Marine Pollution Bulletin xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Review

Recovering microplastics from marine samples: A review of current practices

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ARTICLE INFO

Keywords:
Marine plastic pollution
FTIR
Visual separation
Density flotation
Acid digestion
Enzymatic digestion

ABSTRACT

An important component of microplastic research is development of reproducible methods for microplastic recovery and characterization. Presented is a review of the literature comparing microplastic separation and identification methodologies from seawater, sediment and marine organisms. The efficiency of methods was examined, including processing time, recovery rates, and potential destruction of microplastics. Visual examination and acid digestion were the most common separation methods for seawater samples and organisms, while density flotation was the primary method for sediment. Few studies reported recovery rates, or investigated the physical or chemical impact on plastics. This knowledge gap may lead to misidentification of plastic or unreliable pollution estimates. Further investigation of the impact chemical treatments have on plastic is warranted. Factors, i.e. biomass loading, recovery rates, and chemical compatibility, must be considered to allow for appropriate methodology. Standardizing this will contribute to efficient sample processing, and allow for direct comparison of microplastic contamination across environments.

1. Introduction

Marine plastic pollution has become a global environmental concern and is a growing issue as a result of the exponential increase in the production of plastics. As of 2015, global production of petroleum-based plastics exceeded 300 million metric tons (Avio et al., 2015), with the majority of manufacturing attributed to six main plastic types: polyethylene (PE) (Majewsky et al., 2016), polypropylene (PP) (Majewsky et al., 2016), polyvinyl chloride (PVC), polyurethane (PUR), polystyrene (PS), and polyethylene terephthalate (PET) (Wu et al., 2016). Annual production is estimated to yield a cumulative production of 33 billion metric tons by 2050 (Barrows et al., 2017; Rochman et al., 2013a). One consequence of this mass production is an increased abundance of plastic litter in the ocean and along the shoreline (GESAMP, 2015). It is estimated that 4.8 to 12.7 metric tons of plastic litter enters the ocean environment each year, making this issue one of upmost importance (Andrady, 2011; Barrows et al., 2017). Furthermore, this pollution has the potential to accumulate organic contaminants, such as carcinogenic polychlorinated biphenyls (PCB) (Bellas et al., 2016; Frias et al., 2010; Teuten et al., 2009), polycyclic aromatic hydrocarbons (PAHs) (Rochman et al., 2012; Rochman et al., 2013b) and polybrominated diphenyl ethers (PBDEs) (Tanaka et al., 2012), as well as toxic metals (Nakashima et al., 2011), eventually making its way into and through the marine food web (GESAMP, 2016; Vandermeersch et al., 2015).

Marine plastic pollution has been reported for the past 45 years, and is broadly divided into mega-plastic (> 100 mm diameter), macro-plastic (> 20 mm), meso-plastic (5–20 mm), micro-plastic (< 5 mm) (Barnes et al., 2009; GESAMP, 2016) and nano-plastic (< 100 nm) (Koelmans et al., 2015). Reference to microplastic contamination first appeared in the literature in 1972 (Carpenter et al., 1972), but has only been studied in detail in the past decade or so (Avio et al., 2016; Ivar do Sul and Costa, 2014; Zarfl et al., 2011). The terms 'primary' and 'secondary' microplastics refer to the source, with particles being either specifically manufactured for particular applications (e.g. resin beads, microbeads used in cosmetic products), or produced as a result of fragmentation from larger items (Arthur et al., 2008; GESAMP, 2016). Among the different categories of marine plastic pollution, microplastics are of particular concern due to their ready uptake by marine organisms (Avio et al., 2016; Wright et al., 2013), including some that are consumed by humans, i.e. crabs, oysters, mussels, and fish (Claessens et al., 2013; Cole and Galloway, 2015; Van Cauwenberghe and Janssen, 2014). Indeed, microplastics have been reported from surface waters of every major ocean (Cozar et al., 2014), in sediment types such as intertidal mangroves, beach and deep sea sand (Nor and Obbard, 2014; Quinn et al., 2017; Van Cauwenberghe et al., 2013), and organisms such as bivalves (Li et al., 2016; Vandermeersch et al., 2015) and a wide range of fish species (Guven et al., 2017; Nadal et al., 2016).

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http://dx.doi.org/10.1016/j.marpolbul.2017.08.058
Received 24 July 2017; Received in revised form 28 August 2017; Accepted 28 August 2017
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The body of literature investigating the presence and abundance of microplastics in the marine environment has been growing exponentially since the seminal paper by Thompson et al. (2004). However, methods describing the separation and identification of microplastics from environmental samples are highly variable (Hidalgo-Ruz et al., 2012; Shim et al., 2017) preventing robust comparisons of findings across different studies. Existing separation methods include visual separation (Ivar do Sul et al., 2014; Lusher et al., 2014), flotation separation (Frias et al., 2010; Hall et al., 2015), and acid (Claessens et al., 2013; Desforges et al., 2014), alkaline (Tanaka and Takada, 2016; Zhao et al., 2016), oxidative or enzyme digestion (Cole et al., 2014; Courtene-Jones et al., 2017). Many studies, however, do not report on the exact procedures used, nor do they determine the recovery rate of microplastics from digestion methods that have the potential to damage the structure or physical characteristics of plastic polymers (Cole et al., 2014; Quinn et al., 2017). For identification of microplastics, the current recommended method is attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), due to the simplicity of analysis and diagnostic spectral information that it provides (Shim et al., 2017). However, polymer characterization of microplastics using chemical techniques (i.e. FTIR) does not always occur (Baldwin et al., 2016; Ivar do Sul et al., 2014), and is rarely used in the few studies that report on microplastic recovery rates. Importantly, most studies do not report on details such as the time required to process samples, and to separate and identify microplastics from environmental samples, making it difficult to determine the most (cost-)effective and suitable methods for their processing.

In this study, we 1) review the current methods used to separate and identify microplastics in marine environmental samples, i.e. seawater, sediment and marine organisms, 2) describe the sampling and preservation protocols used, 3) provide a synthesis of the separation and identification methods applied and 4) report on the established recovery rates of microplastics, specifically for the commonly reported chemical separation methods that may have an adverse effect on the structural or chemical integrity of plastic items in environmental samples. We also present recommendations to establish reproducible methodologies, including the need for robust testing of chemical separation methods on common plastic pollutants. Implementation of protocols addressing these factors will contribute towards more efficient processing of microplastics from environmental samples, and allow better comparison of microplastic contamination in seawater, sediment and marine organisms.

1.1. Literature search strategy

A systematic literature review was conducted using the search engine Google Scholar and several online databases: Web of Science, PubMed, ScienceDirect and James Cook University's OneSearch (Proquest's Summon 2.0). The iterative search, conducted between December 2016 and April 2017, used various combinations of the following keywords: microplastics, methodology, extraction, isolation, identification, recovery, chemical, enzymatic, digestion, density, flotation, separation, seawater, sediment, biological organisms, and marine pollution. The specific keyword ‘microplastic’ was the primary inclusion criteria. A detailed review of the reference lists of each retrieved article identified additional articles. In total 71 research articles were included within this literature review.

1.2. Seawater samples

Since the first study in 1972 (Carpenter et al., 1972), microplastic particles and fibers have been documented in the surface waters of every major ocean (Cozar et al., 2014). The primary method used for collecting seawater samples is a neuston net tow through the water (Table 1; Supplementary Material Table 1). Originally intended for plankton monitoring, the use of these nets allows for large volumes of water to be sampled with relative ease. Mesh sizes of nets have varied throughout the literature, ranging from 200 μm (Hall et al., 2015) to the most commonly used size of 333 μm, (Brandon et al., 2016; Carpenter and Smith, 1972; Guven et al., 2017; McCormick et al., 2014; Sutton et al., 2016; van der Hal et al., 2017). A mesh size of 333 μm or smaller significantly increases the amount of plastic particles collected (Barrows et al., 2017; Song et al., 2015) but also increases the entrapment of biological biomass. Sampling has been conducted at the surface, subsurface (at an average depth of 3 m) (Cozar et al., 2014), along the benthos (0–2 m above the bottom) (Lima et al., 2014; Morris and Hamilton, 1974) and from ice cores (Lusher et al., 2015).

Apart from neuston nets, a continuous intake system with a mesh filter size ranging from 250 to 300 μm has been used on larger research vessels like those utilized by Enders et al. (2015), Lusher et al. (2014), and Desforges et al. (2014). This method often requires the water sample to travel through multiple mesh filter sizes. For example, Desforges et al. (2014) initially passed samples through a coarse 5 mm filter to remove large debris and organisms, then consecutively through a series of copper sieves of 250 μm, 125 μm and 62.5 μm aperture size. Wastewater management and monitoring relies on different techniques for sampling, including the use of pumps and sieves with a significantly smaller mesh size (12.5 μm); modified versions of this method have been implemented by Majewsky et al. (2016), Dyachenko et al. (2017), and Mintenig et al. (2017) for seawater samples. The potential for loss of microplastics, i.e. trapped in the mesh filters, has yet to be established, with recovery rates for microplastics at each filtration step largely unknown, although filter specifications may provide some insight. Lusher et al. (2014) did, however, demonstrate that by stacking replicate 250 μm mesh sieves followed by a visual assessment, that a single 250 μm mesh sieve was < 100% effective at removing particles from seawater samples. These results suggest an underestimation of microplastic abundance across samples.

The majority of studies do not mention the use of a preservation method (Dyachenko et al., 2017; Gallagher et al., 2016; Majewsky et al., 2016), or specifically state that samples were processed immediately following collection (Cole et al., 2014; Hall et al., 2015; Lusher et al., 2014). The exclusion of a preservation method for seawater samples is acceptable, especially if the primary focus of the study is to recover microplastics, and not the characterization of the biological material (Government du Québec, 2009). However, this has not always been the aim of investigations that sample marine habitats. Historically, reporting of microplastics from seawater samples has been secondary, with sampling and preservation techniques implemented primarily to obtain information on the biological material (Cole et al., 2013; Frias et al., 2014). Preservation techniques are employed to retard the chemical and biological changes that inevitably continue after the sample is removed from the parent source (U. S. Environmental Protection Agency, 1983). This is in direct contrast to the current research into marine pollution, with the primary concern being to quantify microplastics within samples. Nonetheless, some investigations still include biological preservation methods, since maintaining the integrity of the biological matter may still be crucial to other aspects of the study i.e. to establish microplastic:zooplankton ratios (Frias et al., 2014). In these studies, biological preservation methods are generally applied and include using a 4% formalin solution (Frias et al., 2014; Ivar do Sul et al., 2014). If the identification and characterization of the biological material within a sample is not relevant to the study, simple preservation methods such as refrigeration or freezing could be used, if any, since the degradation of the organic material to liberate microplastics is actually preferred.

The critical aspect of microplastic research relates to the separation of microplastics from the biological biomass (i.e. plankton). Flotation separation methods have been widely used for the isolation of microplastics from seawater samples, either standalone (flotation) (Carpenter et al., 1972), with elutriation (Claessens et al., 2013), combined with a hypersaline solution (density flotation) (Hall et al., 2015; Lima et al.,

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Table 1
Summary of the sampling, separation and identification methods used to collect and characterize microplastics in seawater.

Sampling method	Mesh Size (μm)	Sampling depth (m)	Separation method	Separation details	Identification methods	Location	Reference
Neuston net	333	Surface	Visual	Manual	PC	Atlantic	Carpenter and Smith (1972)
Continuous intake	1	5	Visual	Manual	FTIR	Antarctica	Cincinelli et al. (2017)
Manta trawl	300	N/D	Visual	Manual	PC	Atlantic	Ivar do Sul et al. (2014)
WP2 net, Neuston net, Longhurst plankton recorder	335	0.2 & 25	Visual	Manual	FTIR	Portugal	Frias et al. (2014)
Neuston net	300	Surface	Flotation, visual	NaCl	FTIR	UK	Gallagher et al. (2016)
Cont. intake	250	3	Visual	Manual	Raman	Atlantic	Lusher et al. (2014)
Neuston net	333	N/D	WPO, density flotation	Fe(II), H_2O_2, NaCl	PC	USA	McCormick et al. (2014)[a]
Neuston net	270	Surface/benthos	Visual	Manual	FTIR	UK	Morris and Hamilton (1974)
Neuston net	335	Surface	Visual	Manual	FTIR	Australia	Reisser et al. (2013)
Manta trawl	333	Surface	WPO	Fe(II), H_2O_2	PC	USA	Sutton et al. (2016)
Manta trawl	333	Surface	Visual	Manual	PC	Israel	van der Hal et al. (2017)
Pump	10	N/D	Density flotation, oxidant, surfactant, enzymatic	$ZnCl_2$ & NaOH, H_2O_2, SDS, lipase, cellulase	FTIR	Germany	Mintenig et al. (2017)[b]
Manta trawl	333	N/D	Oxidant	35% H_2O_2	FTIR	Turkey	Guven et al. (2017)
Sieves	12	N/D	Density flotation & oxidant	$ZnCl_2$ & 30% H_2O_2	TGA-DSC	Germany	Majewsky et al. (2016)[b]
Neuston net	333	Surface	Flotation	N/D	FTIR	USA	Carpenter et al. (1972)
Neuston net	200	Surface	Density flotation	NaCl	FTIR	Australia	Hall et al. (2015)
Neuston net	300	Surface/benthos	Flotation	N/D	PC	Brazil	Lima et al. (2014)
Manta trawl	333	Surface	Acid	10% HCl	FTIR	USA	Brandon et al. (2016)
Continuous intake	250	4.5	Acid	5–10% HCl	PC	Pacific	Desforges et al. (2014)
N/D		0.2	Oxidant & acid	30% H_2O_2 & 40% HF	PC	Germany	Dubaish and Liebezeit (2013)
Sieves	12.5	N/D	WPO & catalyst	30% H_2O_2 & $FeSO_4$	FTIR	USA	Dyachenko et al. (2017)[b]
Neuston net	333	Surface	Oxidant & catalyst	30% H_2O_2 & $FeSO_4$	PC	USA	Masura et al. (2015)
Cont. intake	300	3	Surfactant	Sodium dodecyl sulfate	PC	Denmark	Enders et al. (2015)
Plankton net	200 & 500	Surface	Acid, alkaline, & enzymatic	HCl, NaOH, & proteinase-K	FTIR	UK	Cole et al. (2014)
Manta trawl	333	50 cm	Visual	Manual	FTIR	Europe	Maes et al. (2017b)

N/D = not determined or mentioned within literature, PC = physical characteristics.
[a] McCormick et al. (2014) sampled in freshwater.
[b] Majewsky et al. (2016), Dyachenko et al. (2017), and Mintenig et al. (2017) sampled wastewater.

Table 2
Summary of sampling, separation and identification methods used to collect and characterize microplastics in marine sediment.

Depth (cm)	Sediment	Separation method	Separation details	Identification methods	Location	Reference
N/D	Municipal	Pressurized fluid extraction	CH_3OH, hexane & dichloromethane	FTIR	Australia	Fuller and Gautam (2016)
N/D	N/D	Elutriation & density flotation	NaI	Known	Belgium	Claessens et al. (2013)
N/D	Beach	N/D	N/D	FTIR	NZ	Gregory (1977)
N/D	Beach	Density flotation	NaCl	FTIR	UK	Thompson et al. (2004)
3	Beach	Density flotation	NaCl	FTIR	UK	Browne et al. (2010)
2	Beach	Density flotation	NaCl	FTIR	Portugal	Frias et al. (2010)
3–4	Mangrove	Density flotation	NaCl	FTIR	Singapore	Nor and Obbard (2014)
7 & 2	Beach	Density flotation	NaCl	FTIR	Belgium	Claessens et al. (2011)
2	Beach	Density flotation	NaCl	FTIR	Portugal	Martins and Sobral (2011)
Sediment cores	Deep sea	Density flotation	NaI	Raman	Atlantic & Mediterranean	van Cauwenberghe et al. (2013)
3–4	Beach	Density flotation & oxidant	NaCl & 30% H_2O_2	PC	Canada	Mathalon and Hill (2014)
5	Beach	Density flotation	NaCl	PC	Brazil	de Carvalho and Baptista Neto (2016)
3–6	Beach	Density flotation	N/D	FTIR	Mexico	Wessel et al. (2016)
10	River	Density flotation	$ZnCl_2$	Raman	UK	Horton et al. (2017)
5	Shallow & deep	Density flotation & oxidant	NaCl & 30% H_2O_2	PC	Europe	Maes et al. (2017b)
		Density flotation	$ZnCl_2$	FTIR	Lab	Maes et al. (2017a)
N/D	Beach	Density flotation	NaCl, NaBr, NaI & $ZnBr_2$	FTIR	Scotland	Quinn et al. (2017)
5	Beach	Oil extraction protocol (OEP)	Canola Oil, NaI & $CaCl_2$	FTIR	Canada	Crichton et al. (2017)
N/D	Beach	Density flotation, oxidant & catalyst	Lithium metatungstate, 30% H_2O_2 & $FeSO_4$	PC	USA	Masura et al. (2015)

N/D = not determined or mentioned within literature, PC = physical characteristics.

2014) or with a surfactant such as sodium dodecyl sulfate (SDS, 150 g L^{-1}) (Enders et al., 2015). The most commonly used methods typically involve the sample being placed into a hypersaturated saline (sodium chloride, NaCl) solution and either agitated for several minutes via manual stirring (Hall et al., 2015), or left overnight (Majewsky et al., 2016; Masura et al., 2015; Mintenig et al., 2017) to separate. Manual sorting, based on physical characteristics, of the floating particulates is then performed. As of current, we are not aware of any studies reporting recovery of microplastics in seawater samples using any of these flotation methods.

Acidic, oxidative, alkaline or enzymatic digestion methods are also used for separation of microplastics from the organic material in seawater samples, and are often paired with or follow density flotation separation (Guven et al., 2017; Majewsky et al., 2016; Mintenig et al., 2017). Dubaish and Liebezeit (2013) used a two-part digestion starting with 30% hydrogen peroxide (H_2O_2, oxidant and weak acid) followed by treatment with 40% hydrofluoric acid (HF, a strong acid). Majewsky et al. (2016) used a zinc chloride ($ZnCl_2$) solution for initial density flotation separation before oxidizing the organic residue with 30% H_2O_2, resulting in recovery rates of 85% and 91% for PE and PVC particulates, respectively. Both Dyachenko et al. (2017) and Masura et al. (2015) utilized a combination of 30% H_2O_2 and 0.05 M iron (II) sulfate ($FeSO_4$, catalyst). Although Masura et al. (2015) did not report recovery rates, Dyachenko et al. (2017) determined a 87% recovery of PS beads. While chemical digestions can be effective in reducing organic material within samples, they may impact on the structural or chemical integrity of the microplastic. For example, PS and PC particles may not be recovered intact from two-part acid digestions due to their susceptibility towards harsh acids, such as HF (Dubaish and Liebezeit, 2013; Thermo Fisher Scientific, 2017). Furthermore, recovery rates were not reported for these plastic particles. Desforges et al. (2014) and Brandon et al. (2016) both used 10% hydrochloric acid (HCl, a strong acid) to digest the organic material; neither established recovery rates of microplastics. Cole et al. (2014) compared acid (HCl), alkaline (sodium hydroxide, NaOH) and enzymatic (Proteinase-K) digestion methods, both alone and paired with ultrasonication. The enzymatic treatment alone yielded the highest digestion efficiency (88.9% determined by the difference in pre- and post-digestion weight). Although visual inspection confirmed the plankton tissue was fully digested without damaging the physical structure of the plastics, this study did not establish whether the chemical integrity of the plastic polymers was compromised. Given that microplastic identification methods in these studies varied from visually assessing physical characteristics (Desforges et al., 2014; Dubaish and Liebezeit, 2013), to undertaking chemical characterization i.e. FTIR (Brandon et al., 2016; Cole et al., 2014) and thermogravimetry coupled to differential scanning calorimetry (TGA-DSC) (Majewsky et al., 2016), it is not possible to directly compare recovery rates (in those instances where they were established).

The many discrepancies between sampling, separation, characterization and identification methods used across studies into seawater plastic pollution, and the fact that many are time and labour intensive, highlights the need for a single reliable, standardized and efficient approach. Only three studies on seawater samples conducted a recovery check, however, none of these used chemical analytical techniques (i.e. FTIR or Raman spectroscopy, TGA-DSC) both before and after spiking. To establish the most appropriate sample processing method to reproducibly and reliably separate microplastics while retaining structural and chemical integrity of microplastics, standardized spike-and-recovery studies should be performed. Ideally, chemical characterization (i.e. FTIR or Raman spectroscopy) and polymer identification would be done before and after the spiking experiment to monitor for any change in the chemical composition.

1.3. Sediment samples

Analyzing sediment samples for the presence of microplastics began to appear in the scientific literature 15 years ago, and with greater frequency in the last 7 years. Sediment types investigated include deep sea (core) sand, beach sand, river sand, intertidal mangrove mud and municipal soil (Table 2; Supplementary Material Table 2), as a result sampling methods vary greatly (Besley et al., 2017). Maximum depth collected varied from 2 cm (Frias et al., 2010; Martins and Sobral, 2011) to 5 cm (de Carvalho and Baptista Neto, 2016; Wessel et al.,

2016) to sediment cores of unknown depths (Claessens et al., 2011; Van Cauwenberghe et al., 2013). Preservation methods for sediment samples were not mentioned within the literature, most likely due to the low organic loading.

Density flotation methods using either sodium chloride (NaCl, most commonly of 140 g L^{-1}) or sodium iodide (NaI) were widely used, regardless of the sediment type or depth of sampling. Most studies suspended the sediments in hypersaline NaCl solution after which they were allowed to settle (10 min to overnight) (Browne et al., 2010; Claessens et al., 2011; de Carvalho and Baptista Neto, 2016; Frias et al., 2010; Nor and Obbard, 2014), while others (Claessens et al., 2011; Martins and Sobral, 2011) conducted multiple (exhaustive) settlements to ensure all plastics were recovered. Horton et al. (2017) implemented a 3-step procedure involving visual inspection of whole sample, density flotation in $ZnCl_2$, followed by further visual inspection of unfloated sample. This procedure revealed the inefficiency of visual sorting through sediment samples (37% recovery of total plastics), yet the effectiveness of a $ZnCl_2$ density separation (75% recovery). While these recovery rates were not established from spiked samples, the difference demonstrates the importance of density flotation separation when processing sediment samples. Maes et al. (2017a) similarly suggests a $ZnCl_2$ density separation, saying a solution with density of 1.37 g mL^{-1} will allow for the flotation of PA, PS, PVC, PET, PE, and PP. In addition, Maes et al. (2017a) proposed an alternative method allowing for the identification of plastic particles from sediments by staining samples with a Nile Red (NR) acetone solution. While this method proved effective at allowing for slightly faster visual inspection and promises (with further validation) general particle categorization, it is unknown whether this additional step (~60 min) would speed up analysis of samples. In addition, any subsequent FTIR analyses of NR-stained plastic particles is reliant on the use of "very small amounts" i.e. final concentration of 1, 10 or 100 µ g mL^{-1} suspension, and requires adaptation of the FTIR imaging optics (Maes et al., 2017a). Masura et al. (2015) suggested using a commercial separator lithium metatungstate solution as an alternative due to its greater density (1.62 g cm^{-3}) compared to NaCl. This allows for denser particles (i.e. PVC, PET) to be recovered more readily (Quinn et al., 2017).

Claessens et al. (2013) used elutriation, whereby an air stream lifts lower density particles to the surface, followed by decanting and sieving. They suggest the implementation of thorough cleaning, as well as procedural blanks when using an elutriation method for field samples, since there is the potential for contamination during extraction. Wessel et al. (2016) used a custom-made automated density flotation separator with > 35 PSU filtered water, which achieved an average recovery rate of 97.25% (± 2.5) in only 26 min. Crichton et al. (2017) proposed an innovative and cost-effective flotation methodology exploiting the oleophilic properties of microplastics by using retail grade canola oil yielding average recovery rates of 96.1%, and proving a more time efficient method than NaI or $CaCl_2$ methods, although this method will impact on any subsequent chemical analysis, particularly FTIR. More recently, Fuller and Gautam (2016) investigated pressurized fluid extraction using methanol (CH_3OH) and dichloromethane as a means of chemically extracting the microplastics. This extraction procedure dissolved the plastics, producing plastic residues, thereby destroying the morphology of microplastic particles making physical characterization impossible. Only three studies reported using an alkaline, acid or oxidative digestion on sediments (Fuller and Gautam, 2016; Masura et al., 2015; Quinn et al., 2017).

As for seawater samples, FTIR was the identification method of choice in sediment samples, used in over 60% of papers reviewed. Other methods included chemical characterization by Raman spectroscopy (Horton et al., 2017; Van Cauwenberghe et al., 2013), or using physical characteristics to identify plastics (de Carvalho and Baptista Neto, 2016).

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

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

1.4. Biological organisms

Microplastics are ingested by marine organisms (Nadal et al., 2016; Tanaka and Takada, 2016; Taylor et al., 2016), including species consumed by humans (Avio et al., 2015; Foekema et al., 2013; Neves et al., 2015; Possatto et al., 2011; Rochman et al., 2015; Wright et al., 2013). These findings have raised concerns particularly regarding the unknown impact on human health. Subsequently research in this area is on the rise with the majority of literature published since 2015 (Table 3; Supplementary Material Table 3). The blue mussel (*Mytilus edulis*; soft tissue, gills and digestive glands) is the most common organism investigated to date, followed by various fish species (i.e. gut contents of: Atlantic herring *Clupea harengus*, sardine *Sardina pilchardus*, swordfish *Xiphias gladius* and dogfish *Scyliorhinus canicula*), marine invertebrates (digestive tract of sea cucumber *Holothurian spp*., whole zooanthids), and a multitude of bird species (digestive tracts of: common buzzard *Buteo buteo* and black kite *Milvus migrans lineatus*). Organisms were either obtained from the field via bottom trawling (Collard et al., 2015), opportunistic coastal collection (Claessens et al., 2013; Santana et al., 2016), or from aquaculture farms and fishmongers (Vandermeersch et al., 2015). Most studies examined the stomach contents (Bellas et al., 2016; Collard et al., 2015; Zhao et al., 2016) or the gastrointestinal tract (Avio et al., 2015), while others investigated the entire organism (Courtene-Jones et al., 2017; Vandermeersch et al., 2015).

Preservation methods are more commonly used for biological

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Table 3
Summary of sampling, separation and identification methods used to collect and characterize microplastics in marine organisms.

Organism sampled	Tissue Type	Separation method	Separation details	Identification methods	Location	Reference
Birds	Digestive tract	Alkaline	NaI, & 10% KOH	PC	China	Zhao et al. (2016)
Birds	Digestive tract	Visual	Manual	PC	USA	Terepocki et al. (2017)
Bivalves	Gills & digestive glands	Visual	Manual	PC	Germany	von Moos et al. (2012)
Bivalves	All soft tissue	Acid	69% HNO_3	N/D	Belgium	Claessens et al. (2013)
Bivalves	All soft tissue	Acid	69% HNO_3	Raman	Lab	Van Cauwenberghe and Janssen (2014)
Bivalves	All soft tissue	Density flotation & oxidant	30% H_2O_2 & NaCl	PC	Canada	Mathalon and Hill (2014)
Bivalves	All soft tissue	Density flotation & oxidant	30% H_2O_2 & NaCl	FTIR	China	Li et al. (2016)
Bivalves	All soft tissue	Acid	HNO_3	PC	Brazil	Santana et al. (2016)
Bivalves	All soft tissue	Enzymatic	Trypsin, papain & collagenase	FTIR	UK	Courtene-Jones et al. (2017)
Bivalves	All soft tissue	Acid	65% HNO_3 & 68% $HClO_4$	PC	Europe	Vandermeersch et al. (2015)
Bivalves	All soft tissue	Acid, base & enzymatic	HNO_3, NaOH & Corolase 7089	FTIR	UK	Catarino et al. (2017)
Coral	Whole animal	Visual	Manual	PC	Australia	Hall et al. (2015)
Invertebrates	N/D	Visual	Manual	FTIR	UK	Thompson et al. (2004)
Invertebrates	All soft tissue	Acid	65% HNO_3 & 68% $HClO_4$	PC	North Sea	Devriese et al. (2015)
Invertebrates	Digestive tract	Visual	Manual	Raman	Belgium	Remy et al. (2015)
Invertebrates	All soft tissue	Acid, oxidant, alkaline, & enzymatic	10% KOH, pepsin, HCl, 65% HNO_3, 65% $HClO_4$, NaOH & $K_2S_2O_8$	FTIR	Lab	Dehaut et al. (2016)
Invertebrates	Whole animal	Visual	Manual	PC	Indian Ocean	Taylor et al. (2016)
Pelagic fish	Stomach	Visual	Manual	PC	North Pacific Ocean	Boerger et al. (2010)
Pelagic fish	Stomach	Visual	Manual	PC	North Pacific Ocean	Davison and Asch (2011)
Pelagic fish	Gastrointestinal tract	Visual	Manual	FTIR	UK	Lusher et al. (2013)
Sedentary river fish	Digestive tract	Visual	Manual	PC	France	Sanchez et al. (2014)
Pelagic fish	Gastrointestinal tract	Visual, density flotation, acid & oxidant	NaCl, 30% H_2O_2, 22.5 M HNO_3 & 15% H_2O_2	FTIR	Adriatic	Avio et al. (2015)
Pelagic fish	Stomach	Acid	9% NaClO, 65% HNO_3 & 99% CH_3OH	Raman	Lab	Collard et al. (2015)
Pelagic fish	Stomach	Visual	Manual	FTIR	Portugal	Neves et al. (2015)
Pelagic fish	Gut contents	Visual	Manual	FTIR	USA	Phillips and Bonner (2015)
Pelagic fish	Stomach	Visual	Manual	PC	Italy	Romeo et al. (2015)
Pelagic fish	Stomach	Alkaline	NaOH	PC	Spain	Bellas et al. (2016)
Pelagic fish	Gastrointestinal tract	Visual	Manual	PC	Spain	Nadal et al. (2016)
Pelagic & demersal fish	Gastrointestinal tract	Visual	Manual	FTIR	North & Baltic Sea	Rummel et al. (2016)
Pelagic fish	Digestive tract	Alkaline	10% KOH	FTIR	Japan	Tanaka and Takada (2016)
Pelagic fish	Stomach & intestines	Visual	Manual	FTIR	Turkey	Guven et al. (2017)
Lake fish	Gastrointestinal tract	Acid, alkaline & density flotation	NaOH, 65% HNO_3 & NaI	FTIR	Germany	Roch and Brinker (2017)
Pelagic fish	Stomach & intestines	Visual	Manual	PC	Brazil	Vendel et al. (2017)
Pelagic fish	Gastrointestinal tract	Alkaline & Pulsed Ultrasonic Extraction (PUE)	10% KOH	FTIR	Atlantic Ocean	Wagner et al. (2017)

N/D = not determined or mentioned within literature, PC = physical characteristics.

organisms, compared to seawater or sediment samples. The majority of studies froze the samples at − 20 °C (Bellas et al., 2016; Courtene-Jones et al., 2017; Dehaut et al., 2016; Guven et al., 2017; Li et al., 2016; Nadal et al., 2016; Romeo et al., 2015; Tanaka and Takada, 2016; Terepocki et al., 2017; Vandermeersch et al., 2015; Zhao et al., 2016). Several studies preserved specimens in formaldehyde-based fixatives including 10% formalin (Phillips and Bonner, 2015; Vendel et al., 2017), 37% formaldehyde (Collard et al., 2015) or 4% Baker's calcium formol (von Moos et al., 2012). Alternatively, Taylor et al. (2016) stored deep-sea organisms in 70–80% ethanol until processing. Remy et al. (2015) used 99% bidistilled glycerin to preserve invertebrate digestive tracts during visual analysis, however, it was noted that subsequent (undefined) washing techniques to rid plastic fibers of the glycerin were "destructive" hindering the ability to identify microplastics. The method of preservation should be taken into consideration when storing samples for microplastic recovery.

Although visual separation is commonly used to separate microplastics from tissue (Jensen, 2017; Rummel et al., 2016), based on physical characteristics such as size, appearance, shape and color or the 'hot needle test' (Devriese et al., 2015; Hall et al., 2015; Nadal et al., 2016; Romeo et al., 2015; Taylor et al., 2016; Terepocki et al., 2017; Vendel et al., 2017), the likelihood of microplastics being trapped within tissues and therefore not detected is high. As a result, acid, oxidative, alkaline or enzymatic-based digestion of tissue and gut contents is most often employed prior to visual sorting. For acid digestion, 69% nitric acid (HNO_3) is the most widely used (Claessens et al., 2013; Collard et al., 2015; Dehaut et al., 2016; Vandermeersch et al., 2015); other methods include 65% perchloric acid ($HClO_4$, strong acid) (Vandermeersch et al., 2015), or a 4:1 v:v mixture of 65% HNO_3 and 68% $HClO_4$ (Devriese et al., 2015). Oxidative digestion using 30% H_2O_2 (Avio et al., 2015; Li et al., 2016), or 0.27 M peroxodisulfate potassium ($K_2S_2O_8$, oxidizing agent; acidic in water) (Dehaut et al., 2016) is also widely applied. Avio et al. (2015) determined that a combination of NaCl (flotation) and 30% H_2O_2 worked best on gut contents of mullet (*Mugil cephalus*) compared to 69% HNO_3. For alkaline digestion 2–10 M sodium hydroxide (NaOH, alkaline) (Bellas et al., 2016; Dehaut et al., 2016), and 10% potassium hydroxide (KOH, alkaline) (Dehaut et al., 2016; Zhao et al., 2016) are most commonly reported. A two-step alkaline:acid digestion using 9% sodium hyperchlorite (NaClO, alkaline) and 65% HNO_3 (1:10 v/v) with ultrasonication was found to completely digest fish tissues (Collard et al., 2015). Roch and Brinker (2017) suggested a similar method, utilizing a three-step process of exposing fish gut tissues to an alkaline solution (1 M NaOH), acid solution (65% HNO_3), and density flotation (NaI). With recovery rates of PS ranging from 95 to 100% from whitefish (*Coregonus lavaretus* L.) gut contents, and method validation on field samples of round gobies (*Neogobius melanostomus*) and common barbels (*Barbus barbus*), this method proved very effective at separating microplastics from gastrointestinal tracts. Dehaut et al. (2016) compared the digestive efficiency of 10% KOH, 0.063 M HCl, 65% HNO_3, 65% $HClO_4$, 10 M NaOH, and 0.27 M $K_2S_2O_8$ solutions on gut contents of blue mussels (*M. edulis*) and black seabream (*Spondyliosoma cantharus*), with the alkaline methods considered the most effective. Tissue digestion efficiencies of 99.6% for 10% KOH and 99.8% for NaOH:$K_2S_2O_8$ were achieved, based on weight, although microplastic recovery rates were not determined. One study determined that an optimal concentration of 0.3125% trypsin effectively and efficiently digested *M. edulis* tissue (Courtene-Jones et al., 2017) compared to other proteolytic enzymes i.e. papain and collagenase. No changes in overall shape, color or size of microplastics were observed yet no recovery rates were determined. Catarino et al. (2017) compared the efficiency of various concentrations of NaOH, HNO_3 and Corolase 7089 (a neutral protease) to remove PET, HDPE and PA from *M. edulis* tissues. Corolase 7089 ranging from 1 to 100 mL was reported to work best, with recovery rates of 93% for all plastic types, with HNO_3 being the least recommended due to its ability to 'meld together' PET and HDPE fragments, and completely digest PA within spiked samples.

As a complete alternative to visual separation and chemical digestions altogether, Wagner et al. (2017) suggested a methodology utilizing Pulsed Ultrasonic Extraction (PUE), consisting of a series of square envelope bursts modulated by a 39–41 kHz sweep wave form to break apart the tissues of Japanese medaka (*Oryzias latipes)*. When compared to a 10% KOH digestion, this method did not leave behind any tissue residues or reaction products, however, it did effectively break apart the fish gut tissue and allowed for accurate FTIR identification. Wagner et al. (2017) also reports this method is relatively short to implement (~1 h) and eliminates any chemical hazards. While method validation confirmed the applicability of this method to field fish gut samples (family Myctophidae), recovery rates were not reported. Caution should be taken implementing this method, as weathered, more brittle plastics may be susceptible to break apart during ultrasonification.

While FTIR and Raman spectroscopy have been used to chemically identify microplastics in some tissue studies (Guven et al., 2017; Lusher et al., 2013; Remy et al., 2015) microscopy, to determine physical characteristics, such as shape, color, or size is more often employed (Bellas et al., 2016; Hall et al., 2015; Taylor et al., 2016; von Moos et al., 2012). Given that harsh acid treatments can cause discoloration or physical alteration of plastic particles (Claessens et al., 2013; Li et al., 2016; Vandermeersch et al., 2015) relying on visual assessment only means that identification is speculative at best, making comparison between studies difficult.

Although recovery checks are more commonly reported for biological tissues they remain under-reported. Claessens et al. (2013) spiked soft tissues of *M. edulis* with PS and polyamide (nylon) fibers and applied a 69% HNO_3 digestion method. The nylon fibers disintegrated and the PS spheres melted together, indicating that 69% HNO_3 may be too corrosive for the separation of at least some microplastics. On a similar note, Roch and Brinker (2017) reported a method including NaOH, 65% HNO_3 and NaI caused a color change in PET and PVC particles, caused PA to completely dissolved, resulted in LDPE clumping together, and corroded the edges of PS. Similarly, Avio et al. (2015) found that digestion of PE and PS-spiked gastrointestinal tracts of *M. cephalus* with 22.5 M HNO_3 followed by boiling caused dissolution of both polymers (being melted and fused) giving a recovery yield of only 4% while oxidative digestion with 30% H_2O_2 extracted almost 70% of spiked particles, greater than that achieved by visual assessment (~60%). Avio et al. (2015) also acquired FTIR spectra of samples before and after a combined treatment with NaCl (filtered twice) followed by 15% H_2O_2 oxidative digestion of residual organic matter and established that with this protocol the chemical integrity of plastics was not compromised. A spectral similarity of 93% for PE and > 87% for PS was reported. Collard et al. (2015) assessed possible degradation of microplastics subjected to 9% NaClO, 65% HNO_3, and CH_3OH added in succession; the spectra revealed that the chemical composition was not affected. Li et al. (2016) exposed PE and PES fibers to hypersaline NaCl and 30% H_2O_2 (in succession) and reported a 95% recovery rate. However, the 30% H_2O_2 bleached and discolored the microplastics, indicating that the chemical composition of the microplastics might have been compromised. Mathalon and Hill (2014) used the same methodology as Li et al. (2016), with recovery rates not stated and an effect of H_2O_2 not reported. At this time, the effect of H_2O_2 on microplastics is not fully known.

In summary, to initially liberate microplastics from biological tissue, and to reduce the biomass within samples prior to visual separation, alkaline and oxidative digestion methods have proven effective. However, results are inconsistent concerning whether certain digestion treatments (30% H_2O_2 or 69% HNO_3) will chemically alter polymers within samples. For example, Collard et al. (2015) mentioned there was no chemical degradation resulting from a treatment of 65% HNO_3, yet Claessens et al. (2013) noted that nylon fibers and PES fragments were physically altered following exposure to the same treatment. Further

Table 4
Summary of each separation method reviewed within the literature, outlining the advantages and disadvantages of each method.

Method	Advantages	Disadvantages
Visual separation		
Manual sorting	• No chemical hazards • Can be applied to all sample types • Easy methodology • Verified method of all polymer types • Low cost for equipment/tools required • Ability to use FTIR/Raman following separation	• Lengthy time; can take weeks/months to process • Often unreliable due to human error/variation, recovery rates may vary • Potential high cost for employment of visual analyzer
Flotation		
Elutriation	• No chemical hazards • Easy methodology • Recovery rates of 98–100%[f] • Ability to use less NaCl or NaI solution when this step is added • Inexpensive to implement • Verified method for PE, PP, PS, PES, and PA[f] • Ability to use FTIR/Raman following separation	• Relatively time-intensive • Cannot be applied to non-sediment samples
NaCl	• Inexpensive ($118/kg)[a] • Easy methodology • Low chemical hazards • Verified method for PS, PA, PP, PVA, and PE[q] • Ability to use FTIR/Raman following separation • Easily accessible – common in labs	• Multiple density separations must occur to achieve high recovery rates • Can be time-intensive due to multiple density flotations required • Recovery rates only 85%–95%[e] • Applicability to all sample types unknown
NaI	• Easy methodology • Low chemical hazards • Greater density of than NaCl • Ability to use FTIR/Raman following separation • Easily accessible – common in labs	• Relatively expensive ($860/kg)[a] • Recovery rates of only 83%[d] • Applicability to all sample types unknown • Can be time-intensive due to multiple density flotations required • Only verified method for PS, PA and PVC[f]
$ZnBr_2$	• Easy methodology • Low chemical hazards • Relatively inexpensive ($321/kg)[a] • Recovery rates of 99%[e] • Verified for PP, LDPE, HDPE, PE, PS, PVC, PET and PA[e] • Ability to use FTIR/Raman following separation • Easily accessible – common in labs	• Not confirmed for application to non-sediment samples • Can be time-intensive due to multiple density flotations required
Canola oil	• Easy methodology • No chemical hazards • Fast methodology as only one density separation required • Very inexpensive ($4.50/L)[b] • Recovery rates high (96.1%), especially for PVC (high density)[d] • Verified method for PS, PVC, ABS, PES, and PA[d] • Ability to use FTIR/Raman following separation • Easily accessible – common in labs	• Not confirmed for application to non-sediment samples • Additional cleaning step must be applied to allow for FTIR/Raman
Lithium metatungstate	• Easy methodology • Fast methodology • Low chemical hazards • Greater density than NaCl	• Relatively expensive ($650/L)[a] • Recovery rates unknown • Effect on polymer types unknown • Applicability to all samples unknown • Ability to use FTIR/Raman following separation unknown
Sodium dodecyl sulfate	• Easy methodology • Low chemical hazards • Surfactant removes organic materials from the plastic and prevents adherence to collection vessel • Ability to use FTIR/Raman following separation • Short overnight methodology	• Recovery rates unknown • Applicability to all sample types unknown • Effect on polymer types unknown • Relatively expensive ($489/kg)[a]
Alkaline		
NaOH	• Easy methodology • Low chemical hazards • Relatively inexpensive ($206/kg)[a] • Ability to use FTIR/Raman following separation[g] • Easily accessible – common in labs	• Lengthy time; digestion time of 3 weeks[g] • May be required to heat sample, may cause loss of plastics • Applicability to all sample types unknown • Effect on polymer types unknown
KOH	• Easy methodology • Some chemical hazards • Relatively inexpensive ($145/kg)[a] • Ability to use FTIR/Raman following treatment[g] • Short digestion time of only 24 h • Recovery rates show no change in weight[g] • Easily accessible – common in labs	• Recovery rates only reported by weight, not abundance • Applicability to all sample types unknown • Effect on polymer types unknown • Known to leave behind reaction residue on plastics; may hinder FTIR if not cleaned[r]

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Table 4 *(continued)*

Method	Advantages	Disadvantages
Acid		
HCl	• Easily accessible – common in labs • Short digestion time of only 12 h[m] • Easy methodology	• Recovery rates showed a weight change after treatment[g] • Relatively expensive ($650/L)[a] • Ability to use FTIR/Raman following separation unknown • Applicability to all sample types unknown • Effect on polymer types unknown • High chemical hazards - corrosive acid • Often needs to be heated/boiled to digest biologically rich samples, which could result in loss of plastics
HF		• Relatively expensive ($1320/L)[a] • Recovery rates unknown • Digestion times unknown • Ability to use FTIR/Raman following separation unknown • Applicability to all sample types unknown • Effect on polymer types unknown • High chemical hazards - corrosive acid • Often needs to be heated/boiled to digest biologically rich samples, which could result in loss of plastics
HNO_3	• Relatively inexpensive ($264/kg)[a] • Easy methodology • Overnight digestion[f,o] • Ability to use Raman following treatment[o] • Easily accessible – common in labs	• Not applicable to all polymer types - recovery checks show alteration to PS and PA following treatment[f] • Ability to use FTIR/Raman following separation unknown • Applicability to all sample types unknown • High chemical hazards - corrosive acid • Often needs to be heated/boiled to digest biologically rich samples, which could result in loss of plastics
$HClO_4$	• Easy methodology • Overnight digestion[o] • Easily accessible – common in labs	• Recovery rates showed a weight change after treatment[g] • Relatively expensive ($877/L)[a] • Ability to use FTIR/Raman following separation unknown • Applicability to all sample types unknown • Effect on polymer types unknown • High chemical hazards - corrosive acid • Often needs to be heated/boiled to digest biologically rich samples, which could result in loss of plastics
Oxidant		
H_2O_2	• Easy methodology • Relatively inexpensive ($302/L)[l] • Short digestion times of only 30 min[k] to 24 h[j] • Ability to use FTIR/Raman following separation[h] • Recovery rates reported of 85–91%[i] • Can be applied to all sample types • Easily accessible – common in labs	• May discolor or bleach plastics[j] • Effect on polymer types unknown • High chemical hazard - corrosive acid • Often needs to be heated/boiled to digest biologically rich samples, which could result in loss of plastics
$FeSO_4$ (catalyst)	• Relatively inexpensive ($151/kg)[a] • Easy methodology • Short digestion times of < 1 h[k,n] • Recovery rates of 87% reported[k] • Low chemical hazards • Easily accessible – common in labs	• Ability to use FTIR/Raman following separation unknown • Applicability to all sample types unknown • Effect on polymer types unknown • Often needs to be heated/boiled to digest biologically rich samples, which could result in loss of plastics
Enzyme		
Proteinase-K	• Short digestion time of ~3 h[p] • Low chemical hazards • Ability to use FTIR/Raman following separation	• Recovery rates unknown • Applicability to all sample types unknown • Effect on polymer types unknown • Relatively very expensive ($448/100 g)[a] • Methodology more complex than simple acid digestion • Not common in labs
Corolase 7089	• Easy methodology • Fast methodology of ~1 h • Recovery rates of 93%[s] • Verified method for PET, HDPE and PA[s] • Ability to FTIR/Raman following separation • Low chemical hazards	• Needs to heat sample to 60 °C – may result in loss of plastic • Applicability to all sample types unknown • Not common in labs
Trypsin	• Short digestion time of 30 min[l] • Low chemical hazards • No physical alteration of PET, HDPE, PVC, PP, PS and PA confirmed[l]	• Recovery rates unknown • Ability to use FTIR/Raman following separation unknown • Applicability to all sample types unknown • Effect on polymer types unknown • Very expensive ($4210/100 g)[a] • Methodology more complex than simple acid • Not common in labs

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Table 4 *(continued)*

Method	Advantages	Disadvantages
Other		
Pulsed Ultrasonic Extraction	• Easy methodology • Relatively inexpensive • No chemical hazards • Easily accessible – common in labs • Fast methodology (~6 min; fish)[r] • Verified method for PVC, PE, PP, PS, PET & fibers[r]	• Applicability to all sample types unknown • Recovery rates unknown
Dying with Nile Red	• Easy methodology • Relatively inexpensive • Low chemical hazards • Easily accessible • Fast methodology (~1 h)[t] • Ability to FTIR/Raman following separation • Verified for PA and PE[t]	• Not an actual separation method, still need to implement additional techniques • While quick, could add on time to methodology depending on actual separation technique chosen. • Recovery rates unknown • Applicability to all sample types unknown

[a] Prices from Sigma-Aldrich Australia.
[b] Local supermarket price.
[c] lmtliquid.com.
[d] Crichton et al. (2017).
[e] Quinn et al. (2017).
[f] Claessens et al. (2013).
[g] Dehaut et al. (2016).
[h] Avio et al. (2015).
[i] Majewsky et al. (2016).
[j] Li et al. (2016).
[k] Dyachenko et al. (2017).
[l] Courtene-Jones et al. (2017).
[m] Brandon et al. (2016).
[n] Masura et al. (2015).
[o] Vandermeersch et al. (2015).
[p] Cole et al. (2014).
[q] Claessens et al. (2011).
[r] Wagner et al. (2017).
[s] Catarino et al. (2017).
[t] Maes et al. (2017a).

research confirming these impacts on a variety of plastic types is needed.

1.5. Recommendations for future research

One of the major shortcomings in the monitoring microplastics in environmental samples is the varied sampling and separation methods used (summarized in Table 4). With regards to the acid, alkaline, oxidative and enzymatic digestions, in some instances it is not known whether the chemicals used impact on the structural and/or chemical integrity of microplastics, possibly reducing the accuracy of identification. Only a few studies have to date implemented recovery checks to ensure their methods were appropriate for the separation of microplastics, with only two (Avio et al., 2015; Fuller and Gautam, 2016), incorporating FTIR spectroscopy both before and after spiking. While both studies indicated no significant change in chemical composition resulting from separation methods, the same recovery procedure has yet to be conducted for the more commonly used acid (HCl, HNO_3) and enzyme (trypsin) digestions. Multiple studies have noted that the loss of or damage to microplastics is a direct result of the use of acidic solutions in digestion methods (Dubaish and Liebezeit, 2013; Vandermeersch et al., 2015; Dehaut et al., 2016; Li et al., 2016), however, no evidence of chemical degradation was provided.

A major bottleneck in microplastic research at the present is the lengthy time required to manually process the environmental samples (regardless of source), this has the added disadvantage of affecting the reliability and efficiency of the separation of the microplastic particles. Of the papers reviewed here, only Courtene-Jones et al. (2017) and Quinn et al. (2017) have discussed the need for more time efficient processing methods. While both studies present alternatives to previous separation methods (trypsin and $ZnBr_2$, respectively), neither produced evidence that their method allows for accelerated processing of microplastic samples that can also be chemically characterized. It is important to establish a time efficient, reproducible methodology for processing microplastic samples, as this will allow for better comparisons across studies and a more reliable estimation of microplastic contamination in the environment.

The sample preservation technique used should be carefully considered and applied with caution when processing samples, especially if the biological material is to be preserved for analyses other than microplastic separation and identification. For example, Remy et al. (2015) chose glycerin, commonly used to preserve organic material, to store gut contents of macroinvertebrates prior to Scanning Electron Microscopy and microplastic separation. Unfortunately, glycerin coats the particulates and chemically contaminates them, making the spectral interpretation of Raman and similar techniques (FTIR) at best challenging. Furthermore, subsequent washing of glycerin-contaminated cellulose fibers within samples proved destructive (Remy et al., 2015). If chemical characterization of particles found within a sample is desired i.e. by FTIR, preservation methods should consist of a solution that is chemically inert to plastics and which is readily removed by washing or evaporation i.e. ethanol. Indeed, if characterization of the biological material is not relevant to the study, chemical-based preservation methods should be excluded from sampling procedures altogether.

There exists within the literature an inconsistency in the procedures used for microplastic separation methods, limiting the ability to directly compare studies and to accumulate data worldwide. While a density flotation separation methodology utilizing either $ZnBr_2$ or elutriation is recommended for sediment samples, based upon high recovery rates and suitability for chemical characterization, not enough is known regarding the impact of chemical and enzymatic digestion treatments on different polymer types to recommend a universal method for sediment and tissue samples. Indeed, it is advisable to consider the many advantages and disadvantages of the chosen method to ensure it is

compatible with the type of micro particulates under investigation and with the method of identification. The development of a universal protocol allowing for the efficient recovery of microplastics from various sample types (i.e. seawater samples, sediment samples and biological organisms) and for the comparison of results across studies is highly desirable. Ideally, this protocol will rapidly and reproducibly separate microplastics from environmental samples, without altering their structural or chemical integrity, and without introducing additional contamination, allowing for chemical characterization, and accurate estimation of microplastic pollution. Since method choice is currently dependent upon biomass loading, desired polymer types (i.e. fragments, fibers or both), and environmental medium, standardizing methods may be more achievable in the nearby future, than developing a universal technique. Furthermore, development of protocols allowing for the processing of samples regardless of biomass loading, as well as allowing for all polymer types to be recovered. Such protocols will significantly advance research efforts, allowing for more extensive long-term monitoring of microplastic pollution and its effects on our oceans.

Author contributions statement

F.K., C.M. and M.M. developed the idea and project together. M.M. conducted the literature search preliminary drafts. F.K. and C.M. provided edits and contributed literature. All authors discussed and reviewed the manuscript.

Competing financial interests

The authors declare no competing financial interests.

Acknowledgements

Thanks to AIMS@JCU for the Pilot Research Award to M.M. Special thanks to Dr. Kathryn Berry for her critique of the draft manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.marpolbul.2017.08.058.

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