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

Alexandre Dehaut, Ludovic Hermabessiere, Guillaume Duflos

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1 **Title**

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

3 **Authors**

4 Alexandre Dehaut\*, Ludovic Hermabessiere & Guillaume Duflos

5 **Affiliations**

6 ANSES – Laboratoire de Sécurité des Aliments, Boulevard du Bassin Napoléon, 62200 Boulogne-sur-  
7 Mer, France.

8 \*Corresponding Author: Alexandre Dehaut - alexandre.dehaut@anses.fr

9 **ORCID:**

10 Alexandre Dehaut: 0000-0001-5377-995X

11 Ludovic Hermabessiere: 0000-0002-6775-2480

12 Guillaume Duflos: 0000-0003-2820-4411

13 **Abstract**

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

24 **Keywords**

25 Microplastics; fishery products; methods; limits; recommendations

26 **Abbreviations**

27 ATR: attenuated total reflection, DAC: digestion atmospheric control, FAC: filtration atmospheric  
28 control, FAO: Food and Agriculture Organization, FPA: focal plan array, FT-IR: Fourier-transform  
29 infrared, GESAMP: Group of Experts on the Scientific Aspects of Marine Environmental Protection,  
30 H<sub>2</sub>O<sub>2</sub>: hydrogen peroxide, HClO<sub>4</sub>: perchloric acid, HNO<sub>3</sub>: nitric acid, KOH: potassium hydroxide, LC<sub>50</sub>:  
31 lethal concentration 50%, LD<sub>50</sub>: lethal dose 50%, LOAEL: Lowest observed adverse effect level,  
32 MIMS: Minimum Information for publication of Microplastics Studies, MP: microplastic, MSFD:  
33 Marine Strategy Framework Directive, NaClO: sodium hypochlorite, NIC: negative identification  
34 control, NOAEL: No observed adverse effect level, O/SC: operator/solution control, OSPAR:  
35 Convention for the Protection of the Marine Environment of the North-East Atlantic, PCE: positive  
36 control of extraction, PIC: positive identification control, Py-GC/MS: pyrolysis coupled with gas  
37 chromatography and mass spectrometry, SAC: sampling atmospheric control

## 38 1. Introduction

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

45 Consequently, vast quantities of plastic have accumulated in the oceans all around the globe, and these  
46 plastics are subject to degradation through various processes including UV degradation, oxidisation, and  
47 abrasion. The sizes of these pieces of plastics cover nearly 12 log scales from meter to nanometre [3],  
48 facilitating their ingestion by a wide range of marine organisms from the largest such as sperm whales  
49 [4] to the smallest, *i.e.* copepods [5]. Among all these synthetic particles, microplastics (MP) are  
50 commonly defined as plastic items with a size below 5 mm [6].

51 There are many questions surrounding the ecological and human health risks posed by MP. There have  
52 been few studies on the direct, for example physical effects of MP, or indirect harms caused either by  
53 bacteria or by chemicals. Concerning indirect harms, it has been demonstrated that MP are covered by  
54 numerous bacterial genera [7], including pathogenic and non-pathogenic *Vibrio* species [8]. MP are also  
55 a vector of hydrophobic organic compounds (HOC), but the actual risk is a subject of debate [9]. Finally,  
56 another suspected hazard is related to the presence of numerous additives in plastic that can make up  
57 60% of the total weight [10], and that can leach out from the MP [11]. Bacterial and chemical hazards  
58 are thus more related to MP surfaces and volumes; the current definition of MP, solely based on length,  
59 therefore does not seem accurate enough for risk assessment.

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

64 improve and harmonise methods for the future studies, the different steps of MP analysis: sampling,  
65 isolation, quantification, polymer identification, and contamination management were analysed and  
66 compared based on the available literature. For the period from 1973 to April 2018, 64 studies published  
67 were gathered from Scopus and PubMed, based on their main subject dealing with both microplastics  
68 and seafood. These publications were analysed using an *ad hoc* reading grid. This corpus comprises 3  
69 articles published before 2010 [12-14], 5 between 2010 and 2012 [15-19], 7 between 2012 and 2014  
70 [20-26], 12 between 2014 and 2016 [27-38], and 37 between 2016 and 2018 [39-75].

## 71 **2. Definition and size issues**

### 72 **2.1 Current definitions and limits**

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

### 86 **2.2 Limitations of current definitions and proposals for improvement**

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

89 definition is not sufficiently clear and is not suitable for the current challenges in environmental and  
90 human toxicological risk assessment [84]. Moreover, it has been highlighted that regarding particle sizes  
91 and shapes, differences in toxicity are recorded [85]. These limits are illustrated in the first half of [Table](#)  
92 [1](#). Three particles namely a cube, sphere and fibre measuring 5 mm at the largest part of one of their  
93 dimensions are proposed. The cube has a 4-log scale higher volume compared to the fibre. Similarly,  
94 when equal volumes are considered, in the second half of [Table 1](#), the surface area of the fibre is 2-log  
95 scale larger than that of the cube. Due to their morphology, there are considerable differences between  
96 cubes and spheres on the one hand, and fibres on the other. Cubes and spheres are characterised by large  
97 volumes, while fibres have very small volumes but large surfaces of contact. The need for a “three-  
98 dimensional” definition is also echoed in recent studies [86-88]. Indeed, these dimensions will be helpful  
99 to estimate particles weights, depending on polymer types. Weight estimates are requested by scientists  
100 based for different reasons: 1) because plastic inputs in ocean are usually expressed in metric tons, and  
101 2) because identification technology using mass spectrometry, such as pyrolysis coupled with gas  
102 chromatography and mass spectrometry (Py-GC/MS), processes signals related to analytes mass.  
103 Likewise, having an estimate of particle weights coupled with identification of the polymer would be  
104 helpful for toxicological approaches. Clearly, the main toxicological dose descriptors, such as the no  
105 observed adverse effect level (NOAEL), lowest observed adverse effect level (LOAEL), lethal dose  
106 50% (LD<sub>50</sub>) or lethal concentration 50% (LC<sub>50</sub>), are usually expressed as a mass (mg) per mass (kg bw,  
107 kg) or volume (L) per duration (day or hours).

108 **TABLE 1 goes here (if possible)**

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

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

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

### 133 **3. Laboratory environment and prevention of contamination**

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

137 **FIGURE 1 goes here (if possible)**

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

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

### 144 **3.1 Contamination from the operator**

145 With regard to prevention of contamination by the operator, only 36% of the studies reported that the  
146 operators wore cotton lab coats. Nonetheless, behind this overall statistic, it should be noted that  
147 proportions are evolving. Since the first mention of cotton lab coat in a publication in 2014 [29], the  
148 number of publications has increased each year and, in 2018, 58% of publications (n=12) clearly  
149 specified its use [64, 65, 67, 68, 71, 74, 75]. Some interesting additional pieces of information were  
150 provided by two studies. The first, mentioned by Bråte et al. [40], indicates that cotton lab coats were  
151 cleaned with a sticky roller. This detail could be important bearing in mind that lab coats can be cleaned  
152 either at industrial or local laboratory laundries. In the cleaning machines, lab coats can be mixed with  
153 other kinds of fabrics with possible synthetic fibre transfer. The second idea, developed by Kühn et al.  
154 [67], is the use of coloured cotton lab coats. This would help scientists to systematically exclude a given  
155 type of coloured fibre. However, the colour choice has to be oriented towards the less encountered  
156 colours, which is not always easy. Prevention of contamination by the operator could also be considered  
157 through the use of gloves, as operators' fingers can carry fibres (Figure 1 B). Overall, 20% of the corpus  
158 articles (Table 2) clearly mentioned that gloves were worn. Here again, these practices tend to be more  
159 widespread than a few of year. Wearing gloves has two advantages: contamination prevention and  
160 operator protection from harmful chemical products that can be used to destroy biological tissues (see  
161 4.3). Special care should be taken to ensure that no MP are present at the surface of gloves by keeping  
162 them in a protected box, and cleaning them using filtered water/alcohol solutions or compressed air.



### 3.2 Contamination from the work environment

163  
164 Considering the work environment, two parameters should be considered: the place where samples are  
165 handled, and the tools, materials and equipment used to carry out the experiments. Cleaning procedures  
166 are more or less well described in the publications. In all, 61% of the corpus (Table 2) has an explicit  
167 mention of this procedure, with this percentage rising to 80% for the two last years (n=25). Cleaning  
168 procedures are highly diverse, but 87% of them involve the use of liquid rinsing. The solution or  
169 chemicals used for this step are water (tap, deionised, purified, ultrapure, MilliQ), alcohol (either not  
170 defined or ethanol) and acetone. In 35% of these procedures, a combination of solutions or replication of  
171 rinsing with three successive rinsing steps are used. In five studies, cleaning was followed by control  
172 under a stereomicroscope [43, 46, 52, 65, 67]. Only four studies performed solely a stereomicroscope  
173 check, without a rinsing procedure [26, 44, 54, 55]. Finally, a single work used glassware heat treatment  
174 by heating glass at 550°C for 4H before its use [48]. Unfortunately, heating glassware is only feasible  
175 for small containers, while, due to samples sizes, large volumes of solution can be required for the  
176 digestion of tissues.

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

189 (Table 2), since 2017, 61% of the articles did not clearly describe a specific place for carrying out  
190 analyses, and more than half of the studies still do not described a way of preventing contamination in  
191 the working area. This fact raises concerns regarding the levels of MP contamination reported in the  
192 literature. Concerning studies that used strategies to prevent contamination of the work place, only  
193 12.5% (n=8) mentioned the use, even partially, of a type of airflow cabinet during the study on  
194 microplastics in samples [23, 29, 31, 32, 51, 53, 60, 75]. The other most commonly used work areas are  
195 basic cleaned laboratory benches (10.9%; n=7) [26, 44, 45, 55, 57, 68, 72] and fume hoods (10.9%; n=7)  
196 [27, 30, 32, 38, 41, 47, 63]. A single study reported the use of both a fume hood and laminar flow  
197 cabinet [32]. Three studies (4.7%) indicated that analyses were performed in specific laboratories, with  
198 controlled circulation [40, 52, 64]. Finally, one study even used an infant incubator to process samples  
199 [58], which can be considered as a highly protected environment under reserve of sealing the place  
200 where the arms come through.

### 201 **3.3 Contamination from used solutions**

202 Although an essential parameter, the filtration of solutions is mostly not mentioned in publications, as  
203 75% of the corpus (Table 2) did not specify any filtration of the solutions used, even when chemical  
204 approaches were employed to digest tissues. Despite this figure, it should be noted that half of the  
205 experiments using filtered solutions were reported in studies published since 2017. In fact, even the use  
206 of “deionised, purified, ultrapure, MilliQ” water could be subject to recontamination after water  
207 circulation through the filter, membrane and resin. Filtration of solutions, as well as stereomicroscopic  
208 control of used filters, are mandatory for MP studies.

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

### 215           **3.4    Controls of contamination**

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

226    There is a lack of precision and description of controls in the publications; the corpus was only studied  
227    based on “procedural”, “atmosphere” and “observational” controls (Table 2). Before 2014, there is no  
228    mention of blanks or controls. Since 2014, although 73.5% of articles mention a procedural blank, only  
229    18.4% and 10.2% noted the use of atmosphere and observational controls, respectively. A few  
230    publications also propose new controls, namely positive/negative controls [42, 53] or control of sample  
231    containers [71]. Positive controls aim to check whether plastic present in samples is accurately  
232    recovered during the isolation process, whereas negative controls are more difficult to implement, as no  
233    MP-free matrix exists. The number of controls reported in the different studies can vary, and there is no  
234    clear statement on the appropriate number of controls to be performed. Some publications suggest  
235    applying controls to each analysed batch of samples [27, 32, 40], which might be encouraged for future  
236    standardisation. Nonetheless, overall the number of controls should not be too high compared with  
237    samples.

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

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

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

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

### 271 **3.5 Proposals for standardisation**

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

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

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

287 **FIGURE 2 goes here (if possible)**

## 288 **4. Sampling and isolation**

### 289 **4.1 Sampling for studies on MP in seafood**

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

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

297 Certain localisation parameters should also be recorded such as GPS coordinates, catching depths and  
298 types of capture (trawl type, mesh size, etc.). These data are easily recovered for scientific catches [26,  
299 51, 67], but this could be more difficult when samples are purchased directly from fishermen or market  
300 or sold as processed seafood [35, 60, 75].

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

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

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

#### 321 **4.2 Tissue preservation before processing**

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

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

346 **TABLE 2 goes here (if possible)**

### 347 **4.3 Dissection and digestion methods for MP studies**

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

354 Dissection is very easy to set up and implement, and it is not expensive. This could explain why it is  
355 generally preferred for MP studies, and particularly for very large sampling sizes. It accounts for more  
356 than half of the whole corpus (53%) (Table 2). Nonetheless, there are some issues concerning the use of  
357 dissection for MP isolation. The first disadvantage is the sample inspection time, which can be as long as  
358 10 min to 1h30 [39, 45, 57], leading to a high risk of contamination from the work environment. The  
359 second important issue with this methodology is the risk of omitting particles [17, 25]. The first reason  
360 for these omissions is that dissections are sometimes performed with the naked eye, which is not  
361 accurate enough to distinguish all MP. The second reason is that depending on their size and shape, MP  
362 can be difficult to observe among the contents of the alimentary tract.

363 Concerning chemical digestion of tissues, different methods have been described. These include  
364 enzymatic methods (trypsin, proteinase K, mix lipase/amylase/protease), oxidative methods (sodium  
365 hypochlorite (NaClO) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)), acid methods (nitric acid (HNO<sub>3</sub>) or a mixture of  
366 HNO<sub>3</sub> and perchloric acid (HClO<sub>4</sub>)), and basic methods (potassium hydroxide (KOH)). The use of KOH  
367 is the most commonly described of the chemical methods used in the studies of the corpus (17%) (Table  
368 2). This chemical is mostly used at a concentration of 10%, except in one publication where a  
369 concentration of 20% was tested [63]. KOH was used to digest both bivalves [35, 68, 71] and fish



370 tissues [23, 35, 44, 48, 53, 60, 63, 70, 75]. KOH has the advantage of having no deleterious effects on  
371 several polymer types [95, 96]. KOH must, however, be handled with caution, as it is a corrosive  
372 compound. In the corpus (Table 2), eight studies (13%) proposed the use of oxidising solutions such as  
373 9% NaClO on fish stomach contents [51], and 30% H<sub>2</sub>O<sub>2</sub> on bivalves [30, 33, 42, 59, 69] and fish organs  
374 [31, 59, 66]. H<sub>2</sub>O<sub>2</sub> is the second most commonly used technique to digest seafood tissues, mostly  
375 bivalves. The main concern with oxidising solutions is their stability over time. This must therefore be  
376 taken into account in the context of method standardisation. Echoing what was previously discussed (see  
377 3.4) the use of DAC, O/SC and PCE is highly encouraged during the digestion step.

378 A total of eight studies using acid approaches were found in the corpus (Table 2), among which five  
379 (8%) used 69% nitric acid [29, 37, 41, 47, 62] and three (5%) used a 65% HNO<sub>3</sub>: 68% HClO<sub>4</sub> 4:1 (v/v)  
380 mixture [27, 32, 38]. The nitric acid method has only been applied to bivalves, while the acid mixture  
381 was applied to both bivalves and crustaceans. The main drawback of the acid approach is its adverse  
382 effect on polymers, described in numerous studies [95, 97, 98]. Three studies (5%) used enzymatic  
383 digestion for the analysis of bivalve tissues [52, 72] and fish gut [61]. Enzymatic approaches can be  
384 considered mild approaches, and they usually do not require temperatures above 40°C. The second  
385 advantage of this type of approach is that enzymes have no impact on synthetic polymers. One of the  
386 disadvantages of such methods is the use of a multistep analysis, involving multiple solutions with a  
387 higher risk of contamination. As an example, enzymatic digestion was not sufficiently effective on fish  
388 gut [61], and an additional step using 30% H<sub>2</sub>O<sub>2</sub> was necessary. Moreover, these approaches can  
389 sometimes be time consuming. Finally, depending on the method, hazardous reactions can occur, which  
390 is not compliant with good hygiene and safety practices. As an example, H<sub>2</sub>O<sub>2</sub> heating can generate O<sub>2</sub>,  
391 and NaClO should not be put in acidic conditions because of the release of the highly toxic Cl<sub>2</sub>  
392 compound.

#### 393 **4.4 Filtration as the last step in the isolation process**

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

396 corpus (Table 2) (n=31) are 250 µm (3%), 200 µm (6%), 149 µm (6%), 52 µm (3%), 20 µm (6%), 13  
397 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  
398 µm (10%). Regarding these studies, and looking at the filters used, it appears that 61% (n=19) chose a  
399 retention diameter below 10 µm. This makes it possible to retain very small MP below the limit of  
400 detection of certain identification techniques used downstream (see 5.3). Concerning filter composition,  
401 the most common materials are nitrocellulose (29%) and glass fibre (26%). For future studies, a  
402 compromise between filtration efficacy, *i.e.* absence of clogging and absence of interference for  
403 identification, and MP retention needs to be found and then put forward as a standard.

#### 404 4.5 Proposals for standardisation

405 Based on these findings, some advice can be proposed concerning sampling and isolation, in order to  
406 standardise practices. A minimum of 50 individuals must be sampled per studied species (Figure 3),  
407 even though statistical analyses are required to assess whether this is representative of population  
408 variability. It is important to gather metadata as much as possible, including GPS coordinates for the  
409 catch. The second important point to keep in mind is to select individuals that correspond to the  
410 commercial size, if risk assessment for human health is considered. Biometric data, *i.e.* whole size,  
411 whole weight and analysed tissue weight are mandatory for study reports in order to improve  
412 standardisation of digestion techniques. Once sampled, tissues are to be stored as frozen samples, since  
413 the effects of chemical preservatives on polymers are not clearly documented. For particle isolation, the  
414 chemical approach should be given preference (Figure 3) as it ensures low exposure of tissues to  
415 ambient air and recovery of smaller particles. Two methods are popular in the research community but,  
416 to the best of author knowledge, the method using 10% KOH at 60°C for 24H is the one whose impact  
417 has been assessed most closely; additionally, this solution is more stable over time compared to 30%  
418 H<sub>2</sub>O<sub>2</sub>.

419 **FIGURE 3 goes here (if possible)**

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

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

## 432 5. Quantification and identification

### 433 5.1 Quantification strategies for particle isolation in seafood

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

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

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

447 corpus (Table 2). Six publications explicitly mention that fibres were not included in their evaluation  
448 because they were considered laboratory contamination [17, 23, 31, 37, 47, 56]. Nonetheless, it is  
449 important to remember that fibres are produced in high quantities [2] and they are not retained by most  
450 wastewater treatment plants [103]. With this in mind and with the aim of improving prevention of  
451 contamination (see 3.5); it becomes difficult to rule out fibres when studying MP in seafood. Finally,  
452 22% of studies did not mention fibres; however, most of these studies were carried out before 2013. As a  
453 reminder, a list of MP item types was provided by Lusher et al. [104]. It includes fragments, fibres,  
454 beads, foams, and pellets.

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

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

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

## 464 **5.2 Orientation tests: selection of putative polymers**

465 Once quantification has been performed, there is a need for identification in order to discriminate real  
466 MP from non-synthetic particles. Studies that did not perform any identification or orientation represent  
467 16% of the corpus (Table 2). Except for one publication [45], all these works were published before  
468 2015, showing the attention paid in recent years to better characterisation of isolated particles. Since  
469 2015, the absence of identification seems to have been replaced by orientation tests, but it is important to  
470 separate orientation techniques from identification methods. The first indicates the suspected synthetic  
471 nature of a particle, while identification leads to clear determination of the polymer composing the MP.

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

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

### 481 **5.3 Identification methods to ascertain the nature and quantities of true MP**

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

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

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

498 method development, repeatability, and theoretical limits of detection. Such approaches should also be  
499 encouraged for spectroscopic methods allowing for better comparison of results from different studies.  
500 As an example of harmonisation, among all FT-IR studies (n=21), only 52% provided a threshold  
501 beyond which the identification of the polymer's nature is certain, and this threshold varied from 60% to  
502 85%. None of the six studies performing Raman identification provided such information. Working with  
503 the same parameters would help to harmonise identification methods and ultimately enable sharing of  
504 research databases, increasing the diversity of spectra available to everyone, and thereby the power of  
505 identification of the different tools [102]. Finally, it could be suggested to add positive identification  
506 controls (PIC), *i.e.* a single polymer or multiple known polymers, and negative identification controls  
507 (NIC), *i.e.* a single polymer or multiple natural polymers such as cotton or chitin, to ascertain proper  
508 functioning of the analytical device for each batch or particle analysed.

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

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

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

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

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

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

#### 560 **5.4 Proposals for standardisation**

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

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

576 **FIGURE 4 goes here (if possible)**



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

## 583 **6. Minimum information for publication of microplastics studies**

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

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

592 **TABLE 3 goes here (if possible)**

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

## 602 **7. Conclusion**

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

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

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

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

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963

## 964 Captions

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

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

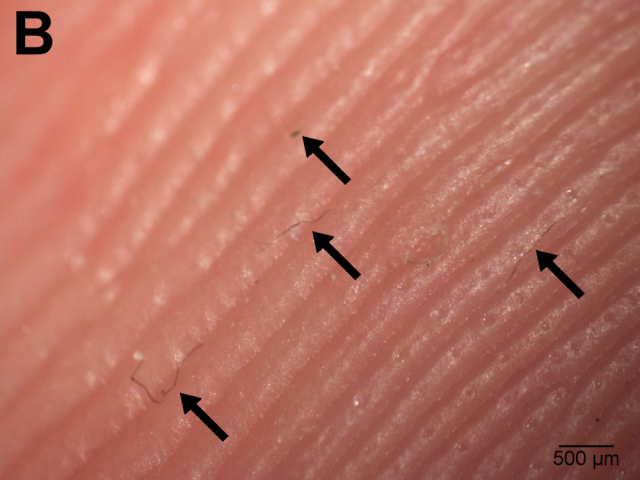
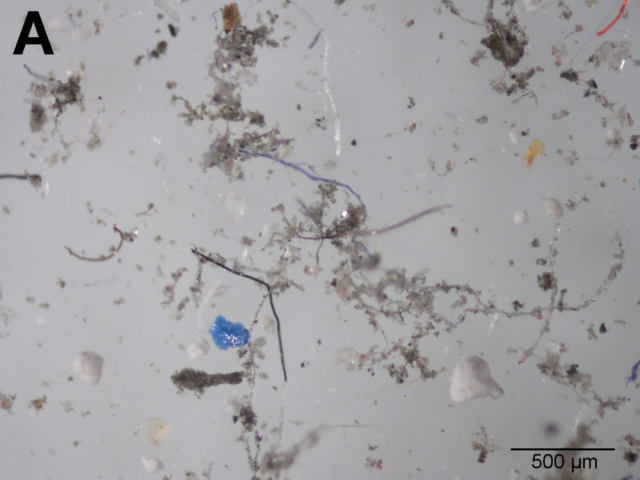
968 **Figure 3: Schematic representation of proposal for standardization of sampling and isolation. SAC: sampling**  
 969 **atmospheric control, PEC: positive extraction control, O/SC: operator/solution control, DAC: digestion atmospheric**  
 970 **control, FAC: filtration atmospheric control.**

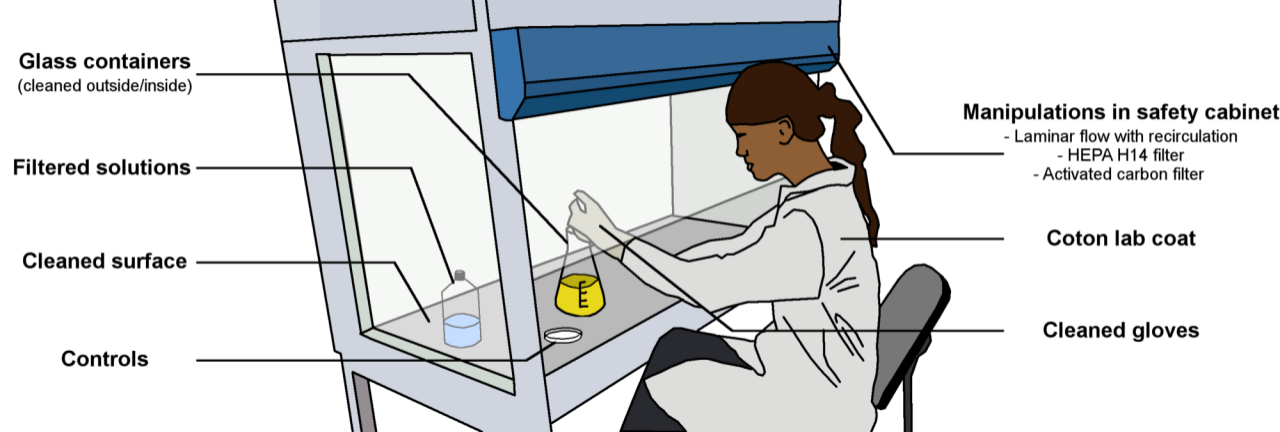
971 **Figure 4: Schematic representation of proposal for standardization of quantification and identification. PE:**  
 972 **polyethylene, PP: polypropylene, PS: polystyrene,  $\mu$ Raman: Raman microspectroscopy,  $\mu$ FT-IR: Fourier-transform**  
 973 **infrared microspectroscopy, Py-GC/MS: pyrolysis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

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

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

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





## SAMPLING

SAMPLES



50 individuals minimum



Tissue digestion

## DIGESTION



6% Ratio  
Tissue:Volume



Time



TC



CONTROLS



BAC



FEC



D/SC



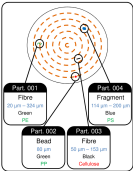
DAC

## FILTRATION

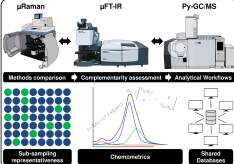


FAC

## QUANTIFICATION (AUTOMATION)



## IDENTIFICATION



**Table 1**

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

<sup>a</sup> Fibre is here considered as a cylinder

<sup>b</sup> 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.

<sup>c</sup> 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 <sup>a</sup>	Cleaning procedure <sup>b</sup>	Sol. filtration	Working place <sup>c</sup>	Blanks <sup>d</sup>	Blanks management	Blank results <sup>e</sup>	Aver. Length <sup>f</sup>	Aver. weight <sup>f</sup>	n <sup>f</sup>	Organism <sup>g</sup>	Method	Concentration	Ratio <sup>h</sup>	Time <sup>i</sup>	T°C <sup>j</sup>	Comp. Treat	Filters <sup>k</sup>	Sizes <sup>l</sup>		Colors	Fibers	Methods <sup>m</sup>
[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.	50	B	HNO <sub>3</sub> : HClO <sub>4</sub>	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 <sub>3</sub>	69 %	7 - 25 mL/individ	O.N	R.T	Yes	5 μm (CN)	Yes	Yes	Yes	μR		
[30]	2014	-	Yes (R)	-	FH	PB (NC)	-	-	Yes	50	B	H <sub>2</sub> O <sub>2</sub>	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 <sub>2</sub> O <sub>2</sub>	20.5 M + 15 %	250 mL (NaCl)	10 min	50°C (H <sub>2</sub> O <sub>2</sub> )	-	8 μm (CN)	Yes	-	Excluded	FT-IR		
[32]	2015	-	Yes	Yes	AFC + FH	PB (B)	Subtraction	-	Yes	165	Cr	HNO <sub>3</sub> : HClO <sub>4</sub>	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 <sub>2</sub> O <sub>2</sub>	30 %	200 mL	24 h + 24 - 48 h	65°C + R.T	Yes	5 μm (CN)	Yes	Yes	Yes	μF		
[34]	2015	Yes + l.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 <sub>3</sub>	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	425	B	HNO <sub>3</sub> : HClO <sub>4</sub>	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 (R)	-	FH	PB + AB (NC)	-	IA/B	Yes	54	B	HNO <sub>3</sub>	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 <sub>2</sub> O <sub>2</sub>	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	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	290	F	Dissection	-	-	-	-	-	-	-	Yes	Yes	Yes	μF (A)(F)	
[47]	2016	Yes	Yes (R)	-	FH	OB (NC)	-	-	Yes	30	B	HNO <sub>3</sub>	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	302	Cr	Dissection	-	-	-	-	-	-	-	-	-	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	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	417	F	Dissection	-	-	-	-	-	-	-	-	Yes	-	FT-IR	
[58]	2017	Yes + l.g.	-	-	II	PB (B)	Exclusion	-	-	1337	F	Dissection	-	-	-	-	-	-	-	Yes	Yes	Yes	FT-IR	
[59]	2017	-	Yes (MR)	Yes	-	PB (NC)	-	IA/B	Yes	378	F	H <sub>2</sub> O <sub>2</sub>	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	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	-	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 <sub>3</sub>	-	-	-	-	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	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	148	Cr	Dissection	-	-	-	-	-	-	-	Yes	Yes	Yes	FT-IR (A)	
[66]	2018	-	Yes (R)	-	-	PB (NC)	Subtraction	-	Yes	160	B	H <sub>2</sub> O <sub>2</sub>	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	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	162	B	H <sub>2</sub> O <sub>2</sub>	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 <sup>a</sup>	Cleaning procedure <sup>b</sup>	Sol. filtration	Working place <sup>c</sup>	Blanks <sup>d</sup>	Blanks management	Blank results <sup>e</sup>	Aver. Length <sup>f</sup>	Aver. weight <sup>f</sup>	n <sup>f</sup>	Organism <sup>g</sup>	Method	Concentration	Ratio <sup>h</sup>	Time <sup>i</sup>	T°C <sup>j</sup>	Comp. Treat	Filters <sup>k</sup>	Sizes <sup>l</sup>	Colors	Fibers	Methods <sup>m</sup>
[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 <sup>a</sup>	Parameter to check	Information <sup>a</sup>
<b>Sampling</b>		<b>Filtration</b>	
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		
Type of catching	D	<b>Counting</b>	
Individual sizes <sup>b</sup>	E	Method used (automatic, stereomicroscope, etc)	E
Commercial size (if risk assessment performed)	D	Counting of particles in controls (SAC, DAC, O/SC, FAC, PCE) <sup>c</sup>	E
Whole and tissues weights	E	Counting of particles in samples	E
Tissue extraction procedure (sample cleaning, organs concerned)	E	Particle shape	E
Time of sample exposition to atmosphere	D	Particle size	E
Conservation method (freezing, chemicals, etc.)	E	Particle colour	E
Proof of innocuousness of this method on MP	E	Particle picture	E
Time of conservation	D		
<b>Work environment</b>		<b>Identification</b>	
Type of lab coat used (cotton or other)	E	Number of analysed particles and proportion compared to total isolated particles	E
Working place (bench, laminar flow cabinet)	E	Identification method used (Raman, FTIR, Py-GC/MS, etc.)	E
Cleaning procedure (chemicals, frequency)	E	Evidence of method performance criterion (optimization, validation, etc.)	D
		Use of standard references	E
		Use of positive/negative controls (PIC/NIC) <sup>d</sup>	D
		Information of identification scores & minimal tolerated value	E
		Identification by second method for unknown	D
<b>Controls</b>		<b>Data analysis</b>	
Description (SAC, DAC, O/SC, FAC, PCE) <sup>c</sup>	E	Availability of the whole results (samples, controls, etc.)	D
Numbers	E	Description of how controls results were taken into account	E
Location and for which step	E	Identification results of PIC/NIC <sup>d</sup>	D
Area covered by controls	D	Identification results (expressed as a % of analysed particle)	E
Time of exposition	D	Information on % misidentified or unidentified results	E
		Clear separation between MP and other particles	E
		Use of adequate units (MP/g & MP/indiv)	E
		Estimation of MP mass based on identification & size	D
<b>Digestion</b>			
Use of filtered reagents exempt of MP	E		
Cleaning procedure of glass and tools	E		
Used chemical	E		
Proof of innocuousness of this method on MP	E		
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		

<sup>a</sup> Essential (E) and desirable (D) information for the MP studies

<sup>b</sup> 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/>)

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

<sup>d</sup> PIC: Positive Identification Control and NIC: Negative Identification Control

