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

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Abstract

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

Keywords

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

Introduction

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

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

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

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

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

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

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

Materials and methods

Sampling

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

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

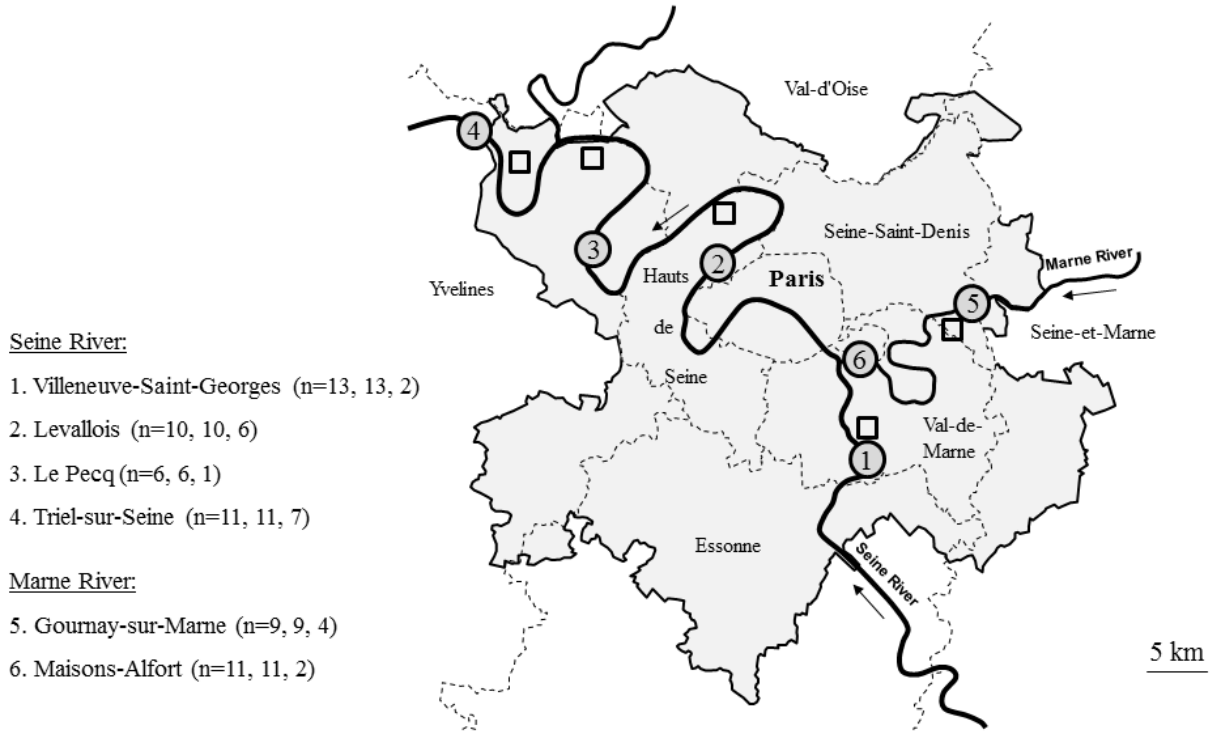

Figure 1. Sampling stations in the Parisian conurbation. Circles represent sampling stations and squares represent wastewater treatment plants. Number of stomach content, liver and muscle samples are shown for each station.

Isolation of APs

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

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

Raman analysis

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

were used to perform matchings: a commercially available database (Omnic Specta software, Thermo Fisher Scientific, U.S.A.) and a personal library, which used the Thermo Specta 2.0 software.

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

Preventing contamination and procedural blanks

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

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

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

Images and measurements of particles

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

Statistics

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

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

Results

Stomach contents (SCs)

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

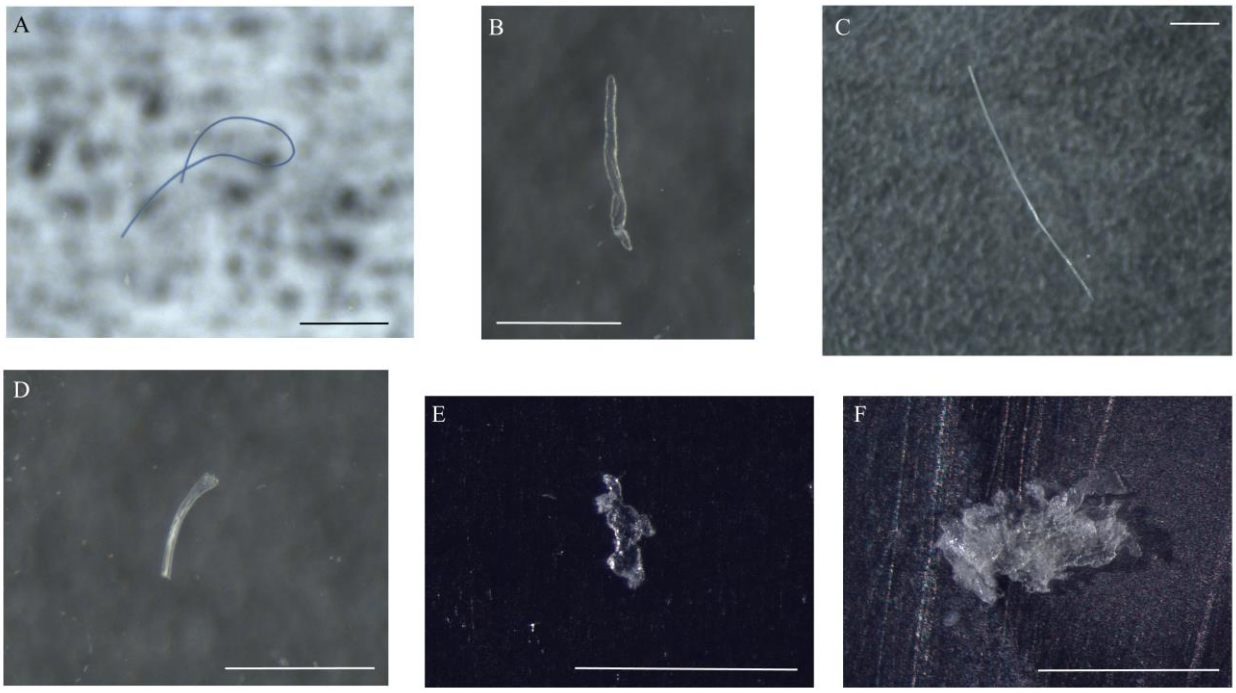

Figure 2. Pictures of APs found in stomach contents (A-D) and livers (E, F) of *S. cephalus*. A: blue dyed fiber, B: polypropylene (PP) fiber, C: polyethylene terephthalate fiber, D: PP fiber, E: polystyrene fragment, F: polyethylene fragment. Scale bar: 500 µm.

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

Station	**Composition**	**Size (mm)**	**Diameter* (µm)**	**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
Villeneuve-Saint-Georges	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
Triel-sur-Seine	Blue dyed particle Blue dyed fiber	1.43 2.21	23 17	FI FI	1.7
	PEVA	6.56	-	FR	0.36
	PP	0.44	-	FR	0.67
Levallois	PET	2.36	56	FI	1.0
	Blue dyed fiber	4.44	22	FI	0.59
Le Pecq	Blue dyed fiber	1.21	31	FI	3.33
	Black dyed fiber	4.79	22	FI	2.0

PET=polyethylene terephthalate, PP=polypropylene, PEVA=poly(ethylene-co-vinyl acetate), PAN=polyacrylonitrile, FI=fiber, FR=fragment.

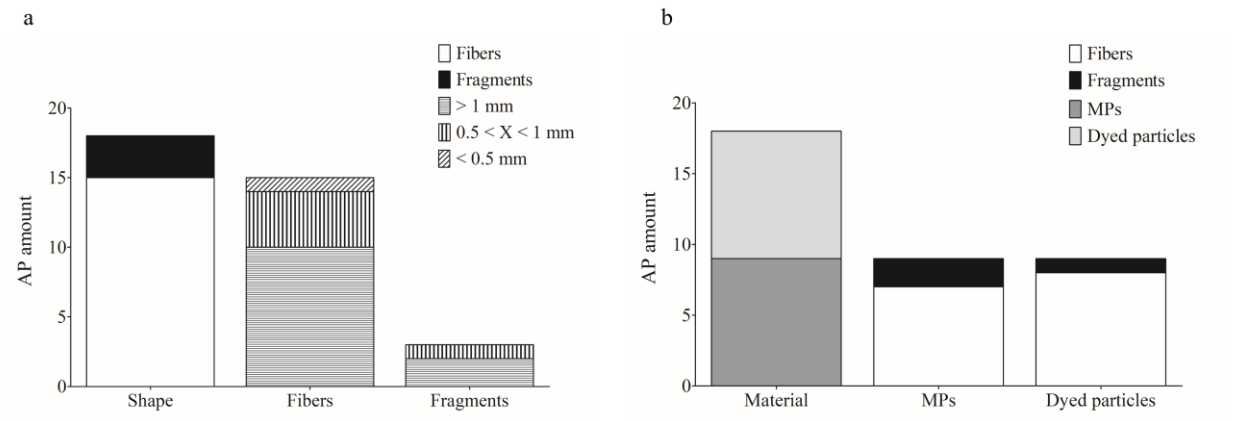

Figure 3. Summary of the main characteristics of APs found in stomach contents. a: total AP amount according to shape, b: total AP amount according to composition.

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

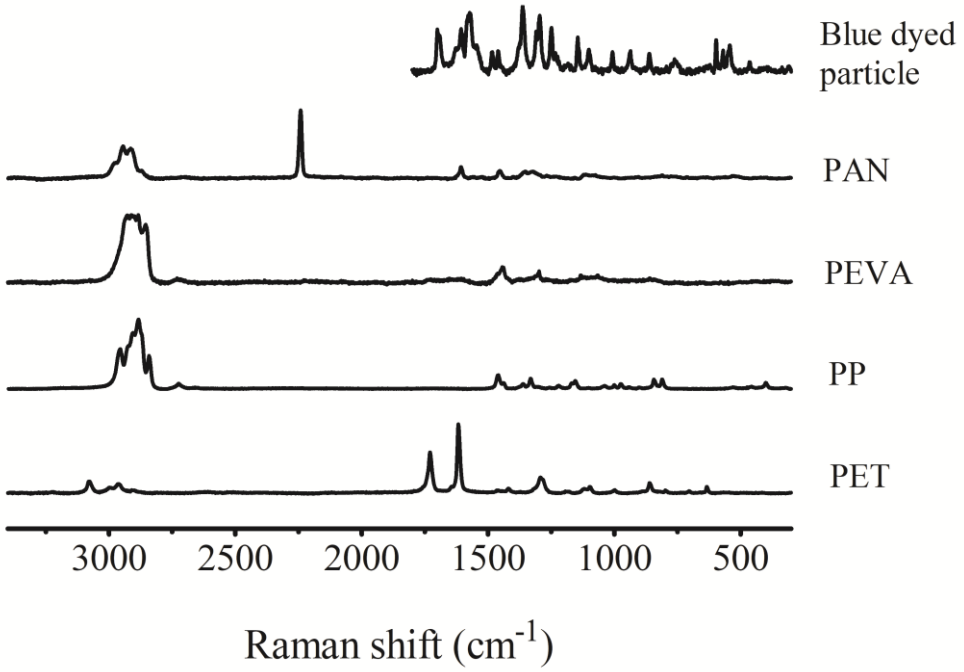

Figure 4. Raman spectra obtained from isolated APs. PET=polyethylene terephthalate, PP=polypropylene, PEVA=poly(ethylene-co-vinyl acetate), PAN=polyacrylonitrile.

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

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

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

Livers & Muscles

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

Table 3. Characteristics of MPs found in livers.

Station	Polymer	Size (mm)	Shape
Levallois	PE	0.567	FR
	PE	0.161	FR
	PE	0.147	FR

Villeneuve-Saint-Georges	PS	0.270	FR

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

No APs were found in muscle tissue.

Discussion

No difference was found between the areas upstream and downstream of Paris. Regarding shape, fibers were dominant, and fibers longer than 1 mm were found in a higher quantity than the two other size classes. The smallest fiber found in SCs was 390 µm long. Smaller particles are unlikely to be ingested because they would probably be rejected with water through branchial structures (Collard et al., 2017b). Although Dris et al. (2015a) did not use any spectroscopic method for identification, these three results are consistent with those highlighted by the previous study in the Seine River water. Their study found an average of 45 fibers/m³ and 0.54 fragments/m³ in the water column but found no difference between the downstream and upstream areas, and fibers longer than 1 mm were predominant. European chubs' stomach contents seem to reflect what is found in the river water. Moreover, since it is not an endangered species in European waters, European chub could then be used as a bioindicator for AP and MP pollution. It is already used as a common bioindicator in European freshwaters for other pollutants, such as musk (Hájková et al., 2007), metals (Dragun et al., 2016), dioxins and polychlorobiphenyls (Pacini et al., 2013).

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

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

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

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

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

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

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

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

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

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

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

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

Conclusions

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

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

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

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