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ORIGINAL ARTICLE

Microplastic occurrence in fish species from the Iquitos region in Peru, western Amazonia

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ABSTRACT

The contamination of aquatic environments by microplastic has become a major threat to biodiversity. The presence of microplastic is documented in the aquatic fauna of the oceans, but, in the Amazon basin, reports on microplastic occurrence are few. The present study surveyed microplastic occurrence in fishes in an area of the Peruvian Amazon. We sampled 61 specimens of 15 commercial species from local markets in the city of Iquitos, Loreto Department. We detected a total of 2337 microplastic particles, 1096 in the gills and 1241 in the internal organs (esophagus, stomach, intestine, liver, gonads, pancreas, swim bladder and heart). The prevalence of microplastic particles was 100% and the overall average abundance was of 38.3 particles per individual (17.9 particles per individual in gills and 20.3 particles per individual in internal organs). Most particles were found in carnivorous fish. There was no correlation of particle abundance with fish standard length and weight. These results provided evidence of the degree of microplastic contamination of the fish fauna in the region of Iquitos.

KEYWORDS: fish diversity; fibers; freshwater conservation; Loreto; river pollution

Ocurrencia de microplástico en especies de peces de la región de Iquitos, Amazonía occidental

RESUMEN

La contaminación de ambientes acuáticos por microplásticos se ha convertido en una gran amenaza para la biodiversidad. La presencia de microplásticos está bien documentada en la fauna acuática de los océanos, pero en la cuenca del Amazonas hay pocos reportes de ocurrencia. En este trabajo se investigó la ocurrencia de partículas de microplásticos en peces de un área de la Amazonía peruana. Se obtuvieron 61 especímenes de 15 especies comerciales provenientes de los mercados locales de la ciudad de Iquitos. Se detectó un total de 2337 partículas de microplástico, 1096 en las branquias y 1241 en los órganos internos (esófago, estomago, intestinos, hígado, gónadas, páncreas, vejiga natatoria y corazón). La prevalencia de partículas de microplástico fue del 100% y la abundancia general de partículas de microplástico fue de 38.3 partículas por individuo (17.9 partículas por individuo en las branquias y 20.3 partículas por individuo en los órganos internos). La mayor cantidad de particulas fué encontrada en peces carnivoros. No hubo correlación entre la abundancia de las particulas y el tamaño estandar y peso de los peces. Estos resultados proporcionan evidencia de los niveles de contaminación por microplásticos en la fauna de peces amazónica en la región de Iquitos.

PALABRAS CLAVE: diversidad de peces; fibras; conservación de ambientes acuáticos; Loreto; contamiancion de ríos

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INTRODUCTION

The United Nations Environment Programme listed plastic pollution as a critical threat comparable to climate change (UNEP 2014). The contamination of aquatic environments by plastics has been intensified in the last years (Peters and Bratton 2016; Lebreton and Andrady 2019; Amobonye *et al.* 2021). The lack of proper waste management leads to the high levels of plastic particles in freshwater sediments (Zhang *et al.* 2020; Yang *et al.* 2021). Primary plastic items such as bottles and bags are a lesser problem than the microplastic particles resulting from plastic degradation in the environment (Eriksen *et al.* 2013, Waldman and Rillig 2020). Plastic fragmentation into smaller pieces is caused by solar radiation, physical mechanisms, oxidation, and atmospheric action (Andrady 2011; Browne *et al.* 2013; Canesi *et al.* 2015; Andrady 2017).

The term microplastics is used to define small plastic particles that are smaller than 5 mm in diameter, and vary in size, shape, color, and chemical composition (Thompson *et al.* 2004; Li *et al.* 2020; Wong *et al.* 2020). Their presence in water bodies is a serious concern, because their ingestion or absorption by aquatic organisms can cause entanglement and blockage of the digestive tract, as well as toxicological effects from released chemicals (Wright *et al.* 2013; Banaee *et al.* 2019; Pannetier *et al.* 2020; Qiao *et al.* 2019).

The fish fauna is highly diverse in the Amazon region (Jézéquel *et al.* 2020; Ribeiro-Brasil *et al.* 2020), and fish are an important item of the human diet in the region, therefore a high contamination rate of fishes by microplastic could be a threat to human health (Yee *et al.* 2021; Pan *et al.* 2021). Therefore, the detection of microplastics in fisheries resources is important for the assessment of the quality of the fish that is being traded and consumed (Cole *et al.* 2011; Barnes *et al.* 2017; Laskar and Kumar 2019; Cera *et al.* 2020; Miller *et al.* 2020; Justino *et al.* 2021), and to identify bioindicator species for the monitoring of microplastic pollution (Salerno *et al.* 2021).

Microplastic occurrence in the aquatic fauna has been studied in water systems of North America (Hurt *et al.* 2020; Peters and Bratton 2016), Europe (Bellas *et al.* 2016; McGoran *et al.* 2017), Asia (Zhang *et al.* 2020; Phuong *et al.* 2022; Piyawardhana *et al.* 2022), Australia (Cannon *et al.* 2016; Su *et al.* 2019), Africa (Biginagwa *et al.* 2016; Naidoo *et al.* 2016) and South America (Silva-Cavalcanti *et al.* 2017; Ribeiro-Brasil *et al.* 2020). In the Brazilian Amazon region, microplastics were reported from the stomach of fishes in Amapá state (Pegado Souza *et al.* 2018), the stomachs of *piranha* and *pacu* fishes from the Xingu River, and the gills and stomsch of stream fish in Pará state (Andrade *et al.* 2019; Ribeiro-Brasil *et al.* 2020). In the Peruvian Amazon, microplastics were reported from the stomachs of *Prochilodus nigricans* Spix & Agassiz, 1829 from a market in Iquitos city (Chota-Macuyama and Chong Mendoza 2020).

In this study, we surveyed several commercial fish species traded in markets in the Peruvian Amazonian city of Iquitos for contamination by microplastic.

MATERIAL AND METHODS

We sampled 61 specimens belonging to 15 commercial fish species (Table 1) from the Iquitos region, in the Peruvian Amazon. We sampled the specimens from May to December 2021. The specimens were obtained from five local markets in the city of Iquitos (3°44'37.23"S, 73°15'5.88"W), Loreto Department, Peru: Mercado de Belén, Mercadillo de Moronacocha, Mercadillo de la Participación, Mercado Modelo and Mercado de Santa Clara (Figure 1). The species were selected due to their high commercial value in this part of the Amazon.

Microplastic particle extraction

First, the gills and internal organs (esophagus, stomach, intestine, liver, gonads, pancreas, swim bladder and heart) of each individual were extracted and placed in separate beakers (one for gills, one for the pooled internal organs) for quantification of microplastic particles. Each sample was immersed in NaOH solution (10 mol L^{-1}) for digestion for five days. The digested samples were filtered using a stainless-steel sieve (0.075 µm). Microplastic particles were identified through visual inspection using a Zeiss SteREO Discovery V12 microscope (blue edition, v2.0) at 6.5× to 50× magnification.

The laboratory working surface where the process of digestion, filtration, quantification, and storage of the microplastic particles was carried out thoroughly disinfected to avoid potential contamination of the samples. Blanks were made before and after analysis to detect contamination. For blanks, a beaker was filled with 20 ml of NaOH solution, and then covered with a glass lid. No contamination was found.

We followed the protocols proposed by Ferreira *et al.* (2019) and Hidalgo-Ruz *et al.* (2012) for microplastic identification. These protocols are useful when it is not possible to use a more precise method such as infrared spectrophotometry with transform Fourier FT-IR. Particles were considered as microplastic when they presented the following characteristics: 1) no cellular or organic structures were identified in the particle or fiber; 2) if the particle was a fiber, it had to be equally thick, not tapered towards the ends, and should not be entirely straight; or 3) smooth particles.

The microplastic particles were categorized by shape according to Justino *et al.* (2020): (i) fibers (filamentous shape); (ii) fragments (irregular shape); or (iii) spherical shape (pellets). We also classified the microplastic particles into seven colors: black, yellow, red, green, brown, blue, and white.

Table 1. Summary information for microplastic contamination in fish acquired from markets in Iquitos (Peru). n = number of fish; Mean = mean number of particles per individual ± standard deviation; Omni = omnivorous; Carn = carnivorous; Herb = herbivorous; Pisc = piscivorous; Ilio = iliophagous.

Order	Family	Species	Feeding habit	n	Number of microplastic particles				
					Total	Mean	Total in gills	Total in internal organs	Average per kg
Characiformes	Anostomidae	*Megaleporinus trifasciatus* (Steindachner, 1876)	Omni	3	93	31.0 ± 7.5	33	60	0.005
	Bryconidae	*Brycon amazonicus* (Spix & Agassiz, 1829)	Omni	1	21	21.0 ± 3.5	8	13	0.012
	Erythrinidae	*Hoplias malabaricus* (Bloch, 1794)	Carn	6	345	57.5 ± 12.4	179	166	0.007
	Hemiodontidae	*Anodus elongatus* (Agassiz, 1829)	Omni	7	207	29.6 ± 12.7	139	68	0.004
	Serrasalmidae	*Myleus schomburgkii* (Jardine, 1841)	Herb	5	102	20.4 ± 3.5	59	43	0.008
		Pygocentrus nattereri Kner, 1858.	Pisc	6	325	54.2 ± 21.9	105	220	0.001
Perciformes	Cichlidae	*Astronotus ocellatus* (Agassiz, 1831)	Omni	4	172	43.0 ± 8.4	113	59	0.007
		Cichla monoculus Spix; Agassiz, 1831	Carn	5	258	51.6 ± 8.8	128	130	0.002
		Satanoperca jurupari (Heckel, 1840)	Omni	1	48	48 ± 16.9	12	36	0.001
Siluriformes	Auchenipteridae	*Ageneiosus inermis* (Linnaeus, 1766)	Carn	1	23	23.0 ± 0.7	11	12	0.010
	Loricariidae	*Pterygoplichthys pardalis* (Castelnau, 1855)	Ilio	5	238	47.6 ± 16.5	78	160	0.005
	Pimelodidae	*Calophysus macropterus* (Lichtenstein, 1819)	Carn	5	102	20.4 ± 3.8	59	43	0.017
		Pseudoplatystoma fasciatum (Linnaeus, 1766)	Pisc	2	65	32.5 ± 11.3	20	45	0.009
		Pseudoplastystoma tigrinum (Valenciennes, 1840)	Pisc	4	190	47.5 ± 9.7	91	99	0.007
		Sorubim lima (Bloch & Schneider, 1801)	Carn	6	148	24.7 ± 4.2	61	87	0.015

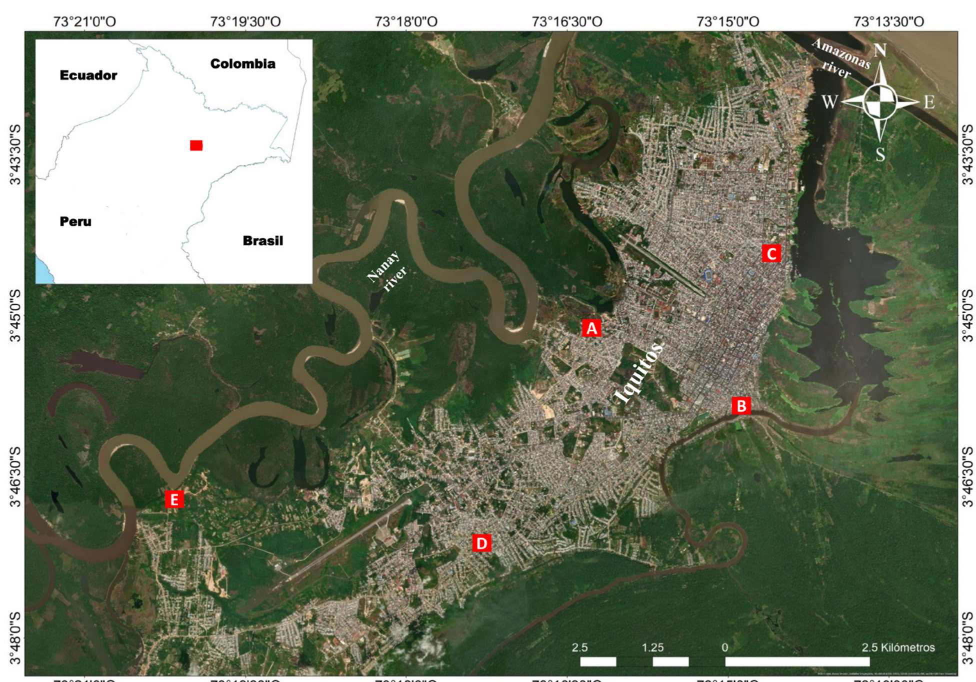

Figure 1. Geographic location of the city of Iquitos in Peru, and of the sampled fish markets in the city of Iquitos. A = Mercadillo de Moronacocha, B = Mercado de Belén; C = Mercado Modelo; D = Mercadillo de la Participación; E = Mercado de Santa Clara. Source: Google Earth pro v.7.3.4.8. This figure is in color in the electronic version.

Fish morphometry and feeding habits

Each sampled fish species was classified by feeding habit following García *et al*. (2018) and measured for standard length (cm) and total weight (g). The feeding habit categories were: carnivores, piscivores, herbivores, omnivores and iliophages. The average number of microplastic particles per individual and per kg of body mass (to standardize for differences in body size) was calculated per species and by feeding habit.

Data analysis

A Mann–Whitney–Wilcoxon test was used to compare the number of microplastic particles in the gills and in the pooled internal organs. A Kruskal-Wallis test and a *post-hoc* pairwise Wilcoxon Rank Sum test were used to compare the number of microplastic particles among the fish of different feeding habits. A Spearman test was used to analyze the correlation of fish standard length and weight with microplastic particle abundance. All statistical analyses were performed using the software R, version 3.6.3 (R Core team 2015) using the standard packages. A significance cut-off of 0.05 was adopted in all analyses.

RESULTS

Microplastic prevalence and distribution in organs

All 61 analyzed fish specimens were contaminated by microplastic particles (Table 1). Overall we detected 2337 microplastic particles, with an average of 38.3 items per individual. The species that presented the highest number of microplastic particles were *Hoplias malabaricus* (345 particles, mean ± SD = 57.5 ± 12.4), *Pygocentrus natteri* (25, 54.2 ± 21.9), *Cichla monoculos* (258, 51.6 ± 8.82) and *Pterygoplichthys pardalis* (238, 47.6 ± 16.5). Most particles were blue (50.7%), followed by black (23.7%), white (14.8%), red (7.4%), brown (3.8%) and green (0.7%). Regarding shape, 99% of the particles were fibers, and only 0.8% were fragments and 0.2% spheres (Figure 2).

When removing the effect of body size, the highest abundance of particles occurred in *Calophysus macropterus* (average of 0.017 particles per kg of body weight), *Sorubim lima* (0.015), *Brycon amazonicus* (0.012), *Ageneiosus inermis* (0.010), *Pseudoplatystoma fasciatum* (0.009) *and Myleus schomburgki* (0.008) (Table 1).

We found 1096 particles in gills, with an average of 17.9 items per individual. The highest particle abundance in gills occurred in *H. malabaricus*, *Anodus elongatus*, *C. monoculos* and *Astronotus ocellatus* (Table 1). In the pooled internal organs, we detected 1241 particles, with an average of 20.3 items per individual. The highest particle abundance in internal organs occurred in *P. nattereri*, *H. malabaricus*, *P. pardalis* and *C. monoculos* (Table 1). There was no significant difference between gills and internal organs in particle abundance (W = 1780, p = 0.68).

Relation to fish size and feeding habits

There was no significant relation between microplastic particle abundance and fish weight (rho = – 0.083; p = 0.51) or fish length (rho = – 0.20; p = 0.11). As there was no correlation, we assumed that particle abundance per individual is comparable among feeding-habit groups.

Particle abundance differed significantly among feeding-habit groups (Kruskal-Wallis chi-squared = 11.07, df = 4: p = 0.025). Significant differences were observed between carnivores and herbivores, and also between piscivores and herbivores (Figure 3). Removing the effect of body size, the highest particle abundance was observed in carnivores and herbivores (average 0.008 particles per kg), followed by iliophages and omnivores (0.005 particles per kg), and piscivores (0.004 particles per kg).

DISCUSSION

Our results indicate that the ingestion of microplastic by fishes in the region of Iquitos is highly prevalent. The average particle abundance in our study (38 particles per individual)

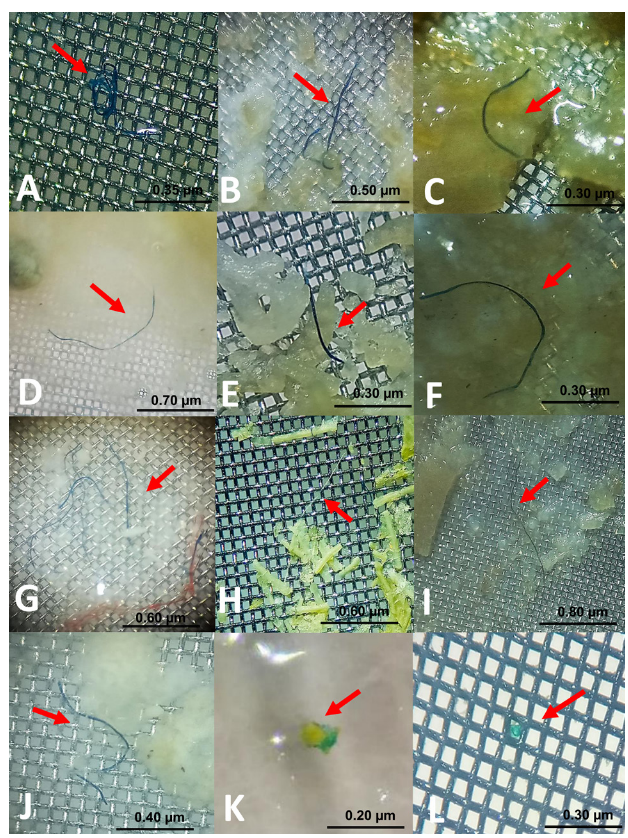

Figure 2. Most common shapes and colors of microplastic particles found in gills and internal organs of 61 fishes from the region of Iquitos (Peru). A-J – fibers; K-L – pellets. This figure is in color in the electronic version.

was considerably higher than that observed in other studies. Andrade *et al.* (2019) reported an average of 6 particles per individual in fish species in the Xingu River in Brazil. Pegado Souza *et al.* (2018) reported an average 1.2 particles per fish in the Amazon River estuary. Chota-Macuyama and Chong Mendoza (2020) reported an average of 17 particles per individual in *Prochilodus nigricans* from Iquitos. Ribeiro-Brasil *et al.* (2020) reported averages of 2.7 particles in gills and 3.0 in gastrointestinal tracts of Amazonian stream fishes in Brazil. The higher number of particles in our study is likely owed to that we analyzed the gills and internal organs of several species, while most of the cited studies analyzed only the stomach (Pegado Souza *et al.* 2018), only one species (Chota-Macuyama and Chong Mendoza 2020) or only two species groups (Andrade *et al.* 2019). Although our analysis of pooled internal organs did not allow to determine in which organ the microplastic particles were lodged, it is probable that most particles were in the gastrointestinal tracts.

The microplastic particles found in the present study were primarily blue fibers. A predominance of blue microplastic fibers in fish was also reported by Chota-Macuyama and Chong Mendoza (2020) for the Iquitos region, and Urbanski *et al.* (2020) for the middle Tietê River basin, in southeastern Brazil. Fibers are considered among the most dangerous forms of microplastic due the ease with which they accumulate in the digestive tract, where they can cause more serious intestinal toxic effects than others forms of microplastic (Qiao *et al.* 2019).

While the presence of microplastic in the stomach of freshwater fish has been widely reported (Phillips and Bonner 2015; Peters and Bratton 2016; Silva-Cavalcanti *et al.* 2017; Pegado Souza *et al.* 2018; Chota-Macuyama and Chong Mendoza 2020), the assimilation of these particles through the gills is poorly studied. The lack of statistical significance indicated that similar levels of microplastic particles accumulate in the gills and in the internal organs. Microplastic enters with the water that circulates in the gills and may accumulate not only when the fish is moving actively, but also when in rest (Azevedo- Santos *et al.* 2021). The adherence of microplastic particles to the gills may decrease oxygen consumption and ion regulation, causing respiratory stress (Watts *et al.* 2016; Abdel-Tawwab *et al.* 2019; Azevedo-Santos *et al.* 2019). The presence of microplastic in internal organs may be attributed to the intentional or accidental intake of microplastics from

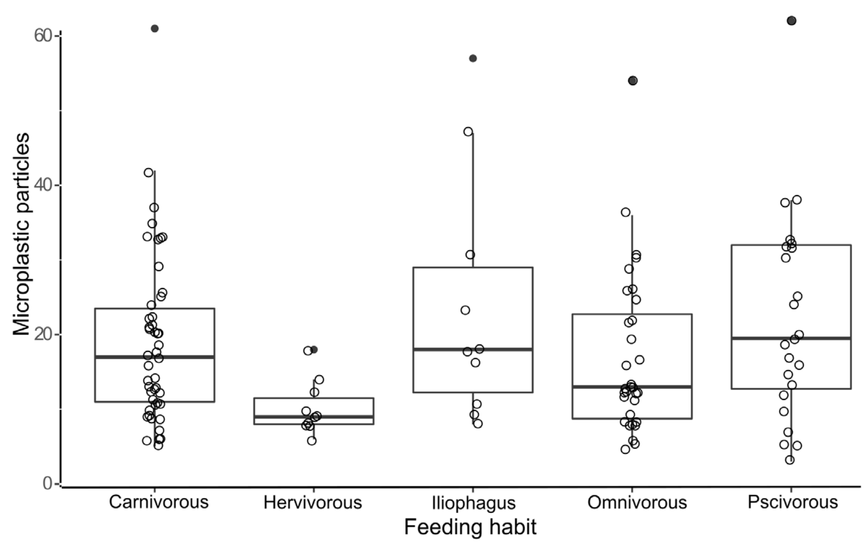

Figure 3. Number of microplastic particles per individual in fish samples grouped by feeding habit. The dots are the raw data, the central line is the mean, the box delimits the 25-75 percentiles, and the bar the minimum and maximum values.

Table 2. Microplastic occurrence in organs of fish of different feeding habits sampled in markets in Iquitos (Peru). N specimens = number of specimens; N species = number of species; Mean = mean number of particles per specimen ± standard deviation; Range = minimum and maximum values found.

Feeding habit	N specimens	N species	Number of microplastic particles			
			Total	Mean	Range	Average per kg
Carnivorous	23	5	876	19.1 ± 11.3	5 – 61	0.008
Herbivorous	5	1	102	10.2 ± 3.6	6 – 18	0.008
Iliophagus	5	1	238	23.8 ± 16.5	8 – 57	0.005
Omnivorous	16	5	541	16.9 ± 10.8	5 – 54	0.005
Psicivorous	12	3	580	24.2 ± 17.1	3 – 73	0.004

water or sediment, or by the ingestion of prey that are already contaminated with microplastic (Jovanović *et al.* 2018; Justino *et al.* 2021). Therefore, microplastic in the gills is primarily due to its presence in the water, while its presence in the internal organs is influenced by feeding habits (Pan *et al.* 2021).

In our study, *Hoplias malabaricus* (Erythrinidae) and *Pygocentrus natteri* (Serrasalmidae) showed the highest abundance of microplastic particles in their organs. Both species are active and voracious predators, and feed primarily on other species of fish (García *et al.* 2018). Predatory fishes can ingest microplastic directly by confusing it with their natural prey and through trophic transference, by ingesting contaminated prey (Farrell and Nelson 2013; Nelms *et al.* 2018; Ory *et al.* 2018; Gouin 2020; Miller *et al.* 2020). Our results agree with Justino *et al.* (2021), that established that microplastic abundance varies among tropical fish of different feeding strategies, and with Azevedo-Santos *et al.* (2019), that reported that a large number of fish species that ingest plastic are carnivores. On the other hand, we point to the fact that strictly non-carnivorous species (herbivores and iliophages) were represented only by one species each and small numbers of individuals in our sample, which may have influenced our results.

The effects of microplastics on fish are still poorly understood, yet, while some studies concluded that microplastic particles have no effect on fish (Schmieg *et al.* 2020), other studies detected significant effects on the development of fish and insect larvae (Stanković *et al.* 2020; Moreno and Cooper 2021), and the branchial function of crabs (Watts *et al.* 2016). Microplastic accumulation in the digestive tract of fishes may also lead to lower energy intake (Salerno *et al.* 2021), malnutrition and eventual starvation (Boerger *et al.* 2010).

Iquitos is the largest urban center in the Peruvian Amazon, with over half a million inhabitants (INEI 2017), where a large part of the population lives without public sanitation and sewage treatment. Most of the fish sold in the sampled markets come from the Nanay and Amazonas rivers in the region around the city, where organic and inorganic waste is dumped into the rivers without any type of treatment. Our results strongly suggest that the uncontrolled dumping of waste material into the rivers has already led to microplastic contamination of fishes throughout the trophic chain.

CONCLUSIONS

The present study showed a higher level of microplastic contamination in fish species used for human consumption in Iquitos than previously detected in the region, and that microplastic is found in the gills in addition to the internal organs. Microplastic particle abundance was independent of fish body size and weight, but varied significantly according to feeding habit. Carnivorous fish presented the larger abundance of particles. Our results suggest that the contamination of fish with microplastic in the influence radius of urban centers in the Amazon region is widespread, and its effect on the aquatic biota and the human population that feeds on it should be monitored.

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