A comparison of microplastic contamination in freshwater fish from natural and farmed sources

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Abstract

Contamination of aquatic systems mainly by urbanization and poor sanitation, deficient or lack of wastewater treatments, dumping of solid residues and run off, has led to the presence of particles, including manmade polymers, in tissues of many marine and freshwater species. In this study, the prevalence of microplastics (MPs) in freshwater fish from farmed and natural sources was investigated. *Oreochromis niloticus* from aquaculture farms in the Huila region in Colombia, and two local species (*Prochilodus magdalenae* and *Pimelodus grosskopfii)*, naturally present in surface waters were sampled. Of the particles identified, fragments were the predominant type in the three tissue types (stomach, gill and flesh) derived from farmed and natural fishes. Micro-FT-IR spectroscopy was conducted on 208 randomly selected samples, with 22% of particles identified as MPs based on a spectra with a match rate ≥70%. A total of 53% of identified particles corresponded to cellophane/cellulose, the most abundant particle found in all fish. Not all fish contained MPs: 44% of *Oreochromis* farmed fish contained MPs, while 75% of natural source fish contained MPs in any of its tissues. Overall, polyethylene terephthalate (PET), polyester (PES) and polyethylene (PE) were the prevalent MPs found in the freshwater fish. A broader variety of polymer types was observed in farmed fish. The edible flesh part of fish presented the lower prevalence of MPs compared to gill and stomach (gut), with gut displaying a higher frequency and diversity of MPs. This preliminary study suggests that the incidence and type of MPs varies in farmed verses natural fish sources as well as across different tissue types, with significantly less detected within the edible flesh tissues compared with stomach and gill tissues.

1. Introduction

There has been a worldwide increase of the concern surrounding microplastics (MPs), particles of a size smaller than or equal to 5mm (Lusher et al. 2017), about their impacts on the environment as well as entering the food chain (Rochman et al. 2015; Van Cauwenberghe & Janssen. 2014) due to its availability for ingestion to a wide range of aquatic organisms. Increasing numbers of studies report their presence in the water column, sediments, and beaches (Bordós et al. 2019) as well as in different animal species and variety of fish tissues around the world (Neves et al. 2015; Biginagwa et al. 2016; Nadal et al. 2016; Jabeen et al. 2017; Pazos et al. 2017; Foley et al. 2018). As an example, in commercially important species, such as *Hoplosternum littorale* a commonly consumed fish in South America, levels of MPs were observed in 83% of the fishes analyzed (Silva-Cavalcanti et al. 2016). Nevertheless, research regarding the abundance, microparticle type and tissue distribution in fish from freshwater ecosystems is more limited compared with marine environments (Horton et al. 2017).

It is yet to be fully elucidated how such MPs reach different aquatic organisms and their tissues, what their fate and biological impacts may be within these, or if there is a relationship between the chemical composition of the ingested MPs and the species that ingest them. Some authors suggest that ingestion of MPs can occur accidentally when the fish is feeding (Bessa et al. 2018), or by the transfer of these MPs within the food chain from prey to predator, where variables such as persistence, concentration of the MPs in preys and the time it takes to eliminate them, if they do so, are factors that must be considered (Santana et al. 2017). Others authors believe that an intentional ingestion may occur when fish confuse the color or shape of the MP with their usual food (Ory et al. 2017). In any case, this will depend on the feeding behaviour of the fish, and on the properties of the MPs (Roch et al. 2020). In terms of the tissue fate of these particles within the organism, there are studies which indicate that some MPs can be stored in the liver of certain species (De Sales-Ribeiro, Brito-Casillas, Fernandez, & Caballero, 2020) but, the greatest concern is the possibility that these compounds may be found in the edible part of fish (Karami, Golieskardi, Ho, Larat, & Salamatinia, 2017), posing a risk for human health when ingested (Akoueson et al. 2020), as well as by the chemical properties of the

MPs (type of polymer and presence of adittives), and the possibility of microbial biofilm growth (Campanale, Massarelli, Savino, Locaputo, & Uricchio, 2020).

The chemical composition of the particles identified as MPs by thechniques such as FTIR or RAMAN in certain freshwater fish species have differed, depending on the species and sampling place, being the polypropylene (PP) (Slootmaekers et al. 2019; Collard et al. 2018) and the polyethylene (PE) (Horton et al. 2018; Biginagwa et al. 2016; Andrade et al. 2019) reported more frequently. Other type of polymers also found less frequently are the polyethylene terephthalate (PET) (Collard et al. 2018), the ethylene-vinyl acetate copolymer (EVA) (Slootmaekers et al. 2019), as well as acrylic fibers (McGoran et al. 2017), and polyester (Bessa et al. 2018). With studies reporting the presence and chemical characterization of MPs in aquaculture being scarce.

Colombia, unlike many countries, has abundant water resources, whereby the average national supply of freshwater is more than 2100 km^3, equivalent to an approximate supply of 50000 m^3 per capita. Nonetheless the distribution of this resource is not equitable throughout the territory, since there are zones of greater exploitation and water demand (Sánchez et al. 2007)(Sánchez Triana, Ahmed, Awe, & World, 2007b)(Sánchez Triana et al. 2007b)(Sánchez Triana et al. 2007b) Among the main uses are agriculture and fish farming. The latter involves an estimated export of Tilapia (*Oreochromis niloticus*) (for 2018) of 46.53 tons of fresh fish, with main destinations being USA and Canada (Departamento Administrativo Nacional de Estadística, 2019), countries where the quality of the product is the main characteristic demanded by consumers. The most productive Department, Huila, is supplied by abundant water bodies, consisting of 40 sub-watersheds and 535 basin areas, plus the Magdalena River, with an average annual temperature of 27 °C. In this region, 58% of the Tilapia that is exported from Colombia is produced (Gómez et al. 2014). Considering these commercial interests in the region, it is important to investigate the presence and prevalence of MPs in fish from such aquaculture sources, as well as placing such information to consumers in perspective by using fish from natural river sources as a comparison.

Herein we report on the prevalence and levels of different types of MPs in various tissue compartments (edible and non-edible) from farmed and wild freshwater fish sources.

2. Materials and Methods

2.1. Sample collection

Methodology used was quantitative, transversal, where adult size *Oreochromis niloticus* (n=18 individuals) were collected from three farms (n=6 each) supplied by surface waters of different origins (namely the Betania Dam, the Magdalena River after its pass through the city of Neiva, and the Bache River) located in the Huila region in Colombia, from March to May of 2018 (Figure 1). In addition, two native species *Prochilodus magdalenae* (n=6 individuals) and *Pimelodus grosskopfii* (n=6 individuals) were obtained from local fisherman in the Magdalena River after its pass through the city of Neiva. The fish (n=6) from each farm sampling site and from the two local species were dissected and ~5 g tissue from muscle, digestive system (stomach) and gill was extracted from each replicate (n=5 sampling sites plus local species, with three tissue types with six 5 g replicates). The fish were transferred to the laboratory of the Exact Sciences Faculty in Universidad Surcolombiana, and were stored at refrigeration (2 ºC) until further analysis (and no longer than 24 h).

2.2. Hydrogen peroxide treatment of soft tissue

The extraction method and analysis of particles from fish was developed according to the protocol described by Li et al. (2016). Each fish unit was rinsed with filtered distilled water, and the length and weight of each were recorded. Approximately 5 g of soft tissues (flesh without skin, gill and gut) was extracted from each individual (procedure performed in extraction cabinet) and placed in a 1 L flask, regarded as one replicate. Six replicates were used for each site and local species. 200 ml of 30% H_2O_2 was added to each flask. The bottles were covered with foil films, and placed in incubator at 65 ºC with frequent manual agitation (each 2 – 3 h/ during the day). The incubation time was between 24 h

(flesh) until 7 d (gill) according on the digestion status of the soft tissue. The digestions were concluded once they appeared clear and no obvious particles were visible. All liquids (distilled water and hydrogen peroxide) were filtered three times with a 1 μm filter paper prior to use to reduce contamination of the samples by airborne microplastic. All of the laboratory materials used were rinsed three times with filtered distilled water. A blank extraction (n=6 replicates), without tissue, was processed simultaneously to identify and characterize any procedural contamination.

2.3. Filtering of particles

Each solution of tissue digested in hydrogen peroxide was filtered through a nitrocellulose filter of 47 mm diameter and pore size of 5 μm (EMD Millipore, Germany) using a vacuum system (Metic-Lab GM-1.00). Next, filters were located in clean petri dishes and remained covered until further analysis.

2.4. Abundance and characterization of particles and MPs in fish tissues

Each filter was observed under a Leica DM 500 microscope equipped with camera ICC 50HD (Leica Camera AG, Germany). A visual assessment (shape and color) and counting was conducted to identify all particles retained on the filters according to the physical characteristics of the particles and based on classification described by Free et al. (2014). The filters were then carried to University of Hull for the validation of the particles. 208 particles were selected from across samples from fish and blanks (approximately 9% of total particles counted), and their identity were confirmed by Fourier-transform infrared microspectroscopy (micro-FT-IR) with a UKAS accredited PerkinElmer Spectrum Spotlight equipped with a mercury−cadmium-telluride focal plane array (FPA) detector (consisting of 16 gold-wired infrared detector elements) cooled with liquid nitrogen (Li et al. 2018). Analysis was conducted in transmittance mode with particles transferred from filters, using either tweezers or a needle, to be diamond mounted. Spectra were acquired with a minimum of 50 scans at a resolution of 4 cm^{-1} and matched using a series of polymer library databases (PolyATR, AR Polymer

Introductory, NDFIBS, RP, CRIME, FIBRES 3, POLY1, POLYADD1) (PerkinElmer, USA), a hit index of at least 70% match was considered acceptable. 161 of the analyzed particles met this threshold.

2.5. Statistical analyses

The normality of variables was tested with Shapiro Wilk, for variables with a normal distribution t-student test was used, and when normality was not observed the Wilcoxon rank sum test was used to compare between natural and farmed source groups. For the comparison of morphology types and abundance, the Wilcoxon rank-sum test was used. Relative number of different type of MPs (composition) in tissue samples was analyzed based on the Odds ratio. Statistical significance was accepted at $p < 0.05$. Statistical analyses of the data was performed using STATA (StataCorp) and SPSS (IBM, USA).

3. Results

A total of 30 individuals (18 *Oreochromis niloticus*, 6 *Prochilodus magdalenae*, 6 *Pimelodus grosskopfii*) were analyzed in our study. Individuals' total length ranged from 23 – 38 cm (mean 25.8 ±0.8 cm for natural fish and mean 30 ±8 cm for farmed fish), and weight from 222 – 1798 g (mean 310.8 ±25.8 g in natural fish and mean 874 ±386 g in farmed) (Table S1), which indicated significant differences in the individual fish sampled according to source. Farmed fish were larger and heavier at the Bache river farm, than fish from other origins. Natural fish were significantly smaller compared to the farmed fish.

3.1 Abundance and morphology of particles in fish

No significant differences between the abundance of particles in the three types of tissues per gram of tissue analyzed was observed, neither between farmed and natural fishes (Figure 2). However, the

amount of particles per gram counted was higher in gill and gut tissues compared with flesh tissues (Figure 2). When microparticles recovered from the three type of tissues were compared to those found in the procedural blanks statistical significant differences were observed for each tissue type (guts p=0.0042, flesh p=0.01, and gills p=0.02).

Different shape types of particles, and their distributions, were detected in gut, gill and flesh tissues. Fragments were the predominant shape of particle identified in the all three tissue types, followed by film and then fiber (Figure 3A-C). No beads were detected. In farmed fish, fragments were the main particle shape observed independent of tissue type. In fish from natural sources, the main type of particle shape depended on the tissue, with a higher prevalence of fragments in gill and flesh, compared with films in gut (Figure 3A-C). When comparing the main particle shapes found in fish from farmed verses natural source, significant differences were observed in the number of fibers detected in gut and gill, with higher counts on farmed fish. The procedural blank samples contained mainly fibers.

3.2 Chemical characterization of particles isolated from fish tissues

A total 208 particles were selected randomly from across all the filters and identified, corresponding to 9% of the total particles isolated from all filters/fish tissues, 161 particles were identified in its chemical composition at a match rate more than 70%. From these, a total of 22% (36) of particles were confirmed as MPs (Figure 4A). Based on this subset of particle characterization, there were individual fish that contained no MPs in any of their tissues. 44% (8 out of 18) of farmed fish sampled had MPs in any of its tissues, while 75% (9 out of 12) of natural source fish sampled presented MPs. It was observed that the individual fish which had MPs presented in average 2.1 ± 1.26 items/individual (calculated by summing number of MPs in the examined tissues). Overall, polyethylene terephthalate (PET) (30.56%) was the dominant polymer type identified, followed by

polyester (PES) (22.22%) and polyethylene (PE) (8.33%); with lower frequencies of other types of polymers (Table S2). The latter comprised plastic additives (10.56%) and materials of other anthropogenic origin (14.29%) (Figure 4A). Other, non-anthropogenic-source, particles with a high frequency of detection were those from biological origins (52.8%) (Figure 4A), being mainly cellulose/cellophane (Table S2). In farmed sourced fish tissues, the main polymer detected was PET (36.4%), PES (22.7%), polyethylacrylate:st:acrylamide and polycarbonate (both 9.1% each). From natural-sourced fish, PET and PES were also the main type of polymer more identified (21.4% each) followed by PE and alkyds (both 14.3%) (Figure 4B). Cellophane and cellulose were the only type of particles found in the procedural blank samples. In terms of tissue distributions, gut tissue displayed a higher diversity of polymer types compared to gill and flesh (Table S2). In the edible flesh tissues of fish, three types of MPs were observed: PET, PP and nylon (Table S2). PET was observed in all three tissue types from farmed fish sources, yet was less abundant in tissues from naturally sourced fish and absent from gut samples (Table S2). PES was identified in gut of natural and farmed fish, but it was not detected in flesh. Alkyd polymers and nylon were only observed in tissues from natural source fish (Table S2). The Odds ratio test indicates that the probability of finding MPs in the flesh of fish from natural source is higher (3.75%) than in the flesh of farmed fish. Nevertheless, the association was not significant.

4. Discussion

The present study provides a report of MPs and other anthropogenic and natural origin particles in freshwater fishes from aquaculture (*Oreochromis niloticus*), farmed in the region of Huila in Colombia, and in two natural freshwater fish species (*Prochilodus magdalenae* and *Pimelodus grosskopfii*) which are locally consumed. This has allowed a comparison of the MPs contamination levels between freshwater fish from farmed and natural sources, as well as the distribution among their different tissues. The fish species analyzed correspond to farmed and wild-caught fish that are

consumed by humans, which is an approach that helps understand potential exposure levels of MPs contamination in the food chain via edible fish tissues.

Our results show that from the particles detected in the different type of tissues, fragments were the main type observed in gut, gill, and flesh similarly for both farmed and wild sourced fish. While acknowledging that this study involves a relatively small sample size, the findings are contrary to many previous studies using fresh and marine water samples and animal species, where the most abundant particle type are fibers (Free et al. 2014; McGoran et al. 2017; Bessa et al. 2018; Li et al. 2018). There are a small number of previous reports, such as Rochman et al. (2015) where 60% of 105 particles of anthropogenic debris recovered from fish sampled in Indonesia corresponded to plastic fragments. Others have similarly highlighted fragments as the most abundant MPs shape in mussels (*Mytilus galloprovincialis*) and fish (*Sardina pilchardus*, *Pagellus erythrinus*, *Mullus barbatus*), with a proportion between 73 – 83% of MPs, classified as fragments (Digka et al. 2018). Finding fragments may reflect either a lack of a proper waste management strategies (Rochman et al. 2015; Schwarz et al. 2019), or the amount of macroplastic inputs via the Magdalena River eventually degrading over time.

Chemical composition analysis of a subset of particles using micro-FT-IR determined that only 22.4% of particles identified (with a match rate ≥70%) were MPs, with a further 10.6% and 14.3% identified as plastic additives and other anthropogenic materials respectively. This contrasts with previously published studies in which 32-55% of debris items identified from the gut tissues of six marine species corresponded to MPs (Digka et al. 2018; Halstead et al. 2018). Cellophane/cellulose were the type of particle with a higher prevalence of identification (52.8%). In this study cellophane was grouped together with cellulose as particles of natural origin, because the identification technique using FTIR for these two polymers are not distinguishable, potentially leading to overestimation of MPs source items otherwise. Cellophane is based on a natural fiber, yet additives cause many authors to classify it as a MPs (Su et al. 2016). Our finding is consistent with Jabeen et al. (2017), where

49.9% of particles identified in 26 marine species and 6 freshwater species corresponded to cellophane. The lower percentage of particles identified as MPs in this study compared to others, can be attributed to the exclusion of cellophane as a synthetic MPs.

The freshwater fish species from natural sources selected in this study are characterized as having benthopelagic habits (Lasso et al. 2011), in contrast to the farmed fish which have epipelagic habits (Lasso et al. 2011). Different habitats of the natural verses farmed fish may account for the higher number of individual wild fish presenting with MPs (75% compared with 44% respectively). Local fishing is common in the Magdalena River, which passes through the city of Neiva, with approximately 500,000 inhabitants and no WWTP. Several investigations relate population density and untreated wastewater discharge, with higher levels of MPs in different matrices (Silva-Cavalcanti et al. 2016; Horton et al. 2017). Yet, in the fewer individual farmed fish, where MPs were observed in their tissues, a greater variety in the composition of the polymers was observed. Differences in the polymers properties such us density, size and area of the MPs (Schwarz et al. 2019), as well as biological interactions (Courtene-Jones et al. 2019), suggest that the epipelagic zone may contain more diversity and availability of MPs than in the benthic zone, also with this MPs having a greater horizontal mobility than vertical movement due to water current.

In terms of the polymers detected in the various fish tissues PET, followed by PES and PE, were the main MPs. This is consistent with studies of fish from Chile whereby PET was identified in 75% of fish sampled from the ocean and 80% from the coast, with PE representing 25% (Pozo et al. 2019). Previously, of 34 studies reviewed by de Sá et al. (2018), PE has been identified as the most common polymer. Similarly in contrast to our findings, PE, polypropylene (PP), and polystyrene (PS) have been highlighted as the dominant polymers in freshwater systems (Ory et al. 2018; Bordós et al. 2019; Schwarz et al. 2019). The type of MPs found in the species analyzed will presumably depend on the quantity and type of polymers most used in the geographical area of study. PE being the most common

plastic used, because of its application in packaging, and PES being a popular material for the manufacture of fishing nets (Pozo et al. 2019).

Finally, the tissue distribution of the MPs detected in this study highlights an important difference. Higher frequencies (25 out of 36 MPs identified) and greater chemical composition diversity of MPs were identified in the gut tissues. This finding is comparable with several studies where presence of MPs in gut of fish from different species is a common denominator (Biginagwa et al. 2016; McGoran et al. 2017). While there were no clear differences in the composition of MPs accumulated in tissues of fish from natural verses farm source, PET was more prevalent in farmed fish within all three tissues, yet absent in gut tissues from natural source fish. Critically, within the typically edible tissue, flesh, PET, PP and nylon MPs were identified (three particles in natural- and two particles in farm-sourced fish), representing a direct exposure route into the human food chain. Also, when looking at the sampling location of the five fish presenting MPs in their flesh tissue, all were obtained from the Magdalena River after its pass through the city of Neiva.

In conclusion, fragment shaped MPs, with a higher prevalence in gut tissues, were identified in fish tissues from natural and farmed sources. Chemical characterization of the MPs isolated from the fish tissues identified PET, PES and PE as the main polymers. A higher number of fish sampled from natural sources presented MPs in their tissues compared to farmed fish, but the individuals cultivated in controlled systems presented a broader variety of MPs according to their chemical composition. Due to the minimal prevalence of MPs in flesh (0.2 MPs/g) we suggest that there is a low exposure risk to human health via the food chain from the consumption of flesh from freshwater fishes of aquaculture and artisanal fishing in the higher part of the Magdalena River. Nevertheless, this preliminary study represents only a small number of fish and further research including analysis of water and sediment samples at sampling sites is now required.

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Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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