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Critical Review

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Plastic particle ingestion by wild freshwater fish: a critical review

France Collard*[1,2], Johnny Gasperi[1,3], Geir W. Gabrielsen[2], Bruno Tassin[1]

[1]Laboratoire Eau Environnement et Systèmes urbains (LEESU), Université Paris-Est Créteil, 61 avenue du Général de Gaulle, 94010 Cedex Créteil, France.

[2]Norwegian Polar Institute, Fram Centre, NO-9296 Tromsø, Norway.

[3]Water and Environment Laboratory (LEE), Geotechnical engineering, Environment, Natural hazards and Earth Sciences Department (GERS), French Institute of Science and Technology for Transport, Development and Networks (IFSTTAR), IRSTV, 44340 Bouguenais, France.

*Corresponding author:

francecollard16@gmail.com

phone: +4777750655

Current address:

Norwegian Polar Institute

Research Department

Hjalmar Johansens gate 14

9296 Tromsø, Norway

25 Graphical abstract

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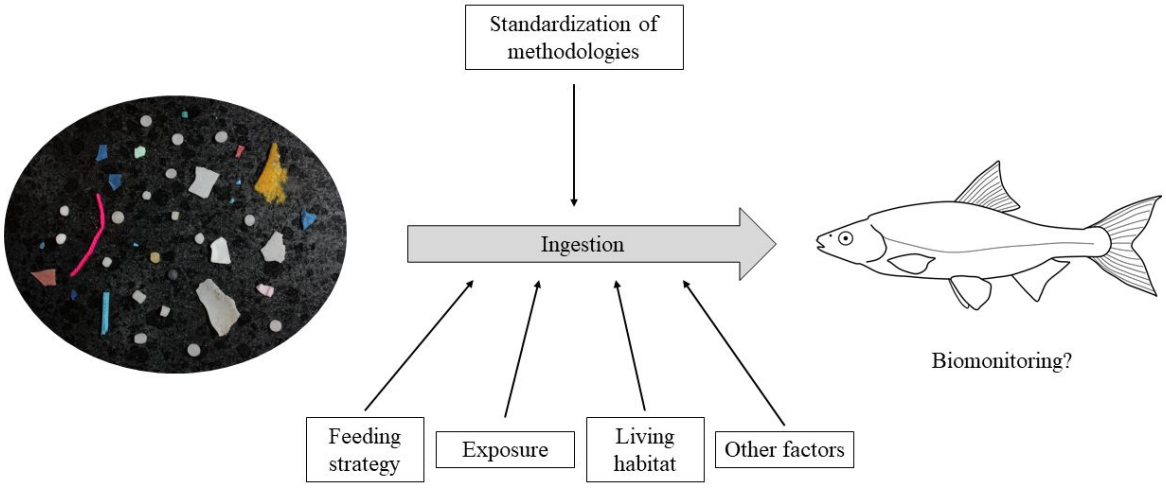

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Abstract

Plastic pollution, especially microplastics (MP) pollution, is a hot topic in both mainstream media and scientific literature. Although rivers are potentially the major transport pathway of this pollution to the sea, plastic contamination in freshwater bodies is comparatively understudied. Microplastic pollution in freshwater fish is of growing interest, and while few studies exist, discrepancies do occur in the sampling, extraction, and identification of MP and in the expression of the results. Even though those differences hamper comparisons between some studies, a comparative work has been performed to identify the factors influencing MP ingestion by fish and consequently to target potential ecological traits that can be used to monitor species. Monitoring plastic ingested by fish will give relevant ecological information on MP pollution. This review focuses on MP ingestion by wild freshwater and estuarine fish. In addition to providing an overview of the existing data concerning contamination levels in wild freshwater fish, we aimed to (1) propose several overall recommendations on the methodologies applicable to all biota, (2) compare MP contamination levels in fish and in their environment, and (3) determine which parameters could help to define fish species for monitoring.

1. Introduction

Due to their exponentially increasing production since the 1950s,[1] plastic materials are polluting all types of environments: marine surface waters,[2,3] deep-sea sediments,[4,5] arctic sea ice,[6,7] soils,[8] and even air.[9,10] As an expected and direct consequence, the number of species or taxa exposed to plastic pollution is alarming.[11] Plastic pieces of all sizes are found in the

environment, and thus are ingested by many different organisms: marine mammals,[12-14] marine and terrestrial birds,[15-17] crustaceans,[18,19] worms,[20] and fish.[21-23] Scientific research is currently more focused on the marine environment. The reasons for this focus are probably due the high economic value of marine resources and abundant funding allocated to marine research and monitoring. In particular, microplastics (MP), which are plastic pieces with sizes less than 5 mm,[24] are well known to pollute seas and oceans. Numerous laboratory studies have shown that this exposure leads to plastic ingestion by various organisms and is associated with some negative impacts,[25] such as neurotoxicity,[26] a change in swimming behavior[27] and reduction of predatory performances.[28]

Human populations are closely linked to water from both marine and freshwater sources. A considerable fraction of the human population lives in the near-coastal zone.[29] Approximately half of the world's population lives within 3 km of a freshwater body and only 10% of the population lives farther than 10 km away.[30] However, given that proximity, between 1.15 and 2.41 million tons of plastics are released into the oceans via rivers each year.[31] Conventional wastewater treatment plants may act as an MP source to rivers,[32,33] and inappropriate waste management[34] combined with a high population density[35] may both be positively correlated with riverine plastic loads.

Increasing number of studies have revealed that freshwaters are contaminated, sometimes largely by plastic, regardless of its size.[36-39] The number of studies about MP pollution and its impacts is growing (in March 2019, searching the Web of Science for "plastic marine pollution" returned 3 results in 2000 and 351 results in 2018), but a small number of these studies deal with a freshwater environment (0 out of the 3 results found in 2000 and 42 out of the 351 results found in 2018). The same observation can be made for fish; searching the Web of Science for the keywords "fish plastic pollution" gave 4 results in 2000 and 99 in 2018, in which 0 and 10 results concern freshwaters, respectively. Despite the large amounts of plastic debris input into

seas and oceans by rivers, the interactions between these debris and the biota of these ecosystems are poorly studied. Nevertheless, some discrepancies in performed protocols do occur. Among others, the sampling size is frequently too small, and MP identification often relies on visual sorting. In addition, the results are reported in one or two units while at least three are used in publications. Those discrepancies lead to difficulties in comparing data.

Fish are the main taxa studied with regard to MP ingestion in freshwater environments[40] and constitute an economically, ecologically and highly diversified group.[41,42] Microplastic ingestion by freshwater fish has been studied worldwide, but these studies have although focused on limited regions (Fig. 1) in comparison with the studies of plastic ingested by wild marine fish.[43] In total, the studies focusing on freshwater fish, while few, have described MP ingestion by more than 200 species. This large number indicates that some studies have collected few samples. Few published papers have studied the interactions between MP and freshwater biota compared to the number of marine studies, although scientific interest in MP ingestion by freshwater fish is rising. We thus believe that a summary of recommendations for the methods and expression of results and factors leading to MP ingestion by freshwater fish would be useful for the scientific community, especially regarding monitoring. Three main factors have been investigated: the MP levels in abiotic compartments, the living habitat and the feeding strategy. Other factors could have been reviewed, but there are too few studies to allow for a reliable discussion. Exposure through abiotic compartments could be directly correlated with the ingestion of MP. As further discussed in this review, MP ingestion does not always depend on abiotic compartment exposure. Additionally, there is not a clear relationship between MP ingestion and the living habitat and feeding strategy. Some studies have found that benthic or demersal fish ingested more MP,[44,45] while others reported the contrary.[22]

Monitoring and assessment are essential steps towards addressing specific questions about marine litter, including MP. Monitoring and assessment are needed to assess the state or level

of pollution and provide objective information to design mitigation measures as well as assess their effectiveness and promote adaptive management.[46] Recommendations for defining indicator fish species are very few but crucial to improve the comparability between studies.

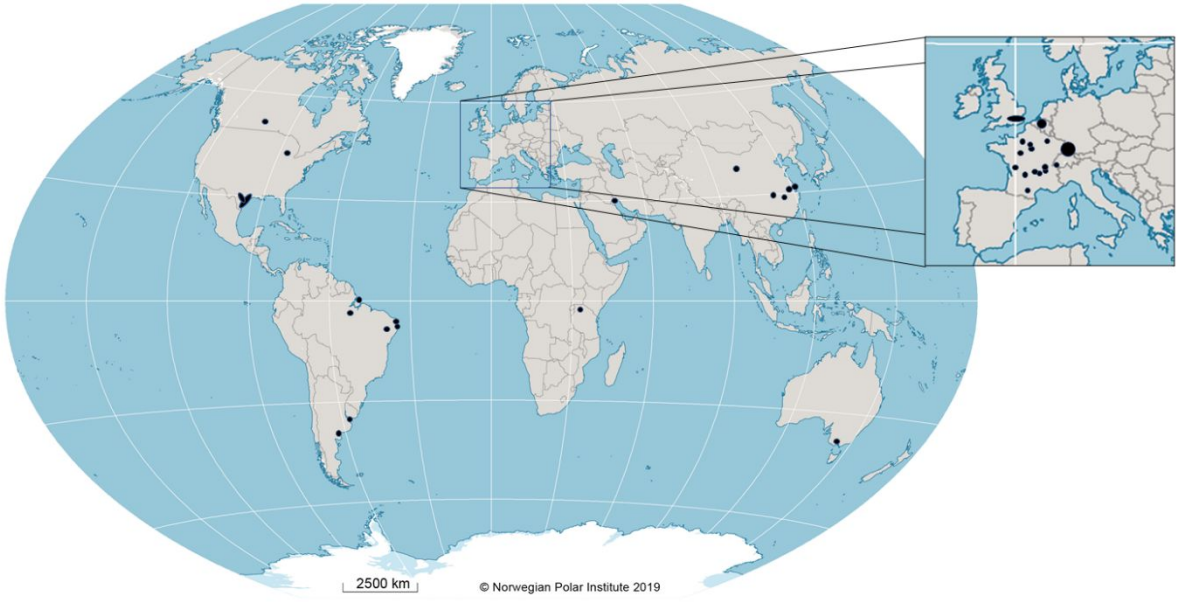

Figure 1. Global overview of the studies performed on MP ingestion by wild freshwater fish.

In this critical review, we aim to (1) propose several recommendations for the harmonization of methodologies, which is applicable not only to MP contamination in freshwater fish studies but also to all biota, (2) compare MP contamination levels in fish and their environment, and subsequently (3) determine which parameters could help to define fish species for monitoring. Our work is based on the occurrence of MP in the guts of fish. The translocation of plastic particles to other organs is beyond the scope of this review but should be considered in further studies.

2. Methodologies for the study of MP contamination in fish guts

Globally, studies on MP ingestion by fish, both marine and freshwater, follow a general pattern regarding their methodology, which include sampling, gut content (GC) extraction, MP extraction (visual sorting or chemical treatment, which is not always performed), and identification (including spectroscopic analyses which are not always performed). No specific method has been defined as the method to be used in fish GC analyses. In addition, this general pattern is not specific to studies about fish, as it is also used for other organisms or even for water or sediment matrices. In the two latter cases, density separation is often chosen to carry out the MP extraction. Whatever the matrix, discrepancies in methodologies occur, including in the study of MP in the GCs of wild freshwater fish. There are variations in all the steps used to extract and identify MP. In the next sections, we highlight the main issues of the key protocol steps used to extract MP from GCs (Fig. 2) and propose some recommendations to increase the comparability of studies for future research. We will discuss the following main steps: the sampling, extraction, and identification of MP and the expression of the results. Within those steps, we highlight some parameters that we think are of great importance including the sampling size, method for digesting the GCs, definition of the size threshold for the extracted particles, spectroscopic analyses, and the way results are provided. In addition, some recommendations for preventing and evaluating the contamination will be proposed. We selected the most important criteria, as other reviews have already focused on methodologies for the sampling, extraction and analyses of MP in biota or abiotic compartments.[40,47,48]

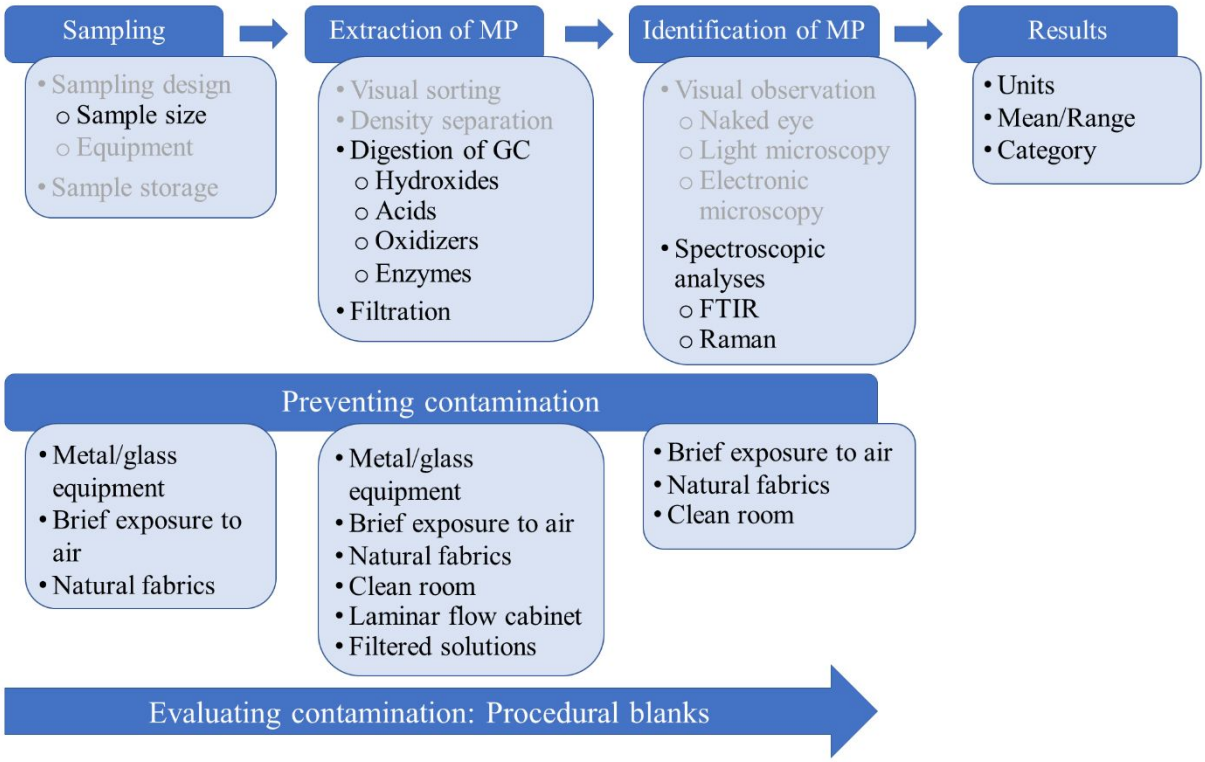

Figure 2. Flowchart of the main steps used to analyze microplastics in fish gut contents. The steps shown in black are discussed in this review. GCs: gut contents, FTIR: Fourier transform infrared.

2.1. Sampling: Sample size

Among all the species studied, more than twenty have been analyzed at least twice, and most of these species have been studied thanks to studies conducted in South America.[49-52] In addition, when considering a sampling of at least 10 individuals per species, only seven out of twenty species can be spatially compared. A review focusing on wild marine fish found that the detection of plastic ingestion was positively correlated with an increased sample size (up to n=10).[43] In many studies reviewed here, the number of individuals representing each species was low (n<10), which is obviously too small to define solid trends in MP contamination. In several publications, data were given for one or two individuals, limiting the representativeness.

Given that a single dataset is available and the number of fish sampled is usually low for most studied species, very local one-time events may influence the results and impair the global understanding of MP ingestion by fish. A large sample size is highly important since it will provide enough data to perform reliable statistical analyses. Previously, a threshold of 50 individuals has been defined as sufficiently reliable regarding to achieve statistical power,[48,53,54] while 40 specimens were considered to be an adequate sample size for the monitoring of plastic ingestion by northern fulmars.[55] Fig. 3 shows that most studies did not sample more than 50 specimens per species. This chart highlights the need for a better sampling design to statistically support the results.

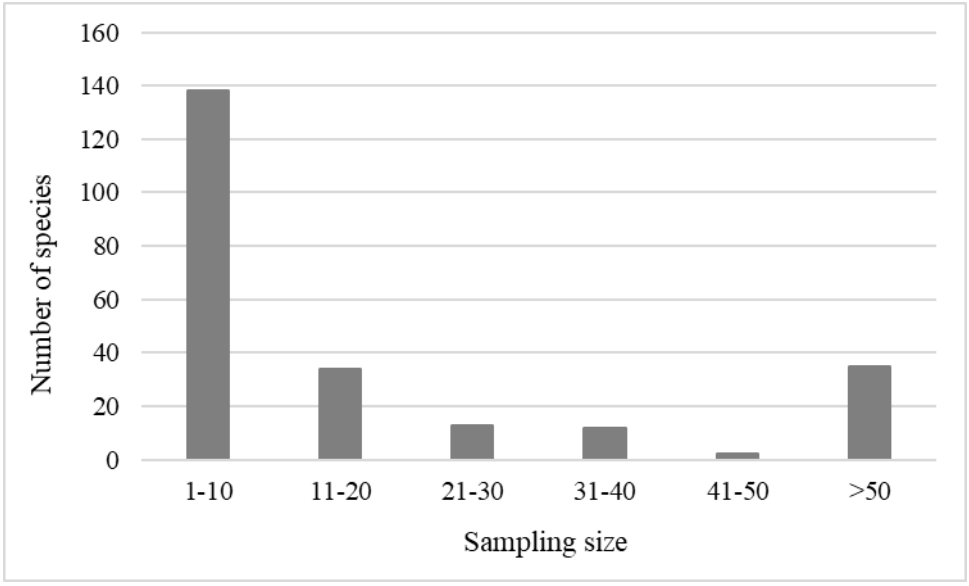

Figure 3. Number of species for each category of sampling size. This chart is based on studies shown in Table 1.

2.2. Extraction of MP: Digestion of fish gut contents

The digestion of organic matter is not mandatory but recommended,[46] especially when aiming to isolate MP from biota. Several methods exist regarding GCs, which are indiscriminate for

174 marine and freshwater fish species. Fig. 4 shows that when present, the most common agents
175 are oxidizers (*e.g.*, H_2O_2 and NaClO)[44,56,57] and hydroxides (*e.g.*, KOH).[58,59] No studies reported
176 acids as the main digesting agent, which is most likely because acids alter some polymers, such
177 as polyethylene terephthalate (PET), high density polyethylene (HDPE) and polyamide (PA).
178 Acids can also lead to suboptimal digestion.[58,60-62] Remarkably, to our knowledge, no studies
179 have used enzymes as digesting agents yet. While the efficiency of such agents has been
180 assessed,[62-64] the cost and duration of this treatment might discourage researchers. Recently,
181 von Friesen et al.[65] proposed a protocol more efficient than that using KOH with the convenient
182 advantages of being less time-consuming and commercially available at a low price. This
183 protocol has been thoroughly tested using bivalves but suits a broader range of organisms
184 according to the authors. No studies on fish GCs have used Fenton's agent either, which is
185 probably due to the efficiency of the previously cited methods. This agent does not affect plastic
186 polymers and reduces the sample preparation time.[66] Both enzymes and Fenton's agent could
187 be a solution to achieving the efficient, rapid and inexpensive digestion of the organic matter in
188 the GCs of freshwater fish but could probably also be used in other biological matrices such as
189 the liver. Some GCs can be very fatty and challenging to digest. No study has identified a means
190 of treating those samples properly. Finding an easy way to process fatty GCs would be useful
191 for further research on fish gut contamination.
192

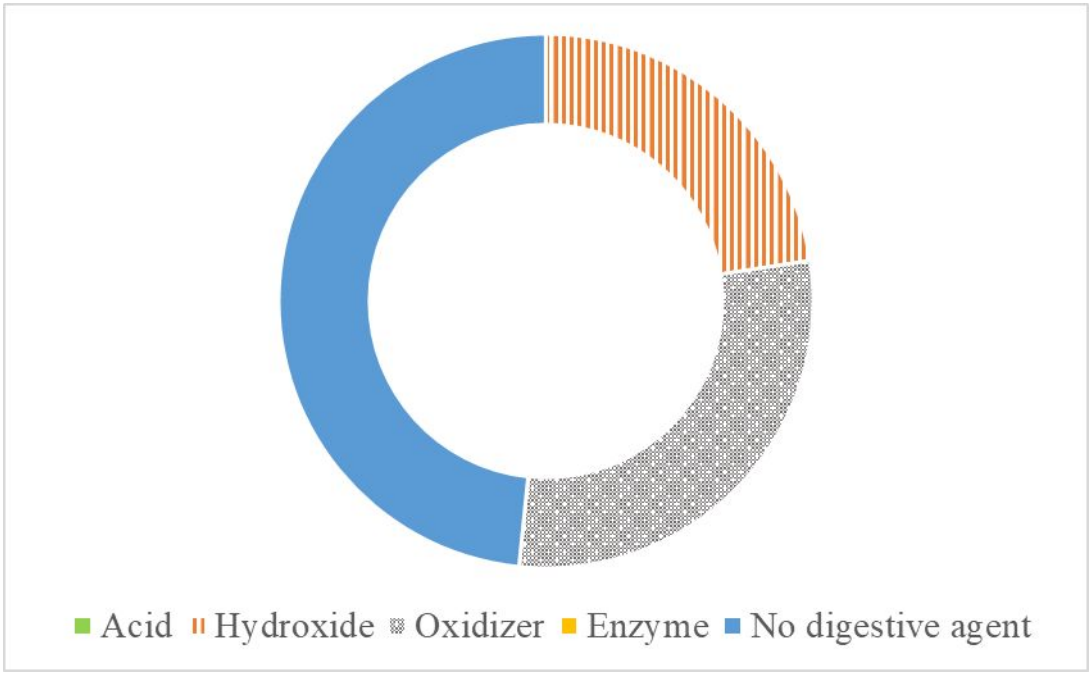

Figure 4. Proportion of studies according to the main digestive agent used.

Only a couple of studies have reported data on all types of anthropogenic particles, *i.e.*, small pieces created or handled by humans, such as plastics, dyed particles or textile fibers.[57,67] Indeed, studies have only focused on plastic materials and have developed protocols for that purpose. To our knowledge, few studies have tested the protocols used to extract MP from biota on natural or modified natural materials such as cellulose or cellulose acetate.[58,68,69] Dehaut et al.[58] tested three extraction protocols on cellulose acetate materials, in which two contained hydroxides (NaOH & KOH) and one contained a combination of a hydroxide and an oxidizer (NaOH & $K_2S_2O_8$). All the protocols led to a mass reduction and a size and shape modification of the cellulose acetate while two of them allowed for identification by Raman spectroscopy. On the other hand, cellulose fibers, along with plastic polymers, were tested with an oxidizing agent and a combination of an oxidizer and an acid (NaClO & HNO_3).[68] Cellulose fibers were left unaffected by both treatments and Raman spectroscopy could successfully be performed. A potassium hydroxide-based protocol has also been tested on several natural materials.[69] Cellulose acetate has been found to lose a significant part of its mass after treatment, which is

similar to the study by Dehaut et al.[58] Some natural materials, such as sheep wool, were dissolved, while others showed a resistance to KOH. The extraction method should then be carefully chosen when textile fibers are targeted.

Data on anthropogenic particles made from natural materials such as wool and cotton should be included in further studies. Laboratory studies have mainly focused on plastic materials. Textile fibers, independent of their composition, are found in high quantities in the environment[3,70] but their impacts are unknown. These particles contain additives and dyes[16,70,71] and may constitute a threat to the environment[72,73], aquatic organisms and human health.[71,74] All anthropogenic particles, regardless of their size, should therefore be studied in both in situ and in vitro studies.

2.3. Extraction of MP: Filtration and targeted MP sizes

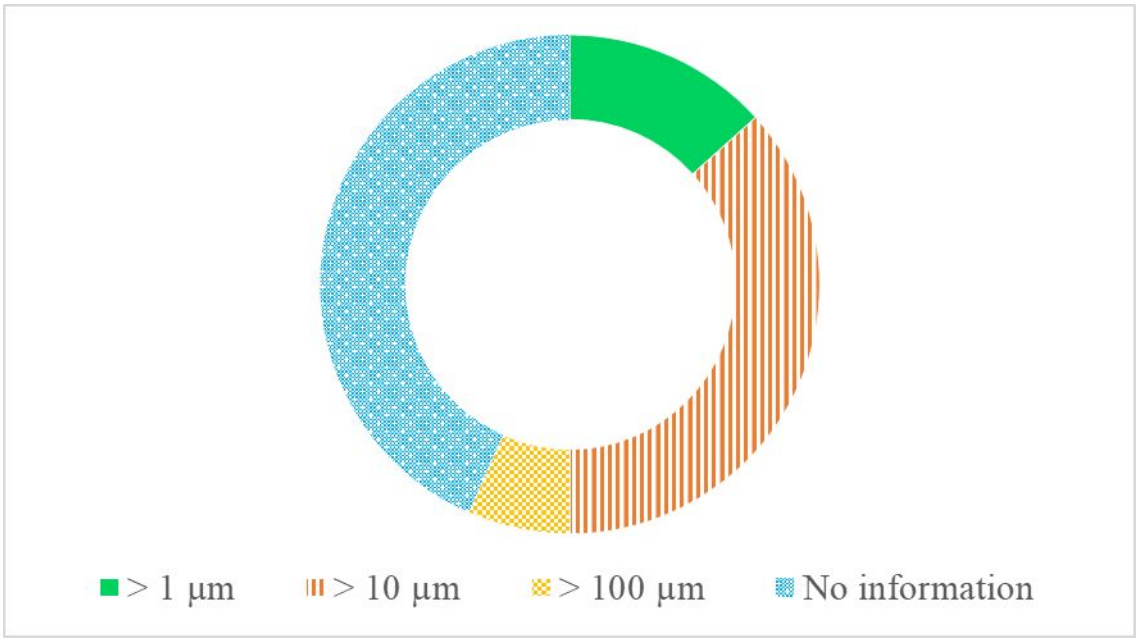

Figure 5. Proportion of studies according to their lowest limit of MP extracted. Based on Table 1.

The minimum size of the targeted particles is not always mentioned (Fig. 5). Generally, the particle isolation process includes a filtration/sieving step, in which a precise mesh size practically defines the threshold for particles to be further analyzed. In several studies, that mesh size is close to the micrometer scale (Fig. 5). Although this mesh size perfectly fits the microplastic size definition, from 5 mm down to a few microns,[75] the limiting factor is then the spectroscopic analyses. Depending on the technique and equipment used, the size threshold allowing for the analysis of a particle varies. For instance, when dealing with particles between 1 and 20 μm in length, Raman spectroscopy is recommended.[76] However, if the aim of the study is to investigate direct ingestion by fish, it might not be worth studying such small MP. Adult fish are unlikely to ingest micron-sized particles because these particles will probably be ejected into the surrounding water from the branchial system, which is not adapted to retain such small particles.[77] The filtration step could then be made easier by using larger mesh sizes depending on the studied species, mainly if no digestion step is performed. However, if the transfer of MP from prey is considered in the study, looking at smaller particles might be of interest. Regarding filtering fish, their filtration system defines the size limit of particles that are retained and further swallowed. For those species, correlating the filtration-limited size to the size of MP ingested might be a way to discriminate MP coming from prey (indirect ingestion) and MP ingested through feeding (direct ingestion). This assumption has to be assessed by future studies. A size of 5 μm could be the lowest limit of investigated MP sizes, as it almost perfectly covers the MP size definition, which comprises direct and indirect MP ingestion and allows for reliable spectroscopic analyses. We encourage authors to provide at least the size classes of the ingested particles, as well as the size distribution. This information will allow for the comparison of those size classes according to the minimal size targeted and will provide baseline data for studies further dealing with the uptake of ingested MP by other organs and tissues.

252 2.4. Identification of MP: Spectroscopic analyses

253

254 Half of the studies on plastics in freshwater fish used spectroscopic analyses to confirm the
255 plastic composition of the particles found, and most of these studies are the oldest (Table 1).
256 When considering other matrices such as sediment or water, such techniques are often set
257 aside.[76] Thus, these techniques only rely on visual observation, which may bias the results.[78-80]
258 Visual observations with a stereomicroscope tend to underestimate the amount of microplastic
259 fragments and, conversely, tend to overestimate the amount of MP fibers compared to the
260 results obtained by Fourier transform infrared (FTIR) analyses.[79] Markic et al.[43] found that
261 plastic was detected more often when a chemical digestion-coupled spectroscopic analyses was
262 performed compared to visual methods alone. The smaller the particles are, the more difficult
263 it is to distinguish them from biological tissues and to assess their synthetic composition with
264 the naked eye. The 'hot point-test' is also sometimes used to determine the plastic composition
265 of particles.[18,81] This test consists of touching a particle with a hot needle and observing whether
266 the needle leaves a mark on the particle.[48,82] Although convenient and cheap, this technique
267 should not be solely used for identification but used as a complement to visual sorting prior to
268 spectroscopic analyses.[48] In a report, the GESAMP group of experts[40] stated that spectroscopy
269 is recommended for particles smaller than 1 mm. Consequently, using either Raman or FTIR
270 spectroscopy when working with microplastic pollution is highly recommended.[46,80] Both
271 techniques usually allow for precisely identify the particle composition to be precisely
272 identified and have the strong advantage of being nondestructive in contrast with other
273 techniques such as pyrolysis-gas chromatography coupled with mass spectrometry (py-GC/MS)
274 or thermogravimetric analysis coupled with solid-phase extraction (TGA-SPE).[83-85] These
275 techniques are complementary, as they give different information but using two or three of these
276 techniques is obviously expensive and time-consuming. Therefore, depending on the

277 information needed or the type of particle, researchers have to choose the best technique. Raman
278 spectroscopy constitutes a better choice if results about additives are needed. Indeed Raman
279 spectroscopy can provide additional information on dyes, for example, and will identify the
280 composition of small particles (< 20 μm) that FTIR will miss.[79,84,85] On the other hand, FTIR
281 spectroscopy is less time-consuming and may allow for the identification of black particles that
282 lead to fluorescence issues in Raman spectroscopy.[84] All these techniques are expensive and
283 time consuming; thus, a random subsample is often collected prior to the analyses, and the
284 results are extrapolated to the total number particles found.[10,44,86-88] This random subsample is
285 usually poorly described and might result from subjective choices, especially regarding the
286 sizes and colors. As selecting the particles may lead to bias, we instead recommend selecting a
287 zone covering both the center and the edge of the filter (a half or a quarter) used during MP
288 extraction.
289 Spectroscopic analyses provide useful and reliable data on particles. However, part of this data
290 is often set aside because studies focus only on plastic polymers (Table 1). As explained in a
291 previous section, we encourage researchers to provide all information, even on natural and
292 semisynthetic materials.
293
294 2.5. Expression of the results
295
296 We recorded three different units of MP contamination in GCs, *i.e.*, the percentage of
297 contaminated individuals, the number of MP per individual and the number of MP in one gram
298 of the GCs. Additionally, units of contamination differ between studies making comparisons
299 impossible. All three units are easy to calculate and, although the most common (Table 2), the
300 percentage of occurrence is not the most representative unit. This unit gives an overview of the
301 number of fish having ingested at least one piece of plastic in a given time but does not give

any valuable quantitative information. The number of MP per gram of GCs could be considered to be the most accurate unit but greatly depends on the fullness of the stomach or gut. As it is not clear whether MP have a greater retention time than food in the digestive tract,[89,90] expressing the quantity of MP per mass of gut contents is not the most consistent option. It is instead preferable to express the results as the number of MP per individual and for comparison purposes, we recommend authors and researchers to report the results with the three units described in this review.

2.6. Procedural blanks

Procedural blanks (PBs), also termed controls or negative controls,[47,91] are used in all procedure steps with the reagents only, and the sample in itself is excluded. Few studies that we have reviewed made PBs although most of the recent studies did. Even if contamination is prevented as best as possible, including PBs is recommended to evaluate possible contamination of the whole treatment protocol[46,48] and, if needed, to adapt the results according to those blank data.[18,82,91] Plastic contamination levels depend on many parameters, including the type of equipment used for sampling, the extraction and the analyses.[92] Given that microplastic fibers are also found in the air,[9,10] tap water[93] and table salt,[94,95] in which the latter is used in laboratories to perform density separation, PBs are necessary to evaluate the contamination levels in environmental samples. Moreover, we recommend including some PBs before starting the sample treatment. This will give an overview about the contamination level in the working environment, which can be then compared to the expected levels in the samples. This step could be critical if minimally contaminated samples are to be processed. In this case, PB levels would easily exceed what may be found in the samples, leading to an impossible interpretation of the results. Depending on the studies and equipment used, PB contamination can be important: 50

327 times higher than plastics within the samples,[96] or low: less than 5% of all plastics found in the
328 samples.[44] However, if PB levels are assessed first, precautions and decisions can be made to
329 prevent the samples from being highly contaminated during the treatment.
330

331 Table 1. Summary of studies reporting MP ingestion by wild freshwater fish and their methodologies.

No. sp.	n/sp.	Country	Water body	Targeted particles		Extracted particle size (µm)	Visual sorting	Digesting agent				Spectroscopic analyses		Procedural blanks	Reference
				AP	PL			Hyd.	Acid	Enz.	Ox.	Rn	FTIR		
3	60, 60, 62	Brazil	E	-	X	?	X	-	-	-	-	-	-	-	Possatto et al.[97]
2	239 & 330	Brazil	E	-	X	?	X	-	-	-	-	-	-	-	Dantas et al.[49]
3	240, 141, 44	Brazil	E	-	X	?	X	-	-	-	-	-	-	-	Ramos et al.[50]
1	186	France	R	-	X	> 1.2	X	-	-	-	-	-	-	-	Sanchez et al.[98]
2	20	Tanzania	L	-	X	> 500	X	X	-	-	-	-	X	-	Biginagwa et al.[99]
4	10	Switzerland	L	-	X	?	X	-	-	-	-	-	-	-	Faure et al.[100]
44	1-67	U.S.A.	R & E	-	X	?	X	-	-	-	-	-	X	-	Phillips & Bonner[101]
1	530	Brazil	E	-	X	?	X	-	-	-	-	-	-	-	Ferreira et al.[102]
2	318 & 118	U.S.A.	R	-	X	> 53	X	-	-	-	-	-	-	-	Peters & Bratton[103]
5	10-75	Canada	R	-	X	> 5	X*	-	-	-	X	-	-	X	Campbell et al.[96]
6	20-40	China	L	-	X	> 5	X	-	-	-	X	-	X	X	Jabeen et al.[44]
2	10 & 66	England	R & E	-	X	?	X	-	-	-	-	-	X	-	McGoran et al.[104]
11	1-21	Argentina	E	-	X	?	X*	-	-	-	X	-	-	-	Pazos et al.[56]
1	48	Brazil	R	-	X	> 63	X	-	-	-	-	-	-	-	Silva-Cavalcanti et al.[105]
27	2-215	Brazil	E	-	X	?	X	-	-	-	-	-	-	-	Vendel et al.[51]
13	1-6	China	R	-	X	> 1.2	X*	X	-	-	-	X	-	-	Zhang et al.[59]
1	60	France	R	X	X	> 5	-	-	-	-	X	X	-	X	Collard et al.[57]
1	64	England	R	X	X	> 1.2	X	-	-	-	-	X	-	-	Horton et al.[67]
46	1-16	Brazil	E	-	X	?	X	-	-	-	-	-	X	-	Pegado et al.[52]
4	4-17	Iran	E	-	X	?	X	X	X	-	X	-	-	X†	Abbasi et al.[106]
11	1-17	U.S.A.	R	-	X	> 0.45	X*	-	-	-	X	-	X	X	McNeish et al.[91]
1	10	China	L	-	X	> 200	X	X	-	-	-	X	-	X	Xiong et al.[107]
16	1-63	Brazil	R	-	X	?	X	-	-	-	-	-	X	-	Andrade et al.[108]
1	78	Belgium	R	-	X	> 8	X	-	-	-	X	X	X	X	Slootmaekers et al.[109]
1	11	China	L	-	X	> 8	X	X	-	-	X	X	-	X	Yuan et al.[110]
14	8-9	China	E	-	X	> 20	X	-	-	-	X	-	X	X	Su et al.[111]

2	265 & 184	Brazil	E	-	X	?	X	-	-	-	-	-	-	Ferreira et al.[112]
1	20	Argentina	E	-	X	> 8	X	-	-	-	X	-	-	-	Arias et al.[113]
22	-	Germany	R & L	-	X	> 20	X	X	X	-	-	-	-	X	Roch et al.[81]
1	180	Australia	W	-	X	> 20	X	X	-	-	-	-	X	X	Su et al.[114]

No.=number, sp.=species, R=river, L=lake, E=estuary, W=wetland, FR=fragments, FI=fibers, AP=anthropogenic particles excluding plastic particles, PL=plastics, Hyd.=hydroxides, Enz.=enzymes, Ox.=oxidizers, Rn=Raman spectroscopy, FTIR=Fourier transform infrared spectroscopy.

*Visual examination after the digestion process. In this table, when the minimum size of the MP was not mentioned in the study, the porosity of the filters was considered. ┼No results for those blanks were provided.

337 Table 2. Summary of plastic characteristics from gut contents of freshwater fish.

Species (n)	Location	Plastic concentration or occurrence			Mean plastic size or range (mm)	Spectroscopic identification?	Reference
		Item/individual	Item/g GC	Contaminated individuals (%)			
Gobio gobio (78)	Several rivers, Belgium	-	-	9	0.67	Yes	109
Gobio gobio (186)	Several rivers, France	-	-	12	-	No	98
Squalius cephalus	Seine River, France	0.16*	0.16*	15	2.67*	Yes	57
22 species	Lakes & rivers, Germany	0.2	-	18.8	0.889	No	81
Alburnus alburnus *Perca fluviatilis* ***Rutilus rutilus (10)*** *Leuciscus leuciscus*	Lake Geneva, Switzerland	3.1 0 0 0.3	-	7.5	-	No	100
Platichthys flesus *Osmerus eperlanus*	Thames Estuary, England	-	-	85 20	-	Yes	104
Rutilus rutilus (64)	River Thames, England	0.69	-	32.8	-	Yes	67
Platycephalus indicus *Saurida tumbil* *Sillago sihama* *Cynoglossus abbreviatus*	Musa Estuary, Iran	2.3 2.8 1.5 2.9	-	-	-	No, SEM/EDS	106
Lates niloticus *Oreochromis niloticus*	Lake Victoria, Tanzania	-	-	20 20	-	Yes	99
Lepomis macrochirus (318) ***Lepomis megalotis (118)***	Brazos River, Texas, U.S.A.	-	-	45	-	No	103
44 species	Several rivers, Texas, U.S.A.	-	-	8.2	-	Yes	101
17 species	Several rivers, U.S.A.	~13	-	85	<1.5	Yes	91
Esox lucius *Catostomus commersoni* *Notropis atheirnoides* ***Pimephales promelas (34)***	Wascana Creek, Canada	-	-	73.5	-	No	96

Eucalia inconstans							
11 species	Rio de La Plata, Argentina	19.2	-	100	0.06-4.7	No	56
Micropogonias furnieri	Bahía Blanca Estuary, Argentina	12.1	-	-	2.4 (median)	No	113
16 species	Xingu River, Brazil	-	-	26.7	1-15	Yes	108
46 species	Amazon Estuary, Brazil	1.2	-	13.7	1.82	Yes	52
Hoplosternum littorale	Pajeú River, Brazil	3.6	-	83	<1-12	No	105
69 species	Paraiba Estuary Mamanguape Estuary, Brazil	0.07 0.12	-	9	-	No	52
Cathorops spixii *Cathorops agassizii* *Sciades herzbergii*	Goiana Estuary, Brazil	-	-	18 33 17	-	No	97
Stellifer brasiliensis (330) *Stellifer stellifer*	Goiana Estuary, Brazil	-	-	6.9 9.2	-	No	49
Eugerres brasilianus (240) *Eucinostomus melanopterus* ***Diapterus rhombeus (44)***	Goiana Estuary, Brazil	-	-	16.2 9.2 11.4	1-5	No	50
Cynoscion acoupa	Goiana Estuary, Brazil	-	-	64.2	<5	No	102
Centropomus undecimalis *Centropomus mexicanus*	Goiana Estuary, Brazil	1.5 1.4	-	-	~1.25	No	112
6 species	Taihu Lake, China	2.4	3.4	95.7	0.4-24.8	Yes	44
14 species	Yangtze Estuary Hangzhou Bay, China	0.3-4.5 0.5-5.3	0.3-6.2 0.1-8.8	-	22-100 25-100	Yes	111
13 species	Xiangxi River, China	-	-	25.7	0.3-1.8	Yes	59
Gymnocypris przewalskii	Qinghai Lake, China	5.4	-	-	-	Yes	107

Carassius auratus (11)	Poyang Lake, China	-	-	91	0.1-1	Yes	110
Gambusia holbrooki	Melbourne Area, Australia	0.60	-	19.4	0.09-4.86	Yes	114

338 GC=gut contents. The bolded items are the species studied in at least two publications with the number of individuals sampled. *Personal unpublished

339 data and the data published Collard et al.[57], which concerned all anthropogenic particles. When the global mean is not provided, we gave the total range.

3. Factors influencing MP ingestion by freshwater fish

Here, we aimed to discuss factors that are likely to influence MP ingestion by freshwater fish foraging for prey. Those factors include specific traits, such as the feeding strategy, and abiotic parameters, such as the environmental MP levels. However, the results in the discussed in situ studies are also influenced by the retention time of MP inside the gut, which is not well understood. The results might be different depending on the size of plastic pieces or the species. Also, the gut content reflects only the instantaneous diet, that is why repeated studies should be performed.

3.1. Levels in abiotic compartments

According to studies, several factors are at play when plastic is ingested by fish. The first factor to consider is probably the exposure. Very few studies have investigated both the abiotic contamination and MP ingestion by freshwater fish. Therefore, this section will give an overview of the contamination levels in those matrices as a first step in determining what induces or prevents MP ingestion.

Many different units are used for both water and sediment concentrations (Table S1), which is also the case for marine environments.[115,116] Regarding water contamination, the results are often given in items per cubic meter of water, and the format “items/volume” is the most commonly used. Regarding the sediment, the results are usually expressed in items per kilogram of sediment (dry weight). Remarkably, all except one study chose to express their results per dry weight. We thus encourage researchers to further express sediment concentration per dry weight too. In addition, some authors calculated the mean concentration while others chose to only show a range. Some authors also excluded values equal to zero from the mean calculation, and others did not give any concentration mean or range. While a global mean value may be very restrictive and not representative of all the parameters such as the different rivers sampled or difference in the sampling locations (close or far

366 from an outlet, close or far from urban activities, etc.), we recommend showing as many values as
367 possible. This will allow further studies to choose the most convenient and adapted value to be
368 compared to.
369 When considering studies that performed analyses in both water and sediment samples obtained from
370 the same place, several trends and questions arise. Regarding the abundances of MP, some studies
371 found different patterns for the two compartments.[59,110,117,118] The authors of these studies discussed
372 those results by mentioning potential factors that could influence MP distribution. MP levels in lake
373 sediments are related to their distance from MP sources.[119,120] Yuan et al.[110] confirmed this fact. The
374 northern and middle regions of Poyang Lake are subject to anthropogenic activities and are the most
375 contaminated parts with regards to the sediment and surface waters, respectively. Sediment and water
376 obtained from the south of Poyang Lake, where less human activities occur, are less contaminated
377 than those of the northern and middle regions. Furthermore, the presence of a wastewater treatment
378 plant in the vicinity of a sampling station might induce higher concentrations of polyethylene
379 terephthalate (PET) fibers.[117,121]
380 The topography of the environment may also be a factor of the concentration or dilution of MP in
381 sediment.[122] In addition, the hydrodynamics, which are influenced by the shoreline morphology, may
382 favor or prevent the deposition of plastics in the sediment; a low velocity environment, such as a
383 harbor will enable plastics to settle.[59,97,120,123]
384 Surprisingly, MP contamination in fish shows different patterns than MP contamination in water or
385 sediment for at least one characteristic (Table S2). Sometimes, a correlation between the water or
386 sediment contamination and fish contamination exists for only one or two parameter(s) (*e.g.*, the
387 shape or the size range) or at one sampling location.[91] Therefore, this shows evidences that exposure
388 is not the only factor involved,[67] and perhaps, that exposure is not a factor at all.[124] Of course, MP
389 ingestion cannot occur if there is not any plastic in the environment, but Kim et al.[124] found that the
390 food concentration is surprisingly more important than the MP concentration in influencing MP
391 ingestion. Kim et al.[124] exposed zebrafish to various mixtures of MP and food and concluded that MP

ingestion increased with increasing concentrations of food. However, in that study, the authors exposed fish to virgin plastic. It is now known that animals react differently to virgin or biofouled plastic. The copepods *Calanus finmarchicus* and female copepods *Acartia longiremis* ingested significantly more biofouled PS beads than virgin PS beads.[125] Similarly, procellariiform seabirds can be fooled by the dimethyl sulfide signature present on marine plastic particles.[126] In 2017, it has also been shown that *Engraulis mordax* anchovies responded to medium and high concentrations of biofouled plastic odors with foraging behaviors.[127] Those behaviors did not appear when these anchovies were exposed to clean plastic particles.

Indirectly, surface runoff and seasons also indirectly act on the exposure level.[112,128] Surface runoff increases the exposure to plastic debris.[112] Consequently, the exposure plays a role, but the ingestion also depends on other parameters, such as the age of the plastic particle or the time of the year.

3.2. Living habitat

Sediment is a sink for microplastics,[4,129] and therefore, demersal and benthic fish could be exposed to more MP than pelagic species. Some studies have focused on both pelagic and benthic or demersal fish with varying results. In China, demersal species ingested more plastics than pelagic species.[44] The same assumption was made by McGoran et al.[104]; a demersal species, *Platichthys flesus* flounder had ingested far more MP than a pelagic species, *Osmerus eperlanus* smelt. McGoran et al.[104] assumed that *P. flesus* had ingested more MP, because it ingested sediment, likely leading to the unintended ingestion of MP. In the marine Scottish waters, demersal fish species have ingested more plastics than pelagic species.[45] They also found a significant difference between coastal and offshore species, in which the former are more contaminated than the latter. Nevertheless, this latter category was only represented by demersal species, which may lead to the wrong conclusions about the involvement of the fish species coastal habitat on MP ingestion. Not all studies focusing on MP ingestion by pelagic and demersal fish found a difference between these two categories.[130] Moreover,

Rummel et al.[22] showed that pelagic feeders had more MP in their stomach than demersal fish. Again, as methods vary from one study to another and as MP ingestion depends on several factors, the results may vary when looking at only one factor.

3.3. Feeding strategy

A more commonly discussed parameter is the feeding strategy of fish and their trophic position. It is thought that (top-)predator fish from both fresh and marine environments might be more at risk than organisms at lower trophic levels because of high energy requirements and trophic transfer.[91,96,102,112,131] Other predators such as marine mammals have ingested MP that are too small to be preyed upon by such larger animals.[13,132,133] This suggests that secondary ingestion has occurred and thus, MP have accumulated through the food chain. The developmental stage of fish might also influence MP ingestion.[67] Indeed, many fish change their diet throughout their development.[134-137] Ferreira et al.[102] studied MP contamination in several ontogenetic phases of *Cynoscion acoupa*. The adults, which are mainly piscivorous, were more contaminated than juveniles and subadults, which exhibit an omnivorous diet. This result leads to the same conclusion previously reported: predator or piscivorous fish are more at risk than fish displaying another type of feeding strategy. Later, Ferreira et al.[112] made the same conclusion for two other species (the snook *Centropomus undecimalis* and *Centropomus mexicanus*) obtained from the same location, the Goiana Estuary. Three ontogenetic phases were sampled and compared. Juvenile snook registered the lowest MP ingestion level while the piscivorous adults, registered the highest. Moreover, peaks of MP ingestion by adults coincide with peak of fish ingestion. Other studies did not find such differences,[108] indicating the possibility for other parameters to be involved. For example, in the marine environment, debris categories were related to the feeding behavior of the sampled fish.[138] Opportunistic feeders ingested all debris categories (i.e., metals, wood, and plastics), while pelagic feeders had only swallowed plastic bags, benthic fish, and hard plastic pieces.

Based on the fish feeding habits, the visual characteristics of plastic pieces may be involved in MP ingestion by particulate-feeders. In contrast to ram-feeding, which consists of passively filtering water and is nonselective, particulate-feeding is a selective mode, where prey are visually detected before their capture.[139] Then, ram-feeders are less likely to be influenced by the visual characteristics of plastics.[77] Yuan et al.[110] noticed that white MP were more frequently found in fish stomachs than in both water and sediment. Yuan et al.[110] supposed that *Carassius auratus* could mistake MP for planktonic food. Ory et al.[140] studied *Seriolella violacea* juveniles in controlled conditions and exposed them to food-shaped and food-sized polyamide particles with different colors. As expected, the juveniles preferentially ingested black particles, which is the same color as food pellets. The other colored particles were supposed to be cocaptured with the food, as they were ingested together with the food pellets only. Nevertheless, attention should be paid to the digesting agent used in the extraction process as some agents, such as NaClO, change or discolor the particles.[68] In a more global framework, the likelihood that beached macroplastics were previously bitten by fish and other organisms depends on the plastic colors.[141] Blue and yellow macroplastics were preferentially more than were other colors. Similarly, plastic threads were the main plastic type ingested by *Cynoscion acoupa* in the Goiana Estuary, while plastic threads represented only 1.4% of all the plastic debris in the water column.[102,142] Several colors of threads were ingested, suggesting that the plastic shape was more important than the color for this species. The same observation was made by Carson,[141] who found that bottle shaped macroplastics displayed more bite marks than other shapes. Visual and chemical cues thus interfere together with the feeding behavior of fish, making the understanding of MP ingestion in fish even more challenging. Further studies that will consider several parameters are needed to understand the different pathways involved. Increasing the knowledge of those factors will help to suggest and define species for the monitoring of plastic pollution in both fresh and marine waters.

4. Freshwater fish for the biomonitoring of MP contamination?

The occurrence of a contaminant in an ecosystem does not mean that the contaminant will cause harm to the populations inhabiting the ecosystem. Biota is needed to link the pollutant levels in abiotic compartments and in organisms' tissue to potential adverse effects.[143] Using fish species for the assessment of the quality of aquatic ecosystems is highly relevant. Fish are found everywhere in aquatic environments and have an intermediate trophic position, linking the lower trophic positions to the higher trophic positions (Beyer 1996 in van der Oost et al.[143]). Nevertheless, attention must be paid when choosing a species for monitoring. The GESAMP[46] report suggested a list of criteria that helps to define good species for monitoring. This species, or group of species, must be representative of different life histories, phylogenies, sizes, ages and developmental stages. General strategies are proposed, which include opportunistic sampling, market sampling of commercial species and targeting biota. In this report, it is stated that fish, as targeted species or those purchased from the markets, could constitute a good monitoring group for the ingestion of MP.[46]

Given the numerous factors influencing the ingestion of MP by fish, choosing only one indicator species or a group of species is challenging and perhaps impossible. The ecology and feeding habits of the chosen species must be known. It is important to know where the fish, as well as all its developmental stages, is located within the food web, for instance. Here, we present a summary of the parameters to be considered when choosing an indicator fish species, for both fresh and marine environments:

- *Trophic position.* As discussed in the previous section, predators, especially top-predators, are more likely to ingest MP through food transfer. However, monitoring MP pollution with top-predators will not accurately indicate the contamination level of the environment (water and sediment) but will give global information on the trophic web and the ecosystem.
- *Feeding strategy.* Ram-feeding fish passively filter water without any visual search for food. They may be good indicators of the MP levels in the surrounding water. In this case, in addition to gut contents, we advise researchers to also examine the gill rakers, which are bony

or cartilaginous structures attached to the branchial arches that retain filtered particles inside the mouth. Those analyses will complete the GC results.

- *Migrations.* Many fish migrate, either vertically (diel migrations) or horizontally (seasonal migrations), and sometimes fish migrate both ways. Depending on the area chosen to be studied, all types of migration can be valuable. Diel migrations could inform researchers about the MP levels in the pelagic zone, as the surface, seasonal and spatial migrations could provide data for the same species but in different areas. Utilizing the same species is important, as it avoids some bias caused by specific related factors, such as the fish length, the mouth structure and the fish vision. Whatever the chosen species and area, the sampling protocol must be designed considering those migration types. Fish must be sampled at the same time of the day or year if only one location or one water column compartment is targeted.
- *Commercial value*. Collecting commercially valued fish is advantageous as this is a cost-effective method for the assessment of human exposure through the chosen species.[46] Indeed, commercial fish can be purchased at markets, which eliminates evident sampling costs. However, it may not be possible to obtain precise information regarding the sampling location.
- *Living habitat*. Benthic and demersal species will provide information about a sea compartment that is different than the sea compartment of pelagic species. Demersal fish will give a less accurate overview of the benthic compartment than benthic fish, as demersal fish are not fully associated with the sediment.
- *Distribution*. For comparison and repeatability purposes, the chosen species should have the widest distribution possible or should at least belong to a higher taxon (genus or family) that is distributed worldwide distributed in order to avoid biases linked to the different species used in various studies.

In summary, the perfect fish species used to monitor MP pollution in fresh and marine water is either a top-predator or a ram-feeding species with a large distribution area and a commercial value. If the

522 species is restricted to a small area, similar species must be found in other areas of the world. The
523 migration of the selected fish must be well known and considered when sampling. Researchers must
524 then carefully choose the correct species according to the information they need.
525
526 5. Perspectives and recommendations
527
528 To provide perspectives for further research, we want to highlight three steps of protocols that are of
529 great importance: the number of samples analyzed, the size limit of the extracted particles and their
530 identification.
531 Sample sizes vary greatly between studies, as sizes vary from 1 to 330 individuals for a single species.
532 A sample size of 50 individuals has been determined to be sufficient for plastic pollution research.[53]
533 A couple of samples is not useful for research; in addition to being statistically irrelevant, time and/or
534 money can be saved by not sampling such small sample sizes and perhaps used for better purposes.
535 Achieving 50 individuals per studied criterion can be challenging, and therefore, we recommend
536 sampling as close to 50 individuals as possible by sampling the same species from a specific location,
537 for example.
538 Studies have rarely identified the target size range of MP extracted from fish gut contents (Table 2).
539 The only information available is the mesh size of the filters used during the extraction step when
540 present. Usually, that mesh size is approximately a micrometer, which allows researchers to extract
541 very small particles, but such particles are hardly observed visually and analyzed spectroscopically.
542 In contrast, focusing on large MP might lead to an underestimation of the number of ingested
543 particles. Both large and small fish are likely to ingest small MP (< 100 μm) either by transfer from
544 prey to predator or through their feeding behavior. It is thus critical to specify which threshold is
545 applied in each study to promote comparability. This threshold must be defined according to the
546 identification method used; FTIR and Raman spectroscopies allow researchers to analyze MP that
547 are smaller than the particles visible by visual observation through a stereomicroscope.

548 MP have often been identified by visual inspection, although the identification techniques used have
549 seemingly improved in recent studies. Only two studies on MP contamination in freshwater fish
550 included all dyed particles, even if the particles were not made of plastic (Table 1). We believe that
551 it is important to report all types of anthropogenic contamination in fish tissues, especially textile
552 fibers, which are usually dyed and released in large amounts into the environment.[144,145]
553 With regards to contamination, we suggest that (1) sample collection is performed with as few plastic
554 materials as possible, (2) all solutions, including tap water, must be filtered with filters having the
555 same porosity as the one used to filter the samples and stored in glass jars, (3) plastic materials should
556 be avoided during sample treatment, (4) if the laboratory work cannot be performed in a clean room,
557 the laboratory should then be ventilated as much as possible, (5) all equipment must be covered with
558 clean non-plastic materials when not in use and (6) the laboratory operator should wear nonsynthetic
559 clothes, a cotton lab coat and gloves. Avoiding plastic during the sampling and treatment steps is not
560 easy, especially regarding the textiles worn, solution storage and use of nonsynthetic gloves, but for
561 the latter at least, an FTIR or Raman spectrum can be recorded and matched with the potential
562 contaminating particles found in the samples. If contamination does occur, those particles can then
563 be excluded from the results.
564 As previously recommended by published reviews,[40,47,48] standardized protocols are required. Thus
565 we briefly recommend an overall optimal methodology that can be used when evaluating MP
566 pollution by working with fish gut samples: (1) the number of sampled individuals should be as close
567 to 50 as possible, (2) a MP target size down to 1 μm should be considered to fit the MP definition,
568 (3) enzymes should be used as digesting agents, (4) identification should be performed by
569 spectroscopy, (5) qualitative observations should be made by light microscopy, (6) as many data as
570 possible (averages and ranges, global contamination or per category, etc.) should be provided, (7) the
571 results should be expressed using several units and per category studied (feeding strategy,
572 developmental stage, etc.), and (8) procedural blank samples should be prepared and analyzed.
573 Spectroscopic analyses can be performed on a subsample of the total amount of extracted particles.

574 However, when focusing on fish contamination, it is uncommon to find tens of particles in a single
575 individual. Therefore, we recommend analyzing all particles extracted from the fish GCs. Visual
576 observations are not recommended for sorting or identifying microplastics but are required to take
577 pictures and to measure the MP found. We encourage researchers to keep that step only for those
578 purposes. The global evaluation of the protocol quality can be made thanks to the quality assessment
579 system proposed by Hermsen et al.[48] This quality assessment has been adapted for MP ingestion from
580 the CRED scoring system[146] and provides a good overview of the protocol strength.
581
582 This review examines MP in the digestive tracts of freshwater fish, but MP appear to translocate to
583 other organs in several aquatic taxonomic groups, including freshwater fish.[57,61,111,147-149] The impacts
584 of MP translocation are not well known, and MP pathways still need to be understood. The liver has
585 primarily been studied, but other tissues are worth investigating. Based on the human health
586 perspective, muscle is obviously the first tissue that comes to mind, as muscle is almost the only part
587 of the fish we consume. Few studies have examined MP in fish muscle,[57,106,111,147] and when MP are
588 found in the muscle, their pathways are unknown. In vitro studies using biofouled
589 plastics are needed to provide more information about the translocation process(es).
590 Selecting one or more species suitable for monitoring is currently challenging. First, studies must be
591 performed on MP ingestion by fish and MP contamination in their nearby environment. Studies
592 should be performed at the same time and, of course, by utilizing proper methods with chemical
593 treatment and identification. This will allow scientists to determine which species reflects the best
594 environmental contamination and thus to decide which species can be defined as an indicator.
595 Furthermore, assessing the species monitoring process is only the first step towards obtaining a more
596 global understanding of MP pollution in biota. To date, most studies on MP ingestion by fish are quite
597 descriptive and contain suggested explanations that are usually not demonstrated, and potential
598 impacts are listed. The quantity and quality of research require improvements to be made to assess
599 the ecological risk posed by MP.[150] A description of the contamination levels is obviously of great

importance to the scientific community, but we think that only studying MP contamination is not sufficient. Microplastics alone have been found to negatively impact fish,[25,151,152] but MPs are part of a cocktail of pollutants containing additives, *e.g.*, phthalates and bisphenols, dyes, organic pollutants, among others. These pollutants are also known to affect fish in several ways.[153-155] Consequently, MP should be monitored concomitantly with other pollutants to more accurately reflect the ecological state of freshwaters with regards to pollution.

In conclusion, this review aims to highlight the need for the standardization of methodologies and to give an overview of the factors involved in the ingestion of MP by freshwater fish. The first step of standardization can be to follow several recommendations, such as suggesting that a sufficient number of individuals be sampled and recommending the systematic use of spectroscopy to identify polymer particles. With regards to biomonitoring, studies should be performed using the same overall methodology and the same or very similar fish species. These species must be chosen for the purpose of monitoring and based on their biological characteristics: top-predator and ram-filtering species provide useful information with regards to plastic contamination.

6. Supporting information

The Supporting Information is available free of charge on the ACS Publications website at DOI: XXX.

An overview of the studies reporting plastic contamination in freshwaters and a summary of data regarding MP contamination in abiotic compartments and fish at the same location.

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