Occurrence of anthropogenic particles in the fish *Squalius cephalus* from the Seine and Marne Rivers

Collard F.[1,*], Gasperı J.[1], Gilbert B.[2], Eppe G.[2], Rocher V.[3], Azımı S.[3] And Tassın B.[1]

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

[2]University of Liege, Inorganic Analytical Chemistry Laboratory, Department of Chemistry, B6c, 4000 Liege, Belgium.

[3]Syndicat Interdépartemental pour l'Assainissement de l'Agglomération Parisienne (SIAAP), Direction du Développement et de la Prospective, 82 Avenue Kléber, F-92700 Colombes, France.

*corresponding author:

e-mail: francecollard16@gmail.com

Abstract

Plastic pollution is a growing concern worldwide but was mainly focused on the marine environment. Few studies dealt with plastic pollution in lakes and rivers and the associated biota. In our study, we aimed to evaluate plastic occurrence in three target tissues (stomach contents, liver and muscle) of a wild freshwater fish (the chub *Squalius cephalus*) in the Seine and Marne Rivers. These rivers are under significant urban pressure with the Parisian conurbation (about 8 million of inhabitants) and low water flows. Several stations were sampled upstream and downstream of Paris conurbation. Fish were dissected and the three tissues were degraded using previous validated protocol. Particles were isolated and were then analyzed by Raman spectroscopy. Preliminary results showed that plastic particles and other anthropogenic particles such as textile fibers were found in several guts at different stations. Observed fibers were made of plastic polymer, such as polyethylene terephthalate or polypropylene, or were probably cellulose, dyed with artificial pigments. Our preliminary results confirmed that *S. cephalus* ingested microplastics, mainly fibers, probably because of the contamination of the river water column.

Keywords: microplastics, Seine River, freshwater fish, *Squalius cephalus*, fiber

1. Introduction

Anthropogenic particles (APs) pollution, including plastic pollution, occurs in each ocean (Cózar *et al*. 2014) and a very large range of marine organisms (Kühn *et al*. 2015). However, little is known about microplastic (< 5 mm, Arthur *et al*. 2009) pollution in freshwater ecosystems such as rivers despite they are a pathway to oceans. Several studies evaluated the amount of plastics in rivers (Dris *et al*. 2015b; Faure *et al*. 2015) such as in the Seine River (Gasperi *et al*. 2014) or in the Danube River (Lechner *et al*. 2014). They showed that, while dynamic, they could also be highly contaminated in plastics. For example, Lechner *et al*. (2014) reported a maximal density superior to 140,000 items/1000 m³ and a global plastic mass for both microplastics (MPs) and macroplastics, higher than fish larvae mass. In the U.S.A., it was estimated that over 4 million particles are released every day from one wastewater treatment plant (Mason *et al*. 2016). In the Seine River, fibers were the dominant shape when a net with small mesh size was used (Dris *et al*. 2015a), with a mean concentration of 30 fibers/m³. The authors also showed a difference of fiber lengths between raw wastewater and treated water, meaning that wastewater treatment is efficient only for biggest fibers (from 1 to 5 mm). Such concentrations could likely lead to plastic ingestion by the biota. These studies are also very scarce. To our knowledge, only five studies, including lake, river and estuarine ecosystems, reported plastic ingestion by wild freshwater organisms, which were fish species (Faure *et al*. 2012; Sanchez *et al*. 2014; Faure *et al*. 2015; McGoran *et al*. 2017) or marine mammal species (Denuncio *et al*. 2011). Similarly to studies on the marine environment, an important variability regarding the frequencies of occurrence of plastic ingestion by fish is found. It varies from 0% in *Rutilus rutilus* or *Perca fluviatilis* (Faure *et al*. 2012; Faure *et al*. 2015) to 85% in *Platichthys flesus* (McGoran *et al*. 2017). The ingestion of plastic debris lead to mechanical harm in marine mammals and birds (Beck and Barros 1991; Jacobsen *et al*. 2010; Barreiros and Raykov 2014; Pierce *et al*. 2004) but also to toxicological impacts, in fish among others (Cedervall *et al*. 2012; Rochman *et al*. 2013). Moreover, MPs can translocate in other organs of fish such as the liver (Avio *et al*. 2015; Collard *et al*. 2017). The chub *Squalius cephalus* has not been studied so far, in the context of plastic pollution despite its ubiquity in European rivers and its abundant population (Freyhof 2014). Generally, they feed on plants, insects (larvae and adults) and small crustacean (Mann 1976; Michel and Oberdoff 1995; Balestrieri *et al*. 2006). However their diet varies with age and seasons. Young fish feed mainly on insects (larvae and adults) (Mann 1976) and adults prefer to feed on other fish, particularly in winter, on plants or crayfishes (Michel and Oberdoff 1995). Our study presents some novelties as it focuses on freshwater fish caught in a megacity, Paris, and its conurbation with high human pressures. Moreover the sampling has been done along the continuum Seine and Marne Rivers allowing the comparison between the upstream and the downstream of Paris. These rivers have already been investigated in the context of anthropogenic fibers (Dris *et al*. 2015a) and for the first time, a first attempt to compare APs contamination in the environment and in fish, can be done. In summary, we aim to (1) evaluate the ingestion of APs by this opportunistic fish species caught within the Parisian conurbation, (2) determine whether microplastics translocate in the liver or muscle of *S. cephalus,* (3) compare APs concentration levels in the environment and amounts of APs ingested by fish at the same location.

2. Methods

2.1. Sampling

Thirty-seven freshwater fish, *Squalius cephalus* (mean length: 293 mm ± 64, mean weight: 331 g ± 214), were electrofished in the Marne and Seine Rivers around Paris, France, during late summer 2016. Dissections for chemical analyses in biomonitoring were performed. The remaining tissues were guts (n=37), livers (n=14) and muscles (n=13). Guts and livers were put into a diluted formaldehyde solution (5%) while muscles were frozen in the lab. All samples were weighed before the isolation process.

2.2 Preventing contamination and procedural blanks

To prevent contamination, latex gloves were worn throughout the whole isolation process. All work surfaces and dissection materials were cleaned with distilled water. To prevent airborne contamination during the drying of ethanol upon stainless-steel plates, they were placed under a metal sifter (36-µm mesh). No fibers smaller than 36 µm in length were found. The sodium hypochlorite solution was filtered before dilution with distilled water. Three procedural blanks of the whole isolation process were performed at the same time than several samples. No anthropogenic particles were found in these blanks.

2.3 Isolating APs

All tissues were degraded and then filtrated following the same methodology previously validated and described by Collard *et al*. (2015). Tissues were put into a NaClO solution overnight. The remaining solution was filtered with a cellulose acetate filter membrane (5 µm porosity) which was rinsed with an absolute ethanol solution. It was then centrifuged at 5,000 rpm for 10 min. The bottom was then collected and deposited on a stainless steel plate for Raman spectroscopy analysis. Three major adaptations have been provided for this study: the concentration of NaClO, its volume and the replacement of methanol 99% by ethanol 99%. The two firsts enhancements were necessary regarding the higher mass of tissues to be degraded. Besides, methanol was replaced by ethanol as methanol degrades polyvinylchloride (PVC).

2.4 Raman analyses

A LabRam 300 spectrometer (Jobin-Yvon) equipped with an Olympus confocal microscope and Andor BRDD Du401 CCD detector was used to analyze particles. A Spectraphysics argon-ion laser (green laser, 514.5 nm) or a Torsana diode laser (red laser, 784.7 nm), and two objectives were used (magnification of ×50 and ×100). The maximum beam laser power on the sample was 5 mW (green laser) and 30 mW (red laser), but several neutral density filters were used most of the time to lower the power, thus avoiding degradation of the sample. The integration times ranged from 5 s to 50 s, depending on the sample. Matching, between recorded spectra and references from commercially available or personal libraries, was performed using the Thermo Specta 2.0 software. Raman analyses allow the identification of materials but also additives such as dyes. After Raman analyses, APs were isolated in 1 ml of 99% ethanol for further observations and measurements.

2.5 Images, measurements and weights of APs

APs were observed on a white filter membrane with a stereomicroscope (Leica MZ12, Leica AG Camera, Germany), photographed and measured with the Histolab software (Histolab Products AB, Sweden).

3. Results

From stomach contents (n=37), 11 APs were ingested. Among them four were made of plastic polymer and the other seven were dyed fibers. Twenty-four percent of individuals had ingested AP(s), and 11% of them had ingested plastic particles. The majority of APs were fibers. The plastic polymers were polypropylene (PP), poly(ethylene terephthalate) (PET) and polyacrylo-nitrile (PAN). Two particles were made of PET. When a dye was recorded by Raman spectroscopy, it was not possible to determine the material (cellulose or other) as dyes gave a strong signal comparing to biological materials. Some natural materials have been found such as carbapatite and cellulose. While cellulose fibers could have an anthropogenic origin (cotton textile fibers), we chose to exclude them from the results because they could come from paper towel used in the lab or simply have a natural origin. No AP was found in liver nor in muscle.

4. Discussion

The percentage of APs found in stomachs is quite similar to previous studies such as Sanchez *et al*. (2014) and Faure *et al*. (2015) who reported 12% of plastic occurrence in *Gobio gobio* and 10% in *Alburnus alburnus*, respectively. The gudgeon, the bleak and the chub are opportunist and have a various diet but the gudgeon stays close to the bottom. The bleaks analyzed came from lakes and not rivers. This highlights the lack of information about plastic and AP ingestion by the fauna in general in freshwaters (rivers, lakes,…). Comparisons are difficult and plastic ingestion might depend on several fish characteristics such as feeding strategies (Rummel *et al*. 2016) or morphological structures (Collard *et al*. 2017). Two rivers have been sampled in Paris, but the number of samples is yet too small to provide a reliable comparison between them and also between the upstream and the downstream of this megacity. Final results will also be discussed with previous studies in the same rivers. Studies reporting AP concentration in both freshwater environment and biota at the same location(s) are very scarce. Our preliminary results showed that the majority of APs and plastics found in the chub were fibers. A previous study in our lab (Dris *et al*. 2015a) confirms the consistency of our first results and attests the high concentrations of fibers in the water column. Plastics found in chubs are common polymers (PET and PP) used in many applications (textiles, household uses, packaging), except the PAN which is used for more accurate applications. While several wastewater treatment plants purify wastewaters from Parisian houses, it seems that fibers are not extracted from wastewater during the process. Washing machines are a major pathway for fibers to reach the environment as a single garment can produce more than 1,900 fibers per wash (Browne *et al*. 2011). Other sources can also contribute to this pollution such as atmospheric fallout (Dris *et al*. 2016). These fibers are ingested by local fauna with unknown consequences.

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