>Fractional Sieving</u> - coarse sediment sieved with interlocking stainless-steel sieves in 3 size fractions (500-µm, 400-µm, 250-µm) - sieve shaken 60 sec - sieve re-shaken 5 sec	- no density separation (i.e. low extraction cost) - 89% recovery rate of spikes - analyzed depositional layers - does not require volume vs. weight decision for inter-study comparisons	- lower size limit 250 µm - underestimate total MP abundance - requires expensive sampling equipment and boat access	Low	Mod	Yes	Martin et al. (2017)

Extraction Method	Design Advantages	Design Limitations	Cont.	Cost	Adapt?	Reference
- ID'd plastic equipment with FTIR to avoid false (+) ID through contamination - Ran continuous background blanks (24 hr) - debris suspected as procedural contamination not included in analysis						

* if more than one extraction technique was trialled, only optimized techniques are discussed.

Table 5. Collection methods for seawater with advantages and limitations. Collection methodology is project specific. Information can be used to make informed decisions that best suit study objectives. MP = microplastic.

Collection Method	Advantages	Limitations	Reference
Subsurface - pumped 4.5 m below surface using saltwater intake system for 10–20 min - sieved (250-µm, 125-µm and 62.5-µm) - preserved 5–10% HCl at 4°C	- cover large survey area - estimating sub-surface plastics - sampling less influenced by weather (i.e. intake system is always submerged) - easy to collect samples - allows choice of mesh sizes	- requires vessel with salt water intake system adapted to filter samples - initial investment costly but reduced if complete numerous studies - potential contamination from boat	Desforges et al. (2014)
Surface microlayer - gentle tapping of SML using metal sieve (depth 150–400 µm) Manta trawl - 10 min trawls at ~2 kn (333 µm mesh)	- estimate MPs at all size fractions along sea surface (preferred) - utilizes common seawater sampling equipment - minimal space required to store samples (no bulk water)	- require optimal conditions to collect samples from side of the vessel	Song et al. (2014)
Manta trawl - 10–16 cm below surface of water (333 µm mesh) - 20 min tow at ~1.2 kn Subsurface - Samples from vessel intake sieved 250 µm (total volume = 2 000 L)	- collected surface and subsurface samples - likely capture particles of varying densities	- denser particles and/or particles entrapped in zooplankton likely lost - underestimated total MP abundance with large mesh sizes	Lusher et al. (2015b)
Neuston net - 350 µm mesh size for 20–30 min at 2–3 kn - stored 4% formalin	- utilizes common seawater sampling equipment - large survey area - high likelihood for inter-study comparisons - minimizes potential for contamination	- large mesh size does not capture particles <350 µm - particles captured influenced by weather conditions	Isobe et al. (2017)

Collection Method	Advantages	Limitations	Reference
Manta trawl - 333 µm mesh - surveyed 60 mins between 1.6 and 8.2 kn - preserved 10% formalin	- large survey area - minimal potential for contamination - high likelihood for inter-study comparisons	- large mesh size does not capture particles <333 µm - particles captured influenced by weather conditions - potential for formalin to interact with some MPs	Maes et al. (2017b)
Manta trawl - mesh size not specified - samples frozen	- sample frozen (no risk MP impact)	- assume relatively large mesh size does not capture particles <300 µm - particles captured influenced by weather conditions	Wagner et al. (2017)
Manta net - 3 m long, 300 µm mesh - rectangular opening 50 cm wide × 15 cm deep - towed 5 min at 5 kn at depth of 15 cm Bongo net - 500 µm mesh, 30 cm diameter - towed 5 min at 5 kn at depth of 1 m Zooplankton nets - 200 and 400 µm mesh - towed from side of moored barge for 5 min Discrete sample - 1 L bottle grab	- manta and bongo nets sample large volume of water - zooplankton nets sample medium volumes of water and capture lower size range of particles (100 µm) - bottle grabs sample micro- and nano-scale particles - capping bottles underwater reduces airborne contamination during sampling (minimal potential for contamination in field)	- preservation method not mentioned (assumed none) - neuston and bongo nets time-consuming to use with higher potential for contamination - zooplankton nets may become clogged leading to biased sample volumes - bottle grabs sample small volumes of water leading to high variability among samples - requires optimal conditions to collect bottles from side of the vessel	Green et al. (2018)
Neuston net - 335 µm mesh Discrete sample - 1 L bottle grab	- bottle grabs collected >3 orders of magnitude more MPs per volume of water than nets - bottle grabs capture smaller size range than nets - bottle grabs can be used in locations where neuston nets are impractical	- bottle grabs sample small volumes of water leading to high variability among samples - bottle grabs need large sample size to be representative	Barrows et al. (2017)

Collection Method	Advantages	Limitations	Reference
	- nets sample large volume of water and captures more large-sized microplastics	- ability of nets to detect small microplastics limited by large mesh size	
Discrete sample - 1 L jars filtered to 8 µm in lab - 10 L metal bucket sieved to 63 µm in field - shore-based sampling methodology	- jar samples capture up to 8.5 times more microplastics per L than bucket samples - jars collect more microfibres than buckets - filtering to 8 µm found higher concentrations of microfibres relative to 63 µm	- small total volume of water sampled could limit diversity of particles collected that are present in the wider sampling area - only particles >100 µm were reliably detected using visual microscopy	Covernton et al. (2019)

* Vessel access with trawling capability assumed.

Table 6. Extraction methods for seawater with advantages and limitations for each, and whether the method is recommended for adaptation (Adapt). Cost = relative cost estimated as high, moderate, or low based on materials required and anticipated labour*; Cont. = relative potential for contamination estimated as high, moderate, or low†; SML = surface microlayer; Raman = Raman spectroscopy; ATR-FTIR = attenuated total reflectance Fourier-transform infrared spectroscopy; MP = microplastic. Note: studies employing digestion methods extract microplastics from seawater matrix + biota in sample.

Extraction Method	Advantages	Limitations	Cont.	Cost	Adapt?	Reference
Acid digest - digested with concentrated HCl (undefined) at 80-90°C for 3 hr - Nile red dye (3 $\mu g \cdot ml^{-1}$) added to solution - filtered 0.45 µm mixed cellulose ester filter paper	- filtered <1 µm - dye easily identifies polymers present at small size fraction	- strong acid likely destroyed sensitive plastics (primarily fibres) - no discussion on contamination protocols or blanks used	High	Mod	No	Desforges et al. (2014)
Filter & H_2O_2 Digest (SML) - filtered directly onto 0.75 µm GF/F - filter dried 60°C (Manta) - 2 week digest in 35% H_2O_2 - filtered 0.75 GF/F - filter dried 60°C	- minimal handling of sample reduces risk of contamination - retain particles down to 1 µm - confirmed particle ID with subset particles using FTIR	- 35% H_2O_2 digest likely impacted polymer size and shape	Low	Low	Yes	Song et al. (2014)
Vacuum Filtration - 24 hr settling period in graduated cylinder (gravity) - supernatant (top layer with MPs) filtered using GF/C Subsurface - filtered GF/C	- minimal steps with inexpensive reagents - analyzed surface and subsurface samples	- denser particles and/or particles entrapped in zooplankton likely lost - underestimated total MP abundancy with large mesh sizes	Low	Low	No	Lusher et al. (2015b)

Extraction Method	Advantages	Limitations	Cont.	Cost	Adapt?	Reference
- no extraction method described - assume direct visual ID - FTIR particles too small to visually ID	- minimal handling time severely minimizes potential contamination - fast and cost effective (for clean water samples such as the Arctic)	- not repeatable for sites with high MPs or areas with high biogenic matter - did not quantify fibres, EPS - chemical polymer ID not a complete subset of MPs counted visually	Low	Low	No	Isobe et al. (2017)
Fractional Sieving - separated sample onto sieves of six size classes (4.79-mm, 2.79-mm, 0.999-mm, 0.709-mm, 0.499-mm) - contents concentrated into petri dish for visual ID	- MPs retained in different size fractions - minimal potential for contamination	- mesh size large and sieve size also large - MP abundances reported >3 330 µm	Low	Low	No	Maes et al. (2017b)
Vacuum Filtration - 1 mm sieve in glass funnel (particles >1 mm collected) - filtered 10 µm PC filter - subset 15 mL sample collected in triplicate - remaining solution filtered 0.1 µm PC filter	- MPs retained from all size fractions - particles isolated for FTIR, SEM and RMS** - sample frozen (no risk MP impact) - isolation technique relatively simple with standard laboratory glassware	- small sample size for polymer ID - moderate sample handling adds some risk for contamination - further information needed to understand colloidal behaviour of MPs in solution (intertwined MFs missed?)	Mod	Low	Yes	Wagner et al. (2017)
Filtration - manta and plankton net samples filtered onto cellulose filter paper (11 µm retention) - bongo net and bottle grab samples filtered through 0.45 µm glass fibre filters (GF/F) - filters dried in clean petri dishes for visual ID	- minimal steps with no reagents - procedural and background blanks conducted during sample processing - FTIR characterization of 10% of particles randomly selected	- preservation method not mentioned - potential for contamination during filtration - length of filtration time and use of vacuum not mentioned	Mod	Low	Yes	Green et al. (2018)

Extraction Method	Advantages	Limitations	Cont.	Cost	Adapt?	Reference
Density Flotation and Vacuum Filtration - for neuston tows: hyper-saline solution (500 mL NaCl per L 0.45 µm filtered seawater) used to separate plastics from biological material - vacuum filtered through 0.45 µm, 47 mm diameter mixed cellulose nitrate filter - filters covered and dried at room temperature	- density flotation step separates water from biota - assessed background contamination through multiple background and procedural blanks - minimal variation in processing technique used for different sample types - retain particles down to 1 µm in size - particle size measured using microscope imaging software	- microplastics less than 100 µm could not be accurately identified visually - no access to chemical identification methods (e.g., FT-IR or Raman) so reported counts are conservative estimates	Low	Low	Yes	Barrows et al. (2017)
Digestion and Vacuum Filtration - vacuum filtered through 8 µm (bottles) or 63 µm (buckets) stainless steel mesh - samples dried at 60°C for 48 hr - 100 mL of 10% KOH added for incubation at 60°C for 24 hr - Vacuum filtered through 47 mm diameter 8 µm polycarbonate membrane filter	- 24 h KOH incubation has minimal effect on most plastic polymers - Glass filter funnels warmed and rinsed to minimize precipitates forming from digstate - Background contamination minimized through series of precautions and blanks	- microplastics less than 100 µm could not be accurately identified visually	Low	Mod	Yes	Covernton et al. (2019)
Collodial Fraction Separation (Nanoplastics) - 1 L seawater filtered 1.2 µm poly (ether sulfone) membrane - Filtered solution concentrated via ultrafiltration - Final retentate volume = 10 mL - ATR–FTIR (using 16 background scans)	- first study examining nanoplastics in seawater - authors used quantitative approach to determine molecules present and identify polymers after filtering through 1.2 µm filter paper	- high number of steps - procedure requires expensive machinery - analysis time is assumed to be high, making low likelihood of high sample throughput	Low	High	Yes	Ter Halle et al. (2017)

Extraction Method	Advantages	Limitations	Cont.	Cost	Adapt?	Reference
- Solution freeze-dried and resultant salt crystals crushed to homogenize; 25 mg lyophilitaze added - Pyrolysis: 700°C for 1 min - Thermodesoption: 300°C for 1 min - Thermochemolysis: 400°C for 1 min - Separated resultant gases using gas chromatography. - Compared mass spectra to library - Determined relative PVC, PS and PET	- procedure is well explained and uses well-established, repeatable methods					

* if more than one extraction technique was trialled, only optimized techniques are discussed.
†Vessel access with trawling capability assumed.

Table 7. Collection methods for zooplankton with advantages and limitations. Collection methodology is project specific. Information can be used to make informed decisions that best suit study objectives. MP = microplastic.

Collection Method	Advantages	Limitations	Reference
- horizontal subsurface tow (no depth specified) - 200 µm and 500 µm plankton nets - transferred samples to insulated containers upon collection then transported to laboratory within 2 hr of trawling - sieved through 200 µm mesh and rinsed Milli-Q (to remove salt) - manually removed macrozooplankton and large debris items	- sampling method similar to other studies	- manually removing objects increases risk of airborne contamination - no sample preservation; death and disintegration of species could have occurred within 2 hr prior to sample processing	Cole et al. (2014)
- vertical tows at 250 m and 10 m off seafloor bottom - Bongo nets with 236 µm mesh size - flowmeter in net opening - stored 10% buffered formalin in seawater for species ID - samples passed through 500 µm sieve - zooplankton individually removed and stored in 20 mL glass vials in deionized water - examined 50 individuals per sampling site, where possible	- measured flow and able to determine volume of water sampled - sampling method similar to other studies - zooplankton individually examined for externally adhered microplastics - separated zooplankton from water; well rinsed/ "cleaned" so could tell difference between consumed MPs vs. in seawater	- preservation technique will impact ability to modify extraction technique using enzymes - manually removing objects increases risk of airborne contamination - clumping and aggregation of material in sieved samples possible	Desforges et al. (2015)
- vertical tows at 200 m and 10 m off seafloor bottom - conical plankton nets with 505 µm and 160 µm mesh - preserved samples 5% formaldehyde solution - samples split equally to calculate species abundances and complete micr analysis	- compared effectiveness between net sizes - replicate samples allows for species ID and MP analysis to be completed independently (i.e. decreasing contamination risk) - sampling method similar to other studies	- preservation technique will impact ability to modify extraction technique using enzymes - manually removing objects increases risk of airborne contamination	Sun et al. (2017)

Table 8. Extraction methods for zooplankton with advantages and limitations for each, and whether the method is recommended for adaptation (Adapt). Cost = relative cost estimated as high, moderate, or low based on materials required and anticipated labour*; Cont. = relative potential for contamination estimated as high, moderate, or low; MP = microplastic.

Extraction Method	Advantages	Limitations	Cont.	Cost	Adapt?	Reference
Proteinase-K - desiccate tissues at 60°C for 24 hr and grind - grind desiccate with mortar and pestle - 15 mL homogenizing solution and homogenize - incubate 15 min at 50°C - add 500 µg·mL^{-1} Proteinase-K per 0.2 g DW - incubate 2 hr at 50°C - 5M $NaClO_4$; agitate 20 min - homogenize and incubate 20 min at 60°C - filter 50 µm mesh-filter - oven dried at 60°C	- enzyme does not affect MPs - no contamination observed in blanks	- expensive enzyme - oily film remains (suggest include Chitinase increasing cost) - small volume tissue digested per trial - grinding tissues poses potential for sample loss - multiple steps increase potential sample loss and/or contamination	High	High	No	Cole et al. (2014)
HNO_3 - fill plate with 1 zooplankton per well - cover individuals with 100% HNO_3 - place lid on well plate and heat 80°C for 30 min - visually examine each plate <30 min - ran procedural blanks (amount not specified)	- dissolved all biological material (including chitin) - no contamination observed - ran blanks for each well plate to estaimte contamination - allowed determination of microplastics to finest possible resolution (individual level)	- strong acid - HNO_3 degrades nylon and PE - underestimated total fibres which constituted >50% MPs identified - visual ID using exposed well plates (assumed) increases contamination risk - placing one zooplankton per well time consuming (assumed)	High	Low	No	Desforges et al. (2015)

Extraction Method	Advantages	Limitations	Cont.	Cost	Adapt?	Reference
HNO_3 - individuals placed in 20 mL scintillation vial - followed procedure described Desforges et al. (2015) within vials - 3 hr digestion in water bath at ~80°C - filtered samples through 0.45 µm mixed-cellulose ester filter paper - ran several procedural blanks (exact number not specified)	- each digestion done in vial to minimize contamination - first report MP abundances in 5 zooplankton groups - no contamination reported in procedural blanks	- use of strong acid underrepresents number of fibres - unknown if number of blanks is appropriate for number of samples run	Mod	Low	No	Sun et al. (2017)

* If more than one extraction technique was trialled, only optimized techniques are discussed.

Table 9. Extraction methods for fish and shellfish, with advantages and limitations for each, and whether the method is recommended for adaptation (Adapt). Cost = relative cost estimated as high, moderate, or low based on materials required and anticipated labour*; Cont. = relative potential for contamination estimated as high, moderate, or low; MP = microplastic.

Method	Advantages	Limitations	Cont.	Cost	Adapt?	Reference
		Shellfish				
HNO_3 - depurate 24 hr - 20 mL HNO_3 (22.5 M) - digest overnight at room temperature - boil 2 hr - dilute to 200 mL with filtered water - filter 5 µm (cellulose nitrate)	- compared 3 digests (HNO_3, H_2O_2, NaOH) - recovered 93–98% PS and large nylon fibre spikes - short digest period - moderate temperature - highly cited in literature	- strong acid digestion - affects pH sensitive plastics - 0% recovery small nylon fibres (30 x 200 µm) - no cont. reporting - high extraction efficiency but, nylon not included	Mod	Mod	No	Claessens et al. (2013)
HNO_3 & $HCLO_4$ - depurate 24 hr - 65% HNO_3 and 68% $HClO_4$ (4:1 v:v) - 500 mL per 100 g tissue - digest overnight at RT - boil 10 min and dilute with 500 mL warm filtered water - boil until fully digested filter (qualitative retention to 10 µm)	- monitored/reported contamination - removed contaminant fibres from analysis - developed Limit of Detection (LOD) for each colour fibre in procedural blanks (aka correction factor)	- strong acid digestion - affects pH sensitive plastics - does not discuss potential for nylon degradation - 24 hr for depuration may not be long enough to expunge environmentally-acquired microplastics	Mod	High	No	De Witte et al. (2014)
H_2O_2 & NaCl - 200 mL 30% H_2O_2 - heat (55–65°C) until H_2O_2 evaporates - 2–3 density separations using 200 mL NaCl (1.2 $g \cdot cm^{-3}$) - magnetic stir 1–2 min - settle 1–2 min - pipette supernatant - filter 0.8 µm (nitrocellulose)	- removed MPs from remnant biological tissues - ran procedural blanks - well cited in literature	- roughly 100 to 150 fibres per filter for blanks not accounted for in analysis - high contamination - potential sample loss for high density MPs (PVC, PET, nylon) - potential loss of sample during pipetting (MPs could stick to sides) - small sample size	High	High	No	Mathalon and Hill (2014)

Method	Advantages	Limitations	Cont.	Cost	Adapt?	Reference
HNO_3 - depurated ½ mussels - followed Claessens et al. (2013) - ID subset MPs via Raman spectroscopy	- controlled contamination	- 0% recovery of nylon fibres	Mod	High	No	Van Cauwenberghe and Janssen (2014)
KOH - 3x volume 10% KOH - incubate 24 hr at 60°C	- successful digestion - controlled/reported contamination	- did not filter digest (manually sorted) - specialized equipment required in fumehood - lower limit 500 µm - did not determine plastic type	Mod	Low	Yes	Rochman et al. (2015)
H_2O_2 - pooled individuals (2–5 per jar) - 200 mL 30% H_2O_2 - oscillate 80 rpm for 24 hr at 65°C - oscillate 80 rpm for 24–48 hr at room temperature - 800 mL NaCl overnight - filter overlaying water 5 µm (cellulose nitrate)	- digested many species - ID MPs down to 5 µm - fibres most prominent - FTIR concluded transparent spheres aluminum silicate	- upper weight limit when using H_2O_2 as a digest - foaming - high temperature - multiple steps - lowwe NaCl density (1.2 $g \cdot cm^{-3}$) than plastics led to potential sample loss - flaking residue left behind	Low	Low	No	Li et al. (2015)
HNO_3 - freeze without depuration - 40 mL (70%) HNO_3 for 4 hr at 90°C - dilute (1:10) 90°C filtered water - filter 1.2 µm (type not specified)	- report MPs weight per gram tissue - report procedural and background blanks	- HNO_3 method outdated - did not explain how account contamination in data analysis - FTIR not completed	Mod	Low	No	Davidson and Dudas (2016)
Corolase (enzyme) - 1 mL Corolase enzyme in 100 mL water - magnetic stir plate at 60°C overnight - filter 0.8 µm (cellulose nitrate) or 1.6 µm glass microfibre (when analyzed via FTIR)	- reported contamination and accounted in data - FTIR spectra unaffected - efficient digestive process - minimal potential airborne contamination - enzyme safe to handle and cost effective	- enzyme availability (from Germany) - magnetic stir bar could break particles apart (overestimate values)	Low	Low	Yes	Catarino et al. (2017)

Method	Advantages	Limitations	Cont.	Cost	Adapt?	Reference
HNO_3 - followed Claessens et al. (2013) - filtered using 2.5 µm cellulose filter paper	- expanded protocol to include blue mussels, Pacific oysters and Manila clams - included 9 procedural blanks	- foaming during heating accounted for potential sample loss - stirring required created avenue for contamination and/or sample loss	Mod	Mod	No	Murphy (2018)
H_2O_2 - soft tissues dissected into flask - followed Li et al. (2015) for digestion of each individual oyster - spiked 10 samples with blue nylon and yellow PP fibres to quantify % recovery rates	- exhibited 91% and 92% recovery for nylon and PP fibres, respectively - reported values in oysters compared to seawater samples collected	- did not discuss contamination procedures in depth (no discussion on use of flow hood, procedural or background blanks) - results less reliable due to lack of contamination discussion	High	Low	No	Waite et al. (2018)
		Fish				
Manual extraction - dissect GI tract and visually sort contents for 10 min (max)	- first record MP ingestion in fish from English Channel - examined multiple species (10 total)	- possible to miss particles among stomach debris - potential for airborne contamination	High	Low	No	Lusher et al. (2013)
KOH - 3x volume 10% KOH - 2–3 week digest at room temperature - filter over 200 µm sieve	- digested biological tissues in multiple species (1 203 fish, 7 species) - low temperature - minimal steps - highly cited method	- long digest period - contamination not monitored (airborne was suspected) - filter limit 200 µm	Mod	Low	Yes	Foekema et al. (2013)

Method	Advantages	Limitations	Cont.	Cost	Adapt?	Reference
NaOH - visual sort (10 min max) - desiccate 24 hr at 90°C - 10 mL NaOH (1M) and shake periodically for 21 d - filtered (size not stated)	- successfully filtered 212 individuals	- long digest period - no blanks reported - potential fibre contamination during visual sorting	High	Mod	No	Bellas et al. (2016)

* If more than one extraction technique was trialled, only optimized techniques are discussed.

Table 10. Comparison of popular techniques to determine microplastic polymer type. The analysis provided below assumes that a subset of particles are identified to determine chemical composition, as opposed to the entire set of particles quantified during visual ID. Cost = relative cost of required reagents and labour intensity (assumes full access to all machinery).

Method	Lower Size Limit	Relative Contamination Potential	Cost
Fourier-transform infrared microscopy (FTIR)	10 µm[1]	Low[2]	High
Raman microscopy (Raman)	1 µm[1]	Mod	High
Scanning electron microscopy (SEM)	nm	Mod	Mod
Pyrolysis–gas chromatography–mass spectrometry (Pyr-GC-MS)	nm	Low	Mod
Atomic force microscopy (Proposed)	nm	NA	NA

[1] Shim et al. (2017)

[2] assuming individual particles are isolated and characterized before identification

Table 11. Comparison of chemical polymer identification techniques. The advantages and limitations provided below are under the assumption that a subset of particles are identified to determine chemical composition (as opposed to the entire set of particles quantified during visual identification). FTIR = Fourier-transform infrared microscopy; ATR-FTIR = attenuated total reflectance FTIR; Raman = Raman microscopy; SEM = scanning electron microscopy; Pyr-GC-MS = Pyrolysis–gas chromatography–mass spectrometry; FPA = focal plane array; AFM = atomic force microscopy; PSXL = cross-linked polystyrene; MP = microplastic.

Method	Advantages	Limitations	Reference
FTIR	- eliminates false positives or false negatives for particle - non-destructive analysis (for specific techniques) - potential automatic mapping using FPA-reflectance mode (for particles 150 – 250 µm)	- machine expensive - particle ID labour intensive - requires contact (ATR) - lower limit 10 µm (compared to Raman) - weathered/small plastics easily lost	Shim et al. (2017)
	- ATR-FTIR optimal for irregular shaped MPs (compared micro-FTIR) - FPA able to cover large surface areas	- ATR-FTIR for MPs >500 µm - classifying polymers in complex environmental samples challenging - manual sorting required before analysis can begin	Mai et al. (2018)
	- label-free technique	- difficult to distinguish cellulose vs. rayon fibres	Comnea-Stancu et al. (2017)
	- collect spectra with Transmittance, Reflectance or Attenuated Total-Reflectance modes - no sample prep required	- requires access to polymer library for reference comparison	Rocha-Santos and Duarte (2015)
	- multiple techniques used for polymers of various shapes and colours - µFTIR transmittance mode = transparent/semi-transparent MPs - ATR-FTIR = opaque MPs	- requires in-depth knowledge of complex machinery	Li et al. (2015)
	- µ-ATR-FTIR - non-destructive - widely independent of particle mass - reliable and fast analysis of polymer type (~1 min for one spectrum)	- size limited (ATR measurement point ~25 µm) - does not provide information on organic additives - contaminations and additives can overlap polymer bands = disturbed identifications - manual sample handling (samples need to be placed on IR microscope stage)	Käppler et al. (2018)

Method	Advantages	Limitations	Reference
Raman	- lower limit 1µm (smaller laser than FTIR) - non-destructive analysis - no contact required	- machine expensive - particle ID labour intensive - pigments impede ID - require access to polymer library for reference comparison	Shim et al. (2017)
	- relatively small lower limit - less interference to non-polar plastic functional groups	- requires relatively pure sample to eliminate false positives - laser more expensive than FTIR ($250k vs. $50k)	Mai et al. (2018)
	- successfully identified PE and PS	- could not distinguish between LDPE vs. HDPE or PS vs. EPS vs. PSXL	Dehaut et al. (2016)
SEM/SEM-EDS	- provides qualitative analysis of physical characters (e.g., cracking) - universities likely have microscope on site (more accessible technology) - compensates for lower magnification limits of optical microscopes that may miss small microplastics	- requires laborious preparation steps	Hidalgo-Ruz et al. (2012)
	- coating not required when working in low vacuum - no sample prep required - chemical and morphological particle characterization	- cannot determine colour of particles for classification	Rocha-Santoz and Duarte (2015)
	- provides high-resolution images and elemental composition signatures - identified adhere silicon oxides on surfaces of microfibres and carbon peaks on foamed granules - stong fluorine peaks on microfilms identified as PTFE microplastics - accurately distinguished between plastic and non-plastic particles in samples - allows calibration of polymer types when combined with spectroscopic techniques to improve plastic ID accuracy	- labourious preparation steps - inconvenient to use before spectroscopic techniques - colours of small particles cannot be determined - only applied to representative microplastic particles rather than entire sample	Ding et al. (2019)

Method	Advantages	Limitations	Reference
Pyr-GC-MS	- provides simultaneous information on polymer type and additives	- destructive method - require access to polymer library for reference comparison - data relatively complex	Shim et al. (2017)
	- no pre-sorting required	- upper limit <1 mm - one polymer identified at a time - multicomponent samples produce only mass results (no particle counts)	Mai et al. (2018)
	- obtain structural information of macromolecules - able to extract organic plastic additive (OPA) information - solvents not required - background contamination low - able to work with particles with masses <350 µg	- heterogeneity of OPAs requires marine MPs be analyzed individually - limited sample mass (0.5 mg) may compromise the representativeness when complex environmental samples are analysed	Fries et al. (2013)
	- successfully identified PE and PS - 80% ID success rate post digestion	- could not distinguish between LDPE vs. HDPE or PS vs. EPS vs. PSXL	Dehaut et al. (2016)
	- widely independent of particle shape and size - inorganic additives do not disturb - simultaneous detailed information about chemical nature of polymer and contained organic additives	- reference polymer databases still under development and not as established as for FTIR so requires literature research or expert experience - inorganic additives (e.g., plastic fillers or pigments) cannot be detected - detection limit depends on polymer type (pg to µg level)	Käppler et al. (2018)
	- optimized method achieved limit of detection <1 µg and 50 µm; thus, applicable to very small and light particles - accurately identified PS, PMMA, PE - verified method with µ-Raman to determine identification rate - identified copolymers (PE-PP or PP-PP-PA6) better than µ-Raman	- sample handling; small particles (<50 µm) difficult to manipulate	Hermabessiere et al. (2018)

Method	Advantages	Limitations	Reference
TED-GC-MS	- used relatively larger sample amounts (up to 100 mg) to increase representativeness of the sample analyzed - rapid analysis (2–3 hr per measurement) - no separation of plastics from environmental matrix required - successfully identified PP, PE and PS particles from bio-gas plant ferment residues, and PE and PS from river water samples	- cannot determine size distribution, colour, or morphology of analyzed particles - pre-treatment (i.e. cutting and milling) may be necessary to reduce natural organic matrix to improve efficacy of identification of microplastics	Dümichen et al. (2017)
AFM (Proposed)	- potential NP analysis (when combined with IR/Raman)	- machinery expensive - method development required - currently difficult to isolate and subsequently find single particles in nm range	Shim et al. (2017)

Table 12. Common microplastic (MP) polymer shape, colour, and type categories, and results presented for reporting microplastic abundances in sediment, seawater, zooplankton, and shellfish. ABS = acrylonitrile butadiene styrene; PAS = Poly(acrylate/styrene).

Matrix	Shape	Colour	Type	Results Presented	Notes	Reference
Sediment	- fibre - foamed - granule - sheet - pellet - fragment	- red - yellow - blue - white - green - dark	- ABS - PVC - EPS - vinyl	- recovery results only	- spike particle types confirmed by FTIR	Crichton et al. (2017)
	- fibre - fragment - sphere - pellet	- blue - transparent - white - red - black - green - grey	- PA - PET - PP - Acrylic	- total MP count by core layer - % MP in water-sediment interface - % MP by shape - % MP by colour - % polymer type identified by FTIR - mean MP by area	- excluded rayon (hard to decipher from cellulose)	Martin et al. (2017)
	- fibre - fragment - sphere	NA	NA	- max and min #MP per kg by site - max and min by particle shape by site - avg by particle shape per kg by site - avg total MP by site - compared #MP to grain size	NA	Maes et al. (2017b)
Seawater	- fibre/filament - fragment	not defined	NA	- MP·m^{-3} by site - min/max MP·m^{-3} for study value - avg particle size (µm) by site - size distribution by site	NA	Desforges et al. (2014)
	- fibre - fragment - film	not defined	- PE, PA, PE - Acrylic - PVC - Cellulose (possibly Rayon) - Unknown origin	- MP·m^{-3} by site - total MP visually identified - % MP by shape - % MP by colour - particle size range - avg particle length - % polymer type identified by FTIR	NA	Lusher et al. (2015b)

Matrix	Shape	Colour	Type	Results Presented	Notes	Reference
	- paint resin - fragment - spherule - sheet - EPS	NA	- PP, PE, PS, PES - Alkyd - PAS - Phenoxy resin - Synthetic rubber	- MP·L^{-1} by site - % particles by category - binned particles by max size - length range by shape - ratio paint to non-paint particles	NA	Song et al. (2015b)
	- fragment - pellet - line - film - foam	- black/gray - blue/green - brown/tan - orange/pink/red - transparent/translucent - white - yellow	NA	- #MP·km^{-2} - total items collected - abundance range - % MP by type - % MP by colour - provided various abundances based on different metric to cover trawl distances	NA	Maes et al. (2017b)
Zooplankton	- fibre - fragment - bead - macroplastic	- white/clear - black - red - orange/yellow - green - blue - brown	- PA, PP, PE - Polyurethane - Acrylic	- #MP·m^{-3} by site - avg MF length - avg MP length - most common colour types MF & MP	NA	Cole et al. (2014)
	- fibre - fragment	not defined	NA	- #MP·m^{-3} for copepods/euphasiids - encounter rate (#plankton per #MPs) - avg plastic size (µm) per group - % fibre of total MP count - corrected for biodilution effect		Desforges et al. (2015)
	- fibre - particle - other	not collected	NA	- #MP·m^{-3} by zooplankton group for Net 1 - #MP·m^{-3} by zooplankton group for Net 1 - % MP shape - avg particle length - encounter rate (%)		Sun et al. (2017)

Matrix	Shape	Colour	Type	Results Presented	Notes	Reference
Shellfish	- fibre - fragment - pellet	- black - red - blue - white - transparent	- PE - PET - PA - Other	- #MP·g^{-1} tissue by species - #MP·ind.$^{-1}$ by species - most common colours - % particle shape by species - % different size classes by species - size range of MPs observed	- only describes shapes and colours observed	Li et al. (2015)
	- fibre - fragment - film	not provided	NA	- #MP·g^{-1} tissue by area - #MP· ind.$^{-1}$ - most dominant MP shape - most common MP colours observed - % MP shape	NA	Davidson and Dudas (2016)
	- fibres - pellet - fragments	- black - red - grey - orange	- PE - PET copolymer - PVC copolymer	- #MP·g^{-1} tissue by location and by species - % MP by size category - % MP by particle shape	- only describes colours observed - identified 6 particles (3 of 6 were confirmed plastic)	Murphy (2018)
	- fibres - beads - fragments	- blue - clear - red - green - black	NA	- #MP· ind.$^{-1}$ - #MP·g^{-1} tissue - % colour by particle type - total MP by shape	NA	Waite et al. (2018)

11.0 FIGURES

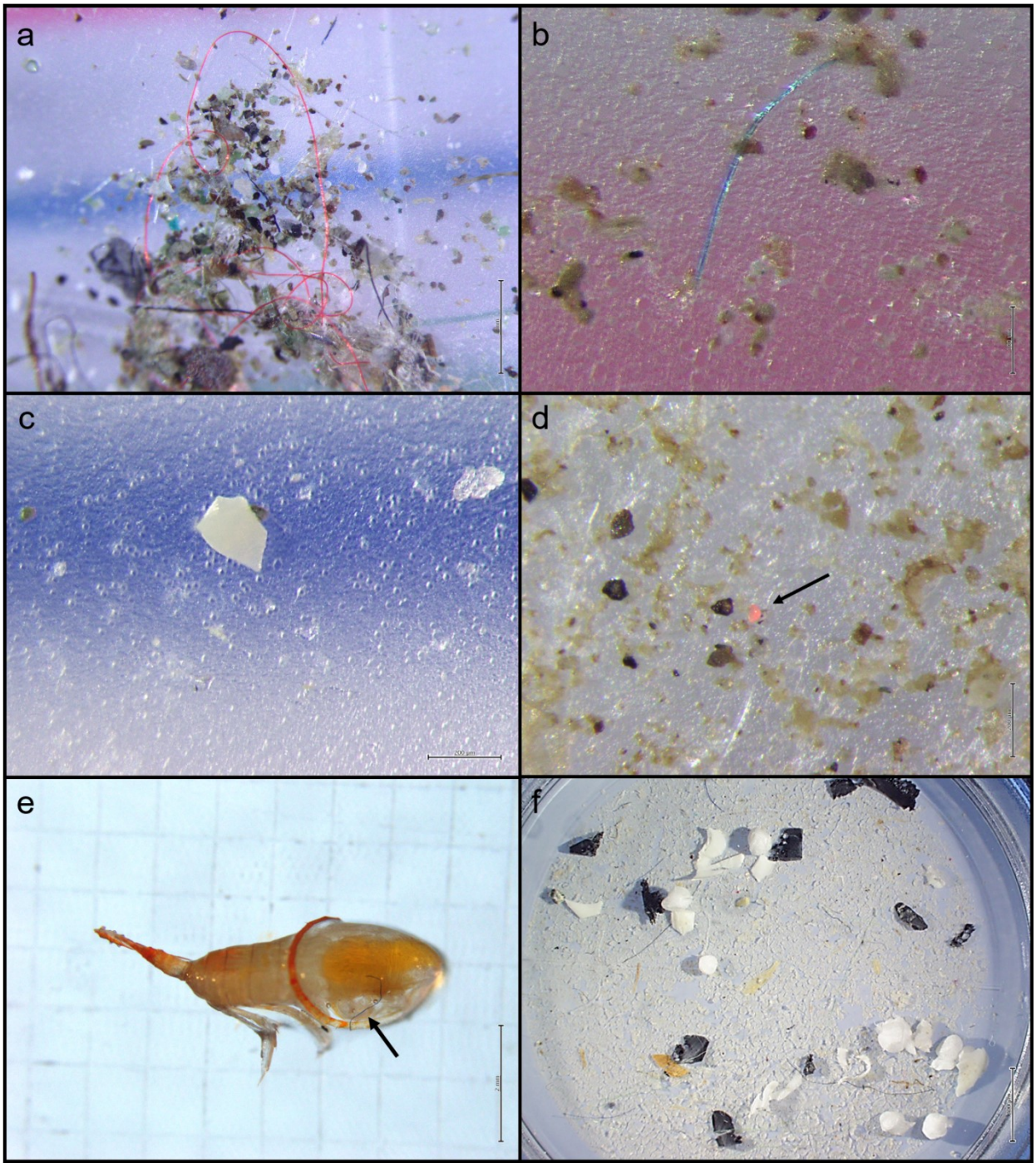

Figure 1. Microplastics extracted from various biological matrices: (a) mass of intertwined fibres from 30 L sieved water sample, Campbell River, B.C.; (b) nylon fibre extracted from blue mussel; (c) polypropylene fragment and (d) polyethylene sphere, extracted from biological matrices; (e) copepod sampled from Chukchi Sea, showing contamination microfibre; (f) petri dish containing extracted microplastic fragments from a spiked seawater matrix, showing flaky residue left behind after KOH digestion (static on filter paper is causing particles to bounce off). Scale bars: (e) 2 mm; (a) 1 mm; (f) 500 µm; (c, d) 200 µm; (b) 100 µm. Photo credits: (a), (b), and (f) by Julie Dimitrijevic; (c) and (d) by Rhiannon Moore (Simon Fraser University/Ocean Wise Conservation Association); (e) by Lauren Howell (Simon Fraser University/Ocean Wise Conservation Association).

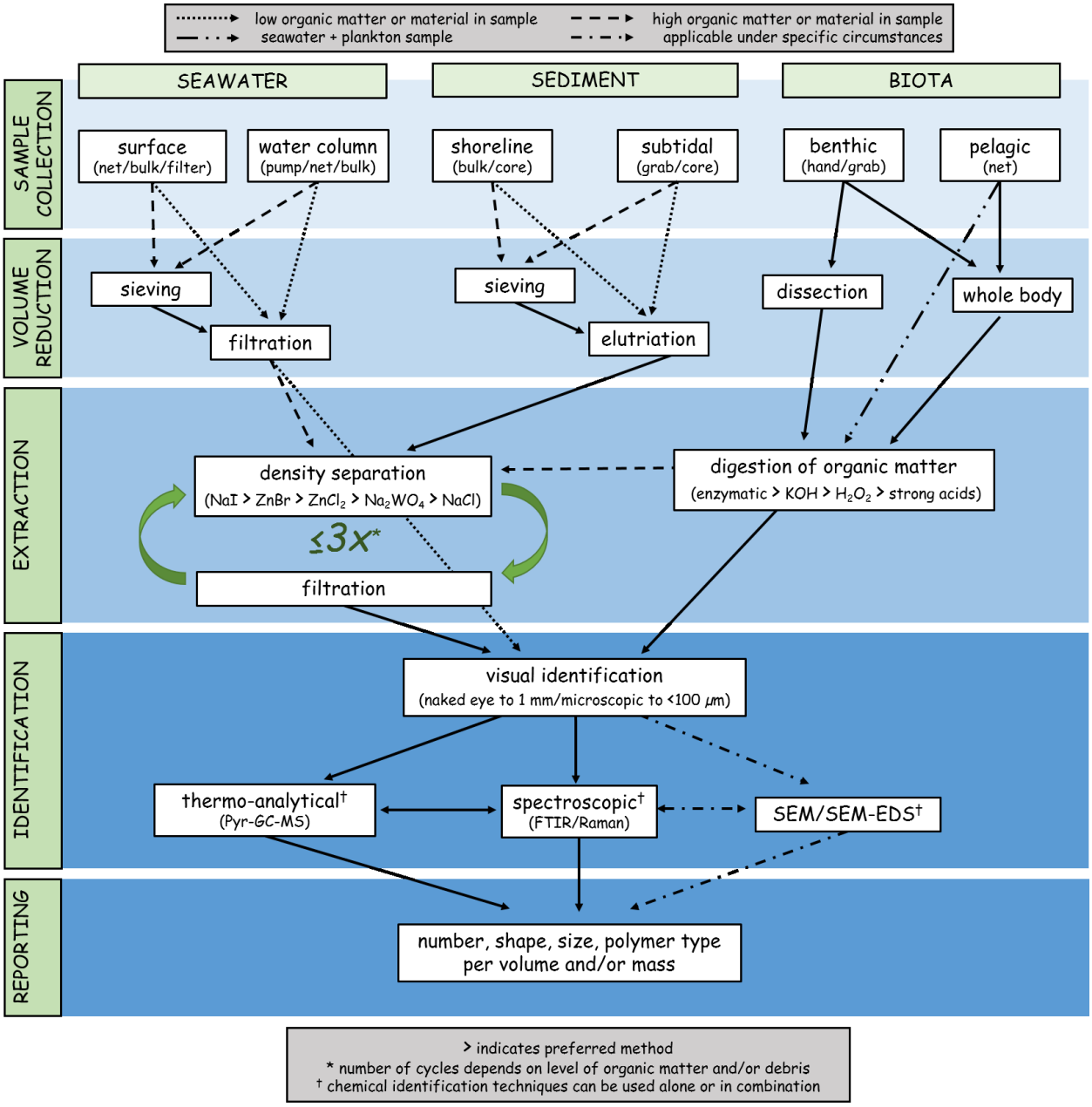

Figure 2. Recommended analytical protocol for microplastics research. Five key stages encompass the collection, volume reduction, extraction, identification, and reporting of microplastics from three different environmental matrices (seawater, sediment, biota). "Benthic" samples include all biota collected from intertidal (e.g., shellfish), subtidal (e.g., epi/infauna), and/or deeper seafloor habitats. See text for details.

APPENDIX I: GLOSSARY

Background blank – empty filter paper which is placed in a petri dish and left open when collecting and/or processing a sample. This provides information on background airborne contamination.

Blind test – during extraction trials, spikes are added to a sample for enumeration after a method is trialled to determine recovery rates. Spikes are added by a second researcher who will not be enumerating the plastics added.

False positive – reporting a microplastic that is either not plastic and/or present in a sample due to contamination.

False negative – failing to report a microplastic that is present within a sample as it is not identified during quantification.

Microfibre – a strand or filament of plastic measured along its longest dimension (i.e. thread-like particles significantly longer in one than wide in two dimensions).

Microplastic – any particle that is made of plastic, this is inclusive of fibres, fragments, foamed, sheet and pellet particles.

Negative blank – see procedural blank.

Procedural blank – used to quantify microplastic contamination that results during the extraction process. All reagents are used (with the absence of a sample) and run through all extraction steps. The resulting filter paper is examined and any contaminant particles quantified.

Recovery rate – the percentage of microplastics recovered after trialing an extraction technique using spikes.

Spike – microplastic particles that are intentionally added to a sample to validate research methods by determining recovery rates and/or damage to individual particles.

APPENDIX II: ABBREVIATIONS

AFM – atomic force microscopy

FPA – focal plane array

FTIR – Fourier-transform infrared spectroscopy

GF/F – glass fibre filter

HDPE – high density polyethylene

IR - infrared

MP – microplastic

MF – microfibre

NP - nanoplastic

PA – polyamide (nylon)

PC – polycarbonate

PE – polyester

PET – polyethylene

Pyr-GC-MS – pyrolysis-gas chromatography-mass spectrometry

PP – polypropylene

PS – polystyrene

PSXL – cross-linked polystyrene

PVC – polyvinyl chloride

RMS – Raman spectroscopy

SEM – scanning electron microscope

SML – surface microlayer