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Original Article

Far from urban areas: plastic uptake in fish populations of subtropical headwater streams

Longe das áreas urbanas: ingestão de plástico em populações de peixes de riachos subtropicais de cabeceira

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Abstract

This study investigated the occurrence of plastic particles in the digestive tracts of fish from headwater streams in a human-thinly populated region of the subtropical Sinos River basin in southern Brazil. In total, 258 individuals from 17 species were collected using electric fishing. Thirty-eight percent (38%) of the specimens contained plastic particles. All of them were fibers, with a maximum count of 43 per individual. Plastic fibers were the fourth most abundant food category. Results showed that the uptake of these plastic particles was proportional to the number of ingested food items. Fiber counts in the guts correlated with the uptake of Trichoptera, which are invertebrates using plastic particles to construct their protective cases. No significant difference in plastic uptake was detected between benthic and water column fish. No evidence of bioaccumulation of plastic particles was found in the intestines. The distance from urban areas was not related to the number of ingested plastic particles, concluding that plastics are ubiquitous and available to biota, even in remote locations. The most probable source of these particles is residences close to the streams which discharge the sewage of washing machines without any treatment.

Keywords: contamination pathways, fibers, freshwater, gut content, sewage.

Resumo

Este estudo investigou a ocorrência de partículas plásticas no trato digestivo de peixes de riachos de cabeceira em uma região de baixa densidade humana da bacia subtropical do rio dos Sinos no sul do Brasil. No total, 258 indivíduos de 17 espécies foram coletados por meio da pesca elétrica. Trinta e oito por cento (38%) dos espécimes continham partículas de plástico. Todos eram fibras, com contagem máxima de 43 por indivíduo. As fibras plásticas foram a quarta categoria de alimentos mais abundante. Os resultados mostraram que a absorção dessas partículas plásticas foi proporcional ao número de itens alimentares ingeridos. A contagem de fibras nos intestinos se correlacionou com a absorção de Trichoptera, que são invertebrados que usam partículas de plástico para construir suas capas protetoras. Nenhuma diferença significativa na absorção de plástico foi detectada entre peixes bentônicos e de coluna d'água. Nenhuma evidência de bioacumulação de partículas plásticas foi encontrada nos intestinos. A distância das áreas urbanas não foi relacionada ao número de partículas plásticas ingeridas, concluindo que os plásticos são ubíquos e disponíveis para a biota, mesmo em locais remotos. A fonte mais provável dessas partículas são as residências próximas aos córregos que descarregam os esgotos das máquinas de lavar sem nenhum tratamento.

Palavras-chave: água doce, conteúdo intestinal, esgoto, fibras, vias de contaminação.

1. Introduction

Plastic pollution is a subject of global concern. The Asian continent is the leader in the world's plastic production around 50% (Plastics Europe, 2021). China is the largest producer with 32%, while Latin America produces 4%. Plastic production at the global level is approximately 367 million tonnes per year. Annual global emissions are expected to increase from 20 million metric tonnes (MT) in 2016 to 60 MT in 2030 under a business-as-usual scenario (Borrelle et al., 2020).

Most studies analyse plastic emissions in the oceans (Wagner et al., 2014). However, concern about plastics in freshwater is increasing (Drago et al., 2020). Microplastics are found in marine water and freshwater from around the world (Chae and An, 2017), even in bottled drinking water (Schymanski et al., 2018).

The number of drifting microplastic particles (MP) in rivers varies drastically. Su et al. (2018) found 0.5 MP/L to 3.1 MP/L in the Middle Yangtze Basin, Moore et al. (2011)

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130 MP/L in surface samples of North American rivers adjacent to urban areas of San Gabriel and Los Angeles, and Leslie et al. (2017) quantified 48 MP/L to 187 MP/L in a water channel of Amsterdam. The plastic concentration in the sediment was even higher. Castañeda et al. (2014) quantified 3,980 MP/L in the St. Lawrence River (Quebec, Canada). Organic sewage contained on average 15,385 MP/L Mahon et al. (2017). Even in very remote areas plastic particles were found. For instance, Free et al. (2014) observed 20,264 MP/km^2 plastic particles in a remote lake in Mongolia with low population density in the catchment area and attributed this result to improper waste management practices.

Plastic debris of all sizes is a risk to biodiversity as it may cause severe effects on the aquatic biota (Mato et al., 2001). The review of Azevedo-Santos et al. (2021), which focused on freshwater biota, described the effects on algae, crustaceans, fishes, amphibians, reptiles, birds and mammals. The major impacts of plastic on larger species are entanglements by ghost nets and the ingestion of particles, which in large quantity may cause false satiation and subsequent starving. Other effects are decreased productivity in algae, decreased survival and reproduction rates, inflammation and injuries of the intestine in crustaceans and fish.

In the Northeast of Brazil, Silva-Cavalcanti et al. (2017) evaluated the stomach contents of 48 individuals of *Hoplosternum littoral*e, a catfish species that inhabits tropical freshwater ecosystems. They found MP in 83% of the analysed specimens, with a maximum of 24 MP/fish. In a study from the Xingu River, a remote part of the Brazilian Amazon basin, 172 individuals of 16 serrasalmid species were analysed. Forty-six stomachs were contaminated with a total of 96 MP. The relation of trophic guilds on MP uptake is not completely clear. Andrade et al. (2019) did not find significant differences in MP uptake between carnivores, herbivores and omnivores, while Azevedo-Santos et al. (2019) found most MP in the intestines of carnivores (54.8%) and omnivores (23.2%).

In the study by Ribeiro-Brasil et al. (2020) in 12 headwater streams of the eastern Amazon basin, 83.3% of analysed individuals had MP adhered to the gills and 88.2% in the guts. The authors suggested that different foraging strategies may be responsible for different levels of MP contamination. In the Uruguay River in southern Brazil, MP were present in 98% of the analysed fish (Santos et al., 2020). Most studies observe higher contamination levels in urbanized areas and relate MP concentrations in water and fish to human population density (Garcia et al., 2020; Silva-Cavalcanti et al., 2017; Tibbetts et al., 2018).

In southern Brazil, the Sinos River basin is crucial for providing water for approximately 1,35 million people. The areas of the headwaters are still in good environmental conditions, with low-intensity agriculture and livestock farming. The lower part of the basin is intensively urbanized and harbours metallurgic and leather processing industries. The Sinos River basin occupies only 1.3% of the area of the state of Rio Grande do Sul but generates approximately 21% of the state´s income (Comitesinos, 2019). Waste management is far from ideal and municipal sewage treatment is precarious. At present only two municipalities out of 30 operate municipal wastewater treatment plants. Microplastic concentration is at high levels with a median abundance of 330 MP/L, drinking water contains 106 MP/L (Ferraz et al., 2020).

The present study focusses on the headwaters of the Sinos River basin. The objective of this work is to quantify the presence of MP in fish stomachs of streams flowing in an area of low human population density, far from urban centers, and to analyse possible pathways of uptake. We addressed the following questions and problems:

- At what frequency MPs are occurring in the intestines of headwater fish?
- As MP might pass through the food chain, we asked, if MP uptake is related with the ingestion of other food items.
- Since several studies showed contradicting results in regard of MP uptake in different guilds, we asked if different trophic guilds take up MPs in different quantities.
- Since the sample sites were located in relatively remote areas, we tried to identify the possible pathways for contamination.

2. Material and Methods

2.1. Study area

The study area is located in the upper Sinos River basin, which is part of Brazil`s southernmost state of Rio Grande do Sul. The basin covers an area of 3,693 km^2, and a water network of approximately 3,400 km (Schulz et al., 2006).

The main uses of water in the basin are irrigation of rice paddies, supply of the industries, and municipal use for drinking water. The most preserved areas are located in the upper part of the basin. The water quality in the lower urbanized sections of the basin is class four, which is the worst on the Brazilian water quality scale [CONAMA 337/35; Brasil (2005)]. The principal problem is the discharge of untreated municipal sewage (SEMA, 2018).

All sample sites were located in 1st order to 4th streams with widths varying between 3 m and 18.5 m.

2.2. Sample collection and laboratory analysis

The fish were collected by electrofishing (750 V unpulsed direct current; EFKO Germany) in seven randomly selected streams, tributaries of the Sinos River, in the upper part of the basin (Figure 1) between December 2017 and November 2018.

The sampling effort was standardized by 30 minutes of electrofishing per site. The captured specimens were euthanized with Eugenol (Lucena et al., 2013) and then fixed in 10% formalin, injecting formaldehyde into the abdomen to neutralize the digestive action and preserve the stomach contents (Silva-Cavalcanti et al., 2017). The collections were authorized by the federal IBAMA license 12430-1 and by the by the local ethics committee for research in animals (process number PPECEUA01.2018).

All individuals were identified, measured (total length in mm), and weighed to the nearest gram. For analysis, the

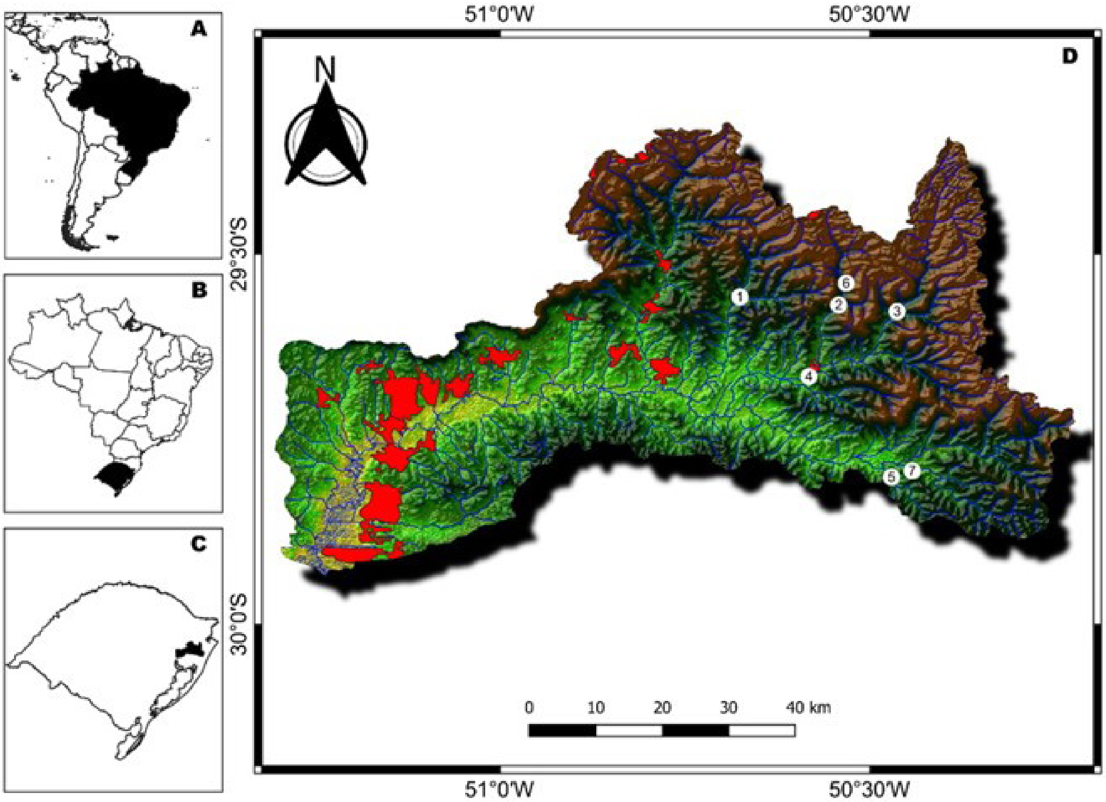

Figure 1. Study area. A. South America and Brazil. B. Brazil and the state of Rio Grande do Sul. C. Rio Grande do Sul and the Sinos River Basin. D. The numbers from 1 to 7 in the white dots show the sampling sites in the upper section of the Sinos River basin. The colour gradient represents the terrain elevation (light green elevations of 30m altitude and dark brown elevations of 980m). The red polygons are the urban areas.

whole intestinal tract was removed from the abdominal cavity.

The food items extracted from the intestine were sorted under a stereo microscope and divided into natural diet items and plastic particles. Natural diet items were composed by plant material and macroinvertebrates, which were counted and identified to the order. Plastic particles per individual were categorized by colour and shape, then measured with a digital caliper (Mitutoyo Absolute 500-172-20B). To ensure the correct identification of synthetic material all particles identified as "plastic" were treated with KOH (15% solution in water) for 36 h.

2.3. Data processing

The absolute frequencies of plastic particles per fish intestine were correlated with the absolute frequencies of the other food items by Pearson´s r to assess if plastic particle uptake is related to particular food categories. Linear regression was performed with the number of plastic particles and the total abundance of food items per fish intestine to analyse if plastic particle uptake is proportional to the overall uptake. To test the eventual accumulation effects of MP we additionally tested the relation between the number of ingested fibers and fish length by linear regression. Mean plastic particle abundances in benthic and water column feeders were tested by the Mann-Whitney-U test to assess the influence of feeding guilds on plastic particle uptake.

To analyse the influence of sewage on plastic uptake, we used the number of residences in the vicinity of sampling sites as a proxy, because most residences near streams discharge their sewage directly without any treatment. The number of residences was counted in 3 km buffer strips upstream and downstream of each sample site by using the satellite images from GoogleEarth Pro (Google, 2020) and the image processing software Qgis 3.10 (QGIS Development Team, 2018). Each strip had a width of 50 m on both stream sides. The number of residences and the mean number of MP's per fish gut were subjected to a Pearson's correlation test. All data were analysed by R software (R Core Team, 2018) and IBM SPSS Statistics 23 (IBM Corporation, 2015).

3. Results

We analysed 258 individuals distributed in 17 species. Thirty-eight percent (n=98) of all investigated intestines were positive for plastic particles. All of them were fibers (Figure 2; Table 1).

The fiber uptake was not species specific. The number of particles observed in each species was highly correlated

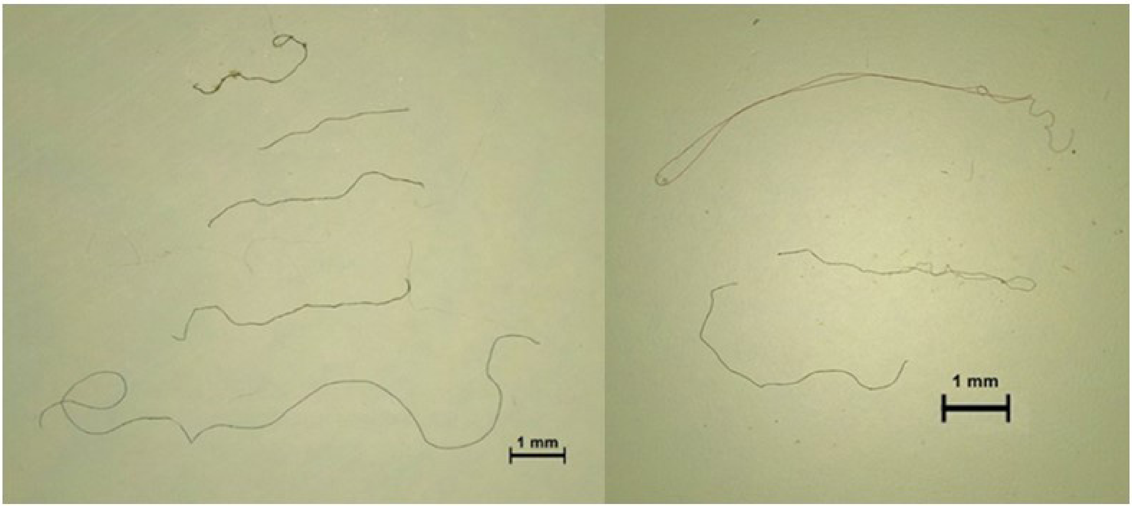

Figure 2. Plastic fibres from fish intestines.

Table 1. Relationship between species, amount of plastic and guild.

Species	Ntot	N with plastics	N fibers	Max	Median	Guild
Ancistrus brevipinnis	35	18	70	10	4	bentic
Astyanax henseli	12	5	16	8	3	water column
Astyanax laticeps	1	0	0	0	0	water column
Bryconamericus iheringii	38	14	39	10	2	water column
Characidium orientale	5	2	2	1	1	water column
Characidium pterostictum	44	19	120	43	6	water column
Crenicichla punctata	2	1	13	13	5	water column
Diapoma speculiferum	2	0	0	0	0	water column
Gymnogeophagus rhabdotus	1	0	0	0	0	bentic
Hemiancistrus punctulatus	51	19	59	11	3	water column
Heptapterus mustelinus	13	5	24	16	5	bentic
Pseudocorynopoma doriae	2	1	1	1	1	water column
Rineloricaria cadeae	2	0	0	0	0	bentic
Rineloricaria malabarbai	3	2	5	4	2.5	bentic
Rineloricaria microlepidogaster	45	12	61	21	5	bentic
Scleronema minutum	1	0	0	0	0	parasite
Synbranchus marmoratus	1	0	0	0	0	bentic
	258	98	410			

Total abundance/species (Ntot), number of individuals with plastic particles (N with plastics), percentage of individuals with fibers (% with fibers), total number of fibers/species (N fibers), maximum number of fibers/individual (Max) and median number and guilds of species.

with species abundance (second and third column of Table 1; R^2=0.936; p<0.001) and reflects different sample sizes per species. No significant difference was detected by comparing the median particle numbers in the guts of benthic (median=3) and water column (median=2) feeders (U= 1.018, p=0.248; Figure 3).

The total number of fibers summed up to 410, with a maximum count of 43 per individual. Median fiber length was 1.81 mm (min: 0.2 mm; max 14.6 mm; Figure 4). The dominant fiber colours were blue (52.9%) and transparent (30.9%).

Considering the total abundance of the food items, Trichoptera and Ephemeroptera were the most abundant. Plastic fibers were the fourth most common category (Figure 5). Plastic particle uptake did correlate significantly with Trichoptera abundance (Pearson´s r=0.32; p<0.001),

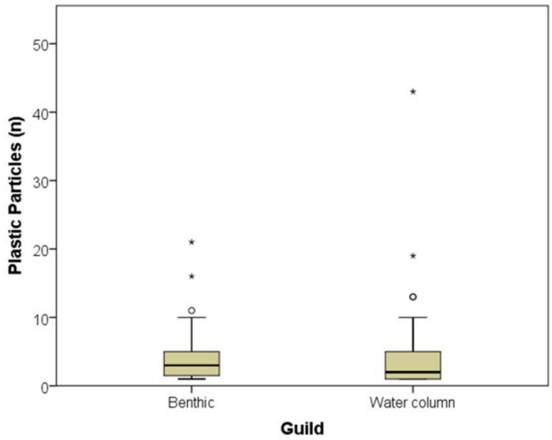

Figure 3. Plastic particles abundances in benthic and water column feeders (whiskers = min - max values, dots outliers, stars extreme outliers, horizontal line median, box 50% tile).

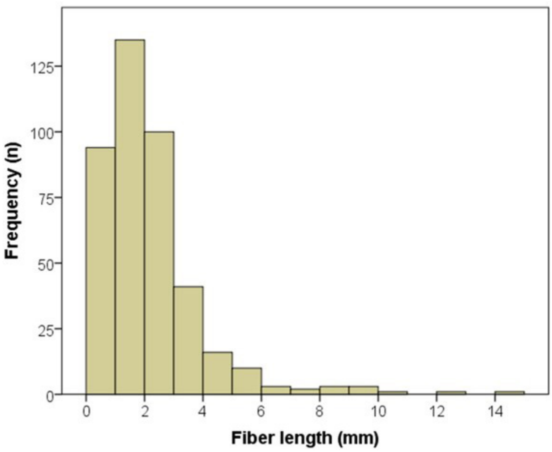

Figure 4. Length frequency distribution of the fibers.

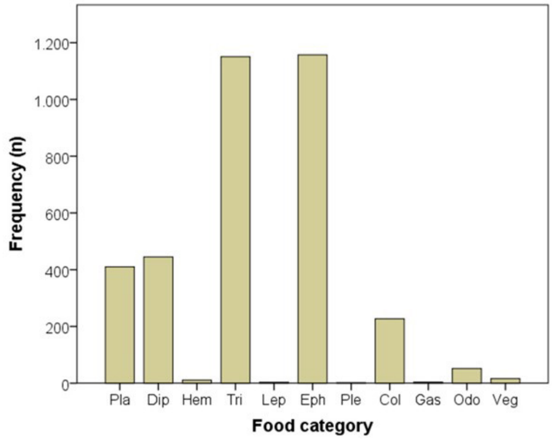

Figure 5. Total abundances of food items per category in comparison with ingested plastic particles abundances. (Pla=Pastics, Dip=Diptera, Hem=Hemiptera, Tri=Tricoptera, Lep=Lepidoptera, Eph=Ephemeroptera, Ple=Plecoptera, Col=Coleoptera, Gas=Gastropoda, Odo=Odonata, Veg= Plant).

but not with Ephemeroptera (r=0.057; p=0.362). Fish that consumed Trichoptera also consumed Ephemeroptera (r=0.611; p<0.001).

Linear regression of plastic particle counts per intestine and the sum of other food items per intestine showed a significant relationship (R^2=0.029; p=0.006), indicating that plastic particle uptake was proportional to the total amount of ingested particles. However, plastic particle uptake was not correlated with fish length in most species, only the Loricariid species *Ancistrus brevipinnis* showed a significant correlation (Table 2).

Fish from all sample sites contained fibers. No clear pattern could be detected considering the distance from urban areas. Neighbouring sites with almost the same distance from urban areas showed low median (ex.: site 5; median=0) and a high median (ex.: site 7; median=2) values (Figure 6). The mean number of fibers in fish guts recorded per sample site did not correlate with the number of residences in the 3 km buffer strips, neither upstream (r=0.094; p=0.84) or downstream (r=-0.333; p=0.465) of the sample sites.

4. Discussion

Out of 258 fish captured 38% of individuals had ingested MPs. These specimens belonged to 11 different species. This result shows inferior numbers of contamination

Table 2. Linear regression of length and number of ingested plastic particles of the five most abundant species.

Species	N	R^2	*p* value
Ancistrus brevipinnis	35	0.197	0.008
Bryconamericus iheringii	38	0.002	0.792
Characidium pterosticum	44	0.087	0.053
Hemiancistrus punctulatus	51	0.007	0.055
Rineloricaria microlepidogaster	45	0.036	0.2012

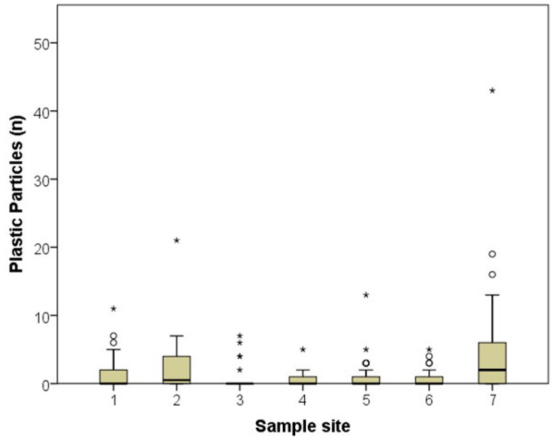

Figure 6. Median plastic particle distribution in fishes per sample site (whiskers = min - max values, dots = outliers, stars = extreme outliers, horizontal line = median, box = 50% tile).

when compared to other studies of MP in freshwater fish. Peters and Bratton (2016) observed that 45% of analysed sunfish (*Lepomis macrochirus* and *Lepomis megalotis*) in the Waco River, Texas, had MPs in their guts, 83% of *Hoplosternum littorale*, a benthic siluriform species from a tropical Brazilian river Silva-Cavalcanti et al. (2017), 80% of serrasalmid species from the Xingu River [Brazilian Amazon Basin; Andrade et al. (2019)] and 98% of stream-dwelling species in the eastern Amazon basin (Ribeiro-Brasil et al., 2020).

All detected particles in our study were fibers and most of them were blue. In his study of MP in the Sinos River main stem, Ferraz et al. (2020) identified fibers as predominant particles shape in water samples. Information about colour was not available, because samples were stained by Nile Red. In a marine environment Steer et al. (2017) observed blue fibers as the most frequently ingested plastic particles in fish larvae. Shape and colour of these were similar to the most abundant particles encountered in water samples, suggesting that fiber uptake is proportional to the occurrence in the environment.

Since intestines were analysed with a stereo microscope, plastic particles smaller than 0.1 mm may have been undetected. But fibers can be considered as surrogates for plastic contamination. Many publications show, that fibers are the most abundant shape of plastic particles in freshwater (Ferraz et al., 2020; Hendrickson et al., 2018) sediment of rivers and (Ballent et al., 2016; Ehlers et al., 2019; Qin et al., 2020) and fish (Calderon et al., 2019; Silva-Cavalcanti et al., 2017; Khan et al., 2024).

The uptake of these particles by fishes may occur by intentional or unintentional ingestion. Carson (2013) noted in his study about food uptake that some marine fish often attack large plastic particles. Out of 5,518 plastic pieces sampled from Hawaiian beaches, 15.8% had bit marks of an animal. Yellow and blue plastic items showed significantly more bite marks than other colours. Unintentional uptake occurs when plastic items are ingested together with natural foods (Peters and Bratton, 2016) or by trophic transfer (Mattsson et al., 2015). The latter study showed that fish feeding on zooplankton contaminated by nano plastic particles had lower predation success.

In the fishes of the Sinos River basin MP uptake seems to be related to the total quantity of ingested particles, as shown by the result of the linear regression of plastic particles and total abundancies of food items per intestine. This relation indicates involuntary ingestion of plastic particles in the water column or on the substrate, as recorded by Santos et al. (2020) for stream fish in the headwaters of the Uruguay River. It appears that fish are not able to distinguish between food items and plastic particles. We suppose that flow velocity is an important factor: At water flow velocities usually ranging from 10 cms^{-1} to 80 cms^{-1} fish that feed in the water column must decide in an extremely short time span whether to take a potential food item or to let it pass.

Many bottom feeders, particularly species of the Loracariid family, are grazers (Delariva and Agostinho, 2001). Their mouths are typically in an inferior position. Most probably, their food uptake does not target particular items, but they "graze" food like biofilm, periphyton, and macroinvertebrates adhering to the bottom substrate or woody debris. Sedimented plastic particles probably are mixed within this "food cocktail" and are involuntarily ingested. Sorption of MP to marine algae was shown by Sundbæk et al. (2018), being of concern in regard to food products based on marine algae.

The significant correlation between the number of ingested Trichoptera and the number of plastic particles may be related to the fact, that Trichoptera are incorporating plastic particles in their protective cases (Ehlers et al., 2019). Trichoptera are bottom dwellers. They use inorganic substrates like sand grains (Okano et al., 2012) or fragments of plant tissue (Moretti et al., 2009) as building materials for their cases. *Lepidostoma basale*, the species analysed by Ehlers et al. (2019), used plastic fibers, fragments, and films that occurred in the water column and sediment.

The number of ingested fibers and fish length did not correlate significantly, except for *A. brevipinnis*. This result suggests that ingested MPs do not accumulate in the intestines. Most probably the major part passes the intestine and is excreted with the faeces. However, Mattsson et al. (2015) observed, that ingested nano plastic particles may end up in the fish`s brain and cause behavioural dysfunctions. This means that plastic particles can pass the intestine-blood barrier. Most probably this process is size dependent, allowing to pass only small particles. The larger particles in the intestine may also harm the fish. MP and the adhering biofilm are potential vectors for heavy metals such as cadmium and mercury (Tang et al., 2015), pesticides like chlorpyrifos (Bour et al., 2020), and other highly toxic organic compounds (Eerkes-Medrano et al., 2015). Additionally, substances with endocrine disruptive properties like unbound monomers and additives like bisphenol A, phthalates, and their metabolites are used during the plastic production process and may leach during the passage of the intestine. Barboza et al. (2020) found bisphenol A concentrations as high as 272 ng g^{-1} dry weight in the muscle of Atlantic chub mackerel (*Scomber colias*). Although it is not straightforward to consider fish as models for other vertebrates, increasing evidence shows, that MP may pass the intestine blood barrier in mice and may cause behavioural disorders including altered locomotion patterns and anxiety levels (Araújo and Malafaia, 2021). A recent publication by Ragusa et al. (2021) describes the occurrence of MP in four out of six analysed human placentas, and Jenner et al. (2022) identified microplastics in seven of 13 lung tissue samples. Most probably future studies will increase concern in relation to MP as important factors influencing public health.

The origin of the fibers most probably are residences near the streams (Ferraz et al., 2020). In rural areas of the Sinos River basin, the wastewater is not collected and treated in municipal treatment plants. Most residences have individual onsite treatment systems composed by septic tanks and drain fields. However, it is very common, that the sewage from washing machines is discharged directly into a nearby stream (Ferraz et al., 2020). Considering that laundering a single garment can produce more than 1,900 fibers per wash (Browne et al., 2011) or that washing a 6 kg load of acrylic fabric may release 700,000 fibers (Napper and Thompson, 2016) the discharge from washing machines is major input source of MP in the headwaters of the Sinos River. The MP input by untreated sewage from washing machines is a highly stochastic process, with the possibility of one residence contaminating extended stream reaches. The stochasticity most probably is responsible for the lack of correlation between the number of residences in the vicinity of the sample sites and the number of MP in fish. Additionally, the upstream transport of fibers by fish may contribute to the presence of fibers in remote areas. Since the movement patterns of small fish species in subtropical streams are very poorly known, it is also possible that fish ingest MP in more densely inhabited areas downstream of the collection sites and transport them upstream during dispersal or migration.

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