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REVIEW

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Plastics in biota: technological readiness level of current methodologies

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Abstract

Plastics are persistent in the environment and may be ingested by organisms where they may cause physical harm or release plastic additives. Monitoring is a crucial mechanism to assess the risk of plastics to the marine and terrestrial ecosystem. Unfortunately, due to unharmonised procedures, it remains difficult to compare the results of different studies. This publication, as part of the Horizon project EUROqCHARM, aims to identify the properties of the available analytical processes and methods for the determination of plastics in biota. Based on a systematic review, reproducible analytical pipelines were examined and the technological readiness levels were assessed so that these methods may eventually (if not already) be incorporated into (harmonised) monitoring programs where biota are identified as indicators of plastic pollution.

Keywords Biota, Technological readiness level, Microplastics, Reproducible analytical pipeline, SWOT

Introduction

The global emissions of plastic litter in rivers, lakes and oceans range from 9 to 23 million metric tons per year (in 2016) [10] and constitute 61% to 87% of the total amount of litter [8, 78]. Because plastic is persistent in the environment, it may take decades to centuries to degrade [17]. Therefore, an immense quantity of plastic can be found in the environment, where it may exert negative effects on the environment and on biota [19]. Furthermore, plastic may fragmentate into microplastic particles through weathering processes [13, 58], but microplastics are also produced on an industrial scale (primary microplastics) with applications in cleaning agents, personal care products, drilling fluids, etc. [28, 35]. Microplastics can further degrade into even smaller nanoplastics [36, 38]. Depending on the size, a broad variety of negative effects may be observed in the marine environment [48, 51, 53, 66]. Therefore, plastic is often categorized according to size in environmental studies [43]. Macroplastics are polymer fragments between 25 mm and 1000 mm, mesoplastics are between 5 mm and 25 mm, while microplastics have a size that ranges between 1 μm and 5 mm [43].

Macrolitter can, once released in the environment cause harm to marine biota and ecosystems [19], by causing entanglement of marine organisms such as birds, mammals, fish and crabs [5, 44]. This can cause death by drowning, suffocation or strangulation [46, 63]. If not lethal, entanglement may also cause injuries and impair movement, reducing the feeding efficiency (starvation) [71] and making the organism an easier prey [3]. When macrolitter is ingested, it may cause lacerations, lesions or blockades. It may also puncture through the gastrointestinal system, resulting in ulcerations and infections [44]. Floating macrolitter can be transported over long distances and can act as a vector for opportunistic

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rafting organisms [68]. When non-indigenous organisms are introduced in a new environment, they may become invasive and cause harm to the marine ecosystem [6, 7].

Similar to macroplastics, microplastics may also be ingested. Although they often remain in the gastrointestinal tract and can be excreted via faeces [91], microplastics can also accumulate in fat tissue [1, 79] and cause physical damage, chronic and even acute toxicity [29, 88]. Due to biomagnification, organisms in a higher trophic level may contain a higher amount of microplastic [22, 42]. Humans may also be exposed to microplastics, especially when seafood is completely ingested [65]. When small microplastics pass through cell membranes and blood barriers, they may cause cell damage, inflammation, induce oxidative stress, etc. [11, 65]. Once microplastics are ingested they could also release plastic additives or adsorbed hydrophobic organic contaminants [25]. The extensiveness of the effects of additives and sorbed chemicals release from (micro)plastics is currently not fully understood and strongly depends on the number of released microplastics, bioavailability and toxicity of the absorbed chemicals/additives [59].

Macro- and microplastics have been detected in a wide variety of organisms [4, 5, 44, 48, 81]. Therefore, monitoring macro- and microplastic pollution is essential. The largest research effort regarding plastic pollution concerns the marine environment, although the number of studies on plastic pollution in freshwater and terrestrial environments has strongly increased over the past years [39, 41]. Despite the great number of publications on plastics in the marine environment, the ecological significance is still unclear, partly due to inadequate sampling and analytical approaches [35]. The Marine Strategy Framework Directive of the EU proposed standardised procedures for sampling and detecting microplastics [32]. Nevertheless, current analytical procedures for the detection and quantification of microplastics are still under development or need further fine-tuning [12, 56]. Additionally, due to a lack of harmonised protocols, it remains difficult to compare different studies with each other [57, 90]. Therefore, there is a need for harmonised methods to assess the quantification of plastic in different (biotic) matrices.

The aim of this study was to identify available analytical methods for macro-, meso- and microplastics ingested by biota and to assess if these methods can be incorporated into plastic monitoring programs. A systematic review (SR) was carried out to identify analytical methods reported in the literature for all plastic size categories in biota. Methodological steps from sampling to data management were arranged in Reproducible Analytical Pipelines (RAPs) and assessed for the Technological Readiness Level (TRL) [2]. Finally, the Strengths, Weaknesses, Opportunities and Threats (SWOT) were established and discussed.

Materials and methods

Systematic review process

A systematic literature search was performed in the framework of the Horizon 2020 project EUROqCHARM. No start time cut-off was defined to avoid bias in paper selection and to allow for the inclusion of historical papers. As the systematic review was performed in the second half of 2021 (and the following years), only publications till the 1st of April 2021 were included. The databases Web of Science and Scopus were used to identify papers on plastic in different matrices (water, soil, air, and biota). Search terms were predefined to retain publications on plastic analysis in the environment, excluding exposure studies. Studies on humans were not considered. The list of the used keywords is provided in the Supplementary materials. In total 3290 publications were extracted, and after title and abstract screening, 2429 papers were retained. After full-text screening, relevant publications were categorised into one or more of the subgroups (water, solids, air and biota). This study only considers publications on biota. In total 498 papers were identified as relevant for biota. Next, 104 papers were further excluded as they did not detect ingested plastics in biota but focussed on entanglement, plastic in nests or rafting species. In total 394 publications were retained by the systematic review for biota. A complete overview of the systematic review process is shown in Fig. 1. Data was collected from these publications after defining a reproducible analytical pipeline as described by [2].

Reproducible analytical pipeline and Technological readiness level

The RAP was identified for plastic monitoring, as described by [2]. It defines six different steps: survey design, sample collection, sample preparation, analytical detection and quantification, quality control and data reporting. Steps two to six were considered for this data collection. As a result, a dataset was constructed bundling information on the used analytical procedures and protocols and other meta information such as the year of publication and place of the study. The biota data was divided into different subgroups according to the organism under investigation (i.e. birds, fish, bivalves, non-bivalves invertebrates, etc.). For each biota subgroup, a RAP was identified if a technique was used in at least 10 different methods. Techniques with an occurrence of less than 10 were grouped as others.

Technological readiness levels were developed by NASA to evaluate if a technology was ready for deployment and were described in the framework of plastic

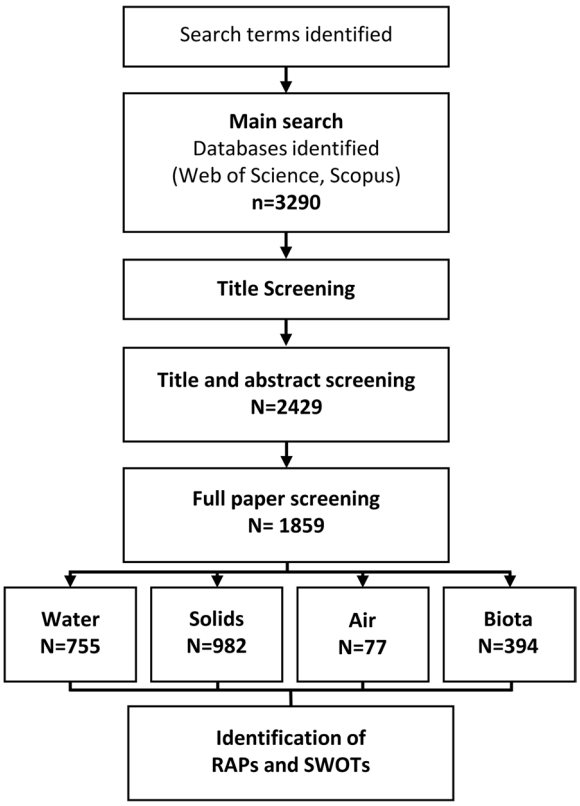

Fig. 1 Overview of the systematic review process used for the production of reproducible analytical pipelines and SWOT analysis. The number of publications retained by the systematic review (N) is reported

monitoring by [2]. These TRLs are shown in Fig. 2. For each publication, the reported method to quantify and or qualify plastics in biota was assigned a TRL.

SWOT analysis

To construct SWOTs the collected data of 394 papers were explored in R (version 4.1.2). For each step of the analytical pipeline, different methods/techniques were identified (e.g. Sample collection: hand collection) and a SWOT was created if the method or technique had been adopted in at least 20 publications. The SWOT results are integrated into the Results and discussion section and presented in detail in the Supplementary materials.

Results and discussion

Over the last decades, the number of environmental studies on plastics has strongly increased. For the entire period before 2011, only 12 publications were retained by our systematic review for biota, whereas for the period of 2011–2015 and 2016–2020, the number of papers increased to 37 and 312, respectively. The remaining publications (33) were published in the first half of 2021. Most of the publications were research-based (90%). Plastic is mostly analysed in fish (173 papers), followed by non-bivalve invertebrates (87 publications), bivalves (60 publications), and birds (59 publications). Although other subgroups, such as mammals, plants, reptiles, among others, were also reported, the number of publications was too limited by the end time of the SR to deduce RAPs (< 10 papers) or perform a SWOT analysis (< 20 papers). Strong regional differences were seen in the choice of matrix selection. For Europe, Asia and South America, the SR revealed that fish was the most studied matrix. In North America and Oceania, relatively more research has been performed on plastics in seabirds.

Both fish and bivalves are of social and economic importance. They can be used to monitor marine as well as freshwater environments but are limited to aqueous environments. Due to the wide diversity of fish species, macro-, meso-, and microplastic can be detected in different functional niches [24]. However, the amount of plastic in fish is often limited [24, 45] and is mainly measured in the gastrointestinal tract. Additionally, certain fish species are not suitable for monitoring purposes due to migration behaviour [24]. Bivalves, in contrast, are sessile organisms allowing for the assessment of microplastics in a local or site-specific area [9, 47]. They are, however, not suitable for meso- and macroplastics and sampling opportunities in open sea are limited. Other invertebrates are also suitable for microplastics in marine as well as freshwater and terrestrial environments, but species are often dependent on a specific habitat, impeding universal applications. The monitoring of plastic in fish and invertebrates may also interfere with the conservation status or stock management [24].

The size of the plastic particles is inherently linked to the biota group under investigation. In birds, mainly particles between 1 and 5 mm are analysed, whereas, for fish, bivalves and non-bivalve invertebrates, a cut-off size < 0.1 mm is often reported (Fig. 3). This also means that for birds, due to the larger plastic fragments, different analytical methods will be applied compared to the three other groups. Therefore, plastics in birds will be discussed separately.

Plastic assessment in birds

A visual representation of the RAPs in birds is presented in Fig. 4. An overview is also reported in Fig. S1 in the Supplementary materials. Plastics are mainly sampled by hand from dead bodies (83%), and assessment should be done carefully, such that it would not endanger colonies (red-listed species). In 81% of the publications, the plastic was extracted from the gastrointestinal tract (GIT) or the stomach only. Other sampling sources (17%) mainly consist of regurgitation and boluses. Because of

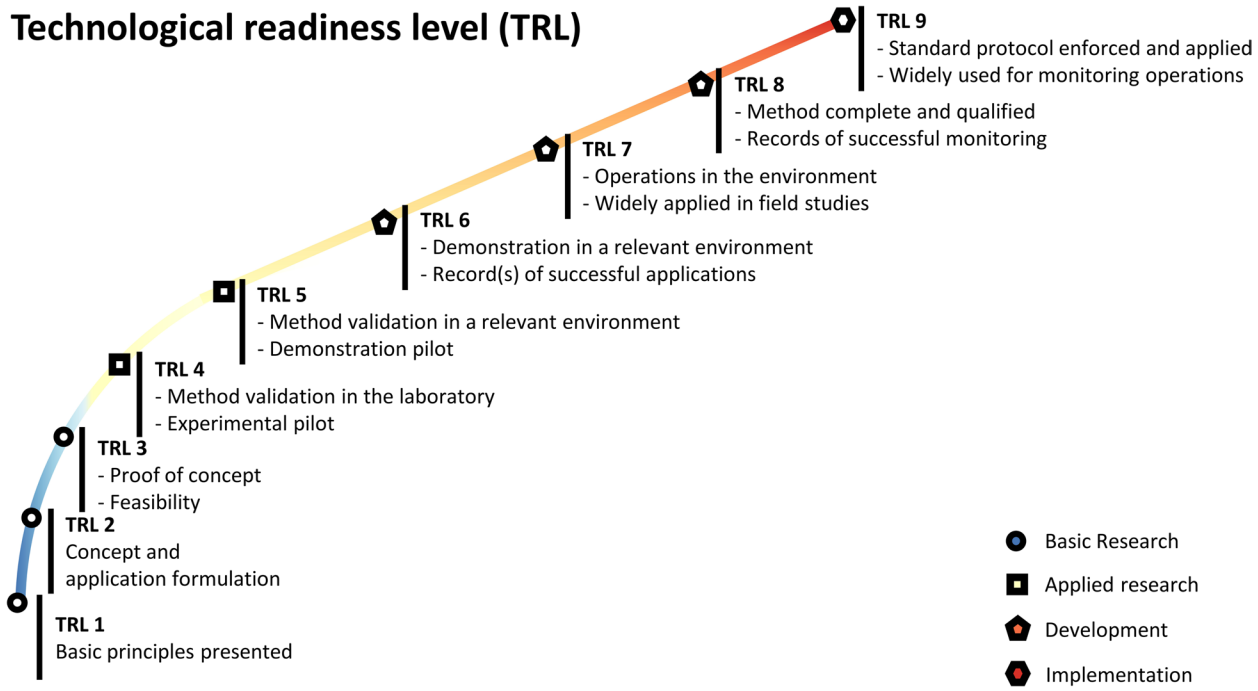

Fig. 2 Overview the the different technological readiness levels as defined by [2]

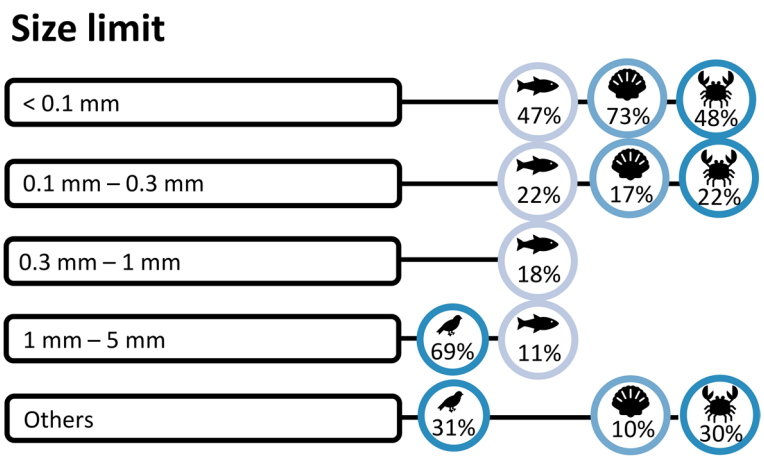

Fig. 3 Overview of the reported size limit of plastic studied in birds, fish, bivalves and non-bivalve invertebrates. Other size limits had less than 10 occurrences and are grouped as others

the migration pattern of birds, the time of exposure and location of exposure are often unknown. Moreover, birds are less relevant to examine the risk of plastics in human consumption.

Seabirds have been recommended for monitoring surface water plastics (>2.5 cm) since 2015 [67]. As a result of this, method development becomes routine, the TRL can be regarded as 9 because of [67, 74, 83]. A visual separation step is standard, as it is sufficient to isolate the larger plastic particles that are often investigated in seabirds, followed by a visual inspection (42% of the studies). Optimal microscopy is also applied in 58% of the studies, but polymer type is only identified in 22% of the cases, mainly by using FTIR.

The use of visual inspection is popular as it avoids the need for (expensive) instruments, resulting in low costs and can be performed directly in the field as well as in the lab. Only limited training is required whereas

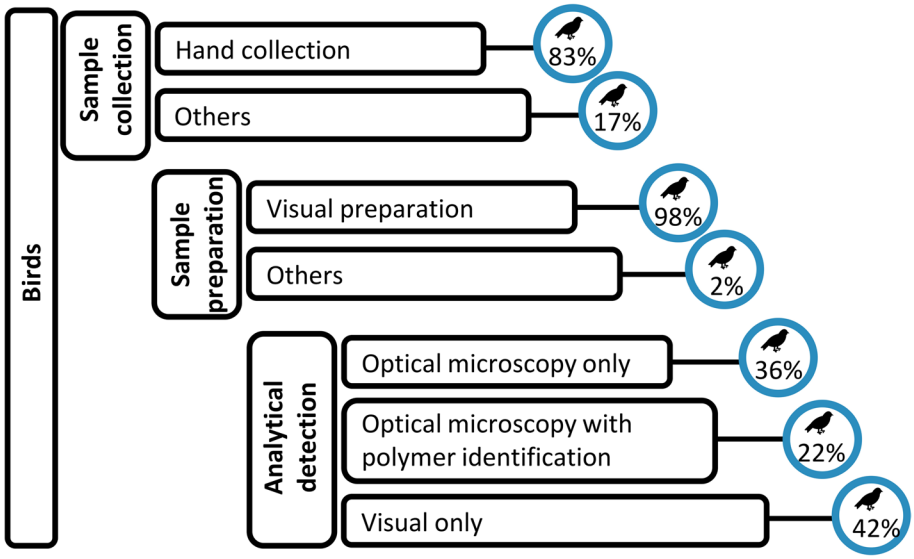

Fig. 4 Overview of the sample collection, sample preparation and analytical detection for plastics in birds. Techniques with less than 10 occurrences are grouped as others

different opportunities arise: broad global applications, increased knowledge base on a broad size range, can be combined with all types of chemical identification techniques and personnel demand can be reduced by artificial intelligence-based image analysis. However, unaided visual detection remains limited to larger plastic particles (> 1 mm) and does not allow for the identification of the polymer [55]. Therefore, there is a danger of under-reporting the real amount of plastic contamination as well as wrong conclusions on the state of pollution and the exposure and impact on the environment. This remains however limited for the analysis of plastics in birds as the plastic size under investigation is often > 1 mm.

Quality assessment is often limited in the assessment of plastics ingested by birds because most methods focus on particles larger than 1 mm. Air blanks, procedural blanks and positive controls were only used in 8%, 5% and 0% of the studies, respectively. Data reporting for birds is mainly based on the work of [83] and [74] and is used in protocols of the OSPAR Regional Sea Convention. Most publications report items per individual (69%) and/or the g of plastic per individual (54%).

Plastic assessment in fish, bivalves and invertebrates

A complete overview of the sample collection, sample preparation, analytical detection methods and the quality assessment and quality control are reported in the Supplementary materials (Fig. S1) for each biota subgroup.

Sample collection

Sample collection requires reproducible methodologies, and background contamination should be minimised [40]. An overview of the techniques for sample collection is shown in the left-hand side of Fig. 5 for fish, bivalves and non-bivalve invertebrates.

A wide range of sample collection methods have been applied. Fish is often caught using active (45%) and passive (hooks/lines (8%), pots/traps (13%)) fishing techniques. The sample collection technique will reflect local fishery activities as sampling is often done in collaboration with local fishermen or existing fishery surveys [24]. When fish is bought from local fishermen, specific information on the used fishing gear may be missing. Regional differences may affect harmonisation as the characteristics (size, feeding behaviour) and habitat of fish species affect the degree of microplastic contamination [30]. Bivalves are mainly collected by hand (65%), but they can also be collected as bycatch in trawls. In contrast to fish and bivalves, non-bivalve invertebrates are a diverse group including worms, crabs and gastropods resulting in a large variety of sampling techniques such as hand-collection (27%), trawling (17%), nets, etc.

The choice of sampled tissue will strongly impact plastic analysis. As in birds, the gastrointestinal tract or GIT is often used for plastic determination in fish and invertebrates. GIT can contain a wide range of plastic sizes (macro/meso/micro) and visual separation is possible for

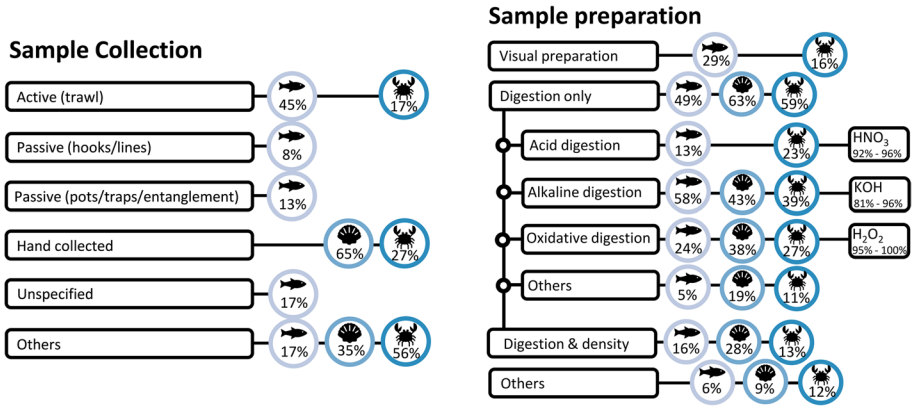

Fig. 5 Overview of techniques for sample collection (left) and sample preparation (right) of plastics in fish, bivalves and non-bivalve invertebrates. For the sample preparation, the reported percentage for the different types of digestion was based on analytical methods including digestion only and digestion in combination with a density separation. For each type of digestion, the most common chemical is reported as well as the range in which it is applied for the different biota subgroups. Techniques with less than 10 occurrences are grouped as others

the larger size (macro/meso). However, the matrix also contains non-plastic particles which may result in mislabelling. The reported amount of plastic in fish is often low [24, 45], may be potentially linked to stomach fullness and may be prone to environmental contamination (also see quality control). All these factors can result in unreliable data. Because the GIT of fish is often not used for human consumption, it is less relevant for assessing the risk of plastic in human consumption.

Bivalves are more relevant for analysing those risks and even allow for the calculation of potential exposure of microplastics in human consumption (e.g. [16, 20]). The entire organism is often analysed, avoiding the need for dissection. This can also be the case for smaller fish [76] or juveniles [64]. However, in all these cases, the specific tissue in which plastic is located remains unknown. Other invertebrates are less relevant for assessing plastic in human consumption, but may have the advantage of containing higher concentrations of plastic and are more commonly spread throughout different habitats (marine, freshwater and terrestrial environments).

Sample preparation

Elements discussed in this section are based on the work of [23, 54, 56]. Visual separation of microplastics from the stomach contents without digestion is commonly used in fish (29%) and non-bivalve invertebrates (16%). It is a simple procedure that can easily be used for the separation of larger plastic fragments (right-hand side of Fig. 5). It is globally accessible and only requires limited training. It does, however, not remove the biological matrix and is limited to identifying macro-, meso- and larger microplastics (> 1 mm). Because the separation occurs visually, non-plastic fragments may be mislabelled, and there may be bias based on the person performing the analysis [55]. Nevertheless, visual separation can easily be used to create large datasets on meso/macro plastic, but due to a lack of harmonisation, it may be difficult to compare different studies with each other.

To quantitatively analyse plastics in biota with limited matrix interference, a digestion step could be used and should have a high efficiency in removing biological tissues without having deleterious effects on the plastic itself, such as changes in shape or size or even complete dissolution [23, 56]. Alkaline digestion with KOH is commonly used in fish (48%), bivalves (40%) and non-bivalve invertebrates (26%), followed by oxidative digestion with H_2O_2 (respectively 23%, 38%, and 22%). Both methods are fit-for-purpose to extract microplastics, with an overall acceptable interlaboratory variability [82], which explains the popularity of their use. Both digestion steps are simple methods but still require trained staff. The step is non-destructive for most polymers at optimal conditions but may result in the degradation of microplastic when parameters are not optimised. Alkaline digestion using KOH is less efficient on fatty matrices, and a balance needs to be found between plastic degradation and matrix removal. Additionally, alkalic digestion with KOH can be prone to interference by inorganic material [70]. Therefore, the incorrect use of alkalic digestion may result in unreliable data. Oxidative digestion using H_2O_2 can be improved by using Fenton's reagent [14, 70], but this will also increase the complexity of the method. Additionally, H_2O_2 is unstable and may result in foaming.

The use of acid and enzymatic digestions is less common (<20 for each sub-compartment), resulting in a TRL below 6. For example, acid digestion with HNO_3 was only used in 14% of the studies for non-bivalve invertebrates.

In contrast to visual separation, digestion leads to matrix removal, which allows for separation and identification of smaller plastic fragments (< 0.1 mm). The removal of the matrix can further be optimized by combining different digestion steps. However, digestion is time-consuming and requires the use of chemicals and skilled lab technicians. During the procedure, there is also a risk for contamination by airborne plastics as well as the destruction of plastic in the sample. Therefore, the abundance of microplastic may be over- or underestimated when the method is applied incorrectly possibly resulting in unreliable data output.

Finally, a digestion step can be combined with density separation to remove high-density particles from the biota matrix such as sediment or soil particles, as observed in 16% (fish), 28% (bivalves) and 13% (non-bivalve invertebrates) of the studies. The density separation can further be optimised by using the appropriate salt. NaCl is the most applied (60% of the density methods), due to its low cost and low toxicity [56]. However, saturated NaCl solutions have a density of ≤ 1.2 g cm^{-3} [31], which hampers the recovery of high-density plastic particles. NaI is applied in 26% of the density methods, has a higher cost and toxicity than NaCl, but offers the advantage of higher recoveries of dense particles due to the high density of saturated NaI solutions (1.8 $g.cm^{-3}$; [31]). When a not-fit-for-purpose density separation is used, microplastic particles with a higher density (compared to the salt solution used for density separation) may not be recovered, resulting in an underestimation of the plastic particles. The use of additional sample preparation steps may also result in non-quantitative recovery, resulting in the underestimation of the microplastic abundance.

Analytical detection and quantification

An overview of the techniques for the detection of plastics in fish, bivalves and non-bivalve invertebrates are given in Fig. 6. Elements discussed in this section are based on [15, 55, 73].

Plastics can be identified using visual detection by optical microscopy, with or- without subsequent polymer identification technique. Optical microscopy alone was used in 41% of the studies on fish, in 36% of the studies on bivalves and in 38% of the studies on non-bivalve invertebrates. Optical microscopy is still a low-cost method and can be applied in the field. Melting point assessments such as the hot needle test may provide an indication if a

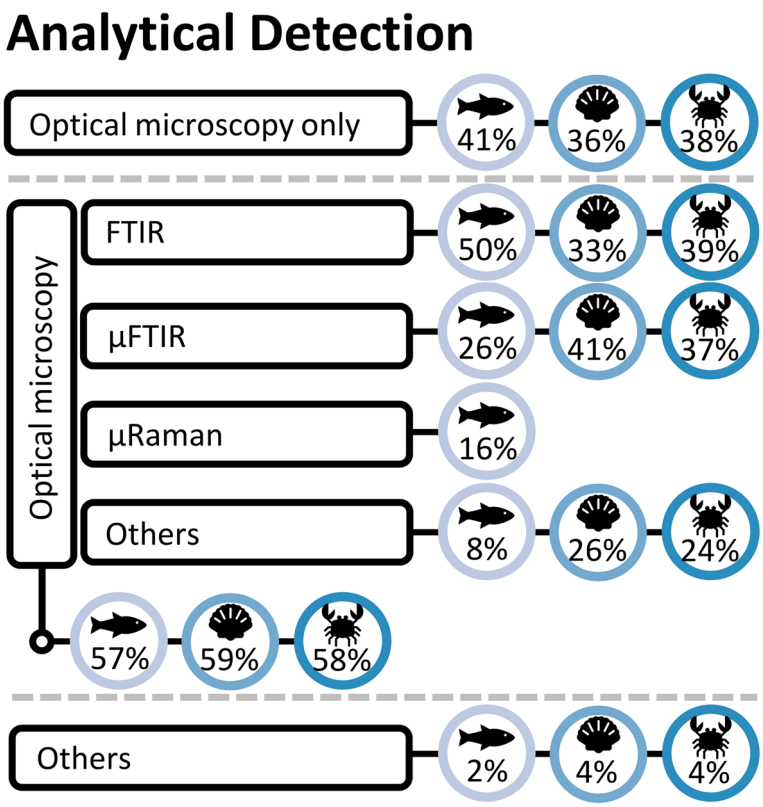

Fig. 6 Overview of techniques for analytical detection and quantification of plastics in fish, bivalves and non-bivalve invertebrates. Techniques with less than 10 occurrences are grouped as others

particle is plastic. This was for example used in 13% of the publications on fish, but has also been reported in studies on other biota. Although optical microscopy, with a resolution of $\geq$ 200 nm, has the same opportunities as listed for visual detection with the naked eye, it still doesn't allow for the identification of the polymer. Additionally, a higher uncertainty can be expected as the count of plastic fragments may differ between persons. The detection of transparent, black or brown polymers remains difficult. Additionally, this technique may be at high risk for false positives and false negatives which may ultimately lead to an over- or underestimation of microplastic abundance.

The combination of optical microscopy with polymer identification (e.g. FTIR, μFTIR and μRaman) has become the state of the art in comparison with optical microscopy only and is used in 57%, 59% and 58% of the cases for fish, bivalves and non-bivalve invertebrates, respectively. The combination of microscopy with polymer identification allows for higher accuracies as non-plastic items are better discriminated and the identification of each plastic particle is possible. This opens interesting opportunities to study the potential source of the pollution and improves the overall assessment of plastic pollution. Polymer identification is, however, time-consuming, and requires analytical techniques with skilled lab technicians. A partial polymer identification can reduce the required analysis time but can result in a biased result if the subsampling is done incorrectly (non-random). This could then result in unrepresentative data leading to incorrect descriptions of potential sources. For fish, the polymer composition was partially or completely identified in 28% and 42% of the cases.

Fourier Transform Infrared Spectroscopy (FTIR) is the most commonly used polymer identification method. It was used in 50%, 33% and 39% of the studies on fish, bivalves and non-bivalve invertebrates respectively where polymer identification was applied. It allows for the identification of particles $\geq$ 0.3 mm. μFTIR can be used for smaller particles $\geq$ 10 μm, but is more expensive than FTIR. μFTIR was used in 26%, 41% and 37% of the studies on fish, bivalves and non-bivalve invertebrates, respectively where polymer identification was applied. Both methods have issues characterising black particles, are time-consuming and can have lower accuracy for weathered particles. RAMAN spectroscopy can deliver high spatial resolution on the sample surface and can easily be combined with other identification methods. Due to the high-energy laser used in RAMAN, sample altering may occur, and the RAMAN spectrum may overlay with the fluorescence spectrum. For smaller particles, down to 1 μm, μRAMAN can be used. This technique even allows for the detection of additives and can be automatised. Unfortunately, the method remains time-consuming, requires expert personnel and high operating cost limits its availability in many labs. μRAMAN was used in 9% of the studies on fish. In the interlaboratory study organized by quasimeme, μRAMAN was only used by one lab, but could correctly identify the polymer type. Raman and ATR-FTIR, used by 3 and 15 labs respectively, misclassified PET and LDPE particles [84]. The performance of μ FTIR was often lower compared to ATR-FTIR.

The use of methods based on pyrolysis-GC-MS (5), LC(sec)HRMS (1), fluorometry (4) or hyperspectral imaging (0) in fish and bivalve samples stays limited. For non-bivalve invertebrates, seven papers that were retrieved by the systematic review applied fluorometric-based techniques for microplastic determination. Fluorometric approaches are cheap to moderate compared to the above-mentioned techniques, allow for rapid identification of the polymer and can be used to guide RAMAN microscopy towards the particles. However, the method can be hampered by the presence of organic matter, resulting in false positives, and non-stainable plastics will be excluded from the results, which may cause an underreporting of the plastic levels. Other techniques such as the use of scanning electron microscopy (SEM) or SEM-energy dispersive x-ray spectroscopy (EDX) are only used in 4% of the publications and are mainly used to aid the particle imaging and material identification, especially when the focus is on particles smaller than 0.1 mm. In the retrieved publications, SEM and SEM-EDX are always used in combination with optical microscopy or optical microscopy with other polymer identification techniques.

For plastic detection by optical microscopy, a large variety of analysis filters have been applied. Glass filters are commonly used in fish and invertebrates (including bivalves) followed by cellulose filters. Other filters such as cellulose acetate, polycarbonate filters and cellulose nitrate are also reported. Some publications do not use filters but determine plastics directly on glassware. The use of FTIR or μFTIR as identification techniques requires compatible filters such as aluminium oxide, silicon or PTFE (polytetrafluoroethylene) filters, and not all filters used for optical microscopy are suitable. Data analysis on applied filters within polymer identification techniques revealed that a large proportion of the publications do not report on the filter type, only report on the filter used for optical microscopy or have unclear descriptions on this part of the method. This hampered the evaluation of the filter type used within polymer identification techniques.

Quality control

To ensure quality within microplastic analysis, publications should provide detailed descriptions of applied

sampling methods, sample size and analytical methods, avoid airborne background contamination, and use negative and positive controls [21, 37]. A procedural blank gives information on the contamination of airborne particles as well as contamination by materials and equipment. There is, however, no uniformity in recalculating the sample result, which may result in data comparability issues. A procedural blank was used in 40%, 68% and 54% of the publications on fish, bivalves and non-bivalve invertebrates, respectively.

To gain information on the recovery and accuracy of the method, a positive control can be used. Positive controls often use artificially mixed spiked materials, which may lack environmental relevance. Additionally, when a non-representative mix is used, an overestimation of the recovery may be obtained. Positive control samples were only applied in 12%, 32%, and 16% of the publications on fish, bivalves and non-bivalve invertebrates, respectively.

Air blanks give information on the background contamination of microplastics. It remains, however, difficult to link direct exposure of plastics to the sample. Air filters may capture airborne particles that were not present in the sample and could therefore be misleading. Air blanks were reported in 38%, 32% and 24% of the publications on fish, bivalves and non-bivalve invertebrates.

Although air filtration systems reduce background contamination, they were often not used or the use was not reported. Fume hoods are common laboratory equipment and can efficiently remove waste stream air. Fume hoods reduce background contamination, although the air of the laboratory may still contaminate samples. Laminar flow can reduce background contamination compared to fume hoods. However, the air from a laminar flow can be recirculated in the lab and may be harmful to operators and other lab staff. A clean room is more effective in reducing background contamination compared to both laminar flow and fume hoods but it is more expensive. Different clean room qualities exist Unfortunately, clean room specifications are not based on standards for microplastic determination. The use of a clean room was reported in 7% of the publications on fish, whilst laminar flow and fume hoods were applied equally frequently (13% of the publications each). In the case of bivalves, a clean room and other air filtration system was only reported in 27% and 33% of the studies, respectively. In some publications on fish, a combined use was reported, such as a clean room with a fume hood [87, 92] or a laminar flow and a fume hood [85].

Data reporting

Microplastics were mainly quantified as items per individual in 80%, 62% and 58% of the studies on fish, bivalves and non-bivalve invertebrates. It is easily interpretable, linked to a count-based analytical method and data can easily be pooled into groups per size category. It also allows for the calculation of the percentage of positive samples. This is frequently calculated for fish and, to a lesser extent for invertebrates. When microplastics were only identified in one or a few organisms, only limited statistical power can be obtained, which could impede conclusion-making. Additionally, count-based methods do not contain information on the total quantification of plastic. Therefore, plastics can also be reported as items per mass. The number of items per mass was reported in 12%, 29% and 68% of the studies on fish, bivalves and non-bivalve invertebrates, respectively. Similar to a count-based system, a mass-based system (e.g. g plastic per individual or g plastic per g biota) can allow for the pooling of data according to size categories. Mass-based data may also provide information on plastic mass fluxes and total plastic input as well as fate- and transport models. A limit below which no presence of plastics can be quantified (count- or mass-based) is seldom applied and was only reported in six publications.

The polymer type was determined in 57%, 69% and 82%, the shape in 90%, 77% and 85% and the colour in 78%, 59% and 63% of the publications on fish, bivalves and non-bivalve invertebrates, respectively. Although identifying the colour and shape of polymer particles is inexpensive and requires only a little training, it may still be time-consuming and be subjected to personal judgment. There is no general use of a common data reporting protocol, and data is generally not reported or in national (1%) or international databases (1%). Publications do not report on the accessibility status of the data (64%) or raw data is not shared (18%). Only in 12% of the cases, data is published using a data repository, can be made available upon request or is published in the Supplementary materials.

Technological readiness level

The TRL of analytical methods for plastic analysis in biota was assessed for the different matrices and different steps of the RAPs. Differentiation in TRL was made based on the degree of implementation in monitoring and the number of publications that implemented defined steps of the analytical procedure. An overview of TRLs is provided in Table 1.

RAPs for plastic determination in birds reach the highest TRL. Existing protocols are in place, which provide information on the minimum size of the plastic to consider, the sample preparation and analysis steps to apply and the procedure for plastic registration. The procedure is based on visual separation and visual analysis and is considered to reach a TRL of 9. For the other matrices (fish, bivalves and non-bivalve invertebrates), there is no

Table 1 Overview of the technical readiness level (TRL) of the different steps in the RAPs for micro- and mesoplastics in biota

	TRL		Sample Collection	Sample Preparation	Analytical detection	Plastic quantification	QA/QC	Data reporting
Plastic ≥ 1mm in biota	Basic Research	1						
		2						
		3						
	Applied research	4						
		5						
	Development	6			Chemical ID with FTIR (ATR, general and microscopy)			
		7		Alkaline and oxidative digestion				Research protocols
	Implementation	8						
		9	Hand collection, nets, hooks/lines	Visual separation	Visual	Guidelines for shapes and colour, Items/individuals, g/individual, %		International protocols (e.g., OSPAR)
Plastic < 1mm in biota	Basic Research	1						
		2						
		3			Hyperspectral Imaging			International databases
	Applied research	4			Pyr-GC/MS	Mass-based units		
		5		Enzymatic and acid digestion	Fluorometric		Field blanks, Positive controls	
	Development	6					Air blanks	
		7		Alkaline and oxidative digestion, density separation	Optical microscopy, FTIR, μ FTIR, RAMAN, μ RAMAN	Counts per individuals, Counts per gram, %	Air filtration systems	Raw data in publications
	Implementation	8					Procedure blanks	
		9	Hand collection, nets, hooks/lines					

single commonly applied protocol in use, although multiple RAPs are well developed and applied, resulting in a TRL of at least 6. Sample collection for fish, bivalves and non-bivalve invertebrates is mostly done by using trawls or other nets or by hand. To analyse (micro)plastics in these sub-compartments, efficient matrix digestion is indispensable which is commonly done with an alkaline digestion with KOH or oxidative digestion with H_2O_2. This is often combined with a density separation with NaCl. For analytical detection and visual identification, the use of optical microscopy alone or in combination with μFTIR, FTIR and μRAMAN are well documented. The use of methods based on pyrolysis-GC-MS, fluorometry or hyperspectral imaging stays limited, indicating that these techniques are currently not at the stage to be applied for routine analysis of plastics in complex matrices such as biota. On the other hand, method developments are still ongoing. Determination of nanoplastics in biota is also still at the stage of basic research, with TRL below 3.

The selection of the best technique is not always straight forward and the monitoring or research goal should always be considered during selection: what is the minimum size of microplastics that should be detected, is it sufficient to analyse common polymers such as polyethylene, PET or PVC, or does the method also has to include more challenging plastics such as tyre wear particles, is there a need to analyse fibers? These goals should also be outweighed against the cost-effectiveness of a method, as large differences between methods exist in

equipment cost, but also in working hours [62]. High-end methods such as pyr-GC-MS or μRAMAN and μFTIR-based methods will allow for low lower size limits and detailed polymer information, but also higher cost than optical microscopy-based methods or polymer identification based on ATR-FTIR [62]. Strengths and weaknesses of different steps of the RAP are provided in the SWOT analysis in Supplementary material and may aid in final RAP selection. Regarding monitoring, steps with high TRL 6 or higher are close to implementation in monitoring schemes.

Recent trends in micro- and nanoplastic analysis

The relative use of conventional methods like FTIR and μRAMAN for the detection of plastics in biota has not changed significantly over the last two years. Di Fiore et al. [26] reported that 47% and 12% of the reviewed papers used respectively FTIR and μRAMAN as detection methods, which coincide with the results reported in Fig. 6 for fish. Three factors are important for the detection of plastics: sensitivity, specificity and throughput [75]. The latter can be increased by automation. This not only reduces analysis time but also characterisation costs and human bias (errors made by human mistake) [62]. Automatisation has already been applied in microscopy-based methods as reported by e.g. [61, 72] and could significantly reduce the processing time of chromatographical methods like GC-MS which is still a challenge [77].

For μRAMAN, lower size limit is often limited to particles ≥ 1 μm. However, this can be overcome to allow detection of nanoplastics (≤ 1 μm). The distribution of labelled nanoplastic particles was studied in bivalves using surface-enhanced RAMAN spectroscopy [27] whereas hyperspectral stimulated RAMAN scattering (SRS) has been deployed to detect environmental nanoplastics [75]. The latter was however performed on bottled water, but this technique has also been deployed in biota samples such as in protozoa [86].

Similar to RAMAN, new developments based on FTIR have been used that could overcome the limited detection threshold for nanoplastics [89]. Super-resolution IR spectroscopy/imaging, for instance, may be promising for the detection of nanoplastics [60, 69]. SEM and transmission electron microscopy (TEM) have been used to visualise the distribution of nanoparticles in e.g. *Ophiactis virens* [80]. Both SEM and TEM have limited use when particles are irregularly shaped or non-doped [60], which may reduce the usefulness of this method for environmental samples.

The use of Pyrolysis-GC-MS for the analysis of plastic in biota remains limited (e.g. [18]), however, this technique was more frequently used in other matrices (e.g. [49, 50, 77]). Other spectrometric methods such as LC-HRMS [33, 52] or matrix-assisted laser desorption ionization time-of-flight mass spectrometry [34] has also been applied for the detection of micro- and nanoplastics in biota.

Conclusion

For plastic analysis in biota, sufficient publications on fish, bivalves, birds and non-bivalve invertebrates were retrieved to examine RAPs. To implement monitoring protocols, studies should select species based on the investigated particle size as well as species characteristics such as geographical distribution, ecological niche and conservational status. The selection of the plastic size class and species proved to be inherently linked, with a higher lower-size limit for methods on plastics in birds (often 1 mm or higher), while methods on plastics in fish, bivalves and non-bivalve invertebrates mostly have a lower size limit below 0.3 mm or even below 0.1 mm. Methods for plastic determination in birds reach the highest degree of standardisation and commonly used protocols are available. For the other matrices, implementation into routine monitoring still requires further steps. However, individual steps of the RAPs can be well developed and frequently applied, such as an alkaline digestion with KOH as sample preparation step, visual determination as analysis step for larger micro-, meso- or macroplastics, and optical microscopy combined with polymer detection techniques such as μFTIR as analysis step for smaller microplastics. Authors should be encouraged to include better-detailed method descriptions. This is especially true for the Quality control section, which is insufficiently described in many papers. Specifically for microplastics, the applied air filtration system is essential information, and the description of procedure blanks and positive controls should be an obligatory part when analysis of microplastics between 1 μm and 1 mm is envisaged. Also the lower size limit, which can depend on the particle shape, is an important characteristic of each method. Although essential for data comparability, this parameter is still too seldom reported. Because methods for nanoplastics determination in environmental biota samples are currently not developed enough, more research is needed before implementation in monitoring programs.

Abbreviations

ATR	Attenuated Total Reflectance
EDX	Energy Dispersive X-ray Spectroscopy
EU	European Union
FTIR	Fourier Transform Infrared
GC	Gas Chromatography
GIT	Gastrointestinal Tract
HRMS	High-Resolution Mass Spectrometry
LC	Liquid Chromatography
LDPE	Low Density Polyethylene

MS	Mass Spectrometry
PET	Polyethylene Terephthalate
RAP	Reproducible Analytical Pipeline
SEM	Scanning Electron Microscopy
SR	Systematic Review
SWOT	Strength, Weakness, Opportunity and Threats
TEM	Transmission Electron Microscopy
TRL	Technological Readiness Level

Supplementary Information

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Supplementary Material 1.

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Authors' contributions
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Availability of data and materials
The data that support the findings of this study will be made available in the near future via Zenodo at 10.5281/zenodo.10680679.

Declarations

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Not applicable.

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