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## **Anthropogenic particles in the stomach contents and liver of the freshwater fish *Squalius cephalus***

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1 Anthropogenic particles in the stomach contents and liver of the freshwater fish

2 *Squalius cephalus*

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4  
5  
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9  
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24 Abstract

25

26 Anthropogenic particles (APs) are a very broad category of particles produced directly or indirectly  
27 by human activities. Their ingestion by biota is well studied in the marine environment. In contrast,  
28 studies on AP ingestion in wild freshwater organisms are scarce despite high contamination levels  
29 in some rivers and lakes. In this study, we aimed to evaluate the ingestion of APs and the possible  
30 occurrence of APs in the liver and muscle of a freshwater fish, *Squalius cephalus*, from the Parisian  
31 conurbation. After isolation, the particles were analyzed using Raman spectroscopy. In sixty  
32 stomachs, eighteen APs were found, half of which were plastics and the other half were dyed  
33 particles. Twenty-five percent of sampled individuals had ingested at least one AP. The mean  
34 length of the APs was 2.41 mm. No significant difference was found between the sites upstream  
35 and downstream of Paris. Additionally, 5% of sampled livers contained one or more APs, which  
36 were characterized as microplastics (MPs). No APs were found in the muscle tissue. The majority  
37 of APs isolated from stomach contents were fibers, which is similar to the findings of a previous  
38 river contamination study. This highlights that fish could be more exposed to fibers than previously  
39 thought and that more studies on the impacts of fiber ingestion are required. Despite their low  
40 occurrence, MPs are reported, for the first time, in the liver of a wild freshwater fish species. While  
41 the pathways and impacts are still unknown, MPs also occur in liver of marine mollusks and fish.  
42 Physiological *in vitro* studies are needed to better evaluate the impacts of such phenomena.

43

44 Keywords

45

46 Microplastics, fibers, Seine River, European chub, muscle, liver

47

48 Introduction

49  
50 Anthropogenic particles (APs) are a very broad category of particles produced directly or indirectly  
51 by human activities, thus showing an anthropogenic origin. In this study, APs are defined as small  
52 pieces – fragments or fibers – with an anthropogenic origin, such as plastic, dyed particles or textile  
53 fibers, regardless of their size. All textile fibers have been handled by humans (e.g., dyed) and thus  
54 have an anthropogenic origin, regardless of whether their basic composition is natural. All seas and  
55 oceans are contaminated by APs, including plastics (Cózar et al., 2014), and so are a large number  
56 of organisms (Kühn et al., 2015). Numerous studies, reviews and books (Bergmann et al., 2015)  
57 report the extent of this phenomenon, particularly plastic pollution and its impact on marine  
58 environments. However, although marine environments have been well studied regarding APs  
59 (mainly plastics), knowledge about freshwater contamination by APs and their ecological impacts  
60 remains scarce (Dris et al., 2015b). Yet, rivers continue to be the main source of microplastics  
61 (MPs) (<5 mm, (Arthur et al., 2009)) to coastal ecosystems (Lima et al. 2014; Lebreton et al. 2017).

62  
63 Several studies conducted in freshwater (Dris et al., 2015a; Faure et al., 2015), such as in the Seine  
64 River (Gasperi et al., 2014), the River Thames (Morritt et al., 2014), the Danube River (Lechner et  
65 al., 2014), the Yangtze and Hanjiang Rivers (Wang et al., 2017), the Laurentian Great Lakes  
66 (Eriksen et al., 2013) and in several Chilean rivers (Rech et al., 2014), showed that freshwater is  
67 contaminated, sometimes highly contaminated, by APs, especially plastics. Among others, a study  
68 by Lechner et al. (2014) calculated that, in the Danube River, the global plastic mass for both MPs  
69 and macroplastics was higher than the fish larvae mass. In the U.S.A., over 4 million particles are  
70 estimated to be released every day from one Californian wastewater treatment plant (Mason et al.,  
71 2016).

72

73 High AP concentrations could likely lead to their ingestion by aquatic organisms. Studies focusing  
74 on freshwater biota contamination are very scarce. Few studies mention AP or MP ingestion by  
75 wild fish species (Faure et al., 2012, 2015; Sanchez et al., 2014; Phillips and Bonner, 2015; Pazos  
76 et al., 2017; Silva-Cavalcanti et al., 2017; Vendel et al., 2017) or marine mammal species  
77 (Denuncio et al., 2011), and even these have focused only on stomach contents.

78 Aquatic organisms can suffer mechanical damage from ingestion of APs, e.g., obstruction or  
79 starvation, as has been shown in marine mammals and birds (Beck and Barros, 1991; Pierce et al.,  
80 2004; Jacobsen et al., 2010). Furthermore, aquatic organisms, including fish, can also be impacted  
81 by toxicological issues including pollutant transfer (Cedervall et al., 2012; Rochman et al., 2013;  
82 Wright and Kelly, 2017) or translocation of MPs into other organs such as the liver (Avio et al.,  
83 2015; Collard et al., 2017a).

84

85 In this study, we analyzed the ingestion of APs by the chub *Squalius cephalus* in two highly  
86 anthropized rivers, the Marne and the Seine Rivers, which both cross the Greater Paris Megacity.  
87 The chub has not been studied so far in the context of AP pollution despite its omnipresence in  
88 European rivers and its abundant population (Freyhof, 2014). Due to its ubiquity, the chub could  
89 be a bioindicator for AP pollution in rivers. This species has a minor economic interest, but its  
90 consumption by Europeans was at its maximum in 2011 (FAO, 2011). Adults are solitary, and  
91 juveniles are gregarious, but both are pelagic. Generally, they feed on insects, plants and  
92 crustaceans such as crayfish (Balestrieri et al., 2006; Mann, 1976; Michel and Oberdorff, 1995).  
93 However, their diet varies with age and season. In the Babuna River (Republic of Macedonia), they  
94 mainly feed on algae (Chrysophyceae) and insect larvae (*Ephemeroptera* and *Plecoptera*) in spring,  
95 on algae (Chrysophyceae) in summer and autumn and on diatoms and other insect larvae

96 (Chironomidae) in winter (Nastova-Gjorgjioska et al., 1997). Young individuals eat primarily  
97 insects (Mann, 1976), while adults generally feed on plants or crayfish and on other fish,  
98 particularly in winter (Michel and Oberdorff, 1995). Moreover, the chub adapts its diet to its habitat  
99 (Piria et al., 2005). For example, in Croatia, depending on the river, the diet -dominant components  
100 are *Diptera* (insects), cladocerans (Djinova, 1976 cited in Piria et al., 2005), detritus (Adamek and  
101 Obrdlik, 1977) or zoobenthos (Losos et al., 1980).

102  
103 AP contamination of the Greater Paris Megacity rivers, e.g., the Seine and Marne Rivers, was  
104 studied during a 19-month period between April 2014 and December 2015, excluding January and  
105 August 2015, from upstream to downstream suburbs crossing the very dense part of the urban area  
106 (Dris et al., 2018). Because of its sprawling population (12 million inhabitants), Greater Paris exerts  
107 a high anthropogenic pressure on rivers and, therefore, is a site of great interest for assessing the  
108 ingestion of APs by *Squalius cephalus*. The sampling was done along the continuum of the Seine  
109 and Marne Rivers, allowing a comparison between the upstream and the downstream suburbs of  
110 Paris. Our objectives were to evaluate the ingestion of APs, including microplastics, by European  
111 chubs caught within the Parisian conurbation and to determine whether APs translocate into muscle  
112 or liver tissue in this species.

113

114 Materials and methods

115

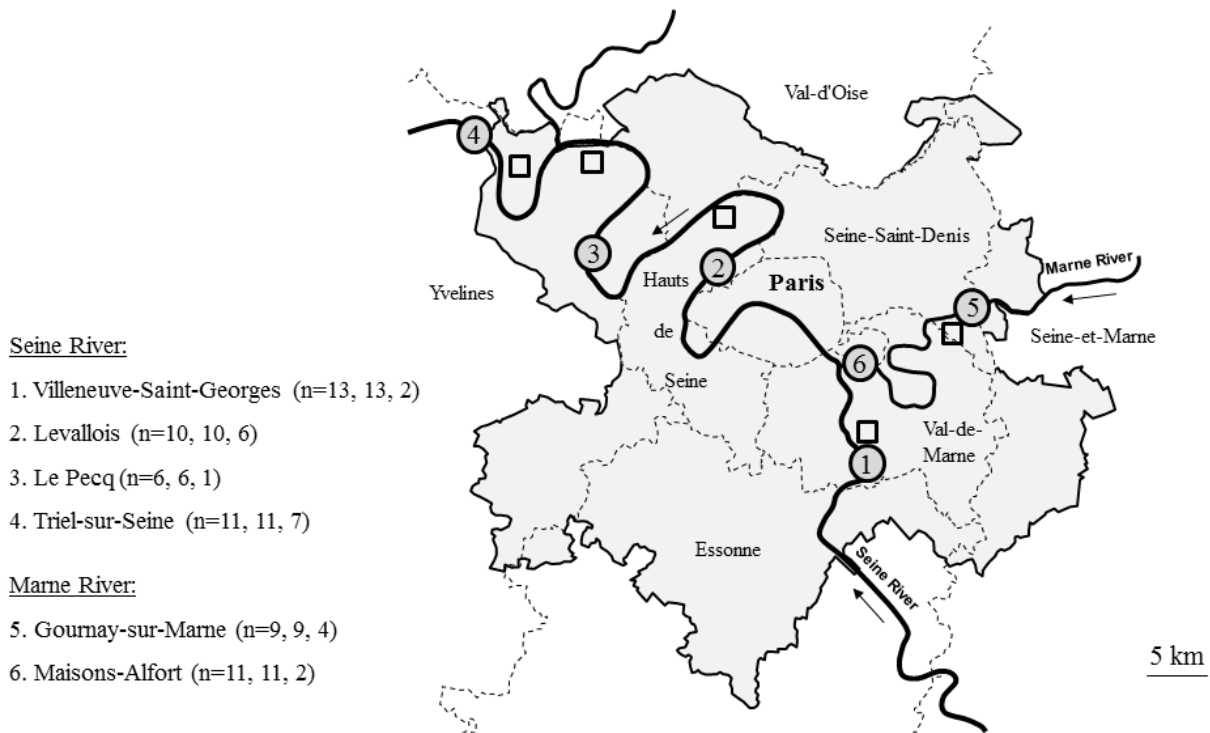
116 *Sampling*

117

118 Sixty freshwater fish of the species *Squalius cephalus* (mean length  $\pm$  SD: 296 mm  $\pm$  62, mean  
119 weight  $\pm$  SD: 340 g  $\pm$  239) were electrofished in the Marne and Seine Rivers around Paris (Fig. 1),

120 France, between 28 August and 2 September 2016. Of the six sampling stations, three were located  
 121 upstream of Paris (Gournay-sur-Marne, Maisons-Alfort and Villeneuve-Saint-Georges), and three  
 122 were located downstream of Paris (Triel-sur-Seine, Levallois and Le Pecq). The three target tissues,  
 123 namely, stomach (n=60), liver (n=60) and muscle (n=22), were immediately removed on board.  
 124 Stomachs and livers were put in distilled water-diluted formaldehyde solution (5%), while muscles  
 125 were packaged in aluminum paper and frozen at -20°C in the lab. All tissues were weighed (wet  
 126 weight) before the isolation process.

127



128  
 129 Figure 1. Sampling stations in the Parisian conurbation. Circles represent sampling stations and  
 130 squares represent wastewater treatment plants. Number of stomach content, liver and muscle  
 131 samples are shown for each station.

132

133 *Isolation of APs*

134  
135 The entirety of the stomach contents and livers were used, while only a small amount of muscle  
136 sample was digested (2-4 g). All tissues were degraded using sodium hypochlorite and then filtered  
137 with a 5- $\mu$ m filtering membrane according to published methodology (Collard et al., 2015).  
138 Nevertheless, methanol (99%), which is typically used for the centrifugation step, was replaced by  
139 ethanol (99%), as methanol degrades polyvinylchloride (Collard et al., 2015). Indeed, ethanol is  
140 commonly used to store biota samples or microplastics once analyzed (Sanchez et al., 2014;  
141 Phillips and Bonner, 2015; Ory et al., 2017).

142 Consequently, in the frame of this study, tissues were put into a 14 g/L NaClO solution overnight.  
143 The NaClO solution was filtered with a cellulose acetate filter membrane (5- $\mu$ m porosity), which  
144 was then rinsed with ethanol. This solution, containing APs, was centrifuged at 5,000 rpm for 10  
145 min. The bottom was then collected and deposited onto a stainless steel plate for Raman  
146 spectroscopy analysis.

147

148 *Raman analysis*

149  
150 All found particles were analyzed using a LabRam 300 spectrometer (Jobin-Yvon) equipped with  
151 an Olympus confocal microscope and an Andor BRDD Du401 CCD detector. Depending on the  
152 color of the particle, either a Spectraphysics argon ion laser (514.5 nm) or a Torsana diode laser  
153 (784.7 nm), with two different objectives (magnification of  $\times 50$  or  $\times 100$ ), was used. The maximum  
154 beam laser powers induced on the sample were 5 mW (green laser) and 30 mW (red laser), but  
155 several neutral density filters were used to decrease the power and avoid degradation of the sample.  
156 The integration times ranged from 5 s to 50 s, depending on the sample. Two spectral databases



157 were used to perform matchings: a commercially available database (Omic Spectra software,  
158 Thermo Fisher Scientific, U.S.A.) and a personal library, which used the Thermo Spectra 2.0  
159 software.

160 After Raman analyses, APs were isolated in 1 ml of 99% ethanol for further observations and  
161 measurements. Only particles whose anthropogenic origin was confirmed by Raman spectroscopy  
162 (plastic particles, dyed particles, etc.) were included in the results. Those particles showing an  
163 anthropogenic origin have been classified as “APs”. Inside the “AP” category, we defined two  
164 subcategories: microplastics and other APs (e.g., dyed particles of an unknown material). In  
165 addition, we classified all APs based on their shape: either fragments or fibers.

166

#### 167 *Preventing contamination and procedural blanks*

168

169 To minimize contamination, white cotton lab coats and latex gloves were worn throughout the  
170 entire isolation process. All work surfaces and dissection materials were rinsed with distilled water,  
171 and all glassware was rinsed with distilled water before each use. Stainless steel plates were placed  
172 under a metal sifter with 36- $\mu$ m mesh to prevent airborne contamination while the plates dried. No  
173 particles smaller than 36  $\mu$ m were found. The NaClO was filtered with a 5- $\mu$ m cellulose acetate  
174 membrane before dilution with distilled water.

175 Three 50-ml volumes of formaldehyde used to store the samples were filtered with the same filter  
176 membrane as that used for the isolation process. No APs were found. In addition, guts and livers  
177 were kept closed in the formaldehyde but were opened under an airflow cabinet just before the  
178 isolation process. Muscles were kept in aluminum, but particles from this material were not found  
179 in any samples.

180 Three procedural blanks were performed, along with runs of several samples, meaning that the  
181 entire isolation process was verified. It began in the formaldehyde solution used to store the  
182 samples and ended with the observation of stainless steel plates using Raman spectroscopy. This  
183 allowed us to evaluate whether solutions, materials or equipment were contaminated by APs in any  
184 way. Two particles made of an unknown organic material and two others made of only cellulose  
185 were found, meaning that those particles were not APs.

186

### 187 *Images and measurements of particles*

188

189 APs were collected from the Raman plates with 99% ethanol solution and then put into a microtube  
190 for further filtration using the same white filter membranes as those used for the isolation process.  
191 APs were photographed on both the stainless steel plate and on the filter membrane using a  
192 stereomicroscope (Leica MZ12, Leica AG Camera, Germany). They were then measured at their  
193 longest dimension using the Histolab software (Histolab Products AB, Sweden).

194

### 195 *Statistics*

196

197 Statistical analyses were performed with the GraphPad Prism software (v5.03, GraphPad software  
198 Inc., California, U.S.A.). Pearson's test was applied to evaluate the correlation between fish and  
199 AP lengths. Normality of the data was checked using the Kolmogorov-Smirnov test prior to  
200 analysis. Fisher's test was applied to compare (1) the number of fish that ingested (or not) at least  
201 one AP, MP, fiber or fragment, with each type of particle considered independently; (2) the number  
202 of all ingested APs versus MPs; (3) the number of ingested fibers versus fragments; and (4) the  
203 concentrations of all APs and MPs in SC, between upstream and downstream of Paris. A Mann-

204 Whitney U test was applied to compare the lengths of ingested APs between the upstream and  
205 downstream suburbs of Paris. All alpha values were set at 0.05. Data are expressed in mean  $\pm$   
206 standard deviation (SD).

207

208 Results

209

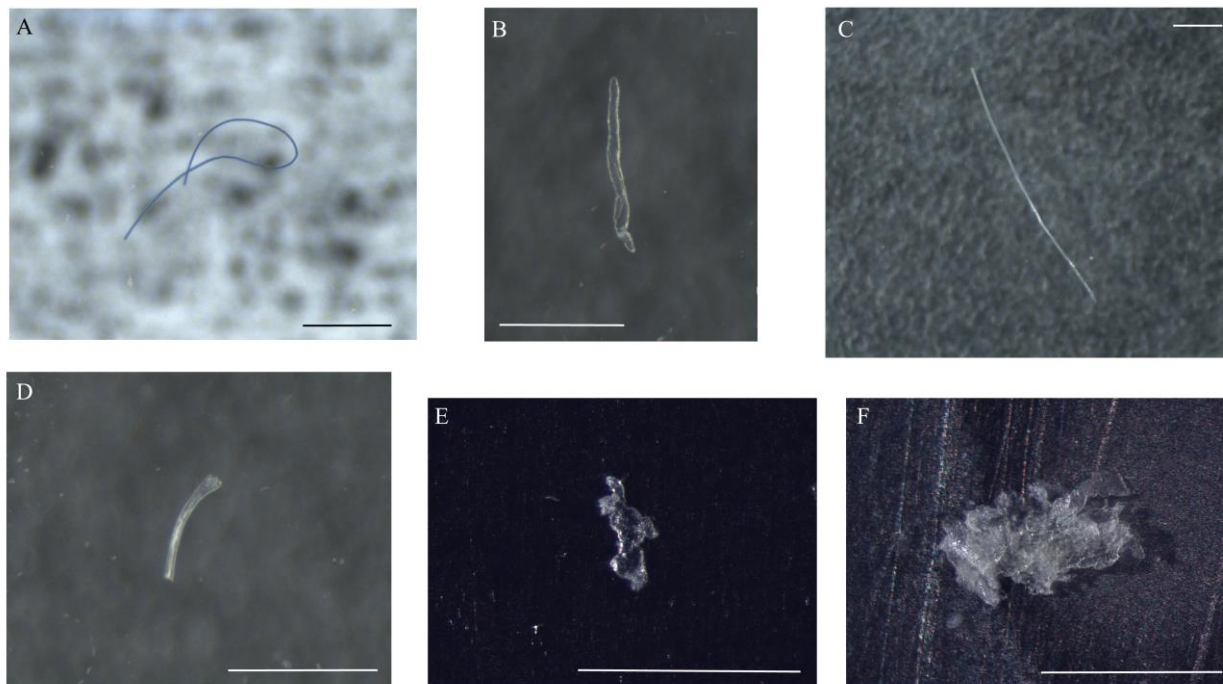
210 *Stomach contents (SCs)*

211

212 All isolated particles were analyzed, and 18 APs out of 70 isolated particles were found (Fig. 2).  
213 The fifty-two other particles were made of natural organic polymers such as cellulose or could not  
214 be identified. Twenty-five percent of fish have ingested at least one AP, and 15% have ingested at  
215 least one plastic particle.

216

217



218  
 219 Figure 2. Pictures of APs found in stomach contents (A-D) and livers (E, F) of *S. cephalus*. A: blue  
 220 dyed fiber, B: polypropylene (PP) fiber, C: polyethylene terephthalate fiber, D: PP fiber, E:  
 221 polystyrene fragment, F: polyethylene fragment. Scale bar: 500  $\mu\text{m}$ .

222

223 Table 1. Characteristics of the 18 APs found in stomach contents (SC). \*Concerns only fibers.

Station	Composition	Size (mm)	Diameter* ( $\mu\text{m}$ )	Shape	Item/g SC
Gournay-sur-Marne	PET	2.96	11	FI	0.38
	Black dyed fiber	0.84	22	FI	2.86
	PAN	7.38	30	FI	
Maisons-Alfort	Blue dyed fragment	2.41	-	FR	0.23

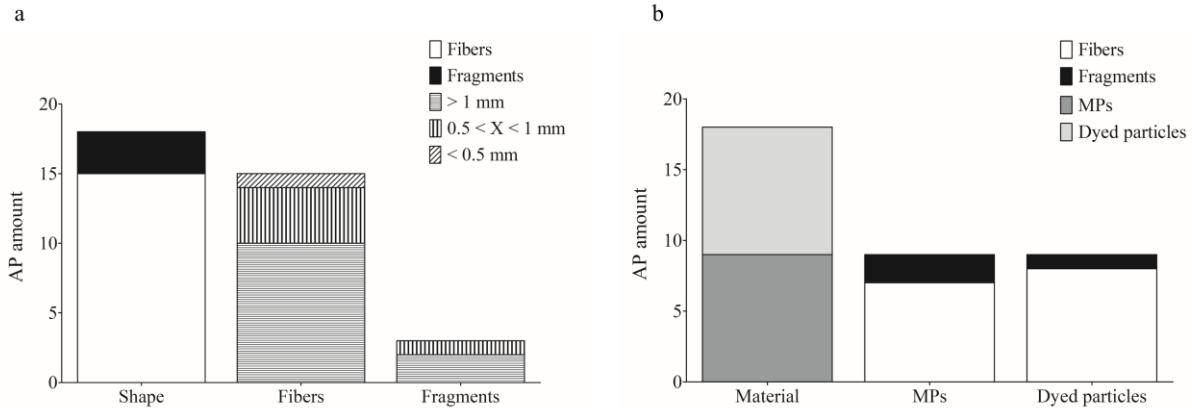
	Blue dyed fiber	0.96	18	FI	
	PET	0.74	12	FI	0.71
<b>Villeneuve-Saint-Georges</b>	PET	2.51	40	FI	2.5
	PP	0.69	59	FI	0.38
	PET	0.39	32	FI	0.67
	Blue dyed fiber	1.08	27	FI	0.38
<b>Triel-sur-Seine</b>	Blue dyed particle	1.43	23	FI	1.7
	Blue dyed fiber	2.21	17	FI	
	PEVA	6.56	-	FR	0.36
	PP	0.44	-	FR	0.67
<b>Levallois</b>	PET	2.36	56	FI	1.0
	Blue dyed fiber	4.44	22	FI	0.59
<b>Le Pecq</b>	Blue dyed fiber	1.21	31	FI	3.33
	Black dyed fiber	4.79	22	FI	2.0

224 PET=polyethylene terephthalate, PP=polypropylene, PEVA=poly(ethylene-co-vinyl acetate),

225 PAN=polyacrylonitrile, FI=fiber, FR=fragment.

226

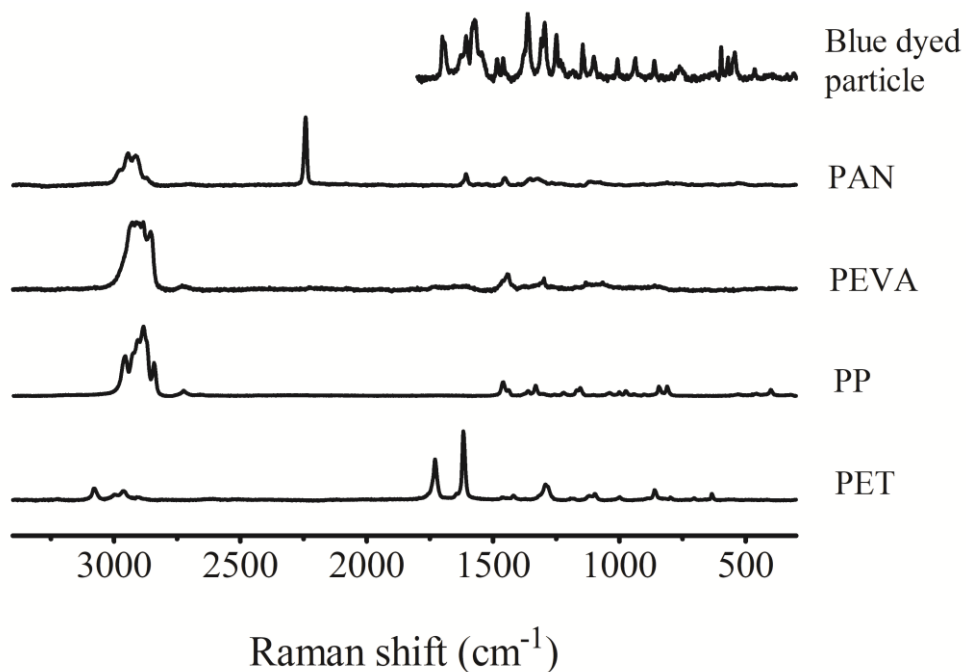
227



228  
 229 Figure 3. Summary of the main characteristics of APs found in stomach contents. a: total AP  
 230 amount according to shape, b: total AP amount according to composition.

231  
 232 Of the 18 APs, 15 were fibers, and 3 were fragments (Fig. 3a). Fibers longer than 1 mm were  
 233 dominant (n=10), followed by fibers ranging from 0.5 to 1 mm (n=4) and by fibers smaller than  
 234 0.5 mm (n=1). The mean diameter of fibers was  $28 \pm 14 \mu\text{m}$ . Among the 9 plastic particles, 7 were  
 235 fibers, and two were fragments. APs measured on average  $2.41 \pm 2.09 \text{ mm}$ , with a minimum length  
 236 of  $390 \mu\text{m}$  and a maximum length of 7.38 mm. Nine APs were made of plastic polymers and nine  
 237 were other kinds of APs, i.e. dyed particles (Table 1, Fig. 3b). Four types of plastic polymers were  
 238 found (Fig. 4): polyethylene terephthalate (PET, n=5), polypropylene (PP, n=2), polyacrylonitrile  
 239 (PAN, n=1) and poly(ethylene-co-vinyl acetate) (PEVA, n=1). Furthermore, twenty fibers of  
 240 cellulose were found in SC samples but were not included in APs, because the anthropogenic origin  
 241 could not be confirmed using Raman spectroscopy. There is no correlation between the length of  
 242 ingested APs and the length of fish ( $R^2=0.0026$ ).

243  
 244



245  
 246 Figure 4. Raman spectra obtained from isolated APs. PET=polyethylene terephthalate,  
 247 PP=polypropylene, PEVA=poly(ethylene-co-vinyl acetate), PAN=polyacrylonitrile.

248  
 249 When comparing the areas upstream and downstream of Paris, no significant difference was found  
 250 regarding the number of individuals having ingested MPs alone, all APs, fibers and fragments; the  
 251 number of ingested APs and MP; the number of ingested fibers and fragments (contingency tables,  
 252  $\alpha > 0.05$ ); and the length of all ingested APs (Mann-Whitney U test,  $\alpha = 0.24$ ). A summary of the  
 253 results is shown in Table 2. Fish from the upstream stations had 0.32 AP/g of SC, while fish from  
 254 the downstream stations had 0.40 AP/g of SC, also showing no significant difference (contingency  
 255 tables,  $\alpha > 0.05$ ).

256  
 257 Table 2. Comparison of different data between the areas upstream and downstream of Paris.  
 258 %=percentage.

	<b>Upstream (n=33)</b>	<b>Downstream (n=27)</b>
<b>% of fish with AP</b>	24%	26%
<b>% of fish with MP</b>	18%	11%
<b>Mean AP size</b>	2.00 mm	2.93 mm
<b>Min-max size</b>	0.39-7.38 mm	0.44-6.56 mm
<b>% of fibers</b>	90%	75%
<b>% of fragments</b>	10%	15%
<b>AP/g of SC (wet weight)</b>	0.32	0.40

259

260

261 *Livers & Muscles*

262

263 Four APs, of 13 total extracted particles, were found in three livers out of the sixty analyzed. All  
 264 were MPs, but three were made of PE, and one was made of polystyrene (PS). Their lengths ranged  
 265 from 147  $\mu\text{m}$  to 567  $\mu\text{m}$  (Table 3). Given the size of these MP fragments, it is very unlikely that  
 266 they come from airborne contamination.

267

268 Table 3. Characteristics of MPs found in livers.

<b>Station</b>	<b>Polymer</b>	<b>Size (mm)</b>	<b>Shape</b>
<b>Levallois</b>	PE	0.567	FR
	PE	0.161	FR
	PE	0.147	FR



<b>Villeneuve- Saint- Georges</b>	PS	0.270	FR
---	----	-------	----

269 FR=fragment, PE=polyethylene, PS=polystyrene.

270

271 No APs were found in muscle tissue.

272

273 Discussion

274

275 No difference was found between the areas upstream and downstream of Paris. Regarding shape,  
 276 fibers were dominant, and fibers longer than 1 mm were found in a higher quantity than the two  
 277 other size classes. The smallest fiber found in SCs was 390  $\mu\text{m}$  long. Smaller particles are unlikely  
 278 to be ingested because they would probably be rejected with water through branchial structures  
 279 (Collard et al., 2017b). Although Dris et al. (2015a) did not use any spectroscopic method for  
 280 identification, these three results are consistent with those highlighted by the previous study in the  
 281 Seine River water. Their study found an average of 45 fibers/ $\text{m}^3$  and 0.54 fragments/ $\text{m}^3$  in the water  
 282 column but found no difference between the downstream and upstream areas, and fibers longer  
 283 than 1 mm were predominant. European chubs' stomach contents seem to reflect what is found in  
 284 the river water. Moreover, since it is not an endangered species in European waters, European chub  
 285 could then be used as a bioindicator for AP and MP pollution. It is already used as a common  
 286 bioindicator in European freshwaters for other pollutants, such as musk (Hájková et al., 2007),  
 287 metals (Dragun et al., 2016), dioxins and polychlorobiphenyls (Pacini et al., 2013).

288

289 Compared to fibers, only a few anthropogenic fragments were found. Fragments may be indirectly  
290 ingested through various pathways: transfer from prey, as shown in experimental studies (Cedervall  
291 et al., 2012; Farrell and Nelson, 2013; Setälä et al., 2014); accidental ingestion from the water  
292 column or from sediments; or intentional ingestion (Ory et al., 2017). The chub is an omnivorous  
293 (Piria et al., 2005) and opportunistic fish (Balestrieri et al., 2006), meaning that everything is  
294 potential prey to feed on, including plastic. However, mechanisms of ingestion cannot be elucidated  
295 in this study.

296  
297 All former studies dealing with AP ingestion by freshwater fish focused on MPs. In comparison,  
298 the next section will also focus on MP ingestion but not on all APs. Few studies exist on MP  
299 ingestion by freshwater fishes, and moreover, their results must be carefully compared. Locations,  
300 species, and methodologies, including characterization of the target particles, differ between  
301 studies, leading to inconsistent comparisons. Here, we chose to provide data in terms of  
302 items/individual, items/g SC and percentage of occurrence. Each of those units has advantages and  
303 disadvantages but will at least allow comparisons between previous and future studies. The  
304 percentage of MP occurrence is often used to make comparisons more reliable. Regarding MP  
305 ingestion by freshwater fish, some of the lowest percentages of MP occurrence are found in fish  
306 from Geneva Lake (Faure et al., 2015), from French rivers (Sanchez et al., 2014), from a Brazilian  
307 estuary (Vendel et al., 2017), and in European smelts *Osmerus eperlanus* from the Thames estuary  
308 (McGoran et al., 2017) (7.5%, 12%, 9%, and 20% of individuals had ingested plastics,  
309 respectively). With an MP occurrence rate of 15%, our study has one of the lowest percentages. In  
310 addition, some studies have reported high MP occurrence percentages of up to 100% (Pazos et al.,  
311 2017). High values were also found in the Thames estuary, where 71% of flounders *Platichthys*  
312 *flesus* have ingested MPs (McGoran et al., 2017). Flounders are a benthic fish, raising questions

313 about the possible difference between pelagic and benthic feeders. According to a study on  
314 European smelts (pelagic) and flounders (benthic), feeding strategies could have an influence on  
315 the ingestion of APs (McGoran et al., 2017). The benthic species had a much higher ingestion rate  
316 than the pelagic one, but this was not statistically assessed. By contrast, Jabeen et al. (2017) and  
317 Murphy et al. (2017) found a significant difference between demersal and pelagic fishes (in  
318 freshwaters and marine waters, respectively). The demersal species ingested more APs. However,  
319 our study concerned a pelagic species, and we found a relatively low rate of MP occurrence  
320 compared to high percentages previously cited. Our percentage of MP occurrence in SC could have  
321 been underestimated. Among the dyed particles found in our study, some may have been made of  
322 a plastic polymer, which cannot be detected using Raman spectroscopy because of the dye coating.  
323 In marine environments, sediments are a sink for microplastics (Woodall et al., 2014). Regarding  
324 freshwaters, the literature lacks information about river sediment microplastic contamination,  
325 highlighting the need for more studies to assess whether freshwater demersal feeders are more  
326 exposed to pelagic ones.

327  
328 In the SC, a higher proportion of fibers was found compared to fragments (83% vs. 17%), as  
329 expected based on other studies (McGoran et al., 2017; Pazos et al., 2017; Silva-Cavalcanti et al.,  
330 2017; Vendel et al., 2017). As the digestion protocol may have bleached some particles because of  
331 the use of NaClO, some dyed cellulosic fibers may have, in actuality, been dyed textile fibers that  
332 were bleached during the isolation process, leading to an underestimation of APs. However, the  
333 results concerning the type of particle ingested by chubs are consistent with what is commonly  
334 found in the Seine and Marne Rivers: fibers are much more abundant than fragments.

335 WWTPs could be sources of fibers in urban rivers. While they help to retain a proportion of them,  
336 e.g., from 83% to 95% (Dris et al. 2015a), WWTP effluents still constitute as a source of fibers

337 (Dris et al. 2015a; Leslie et al. 2017). Paris' WWTPs remove long fibers (> 1 mm), but smaller  
338 fibers can pass through (Dris et al., 2015a). Atmospheric fallout can also be an additional source  
339 of fibers in receiving systems. Indeed, Dris et al. (2016) found that 50% of fibers coming from  
340 atmospheric fallout were longer than 1 mm, which is the dominant size class ingested by chubs. In  
341 that study, 50% of all fibers were natural, 21% were manufactured by transformation of natural  
342 polymers (e.g., rayon), and 29% contain a petrochemical-derived polymer (e.g., mix of cotton and  
343 polyamide, or polyurethane). No information regarding the >1 mm size class was given. Even if  
344 no direct link can be established between atmospheric fallout and the presence of long fibers in  
345 chubs' SC, we speculate that atmospheric inputs in water could be an important source of the  
346 longest fibers, which could then be ingested by freshwater biota.

347 The release of fibers into the environment is already a major concern, since washing machines can  
348 release a high amount of fibers per wash (Hartline et al., 2016; Hernandez et al., 2017). A polyester  
349 garment can release fibers between 0.033% and 0.039% of its weight (Dubai and Liebezeit,  
350 2013), and six identical fleece garments can release fibers from 0.008% to 0.021% of their total  
351 weight (Pirc et al., 2016). Consequently, in urban environments, fibers may be more problematic  
352 than fragments, particularly for filtering organisms that have adapted their morphology to retain  
353 such particles (Collard et al., 2017b).

354  
355 An increasing number of studies report AP, mainly MP, ingestion by fish, but the impacts are  
356 unknown. Studies which focus on wild-caught species probably have data regarding only a  
357 snapshot of the fish's lifetime. After being ingested, MPs are probably excreted (Van  
358 Cauwenberghe and Janssen, 2014; Watts et al., 2014; Grigorakis et al., 2017), but they may have  
359 a longer retention time than food that is typically (Ward and Kach, 2009; Mazurais et al., 2015).  
360 Additionally, no mechanical harm resulting from MPs has been reported in fish, but in birds,

361 plastics can reduce hunger or cause a reduction in assimilation efficiency (Ryan, 1989; Lenzi et al.,  
362 2016). The problem may instead concern pollutants. Microplastic fibers, like other microplastics,  
363 adsorb pollutants present in the surrounding water and contain small amounts of additives. They  
364 can be transferred to organisms once ingested (Teuten et al., 2009), although this is controversial  
365 (Koelmans et al., 2016). Additionally, little is known about adsorption on APs other than those that  
366 are plastic. In our study, 9 out of 18 APs were not made of plastic. How do pollutants interact with  
367 such particles? Did they also leach from particles to organs? Fibers constitute a major part of AP  
368 pollution in freshwater environments, so answering these questions is a priority for AP pollution  
369 research.

370

371 For the first time, MPs have been found in the livers of wild freshwater fish. Translocation also  
372 occurs in Parisian freshwaters, where fragment contamination is lower than fiber contamination  
373 (Dris, 2016). Translocation phenomena of such large MP abundances in fish have already occurred  
374 in both the laboratory (Avio et al., 2015) and the marine environment (Collard et al., 2017a). Other  
375 organisms also exhibit MP translocation (von Moos et al., 2012; Brennecke et al., 2015) but in the  
376 laboratory only. In mammals, the uptake of microparticles from the gut and subsequent passage  
377 through Peyer's patches in the intestine mucosa has been known for several decades (LeFevre et  
378 al., 1978; Jani et al., 1989; Jani et al., 1990). However, fish do not contain Peyer's patches,  
379 (Rombout et al., 1993; Cairn and Swan, 2010), and MP translocation pathways through the  
380 intestinal barrier are unknown in these organisms. Some hypotheses have been proposed, such as  
381 persorption, during which particles pass between cells of the gut epithelium to the circulatory  
382 system (Volkheimer, 1975; Wright and Kelly, 2017); endocytosis, which is an internalization of  
383 particles in cells of the digestive gland and has been demonstrated in mussels (Von Moos et al.,  
384 2012); and, finally, specialized enterocytes called microfold cells may be involved, as suggested

385 by Browne et al. (2008), again, in mussels. The digestive system in mussels differs significantly  
386 from that of fish, but since the literature regarding MP translocation in fish is very scarce, we can  
387 only rely on those few studies to piece together an explanation. Considering the large sizes of MPs  
388 found in livers in this study, the first hypothesis could be the most relevant. Persorption has been  
389 observed for particles of up to 130  $\mu\text{m}$  (Steffens, 1995 cited in Wright and Kelly, 2017). The  
390 remaining possibilities seem unlikely considering the sizes of translocated particles: 3  $\mu\text{m}$  (Browne  
391 et al., 2008) and smaller than 80  $\mu\text{m}$  (Von Moos et al., 2012). However, this should be confirmed  
392 by experimental studies.

393  
394 No APs were detected in muscle tissue despite the use of the sensitive Raman-based method  
395 developed. However, fewer samples, compared to those of SC and liver, were collected, and only  
396 several grams of muscle tissue per individual were taken to limit the amount of solution used.  
397 Consequently, other studies focusing on contamination of muscle tissue, and of other organs, are  
398 needed.

399  
400 Conclusions

401  
402 In conclusion, the opportunistic European chub ingested APs in an amount similar to that observed  
403 in other freshwater species (McGoran et al., 2017). Our results reflected the main ones highlighted  
404 in a study performed in the Seine and Marne Rivers inhabited by chubs. We then suggested that  
405 chubs may be used as an indicator species, as they are not endangered but are in fact common  
406 throughout Europe. Half of the analyzed APs were found to be made of plastic, highlighting that  
407 focusing on plastic particles could underestimate the level of APs ingested, regardless of species.  
408 Fibers were the predominant shape found in stomach contents and are ingested with unknown

409 consequences. Fragments can translocate into other organs by unknown pathways, as shown in  
410 other studies. Until now, this was not observed for fibers.

411 Many questions are yet to be answered. In the field, do MPs translocate into organs other than the  
412 liver? What are the pathways of translocation? How do they impact the organism? Does the MP  
413 contamination level in the environment influence the translocation process? What is the proportion  
414 of ingested plastic that can translocate into the liver? Considering the current global consumption  
415 of fish, humans are directly impacted by those issues. Histological analyses could provide answers  
416 to these questions. Histological analyses could contribute to give answers to these questions. The  
417 next step in AP contamination research could be to focus on *in vitro* studies, which may help to  
418 evaluate impacts and determine what type(s) of particles are likely to be translocated and the  
419 reasons for such translocation (polymer, size, presence of dyes on surface molecules, etc.).

420

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422

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428

429

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