Current frontiers and recommendations for the study of microplastics in seafood

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1 **Title**

2 Current frontiers and recommendations for the study of microplastics in seafood

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Abstract

For seventy years, mass plastic production and waste mismanagement have resulted in huge pollution of the environment, including the marine environment. The first mention of seafood contaminated by microplastics was recorded in the seventies, and to date numerous studies have been carried out on shellfish, fish and crustaceans. Based on an *ad hoc* corpus, the current review aims to report on the numerous practices and methodologies described so far. By examining multiple aspects including problems related to the definition of the term microplastic, contamination at the laboratory scale, sampling and isolation, and quantification and identification, the aim was to point out current limitations and the needs to improve and harmonise practices for future studies on microplastics in seafood. A final part is devoted to the minimum information for publication of microplastics studies (MIMS). Based on the aspects discussed, MIMS act as a starting point for harmonisation of analyses.

Keywords

Microplastics; fishery products; methods; limits; recommendations

Abbreviations

ATR: attenuated total reflection, DAC: digestion atmospheric control, FAC: filtration atmospheric control, FAO: Food and Agriculture Organization, FPA: focal plan array, FT-IR: Fourier-transform infrared, GESAMP: Group of Experts on the Scientific Aspects of Marine Environmental Protection, H₂O₂: hydrogen peroxide, HClO₄: perchloric acid, HNO₃: nitric acid, KOH: potassium hydroxide, LC₅₀: lethal concentration 50%, LD₅₀: lethal dose 50%, LOAEL: Lowest observed adverse effect level, MIMS: Minimum Information for publication of Microplastics Studies, MP: microplastic, MSFD: Marine Strategy Framework Directive, NaClO: sodium hypochlorite, NIC: negative identification control, NOAEL: No observed adverse effect level, O/SC: operator/solution control, OSPAR: Convention for the Protection of the Marine Environment of the North-East Atlantic, PCE: positive control of extraction, PIC: positive identification control, Py-GC/MS: pyrolysis coupled with gas chromatography and mass spectrometry, SAC: sampling atmospheric control

1. Introduction

In 2016, global plastic production, excluding fibres, was estimated at 335 millions metric tons [1]. In less than a century, plastic has become an unavoidable material thanks to its diverse and convenient properties such as durability, resistance and lightweight. However, a major issue concerning plastic is waste management. Since 1950, a small amount of global production has been recycled (9%), most often for a single cycle, while a huge quantity (60%) has been discarded in the environment, and ultimately in the marine environment [2].

Consequently, vast quantities of plastic have accumulated in the oceans all around the globe, and these plastics are subject to degradation through various processes including UV degradation, oxidation, and abrasion. The sizes of these pieces of plastics cover nearly 12 log scales from meter to nanometre [3], facilitating their ingestion by a wide range of marine organisms from the largest such as sperm whales [4] to the smallest, *i.e.* copepods [5]. Among all these synthetic particles, microplastics (MP) are commonly defined as plastic items with a size below 5 mm [6].

There are many questions surrounding the ecological and human health risks posed by MP. There have been few studies on the direct, for example physical effects of MP, or indirect harms caused either by bacteria or by chemicals. Concerning indirect harms, it has been demonstrated that MP are covered by numerous bacterial genera [7], including pathogenic and non-pathogenic *Vibrio* species [8]. MP are also a vector of hydrophobic organic compounds (HOC), but the actual risk is a subject of debate [9]. Finally, another suspected hazard is related to the presence of numerous additives in plastic that can make up 60% of the total weight [10], and that can leach out from the MP [11]. Bacterial and chemical hazards are thus more related to MP surfaces and volumes; the current definition of MP, solely based on length, therefore does not seem accurate enough for risk assessment.

Ingestion of microplastics has been described in numerous marine organisms, including different species of bivalves, crustaceans, cephalopods and fish [12-75]. The number of studies on MP-contaminated seafood has increased exponentially, but there is still no standardised methodology, making it impossible to compare studies. This is a major issue in terms of assessing the risk(s) posed by MP. As a way to

improve and harmonise methods for the future studies, the different steps of MP analysis: sampling, isolation, quantification, polymer identification, and contamination management were analysed and compared based on the available literature. For the period from 1973 to April 2018, 64 studies published were gathered from Scopus and PubMed, based on their main subject dealing with both microplastics and seafood. These publications were analysed using an *ad hoc* reading grid. This corpus comprises 3 articles published before 2010 [12-14], 5 between 2010 and 2012 [15-19], 7 between 2012 and 2014 [20-26], 12 between 2014 and 2016 [27-38], and 37 between 2016 and 2018 [39-75].

2. Definition and size issues

2.1 Current definitions and limits

As previously mentioned, the term microplastic historically refers to “plastic particles smaller than 5 mm” as stated by the international research workshop on the occurrence, effects, and fate of microplastic marine debris [6]. Moreover, this definition is taken up by several international bodies such as the Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) [76], the Food and Agriculture Organization (FAO) [77], the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) [78], and the Marine Strategy Framework Directive (MSFD) [79]. Nonetheless, a minority of researchers prefer to define microplastics as particles with a size < 1 mm [80]. Although the upper limit is well established, its counterpart, *i.e.* the lower limit, is more subject to debate, with different limits being considered: 20 μm and 5 μm [78], 1 μm [81] and 100 nm [82]. Historically, the limit of 100 nm was established based on the definition of nanomaterials. However, nanoplastics result from the degradation of larger pieces and are thus not intentionally produced [81]. Their heterogeneous characteristics such as size distribution, shape, surface charges, stability, ability to form aggregates and porosity, call into question the limit of 100 nm [81].

2.2 Limitations of current definitions and proposals for improvement

More generally, as discussed in the review carried out by Monserrat Filella [83], it is problematic to retain a definition solely based on a size corresponding to the “largest part of the particle”. This

definition is not sufficiently clear and is not suitable for the current challenges in environmental and human toxicological risk assessment [84]. Moreover, it has been highlighted that regarding particle sizes and shapes, differences in toxicity are recorded [85]. These limits are illustrated in the first half of Table 1. Three particles namely a cube, sphere and fibre measuring 5 mm at the largest part of one of their dimensions are proposed. The cube has a 4-log scale higher volume compared to the fibre. Similarly, when equal volumes are considered, in the second half of Table 1, the surface area of the fibre is 2-log scale larger than that of the cube. Due to their morphology, there are considerable differences between cubes and spheres on the one hand, and fibres on the other. Cubes and spheres are characterised by large volumes, while fibres have very small volumes but large surfaces of contact. The need for a “three-dimensional” definition is also echoed in recent studies [86-88]. Indeed, these dimensions will be helpful to estimate particles weights, depending on polymer types. Weight estimates are requested by scientists based for different reasons: 1) because plastic inputs in ocean are usually expressed in metric tons, and 2) because identification technology using mass spectrometry, such as pyrolysis coupled with gas chromatography and mass spectrometry (Py-GC/MS), processes signals related to analytes mass. Likewise, having an estimate of particle weights coupled with identification of the polymer would be helpful for toxicological approaches. Clearly, the main toxicological dose descriptors, such as the no observed adverse effect level (NOAEL), lowest observed adverse effect level (LOAEL), lethal dose 50% (LD₅₀) or lethal concentration 50% (LC₅₀), are usually expressed as a mass (mg) per mass (kg bw, kg) or volume (L) per duration (day or hours).

TABLE 1 goes here (if possible)

This raises the question of how to measure these three dimensions. Some commercial devices propose these expensive configurations, but they usually require a perfectly flat background to serve as a reference of measurement, which is usually not encountered with filters containing MP. Alternative strategies could be used to approximate the volume, such as considering particles as an ellipsoid [86] and using polymer average densities, to estimate particles mass. In this way, authors could propose the contribution of each polymer type to the total mass of MP [86]. The same authors explain that a single

polypropylene MP, with an estimated weight of 4.4 µg, contributes highly to the total mass of the isolated particles. Hermabessiere et al. [87] used a similar approach with pristine MP. It was shown that the volume of microspheres and fibres can be estimated easily by equating them with perfect spheres and cylinders. There are strong concerns regarding fragments with irregular shapes, mostly secondary MP, which probably constitute the largest proportion of MP and are of great concern for the MP community [89]. Here again, approximations can be suggested, such as calculating a mean diameter, assuming that the shape factor of the particle ($4\pi \times \left(\frac{\text{area}}{\text{perimeter}^2}\right)$) is not significantly less than 1 [83]. Among other approximations, Simon et al. [86] proposed to approximate the thickness of the particle. They considered that the ratio linking thickness to the minor dimension of the particle is the same as the average ratio between minor and major dimensions. The average ratio was calculated from the ratios measured for the whole analysed particle. Finally, the particle depth could also be estimated using an ocular micrometre as proposed by Davison & Asch [17], but this would require particle handling which does not meet the current challenges.

Based on these facts, there is a need for the research community working on MP, not only those working on seafood, to reassess the definition of a MP, as the meaning of this term is not the same for everyone. The mass of MP or at least the tri-dimensional structure and the shape of MP have to be considered. As a basis for any study on MP and as the first main element of standardisation this new definition, incorporating lower and upper limits, should be adopted by all scientists.

3. Laboratory environment and prevention of contamination

Microparticles of plastic are ubiquitous and can be collected everywhere in the laboratory environment or on operators (Figure 1). Each type of MP can be found, from fibres that are highly represented, to fragments.

FIGURE 1 goes here (if possible)

The low number of particles in the analysed samples makes the presence of these microparticles in the surrounding environment more than problematic. There is a clear risk of overestimating MP loads in samples, leading to poorly robust results [53].

Based on the corpus (Table 2), the external environment and the prevention of contamination were assessed regarding different parameters such as the operator, the work environment and cleaning procedures, and preparation of solutions, blanks and their management.

3.1 Contamination from the operator

With regard to prevention of contamination by the operator, only 36% of the studies reported that the operators wore cotton lab coats. Nonetheless, behind this overall statistic, it should be noted that proportions are evolving. Since the first mention of cotton lab coat in a publication in 2014 [29], the number of publications has increased each year and, in 2018, 58% of publications (n=12) clearly specified its use [64, 65, 67, 68, 71, 74, 75]. Some interesting additional pieces of information were provided by two studies. The first, mentioned by Bråte et al. [40], indicates that cotton lab coats were cleaned with a sticky roller. This detail could be important bearing in mind that lab coats can be cleaned either at industrial or local laboratory laundries. In the cleaning machines, lab coats can be mixed with other kinds of fabrics with possible synthetic fibre transfer. The second idea, developed by Kühn et al. [67], is the use of coloured cotton lab coats. This would help scientists to systematically exclude a given type of coloured fibre. However, the colour choice has to be oriented towards the less encountered colours, which is not always easy. Prevention of contamination by the operator could also be considered through the use of gloves, as operators' fingers can carry fibres (Figure 1 B). Overall, 20% of the corpus articles (Table 2) clearly mentioned that gloves were worn. Here again, these practices tend to be more widespread than a few of year. Wearing gloves has two advantages: contamination prevention and operator protection from harmful chemical products that can be used to destroy biological tissues (see 4.3). Special care should be taken to ensure that no MP are present at the surface of gloves by keeping them in a protected box, and cleaning them using filtered water/alcohol solutions or compressed air.

3.2 Contamination from the work environment

Considering the work environment, two parameters should be considered: the place where samples are handled, and the tools, materials and equipment used to carry out the experiments. Cleaning procedures are more or less well described in the publications. In all, 61% of the corpus (Table 2) has an explicit mention of this procedure, with this percentage rising to 80% for the two last years (n=25). Cleaning procedures are highly diverse, but 87% of them involve the use of liquid rinsing. The solution or chemicals used for this step are water (tap, deionised, purified, ultrapure, MilliQ), alcohol (either not defined or ethanol) and acetone. In 35% of these procedures, a combination of solutions or replication of rinsing with three successive rinsing steps are used. In five studies, cleaning was followed by control under a stereomicroscope [43, 46, 52, 65, 67]. Only four studies performed solely a stereomicroscope check, without a rinsing procedure [26, 44, 54, 55]. Finally, a single work used glassware heat treatment by heating glass at 550°C for 4H before its use [48]. Unfortunately, heating glassware is only feasible for small containers, while, due to samples sizes, large volumes of solution can be required for the digestion of tissues.

As previously mentioned, the place where handling is carried out should also be considered to avoid airborne MP contamination. In a recent study, Wesh et al. proposed a comparison between different working environments, including a basic laboratory bench, a car in-house laboratory facility, a fume-hood and a clean bench, *i.e.* laminar flow or safety cabinet [90]. Their results showed that the clean bench significantly reduced the number of contaminated samples compared with the other three devices (p -value < 0.01). The use of such devices should therefore be encouraged strongly as a standard practice for the analysis of MP in seafood. A special care would have to be paid to the filters in such devices. Like for microbiological purposes, HEPA H14 0.3 μm filters should be considered as the minimum standard because, based on EN 1822-1, these have an efficiency of 99.995% for particles > 0.3 μm . Considering studies using chemical products and for safety reasons, addition of an activated carbon filter should be recommended for air expelled into the room. The use of horizontal airflow cabinets should be avoided to protect operators from hazardous vapours emanating from digestates. Regarding the corpus

(Table 2), since 2017, 61% of the articles did not clearly describe a specific place for carrying out analyses, and more than half of the studies still do not described a way of preventing contamination in the working area. This fact raises concerns regarding the levels of MP contamination reported in the literature. Concerning studies that used strategies to prevent contamination of the work place, only 12.5% (n=8) mentioned the use, even partially, of a type of airflow cabinet during the study on microplastics in samples [23, 29, 31, 32, 51, 53, 60, 75]. The other most commonly used work areas are basic cleaned laboratory benches (10.9%; n=7) [26, 44, 45, 55, 57, 68, 72] and fume hoods (10.9%; n=7) [27, 30, 32, 38, 41, 47, 63]. A single study reported the use of both a fume hood and laminar flow cabinet [32]. Three studies (4.7%) indicated that analyses were performed in specific laboratories, with controlled circulation [40, 52, 64]. Finally, one study even used an infant incubator to process samples [58], which can be considered as a highly protected environment under reserve of sealing the place where the arms come through.

3.3 Contamination from used solutions

Although an essential parameter, the filtration of solutions is mostly not mentioned in publications, as 75% of the corpus (Table 2) did not specify any filtration of the solutions used, even when chemical approaches were employed to digest tissues. Despite this figure, it should be noted that half of the experiments using filtered solutions were reported in studies published since 2017. In fact, even the use of “deionised, purified, ultrapure, MilliQ” water could be subject to recontamination after water circulation through the filter, membrane and resin. Filtration of solutions, as well as stereomicroscopic control of used filters, are mandatory for MP studies.

There is a real need for an “MP-free” standard for researchers in the field, like what has been developed in molecular biology with the “DNase-free” standard. This call for an “MP-free” standard relates on a broad of range of materials used for MP studies such as solutions, filters, gloves, etc. Unfortunately, laboratory suppliers are not familiar with the topic of MP, and efforts are needed to raise awareness of the analytical constraints involved in microplastics research. These MP-free materials would certainly be more expensive compared to current prices, but they would clearly offer non-negligible efficiency gains.

3.4 Controls of contamination

The last point, concerning contamination prevention, is the use of controls, also referred to as blanks in the publications. Controls aim to ascertain the quantity of MP coming from different sources of contamination; therefore, different types of controls can be used in the studies. They are required whenever analysed tissues are in direct contact with external contamination sources. The main control usually employed in studies is covered by the catch-all term “procedural” blank; the second mentioned term is atmosphere control, and finally the last term is observational control. These controls respectively rely on controls that follow the same process than used for samples, control checking for potential contamination from the ambient air, and lastly is a specific control of ambient air during MP isolation into samples or onto filters. These terms could also refer to different controls at different steps of the analytical process.

There is a lack of precision and description of controls in the publications; the corpus was only studied based on “procedural”, “atmosphere” and “observational” controls (Table 2). Before 2014, there is no mention of blanks or controls. Since 2014, although 73.5% of articles mention a procedural blank, only 18.4% and 10.2% noted the use of atmosphere and observational controls, respectively. A few publications also propose new controls, namely positive/negative controls [42, 53] or control of sample containers [71]. Positive controls aim to check whether plastic present in samples is accurately recovered during the isolation process, whereas negative controls are more difficult to implement, as no MP-free matrix exists. The number of controls reported in the different studies can vary, and there is no clear statement on the appropriate number of controls to be performed. Some publications suggest applying controls to each analysed batch of samples [27, 32, 40], which might be encouraged for future standardisation. Nonetheless, overall the number of controls should not be too high compared with samples.

The first step concerned by control is dissection. This step is mostly subject to contamination by the atmosphere, tools and operator. The main problem at this step concerns the atmosphere, which is why the use of sampling atmospheric control (SAC) is highly encouraged. The second step, if applied, relates

to digestion. It involves possible contamination by the atmosphere, operator and used solutions, and is usually assessed by processing an Erlenmeyer flask without sample, as a flask containing a sample. For this step, two controls can be proposed: a digestion atmospheric control (DAC) to monitor the atmosphere, and a flask that undergoes the same analysis process as a sample, which can be considered an operator/solution control (O/SC). As proposed in a few studies [17, 42, 53], the use of a positive control of extraction (PCE), a flask with a defined number of MP, should be considered in future research. The third step consists in filtration with a risk of contamination by operators and the atmosphere; this risk can be assessed using a filtration atmospheric control (FAC). Finally, the observation step is mostly threatened with contamination by the atmosphere and tools, but most often, filters are protected by the lids of Petri dishes.

Although controls are often referred in scientific studies, there are issues regarding their management and the communication of control results. As an example, since 2014, 49% of the publications did not clearly report how controls results were managed, and only 32.7% of papers reported their blank results. These figures are not surprising as there is no consensus on this topic. Some strategies are not satisfactory as they use systematic exclusion of items, without considering the numbers found in controls vs. those observed in samples. Particles are thus subtracted without considering their colours or shapes, or an average number of particles counted in controls is subtracted. In some studies, it is reported that no particles were observed in the blanks. Based on all the studies analysed in this review, and bearing in mind the environment in which MP analysis is performed, it is justifiable to wonder about the accuracy of these control results. In contrast, the currently most advanced and thorough methodology consists in subtracting control counts, taking particle shape, colour, and synthetic nature into account [46, 72]. This idea could also be combined with other approaches where the notion of control size and contact surface are highlighted [48, 52]. This would help to compare controls and samples if the exposed surface is not the same. Finally, the notion of exposure time could also be taken into account [73] as controls are not always in contact with the atmosphere for a period equivalent to that of the samples. By combining all these ideas, future controls could be expressed as number of items $\text{cm}^{-2} \text{h}^{-1}$. This would enable researchers to accurately subtract items from sampled based on shape and

colour of items, taking into account the surface and exposure time. The scientific community therefore needs to develop new approaches to reach a consensus on how to handle these important issues, and accurately estimate MP loads in seafood.

3.5 Proposals for standardisation

As explained above, preventing contamination in the context of MP analysis is a key factor from the arrival of samples at the laboratory to the reporting of results. Numerous parameters must be managed to perform MP analysis with good quality assurance and good laboratory practices.

Contamination from the operator must be reduced through the use of cotton lab coats and gloves. Concerning the work environment, the use of laminar flow cabinets is strongly encouraged [90]. This has to be accompanied by a cleaning procedure. All solutions used during MP analysis must be filtered to ensure the absence of particles. As contamination cannot be 100% ruled out, the use of different controls is mandatory. These approaches aim to monitor different types of contamination (atmosphere, operator, chemicals) and have to be thoroughly described in publications, together with the way they are incorporated into results.

Some gaps are still seen in published studies, but recent research tends to include improved measures to control contamination. This can be observed when regarding the specific “controls” or “quality assurance-quality control” paragraphs in the materials and methods sections of articles [53, 62, 72]. [Figure 2](#) below illustrates the various parameters that should be applied by scientists for future research on MP in seafood.

FIGURE 2 goes here (if possible)

4. Sampling and isolation

4.1 Sampling for studies on MP in seafood

Sampling is the first step in the process for MP analysis. Preliminary essential data to record in publications are the number of individuals sampled and their scientific names. Overall, these data are suitably presented by authors, as only 7.8% of studies in the corpus ([Table 2](#)) did not explicitly report the

number of individuals. A minimum of $n=50$ individuals has been defined by OSPAR and the MSFD as a limit to obtain adequate sampling [78, 79], although MSFD members recognised that data on variability related to sampling sizes are lacking. It should be noted that this number is not always respected in studies.

Certain localisation parameters should also be recorded such as GPS coordinates, catching depths and types of capture (trawl type, mesh size, etc.). These data are easily recovered for scientific catches [26, 51, 67], but this could be more difficult when samples are purchased directly from fishermen or market or sold as processed seafood [35, 60, 75].

When individuals are still whole, biometric data have to be recorded, such as total or standard length with an ichthyometer or a calliper and weight. Recording whole sample size will help scientists to determine whether this corresponds to a commercial size; as an example, commercial sizes for major European species can be found in Regulation (EC) No 850/98 [91]. Moreover, it could help to estimate the physiological state of studied organisms. Studies on bivalves and crustaceans usually meet this standard, but it is more difficult to find such studies on fish. However, these data are important, especially for risk assessments related to human health. On the basis of the corpus, 64.1% and 42.2% of papers (Table 2) mentioned average size and weight for whole or tissue samples, respectively, which is not satisfactory even in recent years. Less than half of the studies report weights, not always mentioning the one of analysed tissue. Improving these figures is therefore a major challenge for the coming years, particularly when the isolation step involves, as explained below, chemical digestion of tissues that needs to be standardised.

Sample management differs between studies; some research involves direct sampling of tissues on board, while other studies preserve whole organisms until analysis. The procedure for tissue sampling has also to be reported in studies as it could lead to additional contamination of samples, the use of SAC should be encouraged. During tissue recovery, recording the time of sample exposure to ambient air would be an interesting parameter to record. It is especially important if the time factor is taken into account by the controls (see 3.4). Moreover, it is important to rinse parts that are not analysed either for

fish or bivalves as much as possible, so that contamination from other parts of the animal would not occur [27, 53].

4.2 Tissue preservation before processing

There are two methods for storing samples: storage at temperatures below 0°C, *i.e.* samples placed in a freezer, or at temperatures above 0°C in chemical mixtures, *e.g.* formaldehyde and ethanol. These data must also be reported in scientific papers as they could have an impact on the observed results. Together with the method used to preserve samples, the storage time would be of interest, even though it is not essential. To the best of authors knowledge, a single study looked at the impact of preservation methods on microplastics [52], comparing storage at -20°C for ten days to storage in 4% formaldehyde for three days. No effect of the storage method was found by the researchers. Unfortunately, except for two studies that used same concentrations of formaldehyde [18, 67], the others used a concentration of 5% or higher [15, 17, 51, 65, 68]. Likewise, a storage limited to three days in formaldehyde seems to be inconsistent with scientific fishing campaigns that can last weeks [92, 93], with long periods before samples landing. Nonetheless, this type of approach aiming to assess the impact of sample processing on MP integrity is of great interest and should be pursued. Another important area of study is the compatibility of solutions used during samples processing. In the case of formaldehyde, the use of oxidising chemicals or perchloric acid downstream should be prohibited due to the potential development of violent reactions. This shows that sample storage should be taken into account as soon as the study is designed. When possible, sample freezing should be given preference, particularly when chemical digestion is performed downstream. It has been well documented, particularly in fish, that freezing has an impact on muscle constituents. On the one hand, mechanical destruction of tissue can occur due to crystallisation of certain water pools inside muscle, and on the other, protein aggregation and lipid oxidation can occur [94]. These phenomena are all the more likely when freezing kinetics are slow, the freezing time is long, and the freezing temperature is low. Here again, long periods of freezing can occur between sampling and analysis, and it would be of great value to assess whether long freezing

times are an advantage or a disadvantage for both dissection and chemical degradation of seafood tissues.

TABLE 2 goes here (if possible)

4.3 Dissection and digestion methods for MP studies

At this point in the analysis process, there are two main methodologies: dissection and chemical digestion of tissues. Consulting the corpus (Table 2), a trend is emerging, whereby small sampling quantities are more often subject to the chemical approach: 66% of studies (n=29) with sampling ≤ 180 individuals used such an approach. In contrast, 62% of studies (n=29) with sampling > 180 individuals prefer the dissection approach. Methodology can also be governed by the type of studied organism, as an example bivalves are exclusively studied as a whole after a chemical digestion (Table 2).

Dissection is very easy to set up and implement, and it is not expensive. This could explain why it is generally preferred for MP studies, and particularly for very large sampling sizes. It accounts for more than half of the whole corpus (53%) (Table 2). Nonetheless, there are some issues concerning the use of dissection for MP isolation. The first disadvantage is the sample inspection time, which can be as long as 10 min to 1h30 [39, 45, 57], leading to a high risk of contamination from the work environment. The second important issue with this methodology is the risk of omitting particles [17, 25]. The first reason for these omissions is that dissections are sometimes performed with the naked eye, which is not accurate enough to distinguish all MP. The second reason is that depending on their size and shape, MP can be difficult to observe among the contents of the alimentary tract.

Concerning chemical digestion of tissues, different methods have been described. These include enzymatic methods (trypsin, proteinase K, mix lipase/amylase/protease), oxidative methods (sodium hypochlorite (NaClO) and hydrogen peroxide (H₂O₂)), acid methods (nitric acid (HNO₃) or a mixture of HNO₃ and perchloric acid (HClO₄)), and basic methods (potassium hydroxide (KOH)). The use of KOH is the most commonly described of the chemical methods used in the studies of the corpus (17%) (Table 2). This chemical is mostly used at a concentration of 10%, except in one publication where a concentration of 20% was tested [63]. KOH was used to digest both bivalves [35, 68, 71] and fish

tissues [23, 35, 44, 48, 53, 60, 63, 70, 75]. KOH has the advantage of having no deleterious effects on several polymer types [95, 96]. KOH must, however, be handled with caution, as it is a corrosive compound. In the corpus (Table 2), eight studies (13%) proposed the use of oxidising solutions such as 9% NaClO on fish stomach contents [51], and 30% H₂O₂ on bivalves [30, 33, 42, 59, 69] and fish organs [31, 59, 66]. H₂O₂ is the second most commonly used technique to digest seafood tissues, mostly bivalves. The main concern with oxidising solutions is their stability over time. This must therefore be taken into account in the context of method standardisation. Echoing what was previously discussed (see 3.4) the use of DAC, O/SC and PCE is highly encouraged during the digestion step.

A total of eight studies using acid approaches were found in the corpus (Table 2), among which five (8%) used 69% nitric acid [29, 37, 41, 47, 62] and three (5%) used a 65% HNO₃: 68% HClO₄ 4:1 (v/v) mixture [27, 32, 38]. The nitric acid method has only been applied to bivalves, while the acid mixture was applied to both bivalves and crustaceans. The main drawback of the acid approach is its adverse effect on polymers, described in numerous studies [95, 97, 98]. Three studies (5%) used enzymatic digestion for the analysis of bivalve tissues [52, 72] and fish gut [61]. Enzymatic approaches can be considered mild approaches, and they usually do not require temperatures above 40°C. The second advantage of this type of approach is that enzymes have no impact on synthetic polymers. One of the disadvantages of such methods is the use of a multistep analysis, involving multiple solutions with a higher risk of contamination. As an example, enzymatic digestion was not sufficiently effective on fish gut [61], and an additional step using 30% H₂O₂ was necessary. Moreover, these approaches can sometimes be time consuming. Finally, depending on the method, hazardous reactions can occur, which is not compliant with good hygiene and safety practices. As an example, H₂O₂ heating can generate O₂, and NaClO should not be put in acidic conditions because of the release of the highly toxic Cl₂ compound.

4.4 Filtration as the last step in the isolation process

The last step of in the sampling and isolation process involves filtration when chemical digestion of tissues is performed. The use of FAC is required at this step. Filter retention levels mentioned in the

corpus (Table 2) (n=31) are 250 µm (3%), 200 µm (6%), 149 µm (6%), 52 µm (3%), 20 µm (6%), 13 µm (10%), 12 µm (3%), 8 µm (10%), 5 µm (23%), 1.6 µm (3%), 1.2 µm (13%), 0.8 µm (3%), and 0.7 µm (10%). Regarding these studies, and looking at the filters used, it appears that 61% (n=19) chose a retention diameter below 10 µm. This makes it possible to retain very small MP below the limit of detection of certain identification techniques used downstream (see 5.3). Concerning filter composition, the most common materials are nitrocellulose (29%) and glass fibre (26%). For future studies, a compromise between filtration efficacy, *i.e.* absence of clogging and absence of interference for identification, and MP retention needs to be found and then put forward as a standard.

4.5 Proposals for standardisation

Based on these findings, some advice can be proposed concerning sampling and isolation, in order to standardise practices. A minimum of 50 individuals must be sampled per studied species (Figure 3), even though statistical analyses are required to assess whether this is representative of population variability. It is important to gather metadata as much as possible, including GPS coordinates for the catch. The second important point to keep in mind is to select individuals that correspond to the commercial size, if risk assessment for human health is considered. Biometric data, *i.e.* whole size, whole weight and analysed tissue weight are mandatory for study reports in order to improve standardisation of digestion techniques. Once sampled, tissues are to be stored as frozen samples, since the effects of chemical preservatives on polymers are not clearly documented. For particle isolation, the chemical approach should be given preference (Figure 3) as it ensures low exposure of tissues to ambient air and recovery of smaller particles. Two methods are popular in the research community but, to the best of author knowledge, the method using 10% KOH at 60°C for 24H is the one whose impact has been assessed most closely; additionally, this solution is more stable over time compared to 30% H₂O₂.

FIGURE 3 goes here (if possible)

New methodological parameters concerning digestion should be clarified in future studies to improve method standardisation and comparison of results. The main focus should be to estimate the limits of

applicability of each method: species, weight of tissues, etc. Various other parameters also need to be better clarified and defined (Figure 3). As an example, the ratio of solution to tissue weight has to be given [27, 32], instead of highly imprecise information such as the volume of solution without reference to tissue weight [33, 41, 71], or unclear expressions such as “three times the volume of the biological material” [23, 53, 64]. Concerning digestion duration and temperature, certain terms like “overnight” and “room temperature” [27, 32, 37, 38, 47] should also be avoided as they make it difficult for other scientists to reproduce the analysis precisely. Multiple treatments must be thoroughly assessed to avoid unnecessary steps that can lead to contamination, and to determine whether they are necessary for all sample types and sizes. Finally, regarding filtration, harmonisation of filter pores and composition is also needed.

5. Quantification and identification

5.1 Quantification strategies for particle isolation in seafood

The quantification of MP is usually performed by observation under a stereomicroscope. Some studies report observation with the naked eye, but this approach is limited to large MP with sizes above 500 µm. At this step of the analytical process, “MP-like” or “putative MP” are sorted and generally isolated from the dissected tissue while they are directly counted on the digestate filter [44, 64, 66]. This step is highly tedious, and particularly complicated if the filter is loaded or the contents of the dissected tissue are in large volumes.

Currently, automation is theoretically available with both FT-IR and Raman spectroscopy. It has been implemented with both Raman [99] and FT-IR [100] on MP in water and sediment samples. Concerning seafood, automation of particle counting was mentioned in a single publication, without implementation in routine laboratory practice [101], mainly because this approach is still “unreasonably time-consuming” [102] and because of resolution issues.

Preliminary categorisation of items is generally performed based on particle shapes. As a minimum, sorting consists in separating fibres from other items. Fibres are included in 69% of the studies from the

corpus (Table 2). Six publications explicitly mention that fibres were not included in their evaluation because they were considered laboratory contamination [17, 23, 31, 37, 47, 56]. Nonetheless, it is important to remember that fibres are produced in high quantities [2] and they are not retained by most wastewater treatment plants [103]. With this in mind and with the aim of improving prevention of contamination (see 3.5); it becomes difficult to rule out fibres when studying MP in seafood. Finally, 22% of studies did not mention fibres; however, most of these studies were carried out before 2013. As a reminder, a list of MP item types was provided by Lusher et al. [104]. It includes fragments, fibres, beads, foams, and pellets.

A second approach consists in sorting items based on their size. To do this, authors generally assess the frequency of items in the different size classes. Considering the studies in the corpus (Table 2) this strategy was performed in 27% of cases. Once again, the main issue with these studies is that none of them used the same classes, which makes it difficult to compare results.

A third method of sorting is based on particle colour. This approach was adopted in 67% of the articles of the corpus (Table 2). This sorting approach is also of interest because it enables an orientation test before identification of the items (see 5.2). Of course, particles with colours such as pink, red, blue, or yellow have a higher probability of being synthetic compared to transparent, black or white ones [105].

Until clear identification has been carried out, only the terms MP-like, particle or item should be used.

5.2 Orientation tests: selection of putative polymers

Once quantification has been performed, there is a need for identification in order to discriminate real MP from non-synthetic particles. Studies that did not perform any identification or orientation represent 16% of the corpus (Table 2). Except for one publication [45], all these works were published before 2015, showing the attention paid in recent years to better characterisation of isolated particles. Since 2015, the absence of identification seems to have been replaced by orientation tests, but it is important to separate orientation techniques from identification methods. The first indicates the suspected synthetic nature of a particle, while identification leads to clear determination of the polymer composing the MP.

Moreover, it should be mentioned that the sole use of an orientation test is not satisfactory, but it can help to spread non-target particles and avoid overloading analytical devices.

Different orientation tests are proposed in 28% of the corpus articles (Table 2): density tests in solutions with different salinity [22, 44], observation of particle characteristics such as colour, shape, and ability to break [25, 30, 35, 36, 41, 43, 44, 50, 61-63], the use of polarised light microscopy [47], the use of a hot needle that leaves a mark on synthetic particles [27, 32, 38, 39, 61], and finally colouration with Rose Bengal that stains organic particle leaving mineral, chitin and synthetic material unaffected [17]. Rose Bengal is not the unique colouring agent used for orientation, recently some papers mentioned the use of Nile Red [106] to perform such approach [107].

5.3 Identification methods to ascertain the nature and quantities of true MP

Regarding identification, the main technique described in the corpus (Table 2) is Fourier-transform infrared (FT-IR) (48%), as is or adjusted in different configurations: micro FT-IR, attenuated total reflection (ATR), and focal plan array (FPA) (Table 2). The second described technique is Raman microscopy, used in 9% of the studies described in the corpus [18, 29, 37, 51, 60, 75].

The main advantages of such techniques are the ability to analyse small particles such as $< 20 \mu\text{m}$ and the possibility of coupling particle counting and identification, even though particle isolation is usually performed in seafood. Regarding their drawbacks, FT-IR is generally sensitive to moisture content and not able to identify black particles [108]. Raman technology is not able to easily identify fibres, or particles containing pigments [29, 75], and can destroy particles due to the high energy intensity of its laser. Pyrolysis coupled with gas chromatography and mass spectrometry (Py-GC/MS) has not yet been used for seafood studies, contrary to other research fields such as MP in sediment or water. However, a method has been developed and applied on a few MP isolated from bivalves [87]. This technique allows the characterization of the particle core, and recently proved to be efficient in determining polymer composition for samples identified as “pigment” by Raman microscopy [87].

In a context of method comparison and harmonisation of analytical practices, there is a need for method performance assessment. This assessment has been performed for Py-GC/MS [87] with information on

method development, repeatability, and theoretical limits of detection. Such approaches should also be encouraged for spectroscopic methods allowing for better comparison of results from different studies. As an example of harmonisation, among all FT-IR studies (n=21), only 52% provided a threshold beyond which the identification of the polymer's nature is certain, and this threshold varied from 60% to 85%. None of the six studies performing Raman identification provided such information. Working with the same parameters would help to harmonise identification methods and ultimately enable sharing of research databases, increasing the diversity of spectra available to everyone, and thereby the power of identification of the different tools [102]. Finally, it could be suggested to add positive identification controls (PIC), *i.e.* a single polymer or multiple known polymers, and negative identification controls (NIC), *i.e.* a single polymer or multiple natural polymers such as cotton or chitin, to ascertain proper functioning of the analytical device for each batch or particle analysed.

Another issue concerns samples with noise or unidentifiable signals. Among studies having carried out identifications using either Raman or FT-IR spectroscopy (n=37), only 16.2% reported unidentified items [34, 42, 52, 60, 73, 75], nonetheless this information is important and should be detailed. Unidentified particles can be either due to absence of spectrum or a noisy signal; chemometric methods are cutting-edge approaches that can help to solve the latter issue. Briefly, chemometry consists in the application of mathematics and statistics, e.g. multivariate analysis for signal processing and correction. A free online analytical pipeline is available to develop chemometric approaches; the Chemflow project (<https://vm-chemflow-francegrille.eu/>) developed on Galaxy is mostly dedicated to infrared spectrometry. Next steps involve the development of *ad-hoc* analysis pipelines [100], made available to the research community, able to process problematic spectra and improve identification levels. In parallel to the chemometric approach, as proposed by different authors [102, 109], some works must also be undertaken with weathering and analytical specialists to obtain Raman/FT-IR/Py-GC/MS spectra related to weathered polymers. Indeed, most of the particles found in seafood are secondary sourced MP that have been damaged by the action of UV, oxidation and swell. This results in spectra that could be different from pristine polymers conventionally used for databases. Moreover, this has been demonstrated for PVC after analysis by μ -Raman [105] and Karami et al. expected that unidentified

items could partly correspond to weathered polymers [60]. Polymers having known amounts of common additives can also be considered with such approaches to explore whether the plastic additive content could influence identification after weathering processes.

Finally, not all particles are usually analysed with the identification tools; a subset of particles is mostly preferred. Regarding the corpus (Table 2), there is huge diversity in the strategies for studies carrying out identifications (n=37). Five studies (14%) did not report how many particles were analysed or whether there was a selection of items. Seven articles (19%) reported that they analysed all the particles, while 11 (30%) mentioned a fixed number of particles having been analysed, without information on the proportion of isolated particles that it represents. Fourteen studies (38%) mentioned analysis of a subset of particles, among which 10 (27%) provided the number of particles analysed vs. the number of isolated particles [34, 42, 56, 58, 59, 61, 64, 66, 69, 73], this practice should be encouraged for future studies. The MSFD provides a strategy concerning identification, proposing that all the particles < 100 μm should be subject to identification, while for those > 100 μm , only a subset was proposed; as an example, 10% could be analysed in the limit of 50 particles [79]. In a recent review, Hermesen et al. proposed another method consisting in studying a minimum of 100 particles when the number of MP-like is less than 200 and more than 50% of the particles above 200 items [110]. Based on recent studies, it appears that small particles with a size < 100 μm represent the majority of particles [71]. The number of items to analyse is thus increasing with the precision of detection devices, and there is a need to define new rules for particle identification. Unfortunately, the above-mentioned proposals are not strongly supported by statistical data. As a result, there is also a need for statistical work to provide advice concerning subsampling, defining the adequate percentage to analyse and to obtain accurate and representative identification of all particles with a high confidence level. Adjustment of the confidence level should be carefully performed, bearing in mind that very high precision will lead to high costs regarding machine and labour times.

Identification results are generally given as a list of polymers, followed by the percentage for a given polymer. This allows to easily visualise which polymer is mostly identified, but unfortunately not

necessarily the one whose quantity is the highest. This fact was illustrated in a recent study on MP in wastewater [86]. As previously discussed (see 2.2), identification data should be combined with quantification data, especially those on size and estimated volumes, in order to estimate the mass of MP present in seafood. This would be of great value for both environmental and human health risk assessment [84].

Once identification has been performed, and provided that identified sub-samples are representative of the whole sampling, and that controls have been thoroughly taken into account, contamination results can be proposed as average MP indiv⁻¹ and MP g⁻¹ [104]. The latter result is particularly important when assessing risks to human health and exposure to MP through seafood, using total diet studies.

5.4 Proposals for standardisation

Concerning quantification and identification, the first parameter to work on concerns automatic particle counting (Figure 4) in order to lower the risk of contamination and increase the number of particles found compared to manual sorting [109]. Common terminologies need to be adopted by the community for the description of particles. Although the different shapes of particles are well defined [104], the other descriptors need to be standardised further. The use of common terminologies will enable easier comparison of studies, and thereby more efficient risk assessment work.

Regarding identification, the comparison of methods on identical samples is suggested and method performance criteria should be established for all the techniques. This will help to evaluate their complementarity (Figure 4), allowing for the establishment of future common identification strategies, or analytical workflows as recently proposed [88], thus saving time and money. Adding identification controls will help to improve quality assurance and participate in standardisation of practices. There is a need to establish, based on statistical criteria of representativeness (Figure 4), accurate rules to define the size of sub-sampling when used for identification. Ultimately, a link between size and identification should be considered in order to approximate MP mass in samples. This will be helpful in environmental and human health risk assessments.

FIGURE 4 goes here (if possible)

Finally, there are considerable needs concerning approaches in bioinformatics (Figure 4) to enable researchers to identify the currently unidentified spectra. Chemometry and data analysis pipelines would help to remove ambiguities regarding unidentified particles based on statistical tools and enable all scientists to use these difficult methodologies without the need to have extensive knowledge in the field. Finally, the development and pooling of databases, including for weathered polymers, will also improve inter-study reproducibility of identification.

6. Minimum information for publication of microplastics studies

As previously highlighted, the number of publications on MP in seafood has increased at an exponential rate in recent years. Unfortunately, some of these publications do not satisfy minimum criteria that should be required to publish a paper dealing with the identification of MP.

Even though a different methodology was used, authors shared some conclusions with the work of Hermesen et al. [110], *i.e.* the need to work in clean air conditions, the need for contamination controls, or the need for identification to ascertain the polymeric nature of particles. Moreover, authors propose use of recommendation put forward by Lusher et al., concerning, as an example, shape classification and expression of results [104].

TABLE 3 goes here (if possible)

Authors propose the development of a concept that has already been implemented in other sciences, such as molecular biology [111]: the minimum information for publication of microplastics studies (MIMS). This checklist could be used by people involved in publication from authors to reviewers. The list is proposed in Table 3 and is subdivided following the different items presented in this review that represent the different steps of MP analysis from sampling to identification. A distinction was made between essential information that is mandatory to produce an accurate report on a study, and any desirable information that would add value to the work but whose absence would not affect the overall understanding and the ability to reproduce experiments. This table is the fruit of authors' reflexions and could be used and adapted for other studies dealing with MP contamination in seawater or sediments.

7. Conclusion

The study of MP in organisms, including seafood, has essentially developed in the last ten years. That makes it a relatively young field of science. Based on the studies examined in this review, good and less good practices were highlighted. The main information to be drawn from this review is that although in the past much information was missing from the articles, recent studies are more informative concerning for example: contamination prevention, solution filtration, and use of controls. Unfortunately, some practices need to be thoroughly improved such as management of controls, digestion method harmonisation and polymer identification.

There is a need to better structure information to improve standardisation, opening the way for the comparison of studies, which is of particular interest for toxicological risk assessment.

This review was also an opportunity to reflect on the future challenges facing research on microplastics in seafood. These challenges include developing a more accurate definition of MP in regard to risk assessment, better contamination management thanks to airflow cabinets, the need for harmonisation of digestion methods, and the need to accurately identify isolated MP-like particles. Taking into account the needs of standardisation, as well as the challenges, the concept of MIMS is proposed. This checklist ensures that the minimum information would be proposed to publish an accurate report dealing with experiments concerning microplastics.

In the coming years, the above-mentioned challenges should be addressed. This involves interdisciplinary and collaborative work. This will help to improve the quality and accuracy of studies on MP in general, and MP in seafood in particular.

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Captions

Figure 1: Pictures of microparticles and potential MP isolated from a laboratory ventilation grid (A) and the surface of an operator's finger (B) where fibres are pointed out by arrows.

Figure 2: Representation of basic precautions to avoid sample contamination in the context of MP load analysis.

Figure 3: Schematic representation of proposal for standardization of sampling and isolation. SAC: sampling atmospheric control, PEC: positive extraction control, O/SC: operator/solution control, DAC: digestion atmospheric control, FAC: filtration atmospheric control.

Figure 4: Schematic representation of proposal for standardization of quantification and identification. PE: polyethylene, PP: polypropylene, PS: polystyrene, μ Raman: Raman microspectroscopy, μ FT-IR: Fourier-transform infrared microspectroscopy, Py-GC/MS: pyrolysis coupled to gas chromatography and mass spectrometry.

Table 1: Comparison of cube, sphere and fibre lengths, diameters, volumes and areas considering equal maximum size and volumes.

Table 2: Reading grid showing the main points selected from the studies in the corpus.

Table 3: Minimum information for publication of microplastics studies (MIMS).

Glass containers
(cleaned outside/inside)

Filtered solutions

Cleaned surface

Controls

Manipulations in safety cabinet

- Laminar flow with recirculation
- HEPA H14 filter
- Activated carbon filter

Coton lab coat

Cleaned gloves

SAMPLING

DIGESTION

FILTRATION

SAMPLES

CONTROLS

QUANTIFICATION (AUTOMATION)

Part. 001

Fibre

20 μ m – 204 μ m

Green

PE

Part. 004

Fragment

114 μ m – 200 μ m

Blue

PS

Part. 002

Bead

60 μ m

Green

PP

Part. 003

Fibre

50 μ m – 150 μ m

Black

Cellulose

IDENTIFICATION

μ Raman

μ FT-IR

Py-GC/MS

Methods comparison

Complementarity assessment

Analytical Workflows

Sub-sampling
representativeness

Chemometrics

Shared
Databases

Table 1

	Cube	Sphere	Fibre ^a
<i>Equal maximum size</i>			
Lengths (L)	5 mm	-	5 mm
Diameters (D)	-	5 mm	0,05 mm
Areas ^b	150 mm ²	79 mm ²	0,79 mm ²
Volumes ^c	125 mm ³	65 mm ³	0,01 mm ³
<i>Equal volumes</i>			
Lengths (L)	3,7 mm	-	25465 mm
Diameters (D)	-	4,6 mm	0,05 mm
Areas ^b	81 mm ²	66 mm ²	4000 mm ²
Volumes ^c	50 mm ³	50 mm ³	50 mm ³

^a Fibre is here considered as a cylinder

^b Areas were calculated using different formulas: $6 \times L^2$ for the cube, $4\pi \times \left(\frac{D}{2}\right)^2$ for the sphere and $2\pi \times \left(\frac{D}{2}\right)^2 + 2\pi \times \left(\frac{D}{2}\right) \times L$ for the fibre.

^c Volumes were calculated using different formulas: L^3 for cube, $\frac{4}{3}\pi \times \left(\frac{D}{2}\right)^3$ for the sphere and $L \times \pi \times \left(\frac{D}{2}\right)^2$ for the fibre.

Table 2

Ref.	Year	Contamination prevention							Sampling			Isolation					Quantification			Identification				
		Cotton lab coat ^a	Cleaning procedure ^b	Sol. filtration	Working place ^c	Blanks ^d	Blanks management	Blank results ^e	Aver. Length ^f	Aver. weight ^f	n ^f	Organism _s ^g	Method	Concentration	Ratio ^h	Time ⁱ	T°C ^j	Comp. Treat	Filters ^k	Sizes ^l	Colors	Fibers	Methods ^m	
[12]	1972	-	-	-	-	-	-	-	-	270	F	Dissection	-	-	-	-	-	-	-	Yes	Yes	-	FT-IR	
[13]	1973	-	-	-	-	-	-	Part.	-	-	F	Dissection	-	-	-	-	-	-	-	-	Yes	-	-	
[14]	1976	-	-	-	-	-	-	-	-	-	F	Dissection	-	-	-	-	-	-	-	-	Yes	-	-	
[15]	2010	-	-	-	-	-	-	Yes	Yes	670	F	Dissection	-	-	-	-	-	-	-	Yes	Yes	-	-	
[16]	2011	-	-	-	-	-	-	-	-	31	Ce	Dissection	-	-	-	-	-	-	-	No	-	-	-	
[17]	2011	-	-	-	-	PC	-	-	-	141	F	Dissection	-	-	-	-	-	-	-	Yes	Yes	Excluded	Coloration	
[18]	2011	-	-	-	-	-	-	Yes	-	120	Cr	Dissection	-	-	-	-	-	-	-	-	Yes	Yes	μR + SEM	
[19]	2011	-	-	-	-	-	-	Yes	-	182	F	Dissection	-	-	-	-	-	-	-	-	Yes	Yes	-	
[20]	2012	-	-	-	-	-	-	Yes	-	569	F	Dissection	-	-	-	-	-	-	-	Yes*	Yes	Yes	-	
[21]	2012	-	-	-	-	-	-	Yes	-	425	F	Dissection	-	-	-	-	-	-	-	Yes	Yes	-	-	
[22]	2013	-	-	-	-	-	-	Yes	-	595	F	Dissection	-	-	-	-	-	-	-	Yes	Yes	-	Density	
[23]	2013	-	-	-	AFC	-	-	Yes	Yes	388	F	KOH	10 %	3:1 (v/v)	2-3 w	R.T	-	200 μm (S)	Yes	-	Excluded	FT-IR		
[24]	2013	-	-	-	-	-	-	Yes	Yes	19	F	Dissection	-	-	-	-	-	-	-	Yes	Yes	-	-	
[25]	2013	-	-	-	-	-	-	-	-	192	F	Dissection	-	-	-	-	-	-	-	Yes	-	-	O	
[26]	2013	n.g.	Yes (S)	-	CWS	-	-	-	-	504	F	Dissection	-	-	-	-	-	-	-	Yes	-	Yes	FT-IR	
[27]	2014	-	Yes (MR)	Yes	FH	PB (B)	Subtraction	IA/B	Part.	Part.	50	B	HNO ₃ : HClO ₄	65 % & 68 % - 4:1 (v/v)	5:1 (v/w)	O.N	R.T	Yes	13 μm (C)	Yes	Yes	Yes	Hot needle	
[28]	2014	-	-	-	-	-	-	Yes	-	Part.	F	Dissection	-	-	-	-	-	-	-	Yes	-	Yes	-	
[29]	2014	Yes	Yes (MR)	Part.	AFC	PB (NC)	-	Nothing	Yes	-	93	B	HNO ₃	69 %	7 - 25 mL/indiv	O.N	R.T	Yes	5 μm (CN)	Yes	Yes	Yes	μR	
[30]	2014	-	Yes (R)	-	FH	PB (NC)	-	Yes	Yes	50	B	H ₂ O ₂	30 %	15:1 à 20:1 (v/w)	-	55 - 65°C	Yes	0.8 μm (CN)	-	-	Yes	O		
[31]	2015	-	Yes (MR)	Yes	AFC	-	-	Yes	Yes	125	F	NaCl + H ₂ O ₂	20.5 M + 15 %	250 mL (NaCl)	10 min	50°C (H ₂ O ₂)	-	8 μm (CN)	Yes	-	Excluded	FT-IR		
[32]	2015	-	Yes	Yes	AFC + FH	PB (B)	Subtraction	-	Yes	165	Cr	HNO ₃ : HClO ₄	65 % & 68 % - 4:1 (v/v)	5:1 (v/w)	O.N	R.T	Yes	13 μm (C)	Yes	Yes	Yes	Hot needle		
[33]	2015	-	Yes (MR)	Yes	-	PB	-	IN/B	Yes	144	B	H ₂ O ₂	30 %	200 mL	24 h + 24 - 48 h	65°C + R.T	Yes	5 μm (CN)	Yes	Yes	Yes	μF		
[34]	2015	Yes + I.g.	-	-	-	-	-	Yes	Yes	263	F	Dissection	-	-	-	-	-	-	-	Yes	-	Yes	μF	
[35]	2015	-	Yes (MR)	-	-	AB	Exclusion	IA/B	Part.	-	152	M	KOH	10 %	3:1 (v/v)	O.N	60°C	-	-	Yes	-	Yes	O	
[36]	2015	-	-	-	-	-	-	Yes	Yes	22	F	Dissection	-	-	-	-	-	-	-	Yes	Yes	-	O	
[37]	2015	-	-	Part.	-	PB (NC)	-	-	Yes	-	-	B	HNO ₃	69 %	20 mL/3 indiv	O.N	R.T	Yes	5 μm (CN)	Yes	-	Excluded	μR	
[38]	2015	Yes	Yes (MR)	Yes	FH	PB	Exclusion	IA/B (C)	Yes	Yes	425	B	HNO ₃ : HClO ₄	65 % & 68 % - 4:1 (v/v)	5:1 (v/w)	O.N	R.T	Yes	13 μm (C)	-	Yes	Yes	Hot needle	
[39]	2016	Yes	Yes (R)	-	-	OB	-	Nothing	Yes	-	212	F	Dissection + NaOH	1 M	10 mL	21 d	-	-	-	Yes	Yes	Yes	Hot needle	
[40]	2016	Yes + n.g.	Yes (R+S)	-	RAS	OB (B)	-	-	-	302	F	Dissection	-	-	-	-	-	-	-	Yes	Yes	Yes	FT-IR (A)	
[41]	2016	Yes	Yes (R)	-	FH	PB + AB (NC)	-	IA/B	Yes	Yes	54	B	HNO ₃	69 - 71 %	40 mL	4 h	90°C	Yes	1.2 μm (GF)	-	Yes	Yes	O	
[42]	2016	-	Yes (MR)	Yes	-	PB + PC	-	IN/B	S.F	S.F	390	B	H ₂ O ₂	30 %	200 mL	24 h + 24 - 48 h	65°C + R.T	Yes	5 μm (CN)	Yes	-	Yes	μF	
[43]	2016	-	Yes (S)	-	-	-	-	-	-	205	F	Dissection	-	-	-	-	-	-	-	Yes	Yes	Yes	O	
[44]	2016	Yes + g.	Yes (S)	-	CWS	PB (NC) + AB (NC)	Exclusion	IN/B (C)	Yes	Yes	761	F	KOH	10 %	-	2 w	-	-	-	250 μm (S)	Yes	Yes	Yes	O
[45]	2016	Yes	Yes (R)	-	CWS	OB + AB (NC)	-	-	Yes	-	337	F	Dissection	-	-	-	-	-	-	-	-	Yes	-	
[46]	2016	Yes + g.	Yes (R+S)	-	-	PB + AB (NC)	Specif. Subtract.	IN/B (C)	Yes	Yes	290	F	Dissection	-	-	-	-	-	-	Yes	Yes	Yes	μF (A)(F)	
[47]	2016	Yes	Yes (R)	-	FH	OB (NC)	-	-	Yes	-	30	B	HNO ₃	22.5 M	-	O.N	R.T	Yes	0.7 μm (GF)	-	-	Excluded	PLM	
[48]	2016	-	Yes (H)	-	-	PB + AB (NC)	-	IN/B	Yes	-	64	F	KOH	10 %	> 3:1 (v/v)	3 - 4 d	40°C	-	-	Yes	Yes	-	FT-IR	
[49]	2016	-	-	-	-	-	-	-	Yes	-	1450	Cr	Dissection	-	-	-	-	-	-	-	-	Yes	FT-IR	
[50]	2016	-	-	-	-	-	-	-	Yes	Yes	302	Cr	Dissection	-	-	-	-	-	-	-	Yes	Yes	Yes	O
[51]	2017	n.g.	Yes (R)	-	AFC	PB (NC)	-	Nothing	Yes	-	60	F	NaClO + Methanol	9 % + 99 %	-	O.N	-	Yes	5 μm (CA)	Yes	-	Yes	μR	
[52]	2017	Yes	Yes (MR+S)	-	RAS	PB (NC) + AB (NC)	-	Dif. from Samp.	-	-	-	B	Trypsin	0.3125 %	25 mL	30 min	38-42°C	-	52 μm (G)	Yes	Yes	Yes	FT-IR (A)	
[53]	2017	Yes	Yes (R)	-	AFC	PB (NC) + OB + PC + NC	Subtraction	Nothing	-	-	400	F	KOH	10 %	3:1 (v/v)	2 - 3 w	-	-	200 μm (S)	Yes	Yes	-	FT-IR	
[54]	2017	g.	Yes (S)	-	-	-	-	-	-	-	76	F	Dissection	-	-	-	-	-	-	-	-	Yes	Yes	FT-IR
[55]	2017	Yes	Yes (R+S)	-	CWS	AB (NC)	-	-	-	-	212	F	Dissection	-	-	-	-	-	-	Yes	Yes	Yes	μF	
[56]	2017	-	-	-	-	PB (NC)	-	IA/B	Yes	-	20	F	Dissection	-	-	-	-	-	-	Yes	Yes	Excluded	μF FT-IR (A)	
[57]	2017	Yes	Yes (MR)	-	CWS	OB	-	-	Yes	Yes	417	F	Dissection	-	-	-	-	-	-	-	Yes	-	FT-IR	
[58]	2017	Yes + I.g.	-	-	II	PB (B)	Exclusion	-	-	1337	F	Dissection	-	-	-	-	-	-	-	Yes	Yes	Yes	FT-IR	
[59]	2017	-	Yes (MR)	Yes	-	PB (NC)	-	IA/B	Yes	Yes	378	F	H ₂ O ₂	30 %	200 - 400 mL	24 to 72 h	65°C	Yes	5 μm (CN)	Yes	Yes	Yes	μF	
[60]	2017	Yes + n.g.	Yes (MR)	Yes	AFC + CWS	PB	-	Nothing	Yes	Yes	120	F	KOH	10 %	10:1 (v/w)	72 h	40°C	Yes	149 & 8 μm	-	-	-	μR	
[61]	2017	-	Yes (R)	-	-	PB (B)	Aver. subtract.	IA/B	-	S.F	62	F	Proteinase K	3 - 15 U/mg	-	2 h + 20 min	50°C + 60°C	Yes	1.2 μm (GF)	-	Yes	Yes	O	
[62]	2017	-	Yes (R)	-	-	PB (B)	-	IA/B	-	-	26	M	HNO ₃	-	-	-	-	Yes	0.7 μm (GF)	Yes	Yes	Yes	O	
[63]	2017	-	-	-	FH	PB (NC)	Subtraction	-	-	-	62	F	KOH	20 %	3:1 (v/v)	3 w	R.T	-	0.7 μm (GF)	-	Yes	Yes	O	
[64]	2018	Yes	Yes (R)	Part.	RAS	PB + OB	Exclusion	Nothing	Yes	Yes	120	F	Dissection + KOH	10 %	3:1 (v/v)	5 d	60°C	-	1.2 μm (GF)	Yes	Yes	Yes	μF	
[65]	2018	Yes + n.g.	Yes (R+S)	-	-	-	-	-	Yes	Yes	148	Cr	Dissection	-	-	-	-	-	-	Yes	Yes	Yes	FT-IR (A)	
[66]	2018	-	Yes (R)	-	-	PB (NC)	Subtraction	-	Yes	Yes	160	B	H ₂ O ₂	30 %	20:1 (v/w)	-	55 - 65°C	Yes	1.2 μm (GF)	Yes	Yes	Yes	FT-IR	
[67]	2018	Yes	Yes (R+S)	-	-	OB (B)	-	Fibres	Yes	Yes	72	F	Dissection	-	-	-	-	-	-	Yes	Yes	Yes	μF	
[68]	2018	Yes + n.g.	Yes (MR)	Yes	CWS	PB	-	-	-	-	-	B	KOH	10 %	200 mL	24 h + 24 h	65°C + R.T	Yes	20 μm	Yes	Yes	Yes	μF	
[69]	2018	-	Yes (R)	Yes	-	PB	-	IN/B	S.F	S.F	162	B	H ₂ O ₂	30 %	200 mL	24 h + 24 - 48 h	65°C + R.T	Yes	5 μm (CN)	Yes	-	Yes	μF	

Ref.	Year	Contamination prevention						Sampling					Isolation				Quantification				Identification		
		Cotton lab coat ^a	Cleaning procedure ^b	Sol. filtration	Working place ^c	Blanks ^d	Blanks management	Blank results ^e	Aver. Length ^f	Aver. weight ^f	n ^f	Organism _s ^g	Method	Concentration	Ratio ^h	Time ⁱ	T°C ^j	Comp. Treat	Filters ^k	Sizes ^l	Colors	Fibers	Methods ^m
[70]	2018	-	-	Yes	-	PB (NC)	-	Nothing	-	-	533	F	KOH	10 %	5:1 (v/v)	24-36 h	55°C	Yes	1.6 µm (GF)	Yes	-	Yes	µF
[71]	2018	Yes + g.	Yes (R)	-	-	PB + SB	-	Not synthetic	Yes	-	180	B	KOH	10 %	50 mL	24 h	60°C	Yes	12 µm (CN)	Yes	Yes	Yes	µF
[72]	2018	-	Yes (R)	Yes	CWS + RAS	PB (B)	Specif. Subtract.	IA/B	Yes	Yes	450	B	SDS + Biozym F & SE	50 % (5g/L) + 25 % + 25 %	6 mL	48 h	37.5°C	-	20 µm (PN)	-	-	Yes	FT-IR
[73]	2018	-	-	-	-	PB (NC)	-	IA/B	-	-	163	M	Dissection	-	-	-	-	-	-	-	-	Yes	FT-IR
[74]	2018	Yes	Yes (R)	-	-	-	-	-	Yes	Yes	210	F	Dissection	-	-	-	-	-	-	-	Yes	Yes	FT-IR
[75]	2018	Yes + n.g.	Yes (MR)	Yes	AFC + CWS	PB (NC)	-	Not synthetic	-	S.F	198	F	KOH	10 %	10:1 (v/w)	72 h	40°C	Yes	149 & 8 µm	Yes	-	Yes	µR

a: n.g.: nitrile gloves Lg.: latex gloves g.: gloves
b: S: Observation under stereomicroscope R: Rinsing with water or chemical solution MR: Multiple rinsing H: Heat treatment
c: AFC: Airflow cabinet or assimilated CWS: Cleaned work surface FH: Fumehood RAL: Restricted access laboratory or assimilated II: Infant incubator
d: PB: Procedural blank AB: Atmosphere blank OB: Observational blank PC: Positive control NC: Negative control SB: storage blank (B): applied per batch (NC): Not communicated number
e: IA/B: items average per blank IN/B: items number per blank (C) color mentionned
f: Part.: Partially communicated S.F: Communicated in supplementary files
g: F: Fish, Ce: Cephalopods, Cr: Crustaceans, B: Bivalves, M: Multiple organisms
h: Read 3:1 (v/v) as solution added equivalent to 3 x tissue volume and 10:1 (v/w) as solution added equivalent to 10 mL per gram of tissue
i: O.N stands for overnight
j: R.T stands for room temperature
k: s : sieve CA: Cellulose acetate CN: Cellulose nitrate C: Cellulose GF: glass fiber PN : Plankton net G:gauze
l: Asterisk materialize a study where weight of MP where communicated instead of size
m: O: Observation of characteristics µR: µ-Raman spectroscopy µF: µFT-IR Fourier transformed infrared spectroscopy PLM: Polarized Light Microscopy SEM: Scanning Electron Microscopy (A): Attenuated Total Reflectance used (F): Focal Plan Array used

Table 3

Parameter to check	Information ^a	Parameter to check	Information ^a
Sampling		Filtration	
Species names	E	Cleaning procedure of glass and tools	E
Number of individuals	E	Type of filter used	E
Location (GPS)	E	Procedure of filter storage	D
Depth	D	Counting	
Type of catching	D	Method used (automatic, stereomicroscope, etc)	E
Individual sizes ^b	E	Counting of particles in controls (SAC, DAC, O/SC, FAC, PCE) ^c	E
Commercial size (if risk assessment performed)	D	Counting of particles in samples	E
Whole and tissues weights	E	Particle shape	E
Tissue extraction procedure (sample cleaning, organs concerned)	E	Particle size	E
Time of sample exposition to atmosphere	D	Particle colour	E
Conservation method (freezing, chemicals, etc.)	E	Particle picture	E
Proof of innocuousness of this method on MP	E	Identification	
Time of conservation	D	Number of analysed particles and proportion compared to total isolated particles	E
Work environment		Identification method used (Raman, FTIR, Py-GC/MS, etc.)	E
Type of lab coat used (cotton or other)	E	Evidence of method performance criterion (optimization, validation, etc.)	D
Working place (bench, laminar flow cabinet)	E	Use of standard references	E
Cleaning procedure (chemicals, frequency)	E	Use of positive/negative controls (PIC/NIC) ^d	D
Controls		Information of identification scores & minimal tolerated value	E
Description (SAC, DAC, O/SC, FAC, PCE) ^c	E	Identification by second method for unknown	D
Numbers	E	Data analysis	
Location and for which step	E	Availability of the whole results (samples, controls, etc.)	D
Area covered by controls	D	Description of how controls results were taken into account	E
Time of exposition	D	Identification results of PIC/NIC ^d	D
Digestion		Identification results (expressed as a % of analysed particle)	E
Use of filtered reagents exempt of MP	E	Information on % misidentified or unidentified results	E
Cleaning procedure of glass and tools	E	Clear separation between MP and other particles	E
Used chemical	E	Use of adequate units (MP/g & MP/indiv)	E
Proof of innocuousness of this method on MP	E	Estimation of MP mass based on identification & size	D
Recovering rates with the method	E		
Relative proportion chemical/tissue	E		
Model of used devices	E		
Type of heating source	D		
Temperature set to the device	E		
Temperature in the digestate	D		
Temperature monitoring across digestion	D		
Duration of digestion	E		
Agitation speed	E		

^a Essential (E) and desirable (D) information for the MP studies

^b Based as an example on the Ifremer report "Guide for measuring species in fisheries Fishes, mollusks, shellfishes, marine reptiles, marine mammals" (<http://archimer.ifremer.fr/doc/00001/6237/>)

^c SAC: sampling atmospheric control, DAC: digestion atmospheric control, O/SC: Operator/Solution Control, FAC: filtration atmospheric control and PCE: Positive Control of Extraction

^d PIC: Positive Identification Control and NIC: Negative Identification Control