Department
for Environment
Food & Rural Affairs

Evidence Reviews on Analysis, Prevalence & Impact of Microplastics in Freshwater and Estuarine Environments

Evidence Review 1

Are the current sampling and analytical methods scientifically robust and appropriate?

October 2019

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Executive Summary

This Rapid Evidence Assessment used the systematic review procedure to assess the current evidence available on the reliability/robustness of sampling and analytical methodology used to provide data sets describing the presence of microplastics in the environment. A review was conducted of primary literature, including grey literature, which reported sampling and analytical methodologies that have been used to determine the presence of microplastics in aquatic matrices; the abundance, substance, state, morphology, dimensions and sources of microplastics reported to have been found in freshwater and estuarine environments.

Evidence was acquired according to a predefined set of questions, compiled into a database containing full details of the source and its relevance to the project questions. The evidence was analysed, taking into account reporting biases in the literature, to produce a digestible summary of the evidence to answer the main project question and sub-questions, namely,

Are the current sampling and analytical methods used to provide data on the presence of microplastics in freshwater and estuarine matrices as well as other environmental materials scientifically robust and appropriate?

> *a) Are the methods transferable to different sample matrices and can they be used to assess changes to the microplastic profile and properties at different stages from sink to river and river to tap?*
> *b) With samples from different matrices, what pre-processing of samples is used?*
> *c) If the method has been used in marine environments, does it require any modification to be used in equivalent freshwater environments?*
> *d) To what extent can the method provide information on different characteristics of microplastics such as substance, state, morphology and dimensions?*
> *e) Does the sampling method used address spatial and temporal variation adequately?*

A set of pre-defined terms were used to search various databases and 1844 potential evidence sources were identified. Further screening resulted in the identification of 283 sources likely to contain evidence relevant to freshwaters and estuaries. Of these, 209 unique sources were used to provide evidence, with 127 sources providing evidence from freshwaters and 68 from studies on estuaries and 14 providing evidence from both habitats. Several sources contained evidence that was relevant to different habitats within freshwaters.

Are the current sampling and analytical methods used to provide data on the presence of microplastics in freshwater and estuarine matrices as well as other environmental materials scientifically robust and appropriate?

The three main steps in the analysis of microplastics in the environment are,

1. capture of the sample from the environment,

2. preparation of the sample to separate microplastics from other material (including laboratory filtering, density separation and digestion), and
3. quantification and characterisation of microplastic particles (potentially including analytical determination of the polymer).

The approaches used to capture samples of microplastics from water included nets, both static and trawled, bulk water samples and samples of water pumped over sieves. Data were typically reported per unit volume or, occasionally, where surface trawls were used, per unit area. The volume of water sampled varied over eight orders of magnitude in freshwaters and six in estuaries. There was a direct relationship between the size of the smallest particles studied and the volume of water sampled in both freshwater and estuaries: large volumes of water can only be sampled using nets of relatively coarse mesh, which in turn do not capture smaller particles. The mean abundance of microplastic particles reported was inversely correlated with the volume of water sampled. Whilst it is necessary to collect a sufficiently large sample of the matrix to ensure that any estimates of abundance are robust and representative (i.e. sufficient to capture an adequate number of particles to provide a valid estimate of concentration), the mean abundance of particles reported was also inversely related to the size of the smallest particle considered.

Sediments were sampled using a variety of techniques, including grabs, cores, quadrats, scoops and resuspension cylinders. Data were reported per unit volume, per unit sediment mass, or occassionally per unit area (without any indication of depth sampled). To enable comparison, data expressed per unit sediment mass were converted to per unit volume using the density of sediment. The range of volumes of sediment sampled was considerably lower than for water, spanning three orders of magnitude, and with all sample volumes less than 0.1 m^3. The difference in the range of sample volumes used between these two matrices (water and sediment) was likely to be a consequence of the difficulty of sampling large volumes of sediment. The volume of sediment sampled did not appear to be related to the smallest particle size considered in either freshwaters or estuaries, and was more likely to be governed by the practicalities of collecting, transporting and processing large volumes of sediment. Similarly, the mean abundance of microplastic particles did not appear to be related to the volume of sediment sampled or the smallest particle size considered. Nevertheless, due to the limited range of sample volumes used, the evidence available may not be sufficient to robustly test such relationships and it is likely that the inverse relationship between particle size and abundance observed in water also applies to sediment. It is likely that, in many cases, the volume of sediment sampled has been insufficient to provide a robust estimate of larger, and hence less abundant, particles.

The reliability of the evidence provided by the sources was assessed based on ten criteria: (1) sampling method and strategy, (2) sample size, (3) sample processing and storage, (4) laboratory preparation, (5) clean air conditions, (6) negative controls, (7) positive controls, (8) target component (for biota), (9) sample (pre)treatment, and (10) polymer identification. For each criterion, a score of 0, 1, or 2 was assigned to the evidence source under review. Scores signified the following: 2 = reliable without restrictions, 1 = somewhat reliable but with restrictions, 0 = not reliable.

Only 12 studies did not score 0 in at least one category, with the average being 3.77 zeros per study. Overall, the majority of studies of microplastics in freshwaters and estuaries are based on methods that are in some aspects not reliable.

Are the methods transferable to different sample matrices and can they be used to assess changes to the microplastic profile and properties at different stages from sink to river and river to tap?

The standard approach used in marine systems is to tow a net (plankton, manta, neuston or bongo nets) comprised of ≈330 µm mesh over long distances, an approach that has been adopted for sampling both large lakes and rivers. Whilst this method is appropriate for sampling larger particles that occur at low abundance, bulk water samples, either pumped or grab samples, are required to sample smaller sized particles. There was insufficient evidence to draw conclusions regarding the most appropriate way to sample microplastics from freshwater and estuarine sediments.

Direct comparison of concentrations of microplastics among studies that consider different size ranges of particles is not possible, and comparisons should be constrained to studies that consider similar sized particles. However, the log-log relationship between the size of the smallest particles considered and the reported concentration of microplastics should allow cross-study comparison of the relative abundance of microplastics.

With samples from different matrices, what pre-processing of samples is used?

Different methods have been used to separate microplastics from biota, sediment and water, potentially leading to different concentrations and profiles of microplastics being reported from the different matrices. Studies of biota tended to use no separation (other than dissection) more than those of other matrices, and studies of sediment used a combination of density separation and filtration more than those of other matrices. A large proportion of studies did not use any digestion (42% of sources from freshwater [n=141] and 49% of sources from estuaries [n=82]), potentially leading to an overestimate of the abundance of microplastics through the misidentification of non-plastic particles. Digestion with acid or alkali was largely restricted to studies of biota; these more aggressive treatments should be avoided as they lead to degradation of plastic particles and, hence, an underestimate of abundance. Only one study considered particles in the nanoparticle range (≤ 0.1 µm), which used a Triton X-45 (TX-45)-based Cloud Point Extraction technique to separate and capture particles coupled with pyrolysis gas chromatography–mass spectrometry (Pyr-GC/MS) to quantify and characterise them.

If the method has been used in marine environments, does it require any modification to be used in equivalent freshwater environments?

Most evidence from fresh and transitional (estuarine) waters was based on methods that were not modified compared with those used in marine systems (>85% over all evidence sources). Modified methods were more prevalent in studies of sediment (particularly freshwater) than other matrices.

To what extent can the method provide information on different characteristics of microplastics such as substance, state, morphology and dimensions?

Optical microscopy on its own or in combination with Fourier-transform infrared (FTIR) spectroscopy were the most frequently used methods to quantify and characterise microplastics. Of the criteria that form the European Chemical Agency (ECHA) definition of microplastics (substance, state, morphology and dimensions), none of the methods used to quantify and characterise microplastics provided any information on state (solid/semi-solid). Optical microscopy alone provided no information on substance composition (i.e. polymeric form), despite this method being used frequently in both freshwaters (23% of evidence sources [n=141]) and estuaries (39% [n=82]). Optical microscopy on its own or in combination with FTIR or Ramman spectroscopy rarely provided information on the source of particles (primary or secondary particles). The number of particles that were analysed by each evidence source varied from 10 particles throughout an entire study to every particle captured. Hence, the reliability of the information obtained, particularly that describing the profile and chemical properties of microplastic particles, varied considerably among the different evidence sources.

Does the sampling method used address spatial and temporal variation adequately?

The majority of evidence available on microplastics in freshwaters and estuaries was based on spot samples, with no or limited replication in space and time. Overall, 39% of studies were based on unreplicated samples. The lack of robust replication will influence the confidence with which changes can be identified in the profile and properties of microplastics at different stages along the pathway of release and transport.

In conclusion, scientifically robust and appropriate sampling and analytical methods have rarely been used to provide data on the presence of microplastics in freshwater and estuarine matrices. Only 12 studies did not score 0 in at least one of the criteria used assess reliability: the methods used in these 12 studies should be considered as a basis for developing a standardised methodology.

Based on the findings of this evidence review, we recommend that methods should report the size range of particles considered, or better provide estimates of the concentration and properties of microplastics by size class, that these should be based on an appropriate volume of the matrix sampled, and robust replication both in terms of the samples collected from the environment and the proportion of particles characterised. We also recommend a more consistent approach to the separation and digestion stage of processing samples.

1 Introduction

1.1 Background

Plastics are synthetic polymers which can be made into a vast range of inexpensive, light-weight and durable products that bring numerous societal benefits by providing important components for a multitude of applications in modern life. Since the 1950s, the plastics industry has grown exponentially to a global usage of 348 million tonnes annum^{-1} in 2017 (Plastics*Europe* 2018). A great variety of polymers and products are encompassed within the term "plastics", some of which will have a long service life, whereas others (around 40% of all the plastic produced) are used for packaging, which is predominantly single use.

It has been discovered that microscopic particles of plastic (microplastics), have been released into the environment (Thompson et al. 2004). Here we use the European Chemical Agency working definition of microplastic as "any polymer, or polymer-containing, solid or semi-solid particle having a maximum size of 5 mm or less in any dimension" (ECHA 2018). Additionally, the definition includes both those microplastics that have been intentionally created (i.e. primary microplastic), and those that are derived from degradation of larger plastic particles (i.e. secondary microplastic). It is estimated that 12 billion tonnes of microplastic will be discarded globally by 2050 (Geyer et al. 2017), with additional particles derived through degradation of larger material, resulting in impacts on biota predicted to cost in excess of $13 billion annum^{-1} (Nizzetto et al. 2016). Microplastics are now ubiquitous and have been reported from throughout the aquatic environment, from surface freshwaters (Hurley et al. 2018a) to the deepest and most remote oceanic regions (Ivar do Sul and Costa 2014).

As microplastics originate from a variety of sources they comprise a variety of different polymer types, including polyethylene (PE), polypropylene (PP), acrylic, polyacrylamide (PAM), polyamide (PA), polyester (PES), polytetrafluoroethylene (PTFE), and polystyrene (PS) amongst others. Furthermore, as with all particles, microplastics are subject to a number of physical and biologically-mediated processes as they move through the environment. They will be variously affected by these processes such that the concentrations and profile of microplastics may vary substantially both in time and space. A further complication is that various methods have been used to quantify the abundance of microplastics (Mai et al. 2018), thus potentially biasing our perception of the extent of contamination by microplastics and preventing easy comparison between different studies to be able to form a robust evidence base on the prevalence of microplastics in freshwater environments. There is a need to further our understanding of which sources of microplastics are prevalent in freshwater systems, in what forms, and what their potential impacts on freshwater organisms and ecosystems might be. To fully comprehend the prevalence of microplastics it is important to understand the influence of sampling and analysis methods on the data reported describing the concentrations and profile of microplastics in freshwater and estuarine environments.

Within the above wider context, this evidence review is the first of three reviews that aim to provide a robust review of the evidence base for informing policy development. This evidence is needed to inform decision making to effectively manage any potential risks stemming from microplastics.

1.2 Objectives

The overarching aim of this evidence review, commissioned by Defra's Water Quality team, was to improve our understanding of the robustness and appropriateness of current sampling and analytical methods used to provide data sets describing the presence of microplastics in the environment. The evidence available was assessed using the systematic review procedure.

The objectives were to:

undertake a Rapid Evidence Assessment for each of the primary research questions; and

produce a database of assessed evidence.

The objectives of the evidence review were delineated during the production of the evidence review protocol (see Section 2 below) through the following Primary and Secondary questions.

Primary question:

Are the current sampling and analytical methods used to provide data on the presence of microplastics in freshwater and estuarine matrices as well as other environmental materials scientifically robust and appropriate?

Secondary questions:

a) Are the methods transferable to different sample matrices and can they be used to assess changes to the microplastic profile and properties at different stages from sink to river and river to tap?

b) With samples from different matrices, what pre-processing of samples is used?

c) If the method has been used in marine environments, does it require any modification to be used in equivalent freshwater environments?

d) To what extent can the method provide information on different characteristics of microplastics such as substance, state, morphology and dimensions?

e) Does the sampling method used address spatial and temporal variation adequately?

This report forms an objective review of evidence collated relating to the primary and secondary questions above. It may be used to inform future discussions with policy makers, water companies and stakeholders on the robustness and appropriateness of

sampling and analytical methods used to provide data on the presence of microplastics in freshwater and estuarine matrices.

2 Methodology

2.1 Review methodology applied

This evidence review is a Rapid Evidence Assessment (REA) which aims "to provide an informed conclusion on the volume and characteristics of an evidence base together with a synthesis of what that evidence indicates following a critical appraisal of that evidence" (Collins et al. 2015). The first step in undertaking the review was developing a Protocol document to guide the review process, following the methodology outlined in Collins et al. (2015). The Protocol was reviewed and approved by Defra prior to commencement of the REA. The Protocol document included the conceptual framework for the review, the primary and secondary questions to be considered (see Section 1.2), the Population, Intervention, Comparator, Outcome (PICO) elements in Table 2.1 and search terms. It was decided that the REA work would encompass two components, a literature review and interviews with academic experts. Details of the approach for the two REA components are provided in the Sections below.

Table 2.1 REA PICO elements.

PICO element	PICO element for this REA
Population	Microplastics
Intervention	Robust methods used to characterise prevalence of microplastics in freshwaters and estuaries
Comparator	Unreliable methods used to characterise prevalence of microplastics in freshwaters and estuaries
Outcome	Robust evidence base on the prevalence of microplastics in freshwater environments

2.2 Literature Review

The quality of the literature, including grey literature, which reported the sampling and analytical methodologies that have been used to determine the presence of microplastics in aquatic matrices was systematically reviewed and assessed, including the evidence produced using these methods on the abundance, substance, state, morphology, dimensions and sources of microplastics reported to have been found in freshwater and estuarine environments.

2.2.1 Capturing the evidence base

The first step was to assess the overall evidence base detailing research on microplastics in freshwaters and estuarine (transitional) waters. A wide search using population search terms (Table 2.2) was used at this stage to capture as much of the evidence as possible. The results of these searches were saved and interrogated further to answer each of the three more detailed key questions and their sub-questions from the three evidence reviews on microplastics in freshwaters and estuaries (the first of which is reported here)This approach reduced the effort required to establish the evidence base for each ER.

Publications released prior to April 2019 were included in this review. As microplastics have only been studied recently (Thompson et al. 2004), no earliest date was used to define the date range of publications included. An exception on the date range was made to include two works of high relevance to the UK that were released after April 2019, namely Ball et al. 2019 (Sink to River - River to Tap. A review of potential risks from nanoparticles and microplastics. UK Water Industry Research Limited Report No. EQ01A231) and Santillo et al. 2019 (Plastic pollution in UK's rivers: a 'snapshot' survey of macro- and micro-plastic contamination in surface waters of 13 river systems across England, Wales, Scotland and Northern Ireland. Greenpeace Research Laboratories Technical Report 04-2019).

Table 2.2 Population level search terms used with Boolean operators to identify the population of evidence available on microplastics in freshwaters and estuaries.

Population			
plastic*	freshwater*	wetland	potable
micro*	river*	marsh	reservoir
microplastic	stream*	swamp	aquifer
nanoplastic	brook	wastewater*	groundwater
*plastic	lake*	drinking water	sewage
	pool	aquatic	outfall
	pond	ecosystem*	estuar*
			transitional

The databases used for the searches, which encompass both published and grey literature, included:

BioOne, COPAC, DART-Europe E-theses Portal, EBSCO Open dissertations, EThOS: Electronic Theses Online Service, European Commission Research Publications, European Sources Online, GoogleScholar, MedLine, JStor, SciFinder, Open Access Theses and Dissertations, OpenGrey, PubMed, PLoS, Scopus, SciFinder, Web of Science.

To capture grey literature, additional to that included in the list of databases to be searched (i.e. databases detailing unpublished theses and reports) undertook directed searches of holdings of relevant environmental regulators (e.g. Rijkswaterstaat (Dutch water authorities): http://www.rws.nl, Vlaamse Milieumaatschappij (Flemish Environmental Agency): http://www.vmm.be Bundesanstalt für Gewässerkunde (German Federal Institute of Hydrology): http://www.bafg.de RIVM (Dutch Environment Agency): http://www.rivm.nl)

The results of all searches were a) downloaded and saved in a searchable database for use in further searches and b) used to map the evidence record.

The overall evidence base on microplastics in freshwaters captured 3456 unique sources. The search engines Scopas, Scifinder and Web of Science produced the most hits. Some of the terms used produced a large number of hits, e.g. the combination micro AND plastic, but a brief inspection revealed that a large proportion of these sources were not relevant, so these terms were only used further in combination with other qualifying terms. Of the retained searches, *microplastic* produced the most hits (total across all engines 11,636).

To capture the evidence base to address the Primary and Secondary questions of this evidence review, the overall evidence base on microplastics in freshwaters and estuaries captured in the first phase was searched further using search terms specific to the questions of this evidence review (Table 2.3).

The results of all searches were a) saved for further use and b) used to map the evidence record. After each search was undertaken a complete list of all literature records was compiled (with duplicates removed) and transferred to an MS™ Excel spreadsheet, formatted with columns corresponding to information fields relevant to the key question and sub-questions being addressed (see Appendix B: ER1_Capture.xls).

A total of 1844 sources were identified through the searches as potentially relevant to the questions of this review (Fig. 1). The potentially relevant evidence base was divided among the members of the review team in such a way that 10% of records were allocated twice (for quality assurance purposes).

Table 2.3 Search terms used to identify the evidence available on sampling and analytical methods used to characterise microplastics in freshwaters and estuaries.

Population	Intervention	Comparator	Outcome
aggregate*	spectroscop*		count
colloid*	raman		quantif*
floc*	particle analysis		abundance
plankton*	pyrolysis		concentrat*
sediment*	sampl*		density
diet*	separat*		substance
content	identif*		state
*fibre	flotat*		morphology
*fiber	floatat*		dimension
*bead	microscop*		composition
fragment*	digest*		
pellet*	centrifug*		
flake*	buoyan*		
nurdle			
dust			

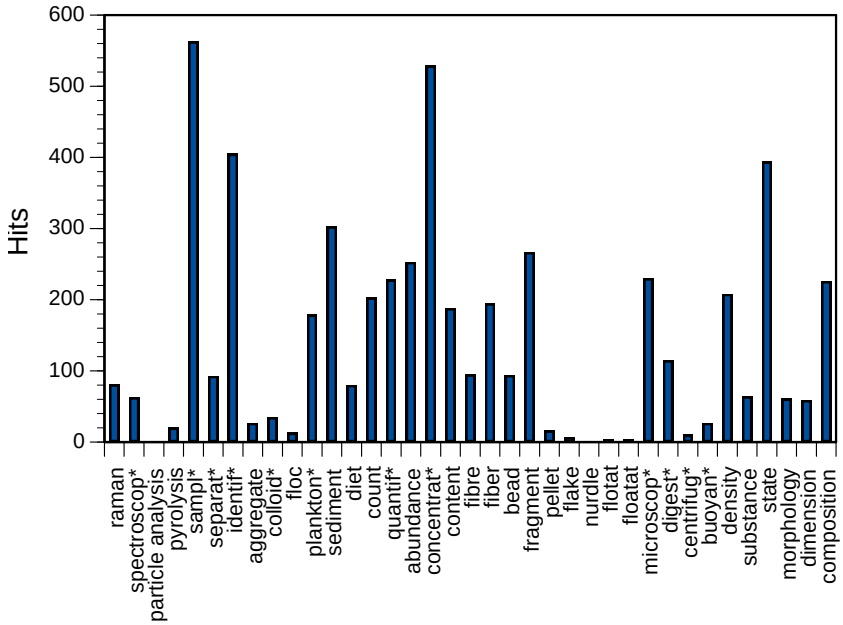

Fig.1. Number of hits for the search terms used in ER1.

Following the methodology outlined in Collins et al. (2015), each reviewer screened the evidence and completed the evidence capture form. The evidence capture form comprised two steps. The first initial screen was to:

a. Identify reviews, which were used for further identification of evidence sources, but not included in data capture per se, unless some novel data were presented.
b. Remove evidence sources not relevant to freshwater or estuarine (transitional) waters.
c. Identify evidence sources that were likely to be relevant to Evidence Review 1 (environmental concentrations and methods), Evidence Review 2 (sources, transport and modification) and/or Evidence Review 3 (biotic impacts, uptake and biological consequences).
d. Of the 1844 evidence sources identified as potentially relevant, the initial screening identified 414 as likely to be relevant to the question of ER1 and, of these, 149 (excluding reviews) likely to contain evidence relevant to freshwaters and 88 (excluding reviews) likely to contain evidence relevant to transitional waters (Fig. 2).
e. Those evidence sources that passed the initial screen were searched in detail to capture the evidence relevant to the question and sub-questions, and any relevant information recorded under the appropriate fields on the evidence capture form (Appendix B: ER1_Capture.xls). In particular, numerical information was captured where effects were quantified in the literature (e.g. volume sampled, concentration of MPs). These evidence sources were supplemented with an additional 33 sources identified as relevant to the questions of this review through the searches undertaken in ER2 (31 sources) and ER3 (2 sources), together with two highly relevant reports that were released after April 2019 (Ball et al. 2019 and Santillo et al. 2019).

Of the sources likely to contain evidence relevant to freshwaters and estuaries, 209 unique sources were used to extract evidence (Fig. 2). Of these, 51 sources contained evidence from standing freshwaters, 67 with evidence from running freshwater, and 35 from other freshwaters, mostly effluent from sewage treatment works but including two where the waterbody type was not given (wild caught fish purchased from markets). 83 unique evidence sources were used where the evidence was from estuaries, with 54 of these sources containing evidence that was relevant to what could be considered transitional waters. Several sources contained evidence that was relevant to both estuaries and freshwaters, as well as different habitats within freshwaters.

After evidence capture, the total evidence base was compiled and quantified.

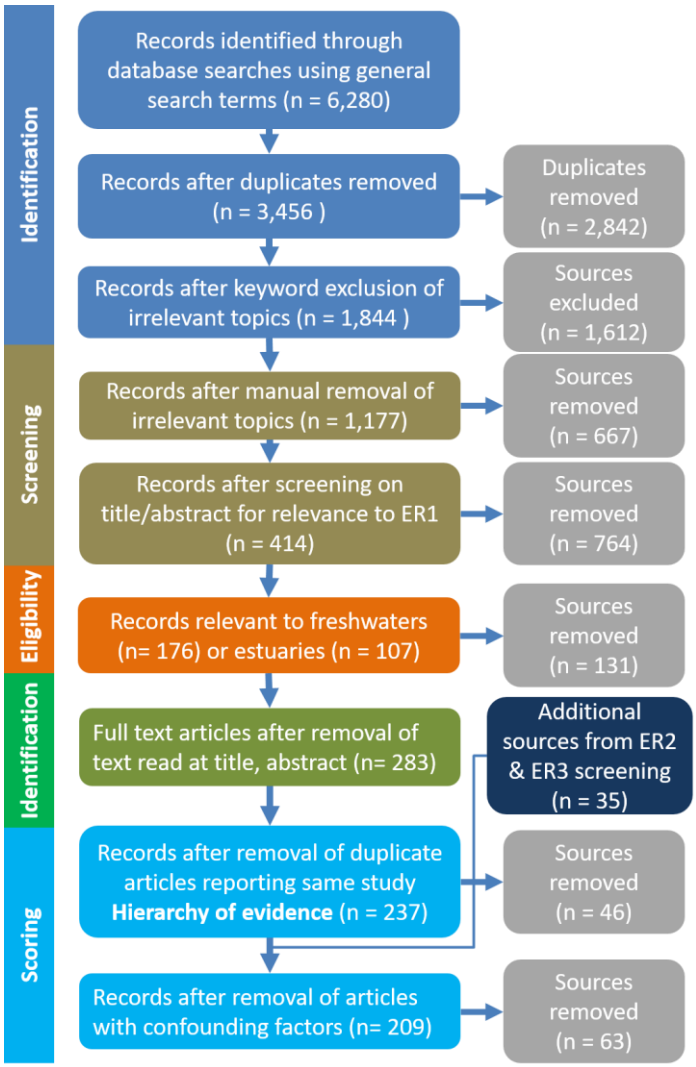

Fig. 2. Map of evidence identified as relevant to ER1 during initial screening.

All the evidence was transferred from the evidence capture form into a searchable MS™ Access relational database, spatially referenced where appropriate (i.e. linked to a GIS data layer illustrating the UK field locations where evidence was obtained from: Fig 3). This database linked literature sources to the key questions and produced extractable summaries of the evidence base underlying each of the key questions and sub-questions.

The evidence considered in this review included 17 studies undertaken in the UK (Fig 3). Of these 11 were from freshwaters and 6 from estuaries.

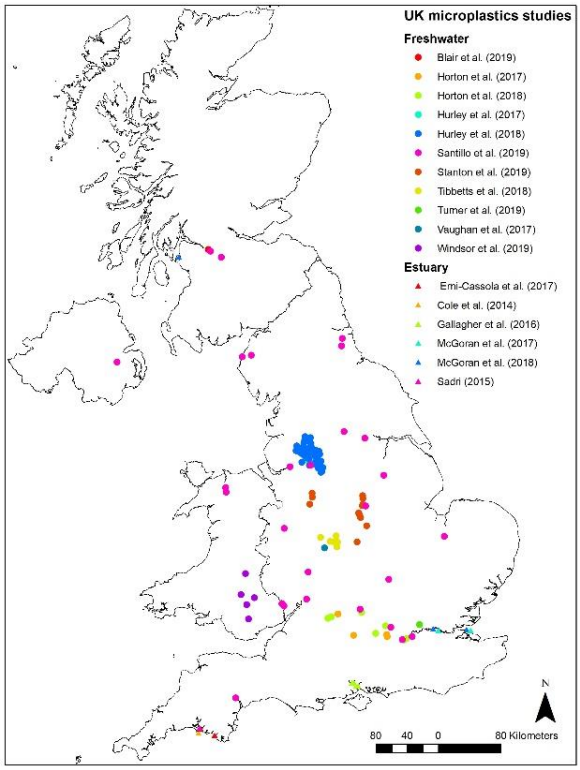

Fig. 3. Location of studies undertaken in the UK considered in this evidence review.

The evidence on microplastics in freshwaters and estuaries identified was restricted to the last decade, with the earliest evidence (from estuaries) from a publication in 2010. Since then there has been an exponential increase in publications (Fig. 4.)

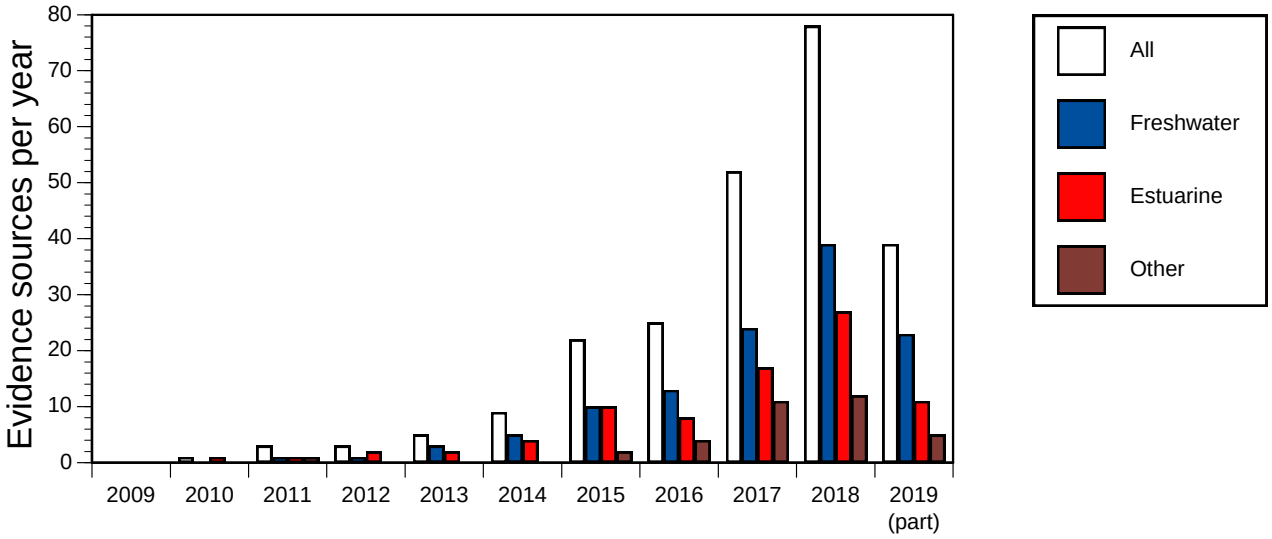

Fig 4. Number of evidence sources per year. NB 2019 only includes publications released prior to April with the exception of Ball et al. 2019 and Santillo et al. 2019.

2.2.2 Reliability scores

Additional information on the reliability of the evidence provided by the source was captured using a separate spreadsheet, based on the methods of Hermsen et al. (2018) and Koelmans et al. (2019). The quality assessment was made up of ten criteria: (1) sampling method and strategy, (2) sample size, (3) sample processing and storage, (4) laboratory preparation, (5) clean air conditions, (6) negative controls, (7) positive controls, (8) target component (for biota), (9) sample (pre)treatment, and (10) polymer identification. For each criterion, a score of 0, 1, or 2 was assigned to the evidence source under review. Scores signified the following: 2 = reliable without restrictions, 1 = somewhat reliable but with restrictions, 0 = not reliable. If information was lacking on certain aspects in the evidence source, this was considered unreliable, leading to a lower score. For each evidence source the Cumulative Score was calculated by adding scores for individual criteria (maximum 18 points for water and sediment, 20 for biota). For the data provided by an evidence source to be considered sufficiently reliable, it should preferably have no 'zero' values for any of the individual scores. To assess the overall reliability of the evidence sources the number of zeros was calculated for each. Furthermore, the product of the scores in all relevant criteria was calculated, following the methods of Hermsen et al. (2018), to give a potential maximum reliability score of 512 (or 1024 for biota), but where any one criterion is evaluated as "not reliable" (0 points) the overall reliability score of the study will be 0.

Table 2.4 Criteria used to assess reliability of evidence sources.

1. Sampling methods	Location - Date - Matrix specific methods should be recorded.
2. Sample size	A suitable sample size - Surface waters: ≥ 500 L, WwTP effluent: ≥ 500 L, Sediment: ≥ 5 L, Biota: ≥ 50 individuals per taxa.
3. Sample processing and storage	Prior rinsing of sample pots in filtered/deionised water. No plastic materials used. Justification for any fixatives added.
4. Laboratory preparation	All materials, equipment, and laboratory surfaces need to be thoroughly washed and rinsed.
5. Clean air conditions	The handling of samples should be performed in clean air facilities.
6. Negative control	A replicate of 3 negative controls is advised that are included for each batch of samples and treated in parallel to the sample treatment.
7. Positive controls	A replicate of 3 is advised in which microplastics of known polymer identity and of targeted sizes are added to "clean" samples, which are then treated and analyzed the same way as the actual samples. The particle recoveries calculated.
8. Target component (for Biota only)	To capture all ingested microplastic, the full gastrointestinal tract (esophagus to vent) of fish and the entire body of smaller species, e.g. bivalves, should be examined.
9. Sample treatment	A digestion step must be included to dissolve organic matter unless from a clean water source, and associated loss of polymers considered. Digestion without such consideration scores 1.
10. Polymer identification	Polymer identify needs to be confirmed by FTIR, Raman or GCMS on at least a representative subsample of ≥ 50 particles or ≥ 25% of filter area. Anaytical techniques to determine polymer identity should not be used on particles smaller than the spatial resolution of the technique. Score 1 if polymer identity was determined on a smaller sub-sample, included excessively small particles or using SEM.

2.3 Interviews

Interviews with academics working in the field of microplastics were conducted to get their expert opinion on the primary and secondary questions. The four academic experts consulted were proposed by Dr. John Iwan Jones (Queen Mary University of London) and Defra. The academics consulted were:

Professor Andrew C Johnson, Centre for Ecology & Hydrology
Dr Claus G. Bannick, Umweltbundesamt, Germany
Professor Dr Stefan Krause, University of Birmingham
Professor Richard Thompson OBE, University of Plymouth

Interviews (lasting 30-45 minutes) were held via phone with all the academics above. During the telephone interviews, the academics were requested to: provide their expert view on each of the primary and secondary questions; comment on key published literature relating to the questions; provide information on ongoing or unpublished work relating to this ER, if applicable. The interviewee responses were recorded as notes during the interviews. The key messages/highlights derived from the interviews are outlined in Section 3.

3 Key messages from interviews with academic experts

Primary question:

Are the current sampling and analytical methods used to provide data on the presence of microplastics in freshwater and estuarine matrices as well as other environmental materials scientifically robust and appropriate?

All four academic experts interviewed noted that a wide variety of methods have been used, with no standardization amongst workers, such that comparison among studies is difficult. In part the lack of a standard definition, particularly of the lower size limit, has added to this lack of comparability. Also, the all four academic experts noted that there is no consensus on what comprises a representative sample volume in order to adequately quantify particles of different sizes. The experts raised concerns regarding the influence of the different sample preparation methods (separation and digestion) used to remove non-plastic material from samples, in particular the effect of digestion on certain polymers. Another substantial concern was the variation, and frequent lack, of measures used to prevent contamination. The four academic experts acknowledged that variety of analytical methods are available, but they are still in a state of flux as the science of microplastics is still in its infancy. Automated methods based on Fourier Transform Infrared Spectroscopy (FTIR) are becoming available which will reduce any potential bias caused by human selectivity when identifying microplastic particles. It was the opinion of the academic experts that human subjectivity has potentially influenced the conclusions drawn to date.

Secondary questions:

a) Are the methods transferable to different sample matrices and can they be used to assess changes to the microplastic profile and properties at different stages from sink to river and river to tap?

The message from the four academic experts interviewed was that, in principle, the analytical methods should be transferable among matrices, but that the preparation of samples would have to be different for the three matrices (water, sediment, biota). The academic experts were of the opinion that appropriate sample preparation is key to ensure that other particles do not confound quantification of microplastics. The experts also stated that variation in methods that have been used to date make comparison among studies difficult, and thus hamper any interpretation of changes to the microplastic profile and properties at different stages along the transport pathway.

b) With samples from different matrices, what pre-processing of samples is used?

The message from the four academic experts interviewed was that digestion is important to remove potentially confounding particles, particularly for spectroscopic methods, but

some processes can degrade plastics, which is rarely taken into account. The experts were of the opinion that use of Fenton's reagent (H_2O_2 and ferrous iron) is the most effective method to remove organic matter. Density separation is necessary for sediment and the effectiveness of separation depends on the granulometry of the sediment. The four academic experts were in agreement that separation of microplastics from the matrix is easiest for water where volume reduction is necessary, and hardest for biota where tissues need to be digested.

c) If the method has been used in marine environments, does it require any modification to be used in equivalent freshwater environments?

The message from the four academic experts was that the methods used in marine environments should be applicable in freshwaters with a little adaptation. However, samples from freshwaters tend to have more organic matter, which has to be removed before quantification

d) To what extent can the method provide information on different characteristics of microplastics such as substance, state, morphology and dimensions?

The message from the four academic experts was that single method does not exist which is suitable for all questions. FTIR and Raman spectroscopy can provide information on polymers, number and dimensions. Thermo-analytic techniques can identify polymers and provide information on mass. FTIR and Pyrolysis–gas chromatography–mass spectrometry can provide information on degradation. Chemical techniques can provide information on contaminants. The experts stated that it may be necessary to use a combination of methods to gain a meaningful and comprehensive picture, but comparison across methods is not feasible. None of the experts suggested methods to establish state (i.e. to distinguish between solid and semi-solid (putty-like) particles).

e) Does the sampling method used address spatial and temporal variation adequately?

The message from the four academic experts was that spatial and temporal variation has not been given sufficient consideration by studies of microplastics in freshwaters and typically, insufficient samples are collected to address variation adequately. Furthermore, the experts noted that data are reported as concentrations rather than loads; the latter are far more informative. It is known that particulate loads are not delivered to rivers evenly. It was the opinion of the four academic experts interviewed that precipitation, discharge and tides will cause more variation in concentrations of microplastics freshwaters and estuaries than in marine waters, and that these variables have to be taken into account when interpreting data.

4 Literature Review

The outcomes of the literature review undertaken are outlined below with the structure being based on the primary and secondary questions. At the end of each question, a summary of the evidence is provided in a text box for clarity. The findings presented are summaries of the evidence available and, therefore, are influenced by the reliability of the primary literature, including grey literature, on which this report is based. An assessment of the reliability of the 209 studies included in this review was undertaken (see section 4.1.2). However, this assessment of reliability was not used to exclude studies from the review, which was based on all 209 evidence sources.

4.1 Primary question: Are the methods transferable to different sample matrices and can they be used to assess changes to the MP profile and properties at different stages from sink to river and river to tap?

In order to address the primary question it was necessary to divide the sampling and analytical methods used to provide data on the presence of microplastics in freshwater matrices into a number of steps,

i) sampling the matrix,
ii) separating microplastic particles from the matrix and other particles,
iii) quantifying the microplastic particles present,
iv) characterising the microplastic particles present.

4.1.1 Sampling the matrix

The methods chosen for the first step, sampling the matrix, is inherently dependent upon the matrix being sampled and methods are not transferable between different matrices. However, the approach to sampling the matrix has the potential to influence the results obtained.

The approaches used to sample microplastics from water included nets, both static and trawled, bulk water samples and samples of water pumped over sieves. Data were typically reported per unit volume or, occasionally, where surface trawls were used, per unit area.

The volume of water sampled varied over eight orders of magnitude in freshwaters and six in estuaries (Fig. 5a).

Sediments were sampled using a variety of techniques, including grabs, cores, quadrats, scoops and resuspension cylinders. Data were reported per unit volume, per unit sediment mass, or occassionally per unit area (without any indication of depth sampled). To enable comparison, data expressed per unit sediment mass were converted to per unit volume

using the density of quartz (2650 kg m^{-3}).and an assumed porosity of 0.4, to give 1590 kg m^{-3} for dry sediment and 1990 kg m^{-3} for wet sediment.

The range of volumes of sediment sampled was considerably lower than for water, spanning three orders of magnitude, and with all sample volumes less than 0.1 m^3 [equivalent to 159 kg dry weight or 199 kg wet weight] (Fig. 5b). The smallest volumes sampled were 0.000028 m^{-3} [equivalent to 45 g dry weight or 56 g wet weight] for freshwater and 0.00005 m^{-3} [equivalent to 80 g dry weight or 100 g wet weight] for estuaries. The difference in the range of sample volumes used between these two matrices (sediment and water) was likely to be a consequence of the difficulty of sampling/extracting particles from large volumes of sediment, which is possible for water through the use of trawled nets.

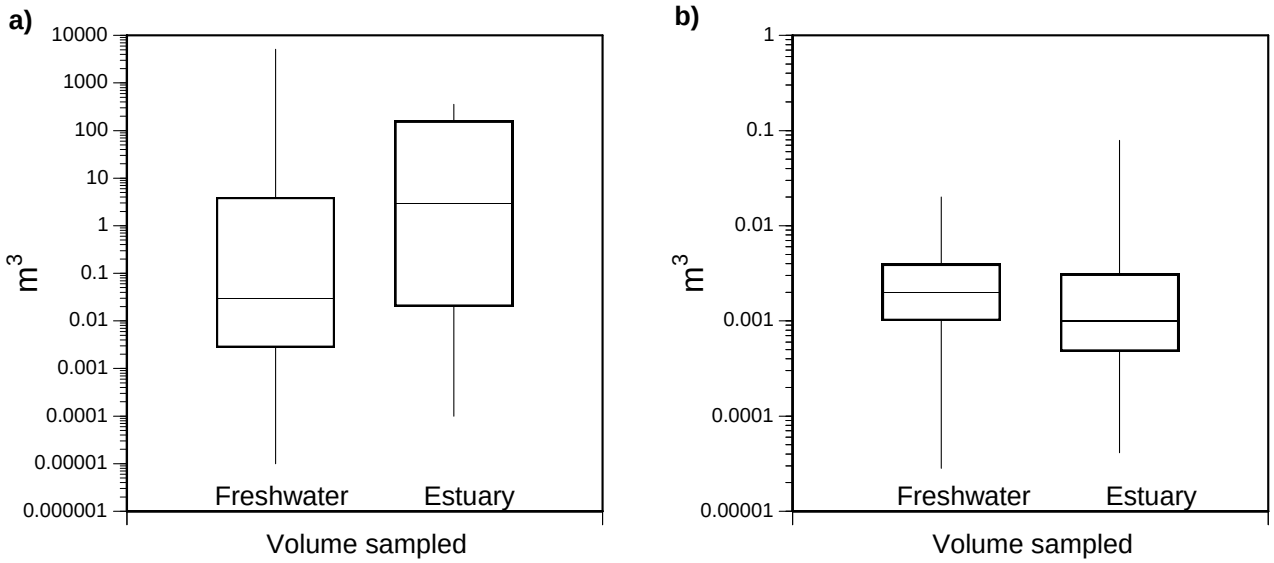

Fig. 5. Range, 25%ile, 75%ile, and median volume of a) water and b) sediment sampled in freshwaters and estuaries.

There are implications associated with the volume of water sampled. Filtration of large volumes of water is only possible through the use of relatively coarse mesh size in nets/filters, as finer mesh sizes are more likely to become clogged, an issue reported by sources that compared the use of nets of different mesh size (e.g. Hohenblum et al. 2015, Dris 2016, Dris et al. 2018). Hence, there was a direct relationship between the size of the smallest particles considered (derived from mesh size of the primary filter/net used) and the volume of water sampled in both freshwater (Fig. 6a) and estuaries (Fig. 6d): large volumes of water can only be sampled using nets of relatively coarse mesh, which therefore, do not capture smaller particles. It is possible that particle morphology may influence the particles retained by filters but no studies considered or quantified such an effect.

In turn, the mean abundance of microplastic particles reported appeared to be inversely correlated with the volume of water sampled (Fig 6b & e). Whilst it is necessary to collect a sufficiently large sample of the matrix to ensure that any estimates of abundance are robust (i.e. sufficient to capture an adequate sample of particles), the mean abundance of particles reported also appeared to be inversely related to the size of the smallest particle considered (Fig. 6c & f). Some studies compared samples of different volumes, but in each case, methodology and the size of particles considered varied as well as volume, so it was not possible to determine the effect of volume *per se* rather than any effect of the size of particles considered. Small particles of microplastic tend to be more abundant than larger particles, as noted by several sources where data were analysed by particle size class (e.g. Estahbanati and Fahrenfeld 2016, Lahens et al. 2018, Di et al. 2019). Hence, those methods of sampling and processing that retained smaller particles returned higher concentrations of microplastic particles.

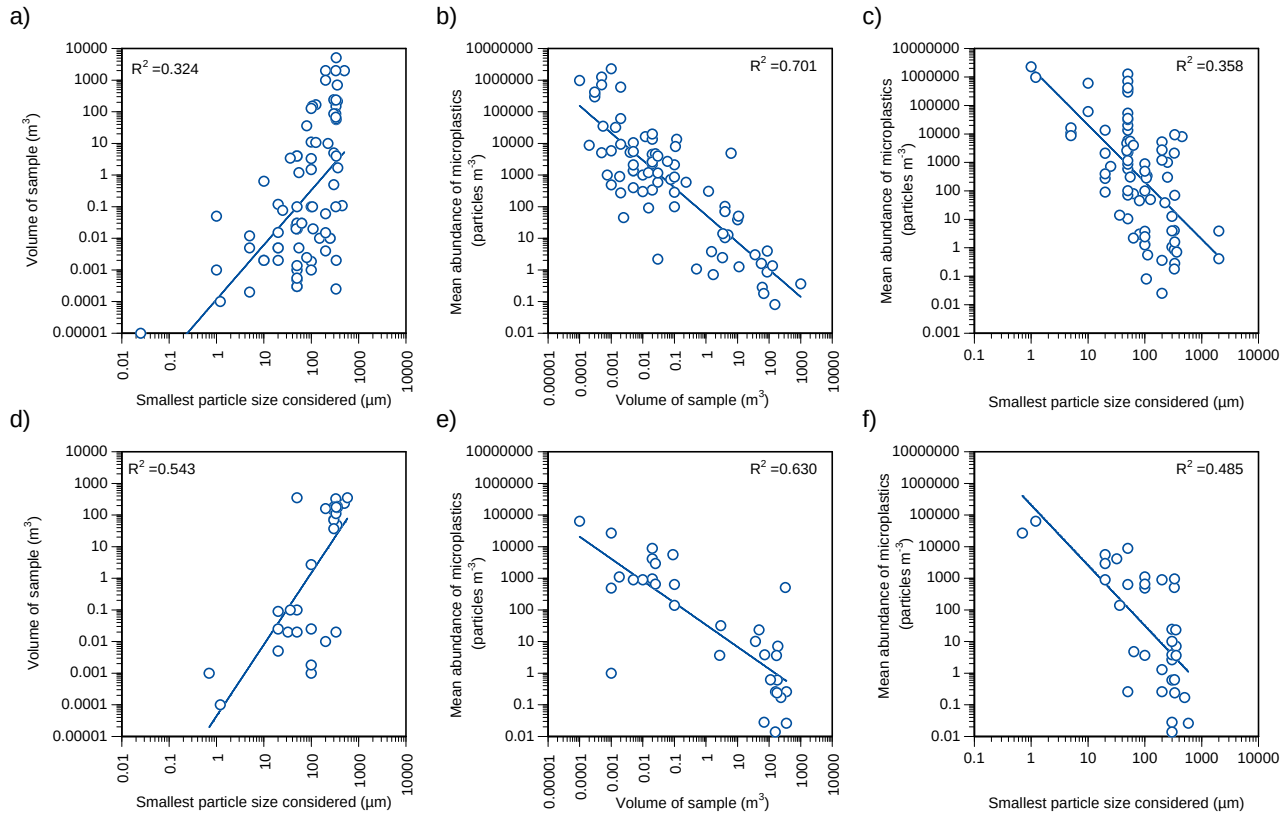

Fig. 6. Relationships among a & d) the volume of water sampled and the smallest particle size considered, b & e) the volume of water sampled and the mean abundance of microplastics recorded, and c & f) the smallest particle size considered and the mean abundance of microplastics recorded, in freshwaters (a-c) and estuaries (d-f). Each point represents values for a different study or sampling technique. Concentrations and volumes sampled standardized to m³.

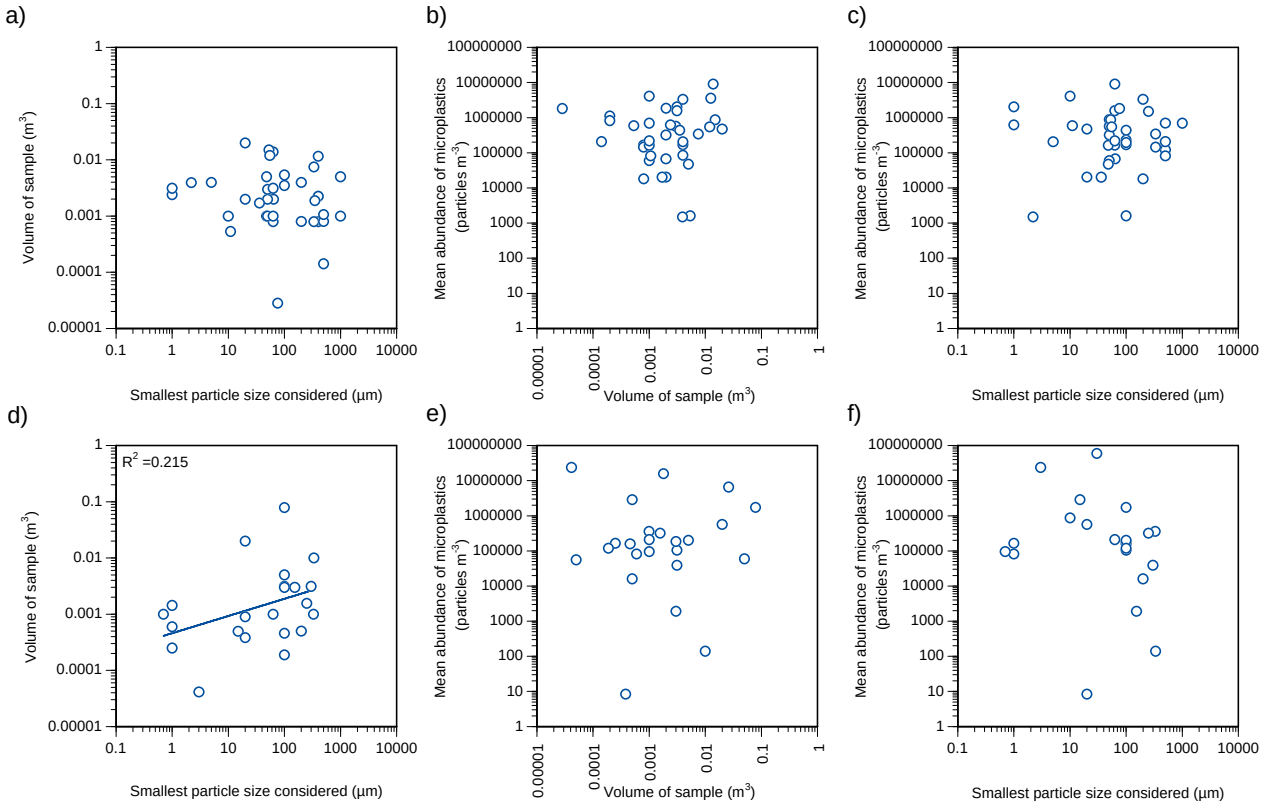

Fig. 7. Relationships among a & d) the volume of sediment sampled and the smallest particle size considered, b & e) the volume of sediment sampled and the mean abundance of microplastics recorded, and c & f) the smallest particle size considered and the mean abundance of microplastics recorded, in freshwaters (a-c) and estuaries (d-f). Each point represents mean values for a different study or sampling technique. Concentrations and volumes sampled standardized to m³.

The standard approach used in marine systems is to tow a net (plankton, manta, neuston or bongo nets) comprised of ≈330 µm mesh over long distances (Bannick et al. 2019), an approach that has been adopted for sampling both large lakes and rivers (e.g. (Dris et al. 2015, Mason et al. 2016, Kapp and Yeatman 2018). Whilst this method is appropriate for sampling larger particles that occur at low abundance (e.g. (Dris 2016), other methods are required to sample smaller particles from water. Bulk water samples, either pumped or grab samples, were typically used by sources that investigated smaller particles (e.g. (Majewsky et al. 2016, Bordos et al. 2019).

The volume of sediment sampled did not appear to be related strongly to the smallest particle size considered in either freshwaters or estuaries (Fig. 7a & d), and was more likely to be governed by the practicalities of collecting, transporting and processing large volumes of sediment. Similarly, the mean abundance of microplastic particles did not appear to be related to the volume of sediment sampled (Fig. 7b & e) or the smallest particle size considered (Fig. 7c & f). Nevertheless, due to the limited range of sample volumes used, the evidence available may not be sufficient to robustly test such relationships. As several sources investigating the relationship between particle size and abundance of microplastics in water have shown that small particles of microplastic tend to be more abundant than larger particles (e.g. Estahbanati and Fahrenfeld 2016, Lahens et

al. 2018, Di et al. 2019), it is likely that an inverse relationship between particle size and abundance also applies to sediment. However, deposition processes may influence the relative abundance of particles in sediment compared with water, as size and density are likely to affect the rate that particles become entrained in sediments.

There was insufficient evidence to draw conclusions on the most appropriate way to sample microplastics from freshwater and estuarine sediments. A range of sample volumes may be necessary to quantify the abundance of different sized particles adequately. More research into appropriate sample volumes for sediment is required.

In summary, the size range of particles captured by the sampling and processing method used influences the mean abundance of microplastic particles reported. Comparison among studies is not possible without consideration of the size of particles considered.

The size range of particles captured by the sampling and processing method used influences the mean abundance of microplastic particles reported.

Comparison among studies is not possible without consideration of the size of particles considered.

The volume of sample required to estimate the concentration of particles reliably is proportional to particle size: larger sample volumes are required to estimate concentrations of larger particles than smaller ones. A range of sample volumes may be necessary to quantify the abundance of different sized particles adequately.

More studies would be needed where standardisation of methods is required.

4.1.2 Reliability

Three measures of reliability were used (see section 2.2.2). The cumulative score (total achieved across all criteria) is a general measure of reliability, the number of zeros is a measure of the number of criteria considered unreliable, and the product of scores in all criteria unambiguously identifies those studies that were reliable across all criteria.

Cumulative reliability scores ranged from 1 to 17 for water and sediment and from 2 to 18 for biota (Fig. 8), with averages close to the middle of possible scores (total possible = 18 for water and sediment, 20 for biota).

The number of reliability categories that scored zero ranged from 8 to 0 per study (Fig. 9), with an average between 3 and 4 zeros per study (out of a possible 9 for water and sediment, and 10 for biota): the overall average was 3.77 zeros per study. A zero score in any criterion indicates it was evaluated as “not reliable”: an average of 3 to 4 zeros per study indicates that most studies were based on methods that were unreliable in several aspects.

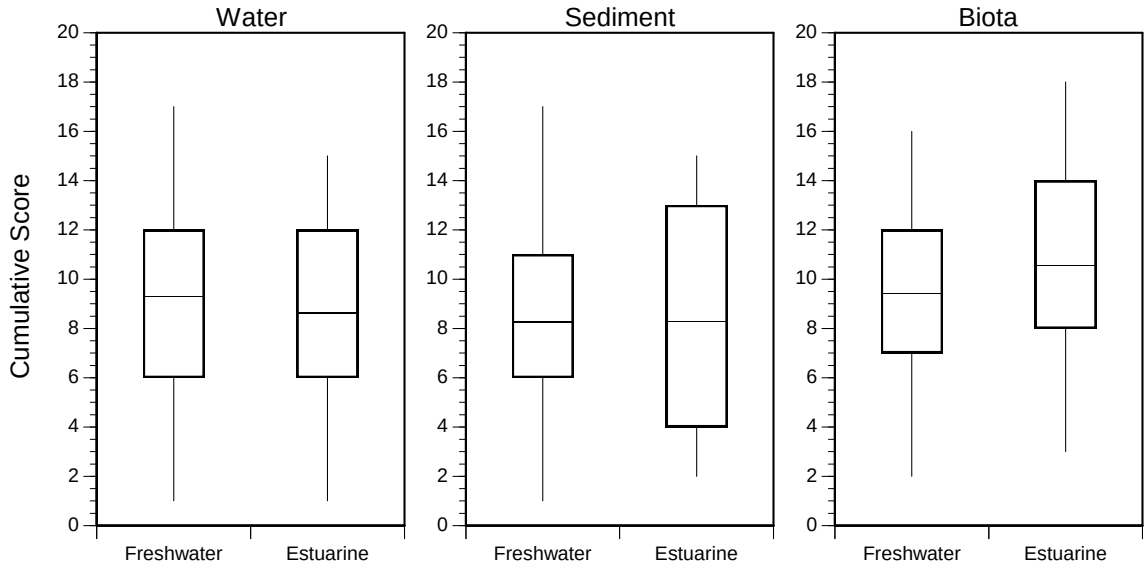

Fig 8. Range, 25%ile, 75%ile, and mean cumulative reliability score of studies of microplastics in freshwaters and estuaries for water, sediment and biota matrices.

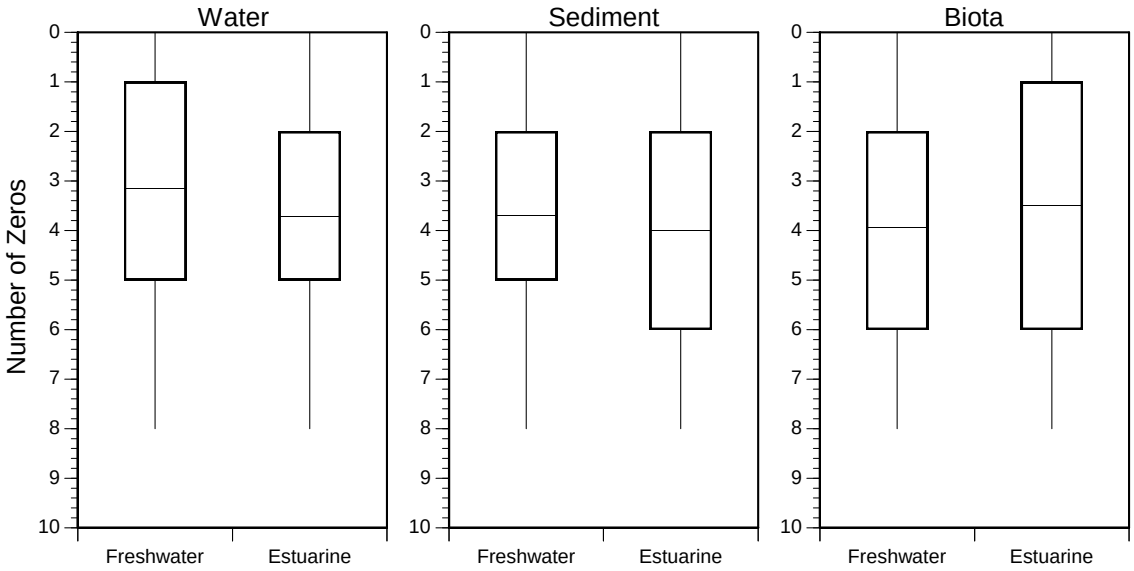

Fig 9. Range, 25%ile, 75%ile, and mean number of zero reliability scores for studies of microplastics in freshwaters and estuaries.

The reliability of studies has improved over time. Whilst there has been little change in the scores achieved by the most unreliable studies over time, the scores achieved by the most reliable studies have improved over time, both in terms of the cumulative score and the number of zeros per study (Fig. 10). These trends suggest that methodological improvements have been made as workers have become aware of the potential pitfalls.

Using a more punitive measure of reliability, the product of the scores in all categories, only 12 studies did not score 0. The methods used in these 12 studies should be considered as a basis for developing a standardised methodology. Nevertheless, the

majority of studies of microplastics in freshwaters and estuaries are based on methods that are in some aspects not reliable.

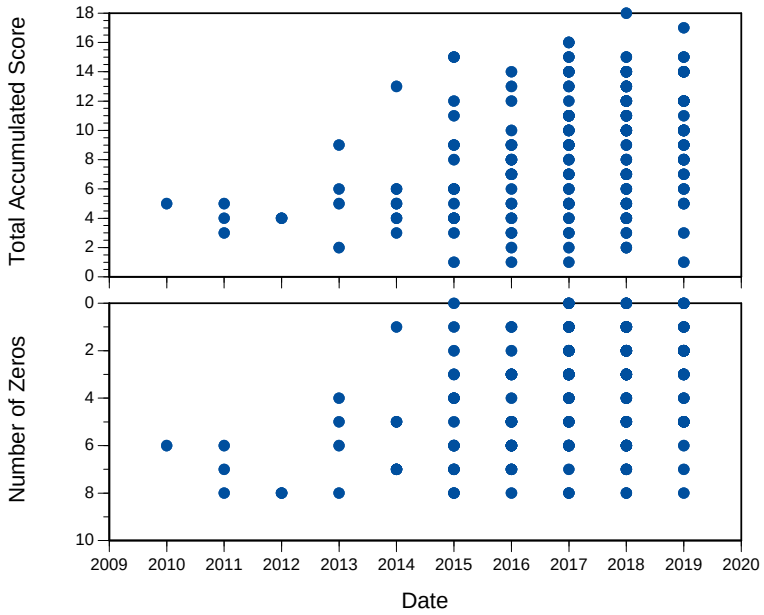

Fig. 10. Change in reliability scores over time.

The majority of studies of microplastics in freshwaters and estuaries are based on methods that are in some aspects not reliable.

The reliability of studies has improved over time.

4.2 Secondary question: Are the methods transferable to different sample matrices and can they be used to assess changes to the microplastic profile and properties at different stages from sink to river and river to tap?

A wide variety of methods have been used to quantify and characterise microplastic particles. As the study of microplastics is relatively new, the advantages and limitations of each method have yet to be fully established.

The techniques most frequently used to quantify and characterise microplastics were optical microscopy on its own and optical microscopy in combination with Fourier-transform infrared (FTIR) spectroscopy (Fig. 11). A wider range of techniques have been used in freshwaters than in estuaries, although combinations of optical microscopy, Raman spectroscopy, FTIR spectroscopy, pyrolysis gas chromatography–mass spectrometry (Py-GC/MS) and/or scanning electron microscopy (SEM) have been used in

both environmental compartments (Fig. 11). Although there was a greater number of studies from freshwaters, the wider range of techniques used in freshwaters appears to suggest more frequent modification of the methods used in this compartment compared with those used in marine systems. Similarly, a smaller proportion of evidence sources from freshwaters used optical microscopy on its own compared with those from estuaries (Fig 11).

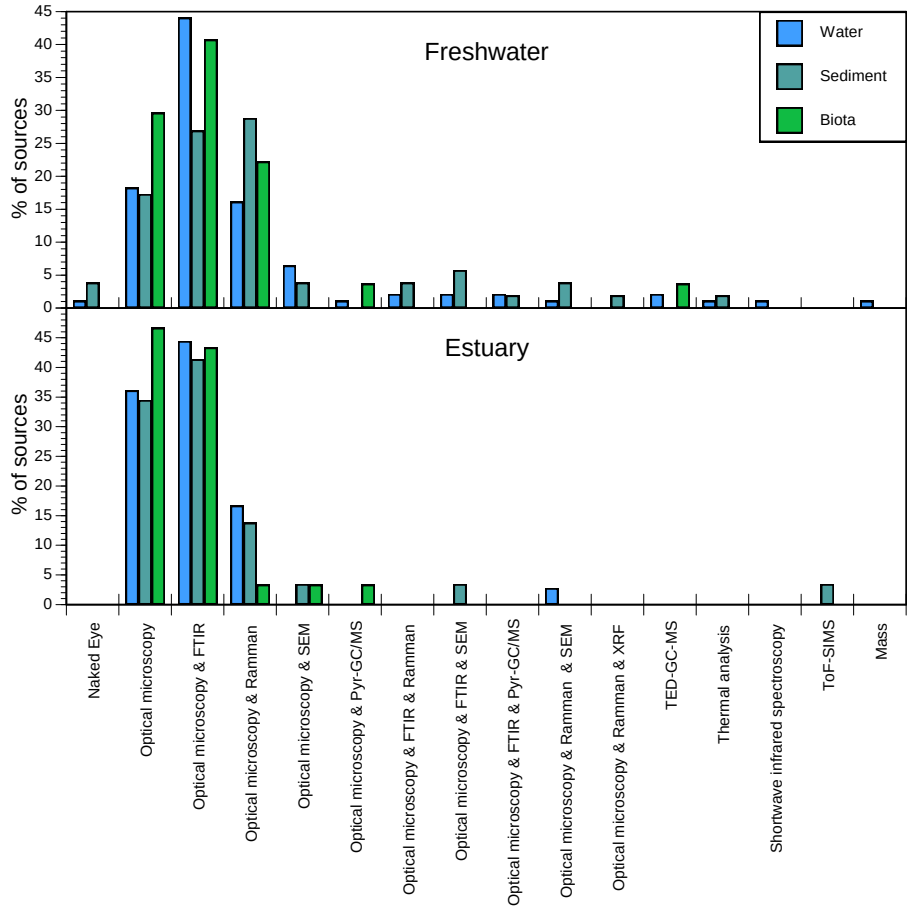

Fig. 11. Techniques used to quantify and characterise microplastic plastic particles in freshwaters and estuaries (number of studies: freshwater: water 94, sediment 52, biota 28; estuary: water 36, sediment 28, biota 30).

It is apparent that the same techniques have been used to quantify and characterise microplastic particles across all three matrices, although the preparation of samples differed (see section 4.3). The size of the smallest particles considered varied with the method used to quantify and characterise the particles. The few sources reporting studies using naked eye to quantify particles only considered particles 2 mm or larger (Fig. 12). The smallest particle size considered by studies in freshwaters using optical microscopy was 1.2 µm, where particles were gathered on grey filters to aid identification (Dubaish and Liebezeit 2013) and 0.7 µm for studies in estuaries (Karlsson et al. 2017). Sources that reported the use of FTIR and Raman spectroscopy, used techniques that captured smallest particles of 0.2 and 1 µm respectively (Fig. 12), although the respective spatial resolutions of FTIR and Raman are approximately 20 and 5 µm. At a spatial resolution smaller than these, the ability to characterise individual particles is compromised as the spectroscopic methods provide an average measure over the area sampled, which will

include both the particle and the background (Balls et al. 2019). It is evident that some evidence sources used sampling/processing techniques that retained microplastic particles that were close to or below the size limit of detection for the technique used to quantify and characterise them. It is likely that the evidence from such sources is an underestimate of the number of microplastic particles present. The only source that considered particles in the nanoparticle range used Triton X-45 (TX-45)-based Cloud Point Extraction technique to separate and capture particles coupled with pyrolysis gas chromatography–mass spectrometry (Pyr-GC/MS) to quantify and characterise them (Zhou et al. 2018).

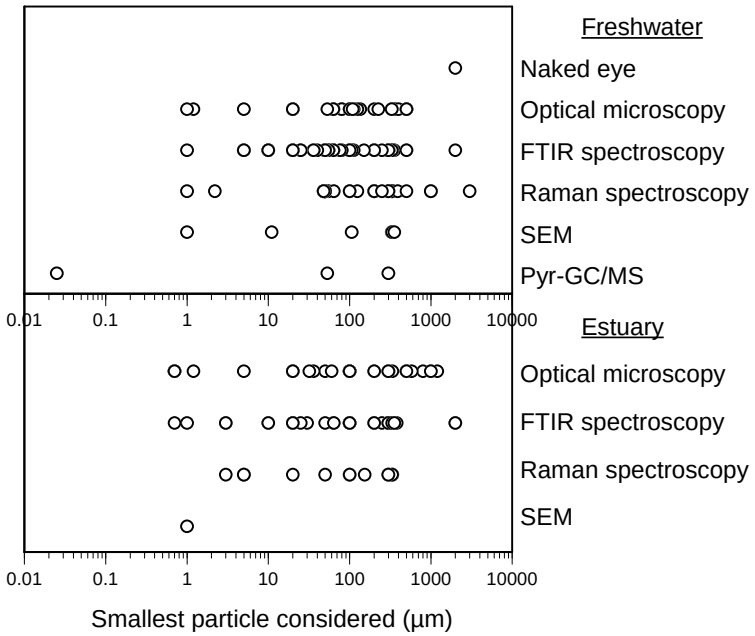

Fig. 12. Size of smallest particle considered by evidence sources and the technique used to quantify and characterise particles in freshwater and estuarine habitats.

The different techniques used can be divided into three categories, namely spectroscopic (FTIR, Raman, near infrared), thermoanalytical (Py-GC-MS, TED-GC-MS) and chemical (ICP-MS) which each return information on different characteristics of the microplastics present in the sample. Details of the preparation, processing time, detection limits and the characteristics recorded for commonly used quantification techniques are given in Appendix A, Table A1. The different techniques used to quantify and characterise microplastic particles each have their own limitations with respect to the size and number of particles considered, the time taken to process the sample, and the information obtained. Currently none of the techniques can be used in the field, as samples need to be prepared to remove all potentially confounding material (especially non-plastic organic matter) before analysis. Handling time is an important consideration; many of the techniques used to date have involved manual selection and separation of particles for analysis. Recent advances include focal plane spectroscopy combined with image analysis which are capable of characterising every particle within the microscope's field of view. The number of particles that were analysed by each evidence source reviewed here varied from 10 particles throughout an entire study (Yonkos et al. 2014) to every particle captured (Lusher et al. 2018). Hence, the reliability of the information obtained, particularly

that describing the profile of microplastic particles, varied considerably among the different evidence sources.

Comparison amongst the different techniques used to quantify and characterise microplastic particles is not possible. Due to the variety of techniques that have been used to quantify and characterise microplastics, as well as variation in the volume sampled and size of particles considered (see Section 4.1.1), it is not possible to assess changes to the microplastic profile and properties at different stages from sink to river and river to tap using the data currently available.

Spectroscopic (FTIR, Raman, near infrared), thermoanalytical (Py-GC-MS, TED-GC-MS) and chemical (ICP-MS) methods have been used to quantify and characterise microplastics in freshwaters and estuaries: most studies have used FTIR or Raman spectroscopy.

The different methods return information on different characteristics of microplastics.

Due to the variety of techniques that have been used to quantify and characterise microplastics, as well as variation in the volume sampled and size of particles considered, it is not possible to assess changes to the microplastic profile and properties at different stages from sink to river and river to tap using the data currently available.

4.3 Secondary question: With samples from different matrices, what pre-processing of samples is used?

In order to quantify and characterise microplastic particles, it is necessary to separate them from the matrix. The processes used to separate microplastic particles from the matrix are relevant to the question "*With samples from different matrices, what pre-processing of samples is used?"* As microplastics have been found in water, sediment and biota, the processes involved in separating microplastics vary dependent on the matrix sampled. This is particularly relevant as the processes used to separate microplastic particles from the matrix may influence how many and which particles, both plastic and non-plastic, are isolated.

Three processes were documented to physically separate microplastic particles from matrices. These were filtration, separation using a density gradient, and dissection, either in isolation or combination. Filtration involves capture of particles on a mesh of various mesh sizes/grades, which influences the size of particles to be trapped (see Section 4.1.1). Density separation involves increasing the density of the solution to separate less dense particles by flotation and the density of the solution chosen can influence the polymers captured dependent upon their density (Quinn et al. 2017). The order of the processes used varied amongst the evidence sources. Both in freshwater and estuaries, a

high proportion of evidence sources reporting investigations into microplastics in water used filtration either alone or with density separation, whereas investigations into microplastics in sediment tended to use density gradients (typically in combination with filtration). Density gradients were established typically by adding either NaCl or $ZnCl_2$ (although other solutions have been used, e.g. NaI, ZnI_2, $Na_6[H_2W_{12}O_{40}]$), with the latter preferable as it produces a larger density gradient and is more likely to separate higher density plastic particles (Quinn et al. 2017). One source detailed the use of canola oil to efficiently separate microplastics from estuarine sediments using the oleophilic properties of microplastics (Crichton et al. 2017). Naturally, dissection was restricted to biota and used frequently (88% of sources from freshwater [n=28], 90 % from estuaries [n=30]) although this process was not always used to separate particles from organisms: some smaller animals were homogenised before particles were separated (e.g. Hurley et al. 2017). When dissection and the order of processes were not considered when calculating percentages (to allow better comparison among matrices), it was apparent that most sources dealing with sediment used a combination of density separation and filtration, and that a large proportion of sources dealing with biota used no separation other than dissection (Fig. 13).

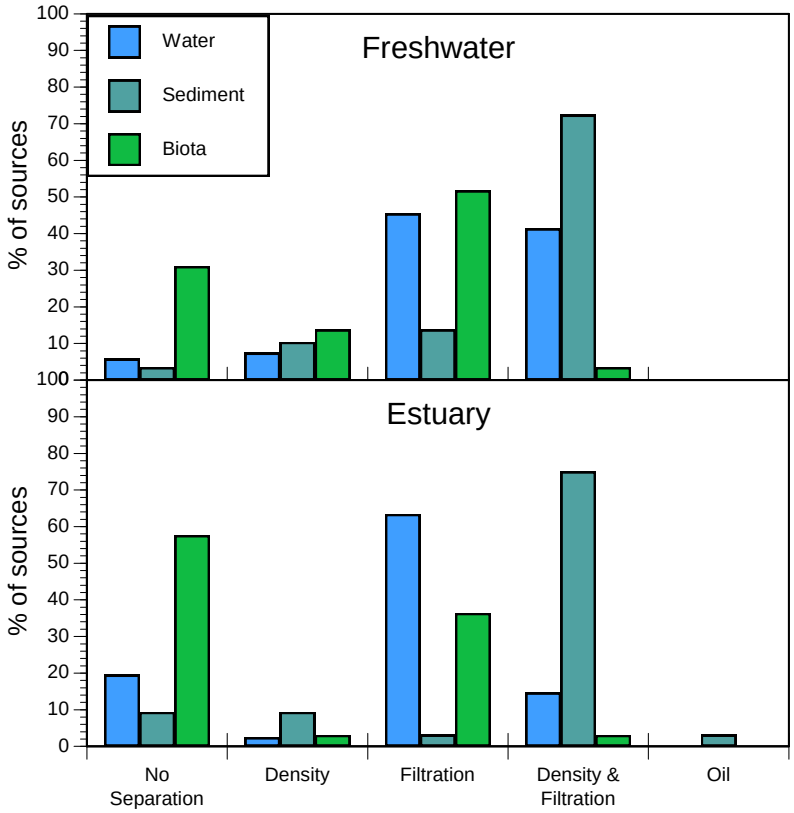

Fig. 13. Techniques used to separate microplastics from the matrix (number of studies: freshwater: water 94, sediment 52, biota 28; estuary: water 36, sediment 28, biota 30).

As sediments are composed of large numbers of particles of varying density, it is not surprising that density gradients were used extensively to separate microplastic particles from this matrix. Similarly, the relative ease of filtering water may explain why this process was used extensively by sources reporting studies of microplastics in water.

Physical separation of particles from the matrix does not necessarily separate microplastic particles from other similarly behaving non-plastic particles. Hence, digestion was frequently used to remove non-plastic particles from the sample before particle quantification, using acid, alkali, oxidation (typically with H_2O_2 or Fenton's reagent: Tagg et al. 2017, Hurley et al. 2018b) or enzymes (Fig. 14). A large proportion of studies did not use any digestion (42% of sources from freshwater [n=141] and 49% of sources from estuaries [n=82]), potentially leading to an overestimate of the abundance of microplastics in these studies. Adequate digestion is required to avoid including non-plastic particles in estimates of abundance. Enzymatic digestion was the method used least (not used in any study of sediment and only used for water in freshwaters). Digestion with acid or alkali was largely restricted to studies of biota: these more aggressive treatments are efficient at digesting large amounts of organic material but lead to degradation of plastic particles and, hence, an underestimate of abundance (Karami et al. 2017, Naidoo et al. 2017, Hurley et al. 2018b). Oxidative digestion is a preferred method as it has less impact on microplastics and was the most frequently used method of digestion in studies of water or sediment (Fig. 14).

Use of a stain to discriminate between plastic and non-plastic particles may aid quantification, particularly of small particles (Erni-Cassola et al. 2017) but requires complete removal of non-plastic organic matter and is rarely used (Fig. 14).

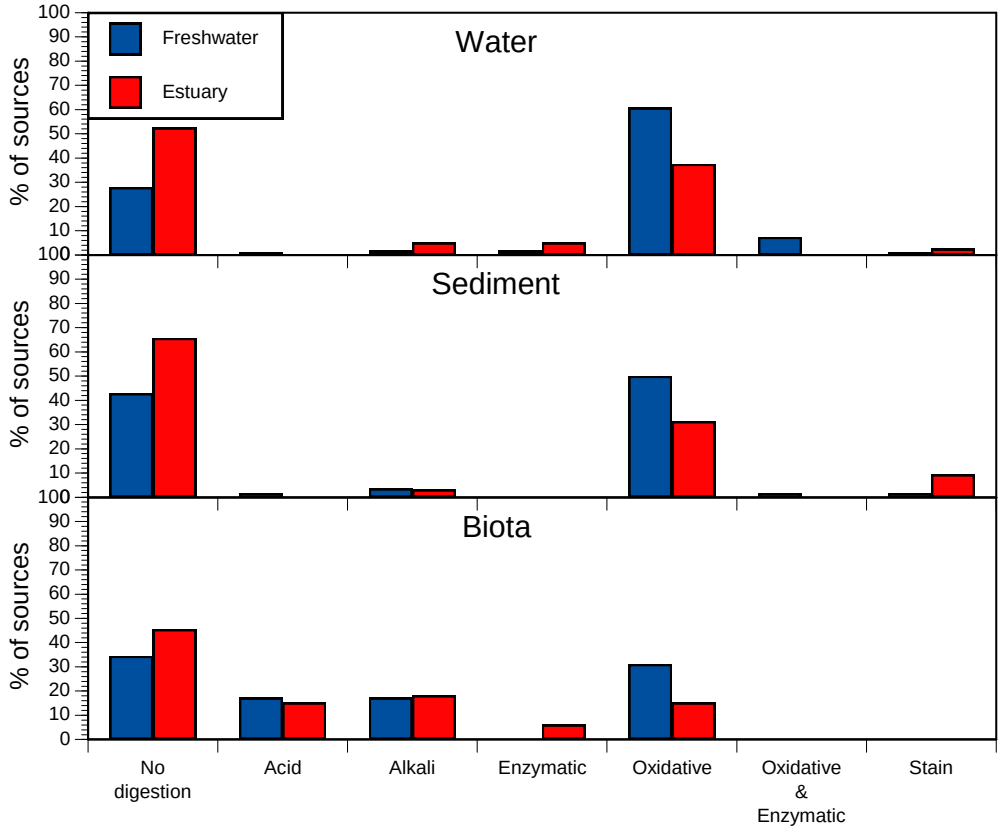

Fig. 14. Methods used for digestion and staining (number of studies: freshwater: water 94, sediment 52, biota 28; estuary: water 36, sediment 28, biota 30).

Physical separation of microplastics is necessary, although not used in all studies.

Most studies have used filtration on its own or combined with density separation.

Digestion of non-plastic organic matter is required to avoid overestimation of the abundance of microplastics, but evidence shows that it has not always been used.

Where organic matter has been digested, oxidative methods are used most frequently although acid and alkali digestions can degrade polymers and lead to an underestimation of the abundance of microplastics.

4.4 Secondary question: If the method has been used in marine environments, does it require any modification to be used in equivalent freshwater environments?

For each evidence source, it was recorded if the methods used to collect and/or quantify the amount of microplastic present were modified compared with methods used in marine systems using a yes/no score. Direct reference to methods used in marine systems was taken as evidence of a lack of modification. Modifications were noted in sampling techniques (novel methods used to collect the matrix, e.g. nets, sediment grabs, pumps), processing (novel methods used to separate microplastics from the matrix, e.g. filtration devices, separation techniques) and quantification (novel methods used to quantify and characterise microplastics). The percentage of evidence sources using modified methods to describe microplastics in biota, sediment and water was calculated.

Most sources reporting evidence from estuaries or freshwaters did not use modified methods (>85% over all evidence sources). It would be preferable if the same methods were used across different habitats to increase comparability of results. Modified methods were used most by sources reporting evidence on the prevalence of microplastics in sediment, particularly freshwater sediment, where 27% [n=52] used modified methods (Fig. 15). No sources reporting evidence on the prevalence of microplastics in biota used modified methods.

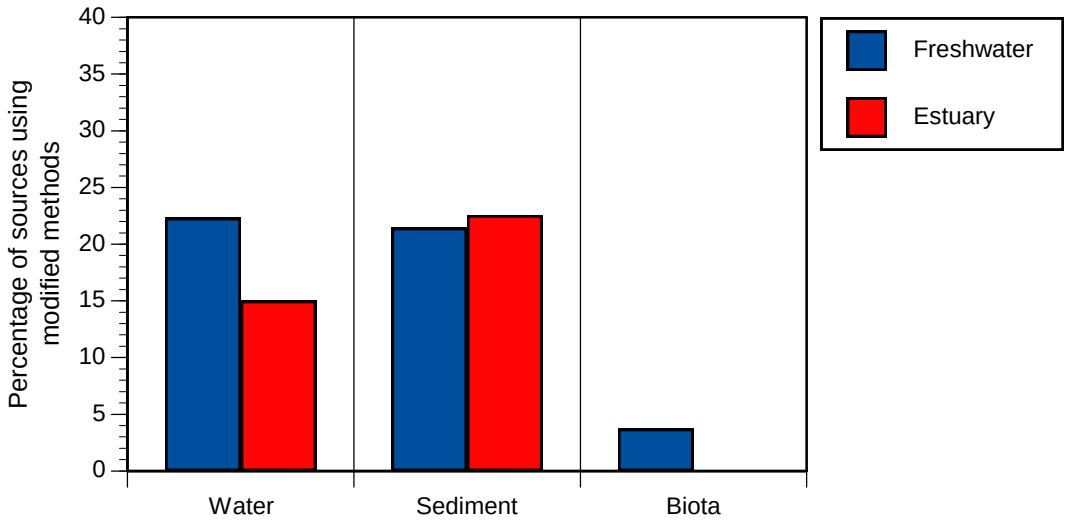

Fig. 15. Percentage of evidence sources that used methods modified from those used in marine studies (number of studies: freshwater: water 94, sediment 52, biota 28; estuary: water 36, sediment 28, biota 30).

Methods used in marine environments do not require substantial modification for use in freshwaters.

4.5 Secondary question: To what extent can the method provide information on different characteristics of microplastics such as substance, state, morphology and dimensions?

The European Chemical Agency (ECHA 2018) definition of microplastics considers four criteria, substance (i.e. which polymer, but potentially also any additives and influence of production), state (i.e. whether solid or semi-solid [putty-like]), morphology (i.e. what shape) and dimensions (i.e. a measure of size). Here, for the more frequently used techniques, the proportion of sources that reported information on these criteria was quantified, together with an additional criterion, the source of the microplastic particle (i.e. discrimination between primary and secondary particles based on surface oxidation/damage).

To enable comparison among the different techniques used to quantify and characterise microplastic particles, the frequency (percentage of evidence sources using the five techniques considered) with which the evidence sources reported information on the five criteria (substance, state, morphology, dimensions and source) was determined. The estimates of frequency provided were more reliable for those techniques that were used more often (see Fig. 16).

None of the evidence sources reported information on the state of particles (i.e. whether solid or semi-solid).

None of the evidence sources that used optical microscopy reported information on substance (Fig. 16a). Evidence sources that used the other techniques (combinations of optical microscopy and FTIR, Raman, Pyr-GC/MS or SEM) all reported information on substance. This is critical to discriminate between plastic and non-plastic particles. Early studies typically isolated individual particles for analysis, with number varying from as few as 10 particles throughout an entire study (Yonkos et al. 2014), although more recent studies use equipment that can analyse every particle captured (Ball et al. 2019). Although not frequently used compared with other techniques (Fig. 11).

Overall, less than a third of the evidence sources reported information on the source of microplastic particles (primary or secondary particles), with the largest proportion being those that used optical microscopy and SEM, where SEM can identify the marks of aging on particle surfaces (Fig. 16b).

With the exception of evidence sources that used optical microscopy and Pyr-GC/MS in estuaries (which were relatively infrequent) evidence sources based on all five of the techniques considered here reported information on the morphology of particles (Fig. 16c). However, there was considerable variation in the terminology used to describe the morphology of particles (e.g. fragments, flakes, beads, spheres). It would be advisable to develop a standard terminology to describe the morphology of particles.

Most evidence sources reported information on dimensions, irrespective of the technique used, with the exception of those few evidence sources that used optical microscopy and Pyr-GC/MS to describe microplastics in estuaries (Fig. 16d). However, the size ranges used to report concentrations varied among studies making comparison difficult. It would be advisable to use standardised size ranges to characterise and report on concentrations, as are used for dust analysis.

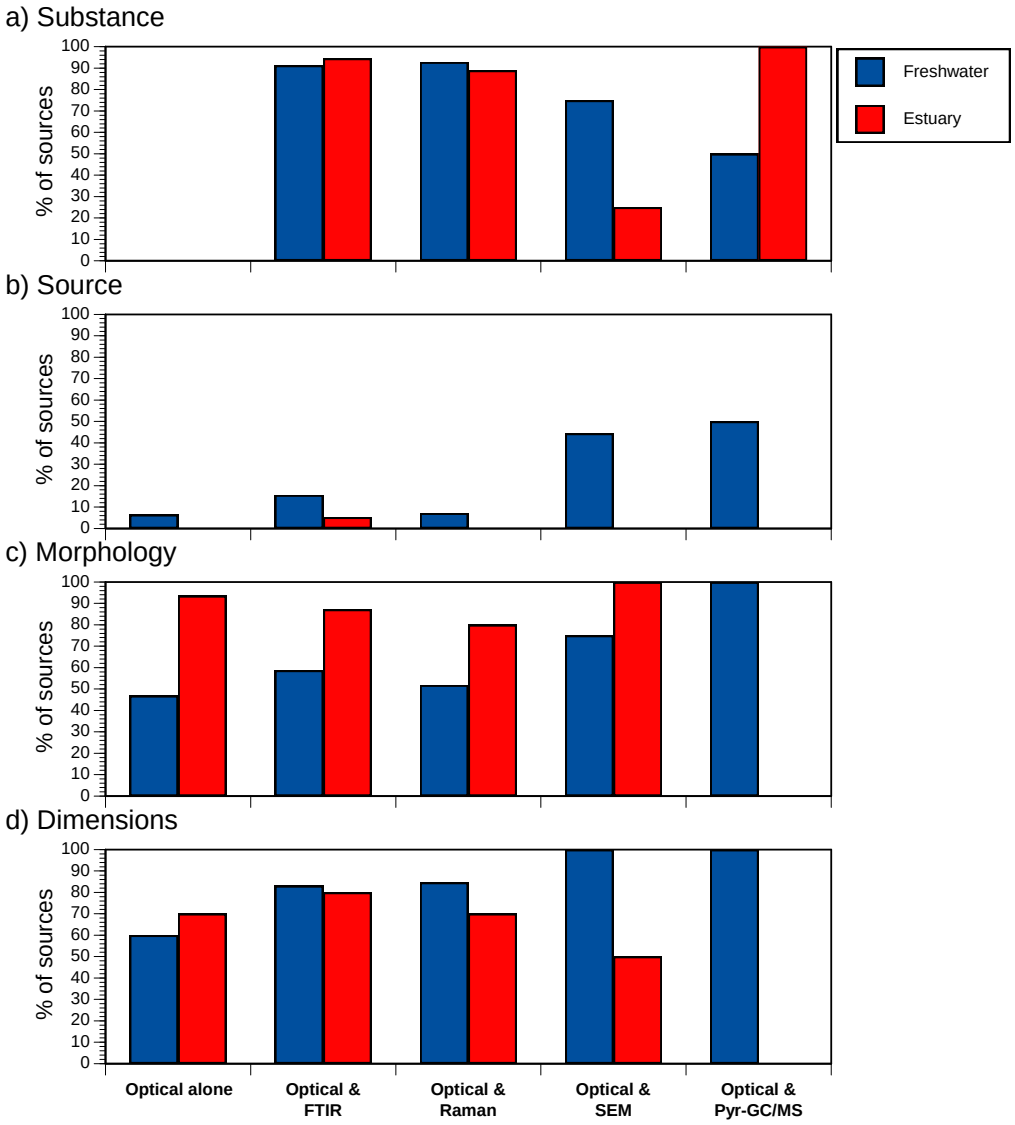

Fig. 16. Influence of methods used to quantify and characterise microplastic particles and the percentage of evidence sources that reported information on a) substance b) source, c) morphology and d) dimensions of microplastic particles in freshwaters and estuaries (number of studies: freshwater 141, estuary 82).

With the exception of sole use of optical microscopy, the methods used to quantify and characterise microplastics provide detail on substance (polymer type), morphology and dimensions.

No methods provide details of state (solid vs semi- solid).

4.6 Secondary question: Does the sampling method used address spatial and temporal variation adequately?

Details of the scale of replication of samples were recorded for each source of evidence where available. The majority of studies used spot samples (where each sample analysed was collected from a single location in space and time) rather than integrated samples (where the sample analysed comprised multiple samples derived from different locations/times and combined) to describe the microplastics present (Fig. 17).

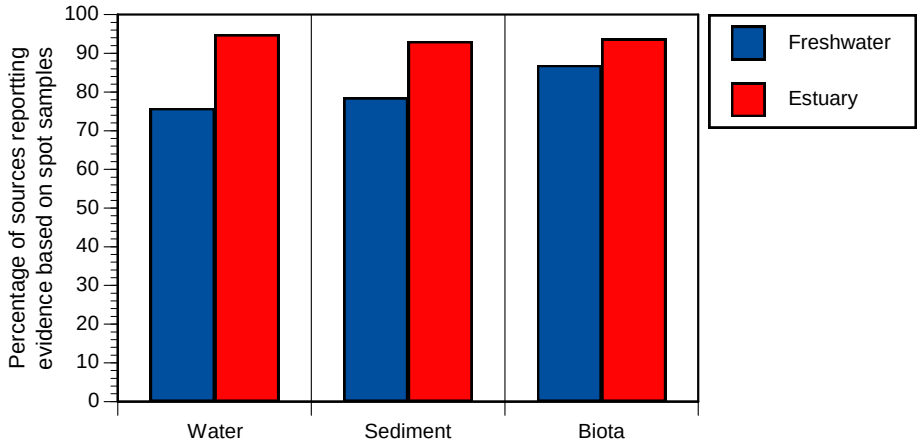

Fig. 17. Percentage of evidence based on spot samples (number of studies: freshwater 141, estuary 82).

Although, concentrations of contaminants are not evenly distributed in space and time in freshwater and estuarine systems (Lloyd et al. 2014), 39% of studies overall [n=209] were based on unreplicated samples with a higher frequency occurring in evidence sources which investigated microplastics in biota (Fig. 18). Spatial replication was more frequently used in studies of microplastics in sediment than other matrices (Fig. 18). Whilst temporal replication was used for all three matrices in estuaries, in freshwaters it was used more frequently in investigations of microplastics in water. Despite studies demonstrating substantial spatial and temporal variation in the abundance of microplastics in water (e.g. Dubaish and Liebezeit 2013, Lima et al. 2015, Rodrigues et al. 2018) and sediment (e.g. Stolte et al. 2015, Hurley et al. 2018a, Imhof et al. 2018) associated with seasonality or rainfall events, spatial and temporal replication was used least, in an average of 5% of studies overall [n=209].

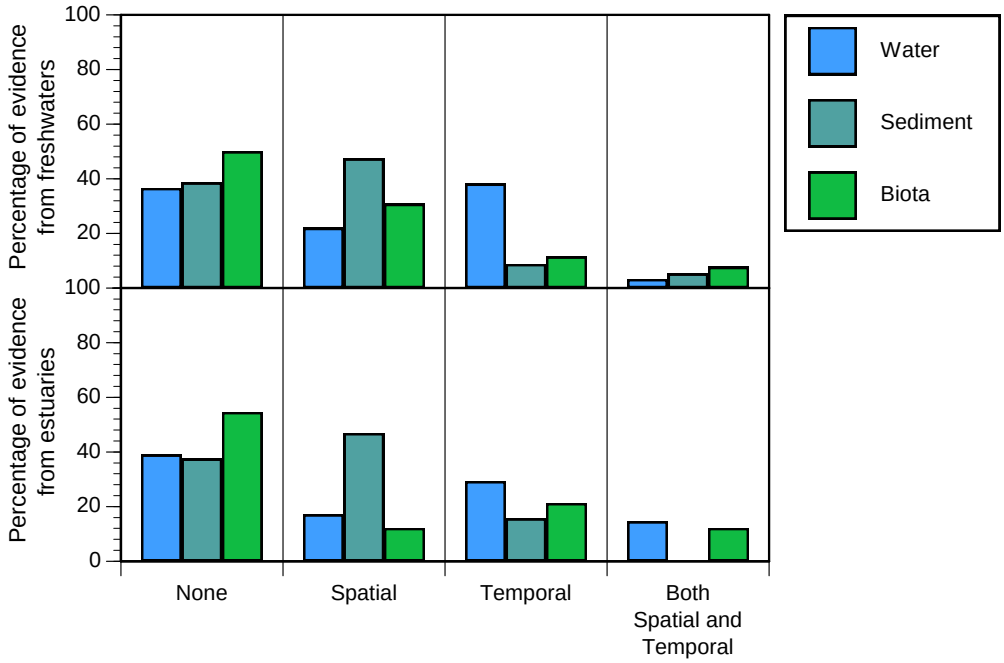

Fig. 17 Scale of replication used by evidence sources for sampling the prevalence of microplastics in freshwaters and estuaries (number of studies: freshwater: water 94, sediment 52, biota 28; estuary: water 36, sediment 28, biota 30).

Most studies have used methods that do not address spatial and temporal variation adequately.

5. Limitations

Key limitations of this review are outlined below and these stem primarily from the facts that:

- This is a relatively new and developing scientific field and methods are developing rapidly.
- There are inconsistencies in the way methods and results are reported in different studies.
- Very few studies have been undertaken that test and report the performance of methods under method validation conditions.

- The findings presented are infuenced by the reliability of the primary literature, including grey literature, on which this report is based. An assessment of the reliability of the studies included in this review was undertaken (see section 4.1.2). However, this assessment of reliability was not used to exclude studies from the review, which was based on all evidence sources.

6. Conclusions

The aim of this evidence review was to address the question "Are the current sampling and analytical methods used to provide data on the presence of MPs in freshwater matrices as well as other environmental materials scientifically robust and appropriate?" using the evidence available from reported studies of microplastics in freshwaters and estuaries. It was clear from this evidence that the methods used to sample and separate microplastic particles from water in both environmental compartments influenced the concentration of microplastic particles reported. In particular, those methods that collected smaller particles from the water reported higher concentrations of microplastic particles, with the relationship between the size of the smallest particle considered and concentration of particles described by a log-log relationship. It is likely that the same is true for the methods used to sample and separate microplastics from sediment, although the practical constraints of collecting large volumes of sediment appears to limit the range of volumes of sediment sampled. It is also likely that, in many cases, the volume of sediment sampled has been insufficient to provide a robust estimate of larger, and hence less abundant, particles.

The standard approach used in marine systems is to tow a net (plankton, manta, neuston or bongo nets) comprised of ≈330 µm mesh over long distances, an approach that has been adopted for sampling both large lakes and rivers. Whilst this method is appropriate for sampling larger particles that occur at low abundance, bulk water samples, either pumped or grab samples, are required to sample smaller particles. This finding is in agreement with the opinion of the experts who noted that there is no consensus on what comprises a representative sample volume in order to adequately quantify particles of different sizes. There was insufficient evidence to draw conclusions regarding the most appropriate way to sample microplastics from freshwater and estuarine sediments.

Different methods have been used to separate microplastics from biota, sediment and water matrices, potentially leading to different concentrations and profiles of microplastics being reported from the different matrices. Studies of biota tended to use no separation (other than dissection) more than those of other matrices, and studies of sediment used a combination of density separation and filtration more than those of other matrices. Studies of either water or sediment used filtration alone more than those of biota. A large proportion of studies did not use any digestion (42% of sources from freshwater and 49% of sources from estuaries), potentially leading to an overestimate of the abundance of microplastics through the misidentification of non-plastic particles. Digestion with acid or alkali was largely restricted to studies of biota; these more aggressive treatments should be avoided as they lead to degradation of plastic particles and, hence, an underestimate of abundance (Karami et al. 2017, Naidoo et al. 2017). The experts expressed concern regarding the influence of the different sample preparation methods on returned concentrations, in particular the effect of digestion on certain polymers. Only one study considered particles in the nanoparticle range, which used a Triton X-45 (TX-45)-based

Cloud Point Extraction technique to separate and capture particles coupled with pyrolysis gas chromatography–mass spectrometry (Pyr-GC/MS) to quantify and characterise them.

Most evidence from freshwaters and estuaries was based on methods that were not modified compared with those used in marine systems (>85% over all evidence sources). It would be preferable if the same methods were used across different habitats to increase comparability of results. No evidence from biota was based on modified methods, whereas modification of methods was most prevalent in studies of sediments: 27% of the evidence from freshwater sediments was based on modified methods. It is likely that the challenges of sampling sediments from freshwaters (compared with marine systems), has resulted in a modification of the methods used.

The majority of evidence available on microplastics in freshwaters and estuaries was based on spot samples, with no or limited replication in space and time. Although microplastics are likely to accumulate in depositional zones, be unevenly distributed through the water column, and vary in concentration dependent on precipitation, overall, 39% of studies were based on unreplicated samples. As the profile of microplastics is likely to be influenced by environmental conditions (e.g. river stage, tidal cycle), the lack of robust replication will influence the confidence with which changes can be identified in the profile and properties of microplastics at different stages from sink to river and river to tap.

Optical microscopy on its own or in combination with FTIR spectroscopy were the most frequently used methods to quantify and characterise microplastics. Optical microscopy on its own is open to the influence of human bias in the recognition of particles, potentially leading to over (inclusion of non-plastic particles) or under (failing to recognize microplastic particles) estimation of concentrations, a point also noted by the experts interviewed. Of the criteria that form the European Chemical Agency (ECHA) definition of microplastics (substance, state, morphology and dimensions), none of the methods used to quantify and characterise microplastics provided any information on state (solid/semi-solid [putty-like]). Optical microscopy alone provided no information on substance, despite this method being used frequently in both freshwaters (23% of evidence sources) and estuaries (39% of evidence sources). Optical microscopy on its own or in combination with FTIR or Raman spectroscopy rarely provided information on the source (primary or secondary) of particles. The number of particles that were analysed by each evidence source varied from 10 particles throughout an entire study to every particle captured. Hence, the reliability of the information obtained, particularly that describing the profile and properties of microplastic particles, varied considerably among the different evidence sources. To avoid false positives or false negatives (inclusion of non-relevant particles, or omission of relevant particles) it is preferable that the composition is determined for all particles, although this presents a methodological challenge with respect to the smallest particles. The experts noted that automated methods based on Fourier Transform Infrared Spectroscopy (FTIR) are becoming available which will reduce any potential bias caused by human selectivity when identifying microplastic particles. It would be appropriate to adopt the limit of detection/quantification approach typically used for analytical determination of contaminants, both in terms of size and concentrations of particles, as described in Ball et al. (2019).

In conclusion, a wide variety of methods have been used, with no standardization amongst workers, making comparison among studies is difficult, a point stressed by the experts interviewed. Scientifically robust and appropriate sampling and analytical methods have rarely been used to provide data on the presence of microplastics in freshwater and estuarine matrices as well as other environmental materials. Only 12 studies did not score 0 in the product of the scores in all categories of reliability assessed: the methods used in these 12 studies should be considered as a basis for developing a standardised methodology.

7. Recommendations

Based on the findings of this evidence review and to work towards an improved level of method standardisation, it is recommend that methods should report the size range of particles being considered. Better still, they should provide estimates of the concentration and properties of microplastics by standardised size classes (e.g. using fractionated filtering as described by Bannick et al. 2019, or through adoption of a system similar to the $PM_{2.5}$ PM_{10} system used for dust analysis). It is also recommend that methods should be based on an appropriate intake volume of the matrix sampled. Following on, robust replication should be practiced both in terms of the samples collected from the environment and the proportion of particles characterised. It is also recommended most strongly that a more consistent approach to the separation and digestion stage of processing samples is adopted, to ensure that the profile and properties of microplastic particles is not influenced. A digestion stage should be included for all material, using a less aggressive oxidation process (such as H_2O_2 or Fenton's reagent), along with a density separation process. Positive controls should be included to assess the influence of separation and digestion on the profile and properties of microplastic particles. Finally, analytical verification of polymer composition should include as much of the sample as possible.

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Appendix A

Table A1. Characteristics of detection methods (modified from Braun et al. 2018)

Characteristic	Spectroscopic						Thermoanalytical				Chemical
	µ Raman	µ FTIR (trans)	FPA FTIR (trans)	µ ATR-FTIR	ATR-FTIR/ Raman	NIR / Hyperspectral imaging	Py-GCMS	Mod. Py-GCMS*	TED-GC MS	DSC	ICP-MS
Specimen mass	ng - µg	ng - µg	ng - µg	mg	mg	mg	µg	mg	mg	mg	mg
Maximum number of measurable particles per sample	10_3 – 10_5	10_3 – 10_5	10_3 – 10_5	1	1	Undefined	1	Undefined	Undefined	Undefined	Undefined
Dimension measuring time (including preparation for measurement)	h - d	d	h	min	min	min	h	h	h	h	min
Detection level (in sample tests)	1-10 µm	20 µm	20 µm	25-50 µm	500 µm	1 %	1-0.5 µg	0.5-2.5 µg	0.5-2.5 µg		ppm
Preparation for measurement	On filter	On special filter	On special filter	Isolated particles	Isolated particles	On filter	Isolated particles	Filtrate or with filter	Filtrate or with filter	Filtrate	Filtrate
Identification of polymer	YES	YES	YES	YES	YES	YES	YES	YES	YES	YES/NO	YES/NO

Mass	NO	NO	NO	NO	NO	NO	NO	NO	YES	YES/NO	YES
Particle size/ number of particles	YES	YES	YES	YES	YES	YES	NO	NO	NO	NO	NO
Additives	Pigments	No	No	No	No	No	Yes	No	No	No	No
State of degradation	Surface oxidation	No	No	Surface oxidation	Surface oxidation	No	Oxidation	No	No	Mol. weight	No
	Sensitive to particle surface			Sensitive to particle surface	Sensitive to particle surface		Very sensitive to polymer compositi on	Very sensitive to polymer composition	Not for PVC	Only for semi-crystaline polymers	Only for soluble polymers

** Depending on the individual design of the pyrolysis unit, larger sample quantities can also be pyrolysed (Curie point filament, Micro furnace).*

µ Raman	Raman microscopy
µ FTIR (trans)	Fourier transformation infrared spectroscopy microscopy in transmission mode
FPA FTIR (trans)	Fourier transform infrared spectroscopy microscopy in transmission mode with focal plane array detector
µ ATR-FTIR	Micro attenuated total reflection Fourier transformation infrared spectroscopy
ATR-FTIR	Attenuated total reflection Fourier transformation infrared spectroscopy
Py-GC-MS	Pyrolysis gas chromatography mass spectrometry
Mod. Py-GC-MS	Pyrolysis gas chromatography mass spectrometry with upstream thermal conditioning of the samples
NIR	Near infrared spectroscopy
TED-GC-MS	Thermal extraction desorption gas chromatography mass spectrometry
DSC	Differential scanning calorimetry
ICP-MS	Inductively coupled plasma mass spectrometry

Appendix B ER1_Capture.xls

See Excel spreadsheet ER1_Capture.xls. Column headers reproduced here for convenience.

Evidence				Waterbody Type	Study Type	Matrix		Bulk sample collection		Volume sampled	
Ref No	Reference	Year	Summary				Other detail		Other detail	Volume	Units
				menu	menu	menu	free	menu	free	free	menu

No Sites	Within site replication		Plastic			Pre-processing Separation		Pre-processing Digestion	Staining
	Sample scale	Replication	Macro-	Micro-	Nano-	Method 1	Method 2	Method	
free	menu	menu	Y/N	Y/N	Y/N	menu	menu	menu	Y/N

Quantification		Characterisation						
Method	Other details	substance	polymer	source	physical state	morphology	dimensions	particle size included
menu	free	Y/N	menu	Y/N	Y/N	Y/N	Y/N	free

Continent Location	UK Location			Reported abundance					
	UK	Lat	Long	Mean	Min	Max	Units	Other details	Comments
menu	Y/N			free	free	free	menu	free	free

Appendix C Evidence Sources Used

Reference	Year	Title	Publication	Vol	Pages
Abbasi, S, Soltani N, Keshavarzi B, Moore F, Turner A and Hassanaghaei M	2018	Microplastics in different tissues of fish and prawn from the Musa Estuary, Persian Gulf	Chemosphere	205	80-87
Abidli, S, Toumi H, Lahbib Y and Trigui El Menif N	2017	The First Evaluation of Microplastics in Sediments from the Complex Lagoon-Channel of Bizerte (Northern Tunisia)	Water, Air, and Soil Pollution	228	
Alam, FC, Sembiring E, Muntalif BS and Suendo V	2019	Microplastic distribution in surface water and sediment river around slum and industrial area (case study: Ciwalengke River, Majalaya district, Indonesia)	Chemosphere	224	637-645
Anderson, PJ, Warrack S, Langen V, Challis JK, Hanson ML and Rennie MD	2017	Microplastic contamination in Lake Winnipeg, Canada	Environ Pollut	225	223-231
Atwood, EC, Falcieri FM, Piehl S, Bochow M, Matthies M, Franke J, Carniel S, Sclavo M, Laforsch C and Siegert F	2019	Coastal accumulation of microplastic particles emitted from the Po River, Northern Italy: Comparing remote sensing and hydrodynamic modelling with in situ sample collections	Mar Pollut Bull	138	561-574
Ball, H, Cross R, Grove E, Horton A, Johnson A, Jürgens M, Read D and Svendsen C	2019	Sink to River - River to Tap. A review of potential risks from nanoparticles and microplastics.	UK Water Industry Research Limited	EQ01A231	
Ballent, A, Corcoran PL, Madden O, Helm PA and Longstaffe FJ	2016	Sources and sinks of microplastics in Canadian Lake Ontario nearshore, tributary and beach sediments	Mar Pollut Bull	110	383-395

Reference	Year	Title	Publication	Vol	Pages
Bannick, CG, Szewzyk R, Ricking M, Schniegler S, Obermaier N, Barthel AK, Altmann K, Eisentraut P and Braun U	2019	Development and testing of a fractionated filtration for sampling of microplastics in water	Water Res	149	650-658
Baptista Neto, JA, Gaylarde C, Beech I, Bastos AC, da Silva Quaresma V and de Carvalho DG	2019	Microplastics and attached microorganisms in sediments of the Vitória bay estuarine system in SE Brazil	Ocean and Coastal Management	169	247-253
Battulga, B, Kawahigashi M and Oyuntsetseg B	2019	Distribution and composition of plastic debris along the river shore in the Selenga River basin in Mongolia	Environmental Science and Pollution Research		
Bessa, F, Barria P, Neto JM, Frias J, Otero V, Sobral P and Marques JC	2018	Occurrence of microplastics in commercial fish from a natural estuarine environment	Mar Pollut Bull	128	575-584
Biginagwa, FJ, Mayoma BS, Shashoua Y, Syberg K and Khan FR	2016	First evidence of microplastics in the African Great Lakes: Recovery from Lake Victoria Nile perch and Nile tilapia	J Great Lakes Res	42	146-149
Blair, RM, Waldron S, Phoenix VR and Gauchotte-Lindsay C	2019	Microscopy and elemental analysis characterisation of microplastics in sediment of a freshwater urban river in Scotland, UK	Environmental Science and Pollution Research	26	12491–12504
Blaskovic, A, Guerranti C, Fastelli P, Anselmi S and Renzi M	2018	Plastic levels in sediments closed to Cecina river estuary (Tuscany, Italy)	Mar Pollut Bull	135	105-109
Blettler, MCM, Ulla MA, Rabuffetti AP and Garello N	2017	Plastic pollution in freshwater ecosystems: macro-, meso-, and microplastic debris in a floodplain lake	Environ Monit Assess	189	581

Reference	Year	Title	Publication	Vol	Pages
Bordos, G, Urbanyi B, Micsinai A, Kriszt B, Palotai Z, Szabo I, Hantosi Z and Szoboszlay S	2019	Identification of microplastics in fish ponds and natural freshwater environments of the Carpathian basin, Europe	Chemosphere	216	110-116
Browne MA, Galloway TS and Thompson RC	2010	Spatial patterns of plastic debris along Estuarine shorelines	Environ Sci Technol	44	3404-9
Browne, MA, Crump P, Niven SJ, Teuten E, Tonkin A, Galloway T and Thompson R	2011	Accumulation of microplastic on shorelines woldwide: sources and sinks	Environ Sci Technol	45	9175-9179
Cable, RN, Beletsky D, Beletsky R, Wigginton K, Locke BW and Duhaime MB	2017	Distribution and modeled transport of plastic pollution in the Great Lakes, the world's largest freshwater resource	Frontiers in Environmental Science	5	
Campbell, SH, Williamson PR and Hall BD	2017	Microplastics in the gastrointestinal tracts of fish and the water from an urban prairie creek	Facets	2	395-409
Caron, AGM, Thomas CR, Berry KLE, Motti CA, Ariel E and Brodie JE	2018	Validation of an optimised protocol for quantification of microplastics in heterogenous samples: A case study using green turtle chyme	Methodsx	5	812-823
Castaneda, RA, Avlijas S, Simard MA and Ricciardi A	2014	Microplastic pollution in St Lawrence River sediments	Can J Fish Aquat Sci	71	1767-1771
Cheung, LTO, Lui CY and Fok L	2018	Microplastic Contamination of Wild and Captive Flathead Grey Mullet (*Mugil cephalus*)	International Journal of Environmental Research and Public Health	15	
Cheung, PK, Fok L, Hung PL and Cheung LTO	2018	Spatio-temporal comparison of neustonic microplastic density in Hong Kong waters under the influence of the Pearl River Estuary	Sci Total Environ	628-629	731-739

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Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C., Galloway, T.S.,	2014	Isolation of microplastics in biota-rich seawater samples and marine organisms.	SciRep.	4	4528
Collard, F, Gasperi J, Gilbert B, Eppe G, Azimi S, Rocher V and Tassin B	2018	Anthropogenic particles in the stomach contents and liver of the freshwater fish *Squalius cephalus*	Science of the Total Environment	643	1257–1264
Collicutt, B, Juanes F and Dudas SE	2019	Microplastics in juvenile Chinook salmon and their nearshore environments on the east coast of Vancouver Island	Environmental Pollution	244	135-142
Corcoran, PL, Norris T, Ceccanese T, Walzak MJ, Helm PA and Marvin CH	2015	Hidden plastics of Lake Ontario, Canada and their potential preservation in the sediment record	Environ Pollut (Oxford, U K)	204	17-25
Courtene-Jones, W, Quinn B, Murphy F, Gary SF and Narayanaswamy BE	2017	Optimisation of enzymatic digestion and validation of specimen preservation methods for the analysis of ingested microplastics	Analytical Methods	9	1437-1445
Cox, K	2018	Distribution, Abundance, and Spatial Variability of Microplastic Pollution in Surface Waters of Lake Superior	MSc University of Waterloo		
Crichton, EM, Noel M, Gies EA and Ross PS	2017	A novel, density-independent and FTIR-compatible approach for the rapid extraction of microplastics from aquatic sediments	Analytical Methods	9	1419-1428
Dantas, DV, Barletta M and da Costa MF	2012	The seasonal and spatial patterns of ingestion of polyfilament nylon fragments by estuarine drums (Sciaenidae)	Environmental Science and Pollution Research	19	600-606

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de Lucia, GA, Vianello A, Camedda A, Vani D, Tomassetti P, Coppa S, Palazzo L, Amici M, Romanelli G, Zampetti G, Cicero AM, Carpentieri S, Di Vito S and Matiddi M	2018	Sea Water Contamination in the Vicinity of the Italian Minor Islands Caused by Microplastic Pollution	Water	10	
Dean, BY, Corcoran PL and Helm PA	2018	Factors influencing microplastic abundances in nearshore, tributary and beach sediments along the Ontario shoreline of Lake Erie	J Great Lakes Res	44	1002-1009
Di, M and Wang J	2018	Microplastics in surface waters and sediments of the Three Gorges Reservoir, China	Sci Total Environ	616-617	1620-1627
Di, M, Liu X, Wang W and Wang J	2019	Manuscript prepared for submission to environmental toxicology and pharmacology pollution in drinking water source areas: Microplastics in the Danjiangkou Reservoir, China	Environ Toxicol Pharmacol	65	82-89
Ding, J-F, Li J-X, Sun C-J, He C-F, Jiang F-H, Gao F-L and Zheng L	2018	Separation and Identification of Microplastics in Digestive System of Bivalves	Chinese Journal of Analytical Chemistry	46	690-697
Domogalla-Urbansky, J, Anger PM, Ferling H, Rager F, Wiesheu AC, Niessner R, Ivleva NP and Schwaiger J	2019	Raman microspectroscopic identification of microplastic particles in freshwater bivalves (*Unio pictorum*) exposed to sewage treatment plant effluents under different exposure scenarios	Environ Sci Pollut Res Int	26	2007-2012
Dris, R	2016	First assessement of sources and fate of macro and micro plastics in urban hydrosystems : Case of Paris megacity : Premières investigation des sources et devenirs des macro et micro plastiques dans les hydrosystèmes urbains : cas de agglomération parisie	PhD Université Paris-Est		

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Dris, R, Tassin B, Gasperi J and Rocher V	2018	Synthetic and non-synthetic anthropogenic fibers in a river under the impact of Paris Megacity: Sampling methodological aspects and flux estimations	Sci Total Environ	618	157-164
Dubaish, F and Liebezeit G	2013	Suspended Microplastics and Black Carbon Particles in the Jade System, Southern North Sea	Water, Air, Soil Pollut	224	1-8
Duemichen, E, Barthel A-K, Braun U, Bannick CG, Brand K, Jekel M and Senz R	2015	Analysis of polyethylene microplastics in environmental samples, using a thermal decomposition method	Water Research	85	451-457
Dyachenko, A, Mitchell J and Arsem N	2017	Extraction and identification of microplastic particles from secondary wastewater treatment plant (WWTP) effluent	Anal Methods	9	1412-1418
Eisentraut, P, Duemichen E, Ruhl AS, Jekel M, Albrecht M, Gehde M and Braun U	2018	Two Birds with One Stone-Fast and Simultaneous Analysis of Microplastics: Microparticles Derived from Thermoplastics and Tire Wear	Environmental Science & Technology Letters	5	608-613
English, MD, Robertson GJ, Avery-Gomm S, Pine-Hay D, Roul S, Ryan PC, Wilhelm SI and Mallory ML	2015	Plastic and metal ingestion in three species of coastal waterfowl wintering in Atlantic Canada	Marine Pollution Bulletin	98	349-353
Eriksen, M, Mason S, Wilson S, Box C, Zellers A, Edwards W, Farley H and Amato S	2013	Microplastic pollution in the surface waters of the Laurentian Great Lakes	Mar Pollut Bull	77	177-182
Erni-Cassola, G, Gibson MI, Thompson RC and Christie-Oleza JA	2017	Lost, but Found with Nile Red: A Novel Method for Detecting and Quantifying Small Microplastics (1 mm to 20 μm) in Environmental Samples	Environmental Science & Technology	51	13641-13648

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Estahbanati, S and Fahrenfeld NL	2016	Influence of wastewater treatment plant discharges on microplastic concentrations in surface water	Chemosphere	162	277-284
Ferreira GVB, Barletta M, Lima ARA, Dantas DV, Justino AKS and Costa MF	2016	Plastic debris contamination in the life cycle of Acoupa weakfish (*Cynoscion acoupa*) in a tropical estuary	Ices Journal of Marine Science	73	2695-2707
Ferreira, GVB, Barletta M, Lima ARA, Morley SA, Justino AKS and Costa MF	2018	High intake rates of microplastics in a Western Atlantic predatory fish, and insights of a direct fishery effect	Environ Pollut	236	706-717
Ferreira, GVB, Lima ARA and Barletta M	2019	Use of estuarine resources by top predator fishesHow do ecological patterns affect rates of contamination by microplastics?	Sci Total Environ	655	292-304
Figueiredo, GM and Pintas Vianna TM	2018	Suspended microplastics in a highly polluted bay: Abundance, size, and availability for mesozooplankton	Marine Pollution Bulletin	135	256-265
Fischer, EK, Paglialonga L, Czech E and Tamminga M	2016	Microplastic pollution in lakes and lake shoreline sediments – A case study on Lake Bolsena and Lake Chiusi (central Italy)	Environmental Pollution	213	648-657
Fok, L and Cheung PK	2015	Hong Kong at the Pearl River Estuary: A hotspot of microplastic pollution	Mar Pollut Bull	99	112-118
Free, CM, Jensen OP, Mason SA, Eriksen M, Williamson NJ and Boldgiv B	2014	High-levels of microplastic pollution in a large, remote, mountain lake	Mar Pollut Bull	85	156-163
Frere, L, Paul-Pont I, Moreau J, Soudant P, Lambert C, Huvet A and Rinnert E	2016	A semi-automated Raman micro-spectroscopy method for morphological and chemical characterizations of microplastic litter	Marine Pollution Bulletin	113	461-468

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Gallagher, A, Rees A, Rowe R, Stevens J and Wright P	2016	Microplastics in the Solent estuarine complex, UK: An initial assessment	Mar Pollut Bull	102	243-249
Garaba, SP and Dierssen HM	2018	An airborne remote sensing case study of synthetic hydrocarbon detection using short wave infrared absorption features identified from marine-harvested macro- and microplastics	Remote Sensing of Environment	205	224-235
Gil-Delgado, JA, Guijarro D, Gosalvez RU, Lopez-Iborra GM, Ponz A and Velasco A	2017	Presence of plastic particles in waterbirds faeces collected in Spanish lakes	Environmental Pollution	220	732-736
Grbic, J, Nguyen B, Guo E, You JB, Sinton D and Rochman CM	2019	Magnetic Extraction of Microplastics from Environmental Samples	Environ Sci Technol Lett		Ahead of Print
Green, DS, Kregting L, Boots B, Blockley DJ, Brickle P, da Costa M and Crowley Q	2018	A comparison of sampling methods for seawater microplastics and a first report of the microplastic litter in coastal waters of Ascension and Falkland Islands	Marine Pollution Bulletin	137	695-701
Guerranti, C, Cannas S, Scopetani C, Fastelli P, Cincinelli A and Renzi M	2017	Plastic litter in aquatic environments of Maremma Regional Park (Tyrrhenian Sea, Italy): Contribution by the Ombrone river and levels in marine sediments	Mar Pollut Bull	117	366-370
Gundogdu, S, Cevik C, Ayat B, Aydogan B and Karaca S	2018	How microplastics quantities increase with flood events? An example from Mersin Bay NE Levantine coast of Turkey	Environmental Pollution	239	342-350

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Gundogdu, S, Cevik C, Guzel E and Kilercioglu S	2018	Microplastics in municipal wastewater treatment plants in Turkey: a comparison of the influent and secondary effluent concentrations	Environ Monit Assess	190	626
Hendrickson, E	2017	Microplastics in the surface water and sediments of western Lake Superior as determined via microscopy, Pyr-GC/MS, and FTIR	MSc University of Minnesota		
Hohenblum, P, Liebmann B and Liedermann M	2015	Plastic and microplastic in the environment	Umweltbundesamt GmbH		
Holland, ER, Mallory ML and Shutler D	2016	Plastics and other anthropogenic debris in freshwater birds from Canada	Sci Total Environ	571	251-258
Horton, AA, Jurgens MD, Lahive E, van Bodegom PM and Vijver MG	2018	The influence of exposure and physiology On microplastic ingestion by the freshwater fish *Rutilus rutilus* (roach) in the River Thames, UK	Environmental Pollution	236	188-194
Horton, AA, Svendsen C, Williams RJ, Spurgeon DJ and Lahive E	2017	Large microplastic particles in sediments of tributaries of the River Thames, UK – Abundance, sources and methods for effective quantification	Marine Pollution Bulletin	114	218-226
Hurley, R, Woodward J and Rothwell JJ	2018	Microplastic contamination of river beds significantly reduced by catchment-wide flooding	Nat Geosci	11	251-257
Hurley, RR, Woodward JC and Rothwell JJ	2017	Ingestion of Microplastics by Freshwater Tubifex Worms	Environ Sci Technol	51	12844-12851
Hylton, LL	2017	Microplastic pollution in Indiana's White River : an exploratory study	MSc Ball State University		
Imhof, HK, Ivleva NP, Schmid J, Niessner R and Laforsch C	2013	Contamination of beach sediments of a subalpine lake with microplastic particles	Current biology : CB	23	R867-868

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Imhof, HK, Laforsch C, Wiesheu AC, Schmid J, Anger PM, Niessner R and Ivleva NP	2016	Pigments and plastic in limnetic ecosystems: A qualitative and quantitative study on microparticles of different size classes	Water Res	98	64-74
Imhof, HK, Wiesheu AC, Anger PM, Niessner R, Ivleva NP and Laforsch C	2018	Variation in plastic abundance at different lake beach zones - A case study	Sci Total Environ	613-614	530-537
Jabeen, K, Su L, Li J, Yang D, Tong C, Mu J and Shi H	2017	Microplastics and mesoplastics in fish from coastal and fresh waters of China	Environ Pollut (Oxford, U K)	221	141-149
Jian, M, Zhou L, Yu H and Liu S	2018	Separation and microscopic study of microplastics from the sediments of the wetland in the estuary of Raohe River of Poyang Lake	Huanjing Kexue Xuebao	38	579-586
Jiang, C, Yin L, Wen X, Du C, Wu L, Long Y, Liu Y, Ma Y, Yin Q, Zhou Z and Pan H	2018	Microplastics in Sediment and Surface Water of West Dongting Lake and South Dongting Lake: Abundance, Source and Composition	International Journal of Environmental Research and Public Health	15	
Jungnickel, H, Pund R, Tentschert J, Reichardt P, Laux P, Harbach H and Luch A	2016	Time-of-flight secondary ion mass spectrometry (ToF-SIMS)-based analysis and imaging of polyethylene microplastics formation during sea surf simulation	Sci Total Environ	563-564	261-266
Kang, J-H, Kwon OY, Lee K-W, Song YK and Shim WJ	2015	Marine neustonic microplastics around the southeastern coast of Korea	Mar Pollut Bull	96	304-312
Kapp, KJ and Yeatman E	2018	Microplastic hotspots in the Snake and Lower Columbia rivers: A journey from the Greater Yellowstone Ecosystem to the Pacific Ocean	Environ Pollut	241	1082-1090

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Karakolis, EG, Nguyen B, You JB, Graharn PJ, Rochman CM and Sinton D	2018	Digestible Fluorescent Coatings for Cumulative Quantification of Microplastic Ingestion	Environmental Science & Technology Letters	5	62-67
Karami, A, Golieskardi A, Choo CK, Romano N, Bin Ho Y and Salamatinia B	2017	A high-performance protocol for extraction of microplastics in fish	Science of the Total Environment	578	485-494
Karlsson, TM, Arneborg L, Broström G, Almroth BC, Gipperth L and Hassellöv M	2018	The unaccountability case of plastic pellet pollution	Marine Pollution Bulletin	129	52-60
Karlsson, TM, Vethaak AD, Almroth BC, Ariese F, van Velzen M, Hassellov M and Leslie HA	2017	Screening for microplastics in sediment, water, marine invertebrates and fish: Method development and microplastic accumulation	Marine Pollution Bulletin	122	403-408
Kataoka, T, Nihei Y, Kudou K and Hinata H	2019	Assessment of the sources and inflow processes of microplastics in the river environments of Japan	Environ Pollut	244	958-965
Kazour, M, Jemaa S, El Rakwe M, Duflos G, Hermabassiere L, Dehaut A, Le Bihanic F, Cachot J, Cornille V, Rabhi K, Khalaf G and Amara R	2018	Juvenile fish caging as a tool for assessing microplastics contamination in estuarine fish nursery grounds	Environ Sci Pollut Res		Ahead of Print
King, D, Boneillo G and Guentzel J	2016	Abundance and distribution of microplastic particles in Winyah Bay, South Carolina	Honours Thesis Coastal Carolina University		

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Klein, S	2015	Microplastics in Freshwater Systems	PhD Technische Universität Dresden		
Lahens, L, Strady E, Kieu-Le TC, Dris R, Boukerma K, Rinnert E, Gasperi J and Tassin B	2018	Macroplastic and microplastic contamination assessment of a tropical river (Saigon River, Vietnam) transversed by a developing megacity	Environ Pollut	236	661-671
Lares, M, Ncibi MC, Sillanpaa M and Sillanpaa M	2018	Occurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology	Water Res	133	236-246
Lasee, S, Mauricio J, Thompson WA, Karnjanapiboonwong A, Kasumba J, Subbiah S, Morse AN and Anderson TA	2017	Microplastics in a freshwater environment receiving treated wastewater effluent	Integr Environ Assess Manag	13	528-532
Lechner, A, Keckeis H, Lumesberger-Loisl F, Zens B, Krusch R, Tritthart M, Glas M and Schludermann E	2014	The Danube so colourful: A potpourri of plastic litter outnumbers fish larvae in Europe's second largest river	Environmental Pollution	188	177-181
Lee, H and Kim Y	2018	Treatment characteristics of microplastics at biological sewage treatment facilities in Korea	Mar Pollut Bull	137	1-8
Leslie, HA, Brandsma SH, van Velzen MJ and Vethaak AD	2017	Microplastics en route: Field measurements in the Dutch river delta and Amsterdam canals, wastewater treatment plants, North Sea sediments and biota	Environ Int	101	133-142
Li, H-X, Ma L-S, Lin L, Ni Z-X, Xu X-R, Shi H-H, Yan Y, Zheng G-M and Rittschof D	2018	Microplastics in oysters *Saccostrea cucullata* along the Pearl River Estuary, China	Environmental Pollution	236	619-625

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Li, R, Zhang L, Xue B and Wang Y	2019	Abundance and characteristics of microplastics in the mangrove sediment of the semi-enclosed Maowei Sea of the south China sea: New implications for location, rhizosphere, and sediment compositions	Environ Pollut	244	685-692
Liedermann, M, Gmeiner P, Pessenlehner S, Haimann M, Hohenblum P and Habersack H	2018	A Methodology for Measuring Microplastic Transport in Large or Medium Rivers	Water	10	
Lima, AR, Costa MF and Barletta M	2014	Distribution patterns of microplastics within the plankton of a tropical estuary	Environ Res	132	146-155
Lima, ARA, Barletta M and Costa MF	2015	Seasonal distribution and interactions between plankton and microplastics in a tropical estuary	Estuarine Coastal and Shelf Science	165	213-225
Lin, L, Zuo LZ, Peng JP, Cai LQ, Fok L, Yan Y, Li HX and Xu XR	2018	Occurrence and distribution of microplastics in an urban river: A case study in the Pearl River along Guangzhou City, China	Sci Total Environ	644	375-381
Lo, H-S, Xu X, Wong C-Y and Cheung S-G	2018	Comparisons of microplastic pollution between mudflats and sandy beaches in Hong Kong	Environ Pollut (Oxford, U K)	236	208-217
Long, Z, Pan Z, Wang W, Ren J, Yu X, Lin L, Lin H, Chen H and Jin X	2019	Microplastic abundance, characteristics, and removal in wastewater treatment plants in a coastal city of China	Water Research	155	255-265
Luo, W, Su L, Craig NJ, Du F, Wu C and Shi H	2019	Comparison of microplastic pollution in different water bodies from urban creeks to coastal waters	Environ Pollut (Oxford, U K)	246	174-182
Lusher AL, Buenaventura NT, Eidsvoll DP, Thrane J-E, Økelsrud A and Jartun M	2018	Freshwater microplastics in Norway: A first look at sediment, biota and historical plankton samples from Lake Mjøsa and Lake Femunden	NIVA Technical Report 7326-2018	7326 - 2018	16803

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Lv, W, Zhou W, Lu S, Huang W, Yuan Q, Tian M, Lv W and He D	2019	Microplastic pollution in rice-fish co-culture system: A report of three farmland stations in Shanghai, China	Science of the Total Environment	652	1209-1218
Magni, S, Binelli A, Pittura L, Avio CG, Della Torre C, Parenti CC, Gorbi S and Regoli F	2018	The fate of microplastics in an Italian Wastewater Treatment Plant	Sci Total Environ	652	602-610
Majewsky, M, Bitter H, Eiche E and Horn H	2016	Determination of microplastic polyethylene (PE) and polypropylene (PP) in environmental samples using thermal analysis (TGA-DSC)	Sci Total Environ	568	507-511
Manalu, AA, Hariyadi S and Wardiatno Y	2017	Microplastics abundance in coastal sediments of Jakarta Bay, Indonesia	AACL Bioflux	10	1164-1173
Mani, T, Blarer P, Storck FR, Pittroff M, Wernicke T and Burkhardt-Holm P	2019	Repeated detection of polystyrene microbeads in the Lower Rhine River	Environ Pollut	245	634-641
Mason, SA, Garneau D, Sutton R, Chu Y, Ehmann K, Barnes J, Fink P, Papazissimos D and Rogers DL	2016	Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent	Environ Pollut	218	1045-1054
Mason, SA, Kammin L, Eriksen M, Aleid G, Wilson S, Box C, Williamson N and Riley A	2016	Pelagic plastic pollution within the surface waters of Lake Michigan, USA	J Great Lakes Res	42	753-759
McCormick, AR, Hoellein TJ, London MG, Hittie J, Scott JW and Kelly JJ	2016	Microplastic in surface waters of urban rivers: concentration, sources, and associated bacterial assemblages	Ecosphere	7	e01556

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McGoran, AR, Clark PF and Morritt D	2017	Presence of microplastic in the digestive tracts of European flounder, *Platichthys flesus*, and European smelt, *Osmerus eperlanus*, from the River Thames	Environ Pollut	220	744-751
McGoran, AR, Cowie PR, Clark PF, McEvoy JP and Morritt D	2018	Ingestion of plastic by fish: A comparison of Thames Estuary and Firth of Clyde populations	Mar Pollut Bull	137	12-23
Miller, RZ, Watts AJR, Winslow BO, Galloway TS and Barrows APW	2017	Mountains to the sea: River study of plastic and non-plastic microfiber pollution in the northeast USA	Mar Pollut Bull	124	245-251
Mintenig, SM, Bauerlein PS, Koelmans AA, Dekker SC and van Wezel AP	2018	Closing the gap between small and smaller: towards a framework to analyse nano- and microplastics in aqueous environmental samples	Environmental Science-Nano	5	1640-1649
Mintenig, SM, Int-Veen I, Loder MGJ, Primpke S and Gerdts G	2017	Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging	Water Res	108	365-372
Murrell, KA, Ghetu CC and Dorman FL	2018	The combination of spectroscopy, microscopy, and profilometry methods for the physical and chemical characterization of environmentally relevant microplastics	Anal Methods	10	4909-4916
Naidoo, T, Goordiyal K and Glassom D	2017	Are nitric acid (HNO3) digestions efficient in isolating microplastics from juvenile fish?	Water Air and Soil Pollution	228	470
Naidoo, T, Smit AJ and Glassom D	2016	Plastic ingestion by estuarine mullet *Mugil cephalus* (Mugilidae) in an urban harbour, KwaZulu-Natal, South Africa	African Journal of Marine Science	38	145-149
Nel, HA, Dalu T and Wasserman RJ	2018	Sinks and sources: Assessing microplastic abundance in river sediment and deposit feeders in an Austral temperate urban river system	Sci Total Environ	612	950-956
Nel, HA, Dalu T, Wasserman RJ and Hean JW	2019	Colour and size influences plastic microbead underestimation, regardless of sediment grain size	Science of the Total Environment	655	567-570

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Nocoń, W, Moraczewska-Majkut K and Wiśniowska E	2018	Microplastics in surface water under strong anthropopression	Desalination and Water Treatment	134	174-181
Olivatto, GP, Martins MCT, Montagner CC, Henry TB and Carreira RS	2019	Microplastic contamination in surface waters in Guanabara Bay, Rio de Janeiro, Brazil	Mar Pollut Bull	139	157-162
Oztekin, A and Bat L	2017	Microlitter Pollution in Sea Water: A Preliminary Study from Sinop Sarikum Coast of the Southern Black Sea	Turkish Journal of Fisheries and Aquatic Sciences	17	1431-1440
Pagter, E, Frias J and Nash R	2018	Microplastics in Galway Bay: A comparison of sampling and separation methods	Mar Pollut Bull	135	932-940
Pazos, RS, Bauer DE and Gomez N	2018	Microplastics integrating the coastal planktonic community in the inner zone of the Rio de la Plata estuary (South America)	Environ Pollut	243	134-142
Pazos, RS, Maiztegui T, Colautti DC, Paracampo AH and Gomez N	2017	Microplastics in gut contents of coastal freshwater fish from Rió de la Plata estuary	Mar Pollut Bull	122	85-90
Pegado, T, Schmid K, Winemiller KO, Chelazzi D, Cincinelli A, Dei L and Giarrizzo T	2018	First evidence of microplastic ingestion by fishes from the Amazon River estuary	Mar Pollut Bull	133	814-821
Peng, G, Xu P, Zhu B, Bai M and Li D	2018	Microplastics in freshwater river sediments in Shanghai, China: A case study of risk assessment in mega-cities	Environ Pollut	234	448-456
Peters, CA and Bratton SP	2016	Urbanization is a major influence on microplastic ingestion by sunfish in the Brazos River Basin, Central Texas, USA	Environ Pollut	210	380-387

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Phillips, MB and Bonner TH	2015	Occurrence and amount of microplastic ingested by fishes in watersheds of the Gulf of Mexico	Mar Pollut Bull	100	264-269
Pivokonsky, M, Cermakova L, Novotna K, Peer P, Cajthaml T and Janda V	2018	Occurrence of microplastics in raw and treated drinking water	Sci Total Environ	643	1644-1651
Possatto, FE, Barletta, M, Costa, MF, do Sul, JAI and Dantas, DV	2011	Plastic debris ingestion by marine catfish: An unexpected fisheries impact	Marine Pollution Bulletin	62	1098-1102
Ramos, JAA, Barletta, M and Costa, MF	2012	Ingestion of nylon threads by Gerreidae while using a tropical estuary as foraging grounds	Aquatic Biology	17	29-34
Ravit, B, Cooper K, Moreno G, Buckley B, Yang I, Deshpande A, Meola S, Jonesand D and Hsieh A	2017	Microplastics in urban New Jersey freshwaters: distribution, chemical identification, and biological affects	Aims Environmental Science	4	809-826
Renner, G, Schmidt TC and Schram J	2017	A New Chemometric Approach for Automatic Identification of Microplastics from Environmental Compartments Based on FT-IR Spectroscopy	Analytical Chemistry	89	12045-12053
Revel, M, Yakovenko N, Caley T, Guillet C, Chatel A and Mouneyrac C	2018	Accumulation and immunotoxicity of microplastics in the estuarine worm Hediste diversicolor in environmentally relevant conditions of exposure	Environ Sci Pollut Res		Ahead of Print
Reynolds, C and Ryan PG	2018	Micro-plastic ingestion by waterbirds from contaminated wetlands in South Africa	Marine Pollution Bulletin	126	330-333

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Roch, S and Brinker A	2017	Rapid and Efficient Method for the Detection of Microplastic in the Gastrointestinal Tract of Fishes	Environ Sci Technol	51	4522-4530
Rodrigues, MO, Abrantes N, Goncalves FJM, Nogueira H, Marques JC and Goncalves AMM	2018	Spatial and temporal distribution of microplastics in water and sediments of a freshwater system (Antua River, Portugal)	Sci Total Environ	633	1549-1559
Rodrigues, MO, Goncalves AMM, Goncalves FJM, Nogueira H, Marques JC and Abrantes N	2018	Effectiveness of a methodology of microplastics isolation for environmental monitoring in freshwater systems	Ecol Indic	89	488-495
Rodrigues, SM, Almeida CMR, Silva D, Cunha J, Antunes C, Freitas V and Ramos S	2019	Microplastic contamination in an urban estuary: Abundance and distribution of microplastics and fish larvae in the Douro estuary	Sci Total Environ	659	1071-1081
Sadri, SS	2015	Investigation of microplastic debris in marine surface waters using different sampling methods	PhD Thesis University of Plymouth		
Sanchez, W, Bender C and Porcher J-M	2014	Wild gudgeons (*Gobio gobio*) from French rivers are contaminated by microplastics. Preliminary study and first evidence	Environ. Res.	128	98-100
Santana, MF, Ascer LG, Custodio MR, Moreira FT and Turra A	2016	Microplastic contamination in natural mussel beds from a Brazilian urbanized coastal region: Rapid evaluation through bioassessment	Mar Pollut Bull	106	183-189
Santillo, D, Brigden, K, Pasteur, V, Nicholls, F, Morozzo, P and Johnston P	2019	Plastic pollution in UK's rivers: a 'snapshot' survey of macro- and micro-plastic contamination in surface waters of 13 river systems across England, Wales, Scotland and Northern Ireland	Greenpeace Research Laboratories Technical Report 04-2019, June 2019	4	30

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Schessl, M, Johns C and Ashpole SL	2019	Microbeads in Sediment, Dreissenid Mussels, and Anurans in the Littoral Zone of the Upper St Lawrence River, New York	Pollution	5	41-52
Schmidt, LK, Bochow M, Imhof HK and Oswald SE	2018	Multi-temporal surveys for microplastic particles enabled by a novel and fast application of SWIR imaging spectroscopy - Study of an urban watercourse traversing the city of Berlin, Germany	Environ Pollut	239	579-589
Shim, WJ, Song YK, Hong SH and Jang M	2016	Identification and quantification of microplastics using Nile Red staining	Marine Pollution Bulletin	113	469-476
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Talvitie, J, Mikola A, Koistinen A and Setala O	2017	Solutions to microplastic pollution - Removal of microplastics from wastewater effluent with advanced wastewater treatment technologies	Water Res	123	401-407
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Tan, X, Yu X, Cai L, Wang J and Peng J	2019	Microplastics and associated PAHs in surface water from the Feilaixia Reservoir in the Beijiang River, China	Chemosphere	221	834-840
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Vaughan, R, Turner SD and Rose NL	2017	Microplastics in the sediments of a UK urban lake	Environ Pollut	229	10-18
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Zbyszewski, M and Corcoran PL	2011	Distribution and Degradation of Fresh Water Plastic Particles Along the Beaches of Lake Huron, Canada	Water Air and Soil Pollution	220	365-372
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