

Article

Occurrence of microplastics in Fish from Mendoza River: First Insights into Plastic Pollution in the Central Andes, Argentina

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Abstract: The widespread use of plastic products in our modern life represents a serious threat to aquatic environments and wild animals that are exposed to plastic waste. Although microplastics (MPs) have been reported in fish from several freshwater environments around the world, mountain environments have been little studied so far. The occurrence of MPs was assessed in the gastrointestinal tracts (GITs) of non-native (rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta*) and native (torrent catfish *Hatcheria macraei*) fish from the Mendoza River in the Central Andes, Argentina. Fibers (85%) were the main MPs type recovered from the fish here analyzed, followed by fragments (15%). Blue fibers were the main type of MPs in analyzed specimens: brown trout (50%), rainbow trout (71%), and torrent catfish (63%). Significant differences in the median total MPs' abundance and median total fiber abundance were observed among fish species. The highest MPs' abundance was found in the GITs of brown trout followed by rainbow trout, while the lowest was found in the GITs of torrent catfish. This study represents a baseline for the occurrence and characteristics in terms of shape and color of MPs in freshwater fish collected from a mountain river of the Central Andes.

Keywords: Central Andes; fish; freshwater; microplastics



Citation: Ríos, J.M.; Teixeira de Mello, F.; De Feo, B.; Krojmal, E.; Vidal, C.; Loza-Argote, V.A.; Scheibler, E.E. Occurrence of microplastics in Fish from Mendoza River: First Insights into Plastic Pollution in the Central Andes, Argentina. *Water* **2022**, *14*, 3905. <https://doi.org/10.3390/w14233905>

Academic Editor: Reinaldo Luiz Bozelli

Received: 8 November 2022

Accepted: 28 November 2022

Published: 1 December 2022

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1. Introduction

The widespread presence of microplastics (MPs) in natural environments has caused an increase in scientific and public attention on this issue over the last decade. Despite the benefits of plastics, with multiple functions and utilities such as versatility, durability, and resistance [1,2], the omnipresence of plastic particles in the environment represents a global threat [3]. It is estimated that the growth in the production of plastics in the last 65 years has outperformed any other manufactured material [4], with a global production close to 367 million metric tons [5].

Plastic debris can be classified according to its size. According to GESAMP 2019 [6], size classes are typically attributed to the nomenclature of nano- (<1 µm), micro- (<5 mm), meso- (5–25 mm), and macroplastics (25–1000 mm). Particle size will be of major ecological relevance because it is an important factor determining the item's interaction with biota and its environmental fate [7]. Irrespective of size, plastic debris can also be classified according to its origin into primary (those that are a small size at the time of manufacture, e.g., pellets and microbeads found in personal care products) [8] or secondary (those that originate from the degradation of larger particles) [9]. Microplastics vary in form and origin often resulting from the breakdown of macroplastics through ultraviolet, microbial, and physical degradation [10]. Further categorization includes grouping based on shape, as fibers, fragments, pellets, foams, and films [7]. Microplastics have been reported in

different fish species from marine and freshwater environments [2]. One of the aspects linked to the presence of MPs in aquatic ecosystems is their potential negative impact on aquatic organisms. Among the biological responses reported after the ingestion of MPs by freshwater fish are alterations in swimming performance [11] and digestive tract blockage [12]. In addition, harmful contaminants may be adsorbed onto microplastics; thus, aquatic organisms can potentially consume and accumulate these chemical compounds with the microplastics [13]. Furthermore, microplastics and harmful contaminants possibly accumulate in the food web as the lower trophic level organisms are consumed by fish [14]. Therefore, the ecotoxicity of microplastics in aquatic organisms is now an important field of research.

The Central Andes (South America) has the highest peaks (e.g., Mount Aconcagua, 6962 masl) of the Andes range and represents the second-most important glacier area in Argentina, after the Patagonian Andes. Management plans (Argentinian law 26.639, enacted in 2010 for the preservation of glaciers and the periglacial environment) have been implemented to control water reserves for human use and biodiversity protection [15]. In this way, there is increasing interest in knowing the biotic interactions in Andean freshwater environments to build biomonitoring plans for water-quality assessments. Mountain rivers play a key role in the transport of MPs' pollution, with fluvial dynamics expected to influence biotic interactions, particularly for fish [16]. For example, the Mendoza River basin, located in central-west Argentina, is suspected of having MPs exposure due to a high density of likely plastic waste sources such as an oil refinery and hydroelectric power plant, agricultural practices, sport fishing, and a rise in urbanization and tourism [17,18].

Currently, few field reports have looked at MPs in wild freshwater salmonids worldwide, with three of these reports including wild brown trout [16,19,20] and just one including wild rainbow trout [21]. Fish biological traits (e.g., fish body size) have been evaluated as factors that influence MPs' intake for mountain-dwelling fish species from other regions of the world, e.g., [16,22–24]. Nevertheless, there is a lack of information for fish from the Andes range. The aims of this study were to: (i) investigate the occurrence (abundance per individual) and prevalence (percentage of occurrence) of MPs in fish GITs from the middle section of Mendoza River; (ii) analyze possible relationships between MPs' occurrence and biological traits (e.g., fish body size); and (iii) identify suitable bioindicator species for future MPs assessments in this mountainous region.

2. Materials and Methods

2.1. Study Site

The fish specimens analyzed in this study were collected from the middle section of the Mendoza River basin (32°52'29" S; 69°15'49" W) in the Central Andes range in the northwest of Mendoza Province (Figure 1). Mendoza River is formed from the confluence of the Cuevas, Horcones, Tupungato, and Vacas rivers at the locality of Punta de Vacas (2394 masl). The river runs 300 km until draining into the Guanacache and del Rosario plain systems. The waters of the Mendoza River originate mainly from glaciers and snow melting from the Andes mountains. The Mendoza River has an average annual discharge of $50.6 \text{ m}^3 \text{ s}^{-1}$, which increases between December and February (austral summer) due to melting snow, reaching values between 90 and $120 \text{ m}^3 \text{ s}^{-1}$. Rainfall occurs in spring and summer with an annual average of 250 mm, making the contribution of rainwater to the Mendoza River negligible. The regional climate can be defined as arid and typically temperate. The climate characterizing the middle basin is typical of mountain areas, with very cold winters when air masses from the Pacific Ocean produce the main meteoric contribution in the form of snow between June and September. Environmental moisture is overall low, and the thermal regime is characterized by strong seasonal and daily fluctuations [25]. Different human activities, such as agriculture, oil industry, hydroelectric power, tourism, truck transport, and fishing [17,18], are located across the gradient of the Mendoza River basin with the international Bi-oceanic Highway between Argentina and Chile, all act as possible sources of plastic waste.

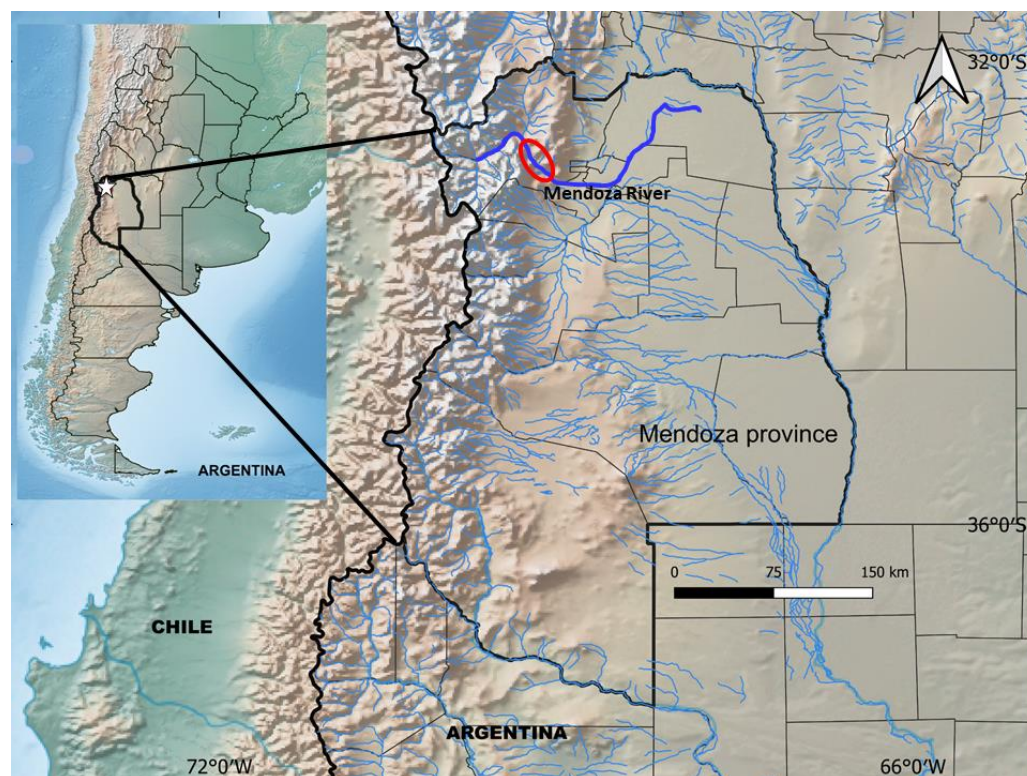


Figure 1. Map of the Central Andes range (Argentina). The red ellipsis encloses the section of the river (the middle section of the Mendoza River basin) where the fish were collected.

2.2. Fish Collection and Morphometry

The rainbow and brown trout are salmonids fish species native to North America and Europe, respectively. Both non-native salmonids species have been introduced in the Andean freshwater ecosystems of the province of Mendoza for gastronomy and sport fishing since 1957 [26–28]. The official introduction of these salmonids from San Carlos de Bariloche (Argentinean Patagonia) to Mendoza was performed through procedures developed in the El Manzano hatchery [26], located in the middle zone of the Central Andes. Specifically, these procedures included incubating, rearing, and stocking fry. Since 1957, the release of fry has taken place regularly in several streams and rivers within the Mendoza Province in the Central Andes region [29]. On the other hand, the torrent catfish is a native species to the Mendoza River in the Central Andes range [26,27]. According to our research authorization, all fish specimens were adult and caught using conventional fishing with full metal lures (Mepps fishing lures Co, Antigo, WI, USA).

Native torrent catfish *Hatcheria macraei* (Girard, 1855) were captured during the austral winter season of 2016, and non-native rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) and brown trout *Salmo trutta* (Linnaeus, 1758) were captured during the austral winter season of 2021. None of the mentioned species is threatened according to the IUCN Red List of Threatened Species™ (status: ongoing). A total of 46 specimens of fish (rainbow trout: $n = 12$; brown trout: $n = 21$; and torrent catfish: $n = 13$) were collected. Immediately after capture, fish specimens were transported in coolers with ice to the laboratory, where the samples were weighed and sized before dissection. The catch dates, number of individuals, fishing methodology, and sacrifice method were carried out according to the Directorate of Renewable Natural Resources' permission (Government of Mendoza, Argentina, research permit #420). Fish were weighed (fresh total weight; to the nearest 0.01 g) and measured (standard length; to the nearest 0.1 cm). After dissection, gastrointestinal tract (GIT) of each fish was carefully removed with surgical tweezers and scissors, then weighed and stored at $-18\text{ }^{\circ}\text{C}$ until analysis.

2.3. Determination of Plastic Consumption by Andean Fish

Each GIT was digested using a modified alkaline digestion process from Dehaut (2016) [30]. For this, each GIT was placed in a 250 mL Erlenmeyer (previously rinsed with Milli-Q water and covered with aluminum foil until used) and 50 mL of 10% KOH solution added to each one (the sample was submerged entirely), and then these Erlenmeyers (covered with aluminum foil) were placed in an oven at 60 °C for 12 h [31]. Subsequently, the contents of the Erlenmeyers were filtered using a 100-micron mesh, and the retained material was added to glass Petri dishes (using Milli-Q water) to be observed under a stereomicroscope (SZX7, 56×; Olympus, Tokyo, Japan).

The observation was carried out keeping the Petri dishes closed at all times. Each potential plastic particle was measured and photographed. Later, the particle was extracted with stainless steel tweezers, analyzed under a 50i Eclipse microscope (type 104; Nikon, Tokyo, Japan) equipped with a polarized light filter (Ascer 2015; Nikon) to confirm that it was indeed an MP, and stored in 2 mL Eppendorf tubes with Milli-Q water [31]. For plastic identification, exposure to polarized light can be used as a tool for plastic detection [32]. Polarized light allows us to identify polymers such as polyethylene, polypropylene, and polyethylene terephthalate since they are anisotropic materials and shine when exposed to polarized light (i.e., are optically active). In the case of particles that were not optically active under polarized light, the 'hot needle' technique was also used to assess their response [32]. Once confirmed as MPs, the particles were classified according to shape and color.

In the laboratory, all work surfaces and materials were cleaned with ethanol and Milli-Q water to prevent potential airborne plastic contamination. Further, each sample was kept in a closed Petri dish and only handled with gloves and cotton clothes. During sampling analyses, the laboratory doors and windows remained closed, and all work was conducted without using air conditioners to prevent particle movements. In addition, control Petri dishes containing Milli-Q water were set up during the chemical digestion described above and later when the samples were observed under stereoscope and microscope. If any plastic item was found in the control, it was counted, photographed, measured, and classified (shape and color). In the case of observed coincidences (shape and color) between plastic items in the sample and the control, such particles were discounted from the sample [31].

2.4. Statistical Analysis

Non-parametric statistics were used since the raw data did not fit a normal distribution (Shapiro–Wilk *W* test). To test for differences in MPs content (i.e., abundance per fish) in the GITs of fish species, Kruskal–Wallis *H* tests (KW) followed by a posteriori multiple comparisons of mean ranks for all groups were applied. Fish size (weight and length) is often assessed as a predictor of microplastics abundance in fish studies (e.g., [16,33–36]). A Kendall rank correlation (Kendall's tau coefficient) was performed to analyze whether there was any intraspecific relationship between total MPs' occurrence and the fish, as it provides a stronger association than Spearman's rho when there are smaller sample sizes and is less sensitive to error [37]. Statistical analysis was conducted using the InfoStat 2011 software, version 2011p [38]. For all statistical comparisons, a *p* value ≤ 0.05 was considered significant and $0.06 \leq p \leq 0.10$ was taken to indicate a trend [39].

3. Results

3.1. Microplastics Occurrence and Prevalence in Andean Fish

The analysis of MPs in fish GITs allowed the identification of plastic fibers and fragments. No foams, rubber, pellets, beads, or films were found. In the case of fibers, the colors detected were red, yellow, white, black, and blue, the latter being dominant. In the case of fragments, the colors detected were green and white. In the control samples, a maximum of three fibers per session were found and no fragments were recorded. Overall, the analysis found that fibers were present in 65% of the samples, while fragments were found in 10% of the analyzed specimens. A total of 88 MP items were counted, corresponding to 80% fibers and 20% fragments. Blue fibers (57.75%) were the most common, followed by black

(26.76%), red (7.04%), white (5.63%), and yellow (2.81%) fibers in the analyzed specimens. The fiber sizes ranged between 0.4 and 5 mm, while the sizes of the fragments ranged between 0.1 and 1 mm.

The percentage contribution and the total number of MP fibers in non-native fish and the native freshwater fish of the Mendoza River are shown in Tables 1 and 2, respectively. Green and white fragments were found in the brown trout and torrent catfish, respectively. Specifically, fourteen green fragments (82%) were found in the GITs of three specimens of brown trout, while three white fragments (18%) were found in the GITs of two specimens of torrent catfish.

Table 1. Percentage contribution and total number of MP fibers in the GITs of the non-native rainbow trout and brown trout collected from the Mendoza River.

MP Color	Rainbow Trout (<i>n</i> = 12) (<i>Oncorhynchus mykiss</i>)		Brown Trout (<i>n</i> = 21) (<i>Salmo trutta</i>)	
	Contribution (%)	Number of Fibers	Contribution (%)	Number of Fibers
blue	71	15	50	21
black	19	4	31	13
red	5	1	7	3
white	5	1	7	3
yellow	-	-	5	2
fibers (total)		21		42

Table 2. Percentage contribution and total number of MP fibers in the GITs of the native torrent catfish collected from the Mendoza River.

MP Color	Torrent Catfish (<i>n</i> = 13) (<i>Hatcheria macraei</i>)	
	Contribution (%)	Number of Fibers
Blue	63	5
Black	25	2
Red	12	1
White	-	-
Fibers (total)		8

A significant difference was found among the fish species ($KW H = 7.68, p = 0.02$) when comparing the median of the total MPs content (i.e., abundance per fish) in the GITs of the analyzed specimens. The highest MPs content was found in the GITs of brown trout followed by rainbow trout, while the lowest MPs content was found in the GITs of torrent catfish specimens (Figure 2). The a posteriori multiple interspecific comparisons revealed that the MPs' abundance in the GITs of brown trout was statistically different from that found in torrent catfish specimens. No significant difference was detected between brown trout and rainbow trout specimens when comparing the total MPs abundance (Figure 2). Multiple interspecific comparisons also revealed that the MPs' abundance in the GITs of rainbow trout was not statistically different from that found in torrent catfish specimens (Figure 2).

A slight, but statistically significant, difference was found among fish species ($KW H = 5.93, p = 0.05$) when comparing the median values of total fiber abundance in the GITs of the analyzed specimens. The highest fiber abundance was found in the GITs of brown trout followed by rainbow trout, while the lowest abundance of fibers was found in the GITs of torrent catfish specimens (Figure 3). A posteriori multiple interspecific comparisons revealed that the abundance of fibers in the GITs of brown trout was statistically different from that found in torrent catfish specimens. No significant differences were detected between brown trout and rainbow trout specimens when comparing the abundance of fibers (Figure 3). Multiple interspecific comparisons also revealed that the abundance of fibers in the GITs of rainbow trout were not statistically different from those found in torrent catfish specimens (Figure 3). Although there was no difference ($KW H = 5.27, p = 0.07$) in

the blue-fiber abundance in GITs among the fish species, the brown trout and rainbow trout showed a trend towards higher blue-fiber consumption than the torrent catfish (Figure 3).

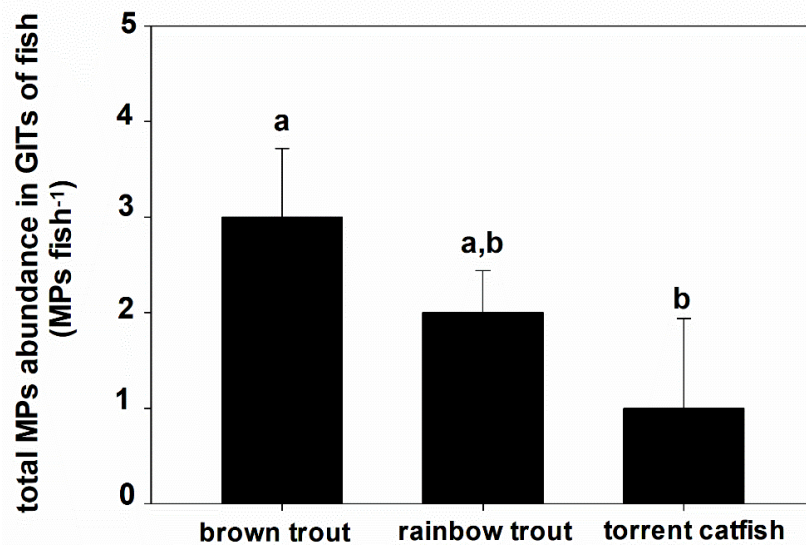


Figure 2. Total MPs abundance in fish GITs from Mendoza River in the Central Andes range. Bars represent medians and the whiskers standard errors. Different lower-case letters indicate significant differences in the fiber content among fish species (Kruskal–Wallis H test (KW), followed by a posteriori multiple comparisons of mean ranks for all groups).

3.2. Associations between MPs Content in Fish GITs and Body Size

Details of fish morphometry are included in Table 3. Kendall rank correlations were used to explore possible intraspecific associations between total MPs abundance in GITs and fish body size. In this sense, total MPs abundance in GITs was correlated with fish weight and length. Kendall's tau correlation coefficients indicated that there were no significant associations between the total MPs in GITs and the fish weight and length for brown trout (Kendall's tau = 0.28, $p = 0.12$; Kendall's tau = 0.15, $p = 0.40$, respectively), rainbow trout (Kendall's tau = -0.10 , $p = 0.70$; Kendall's tau = -0.16 , $p = 0.53$, respectively), or torrent catfish (Kendall's tau = 0.00, $p = 1.00$; Kendall's tau = 0.00, $p = 1.00$, respectively).

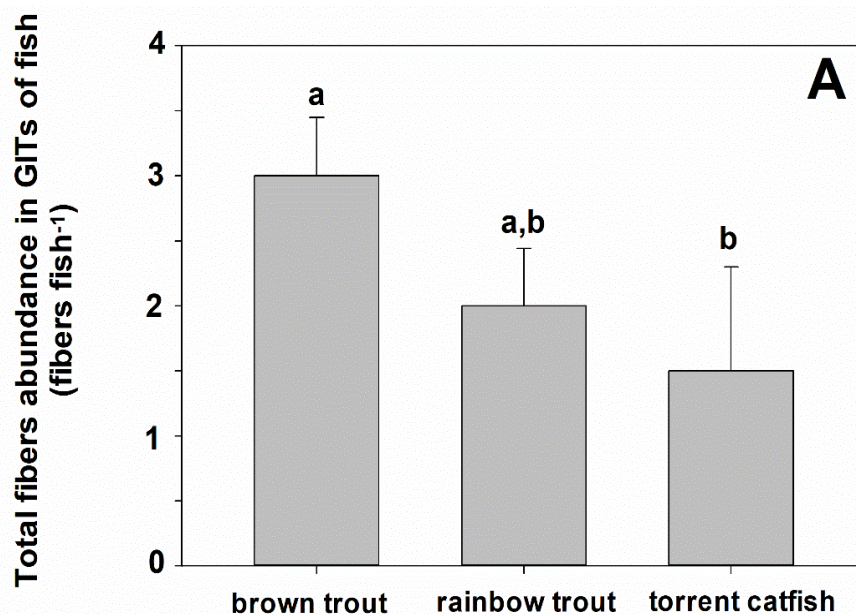


Figure 3. Cont.

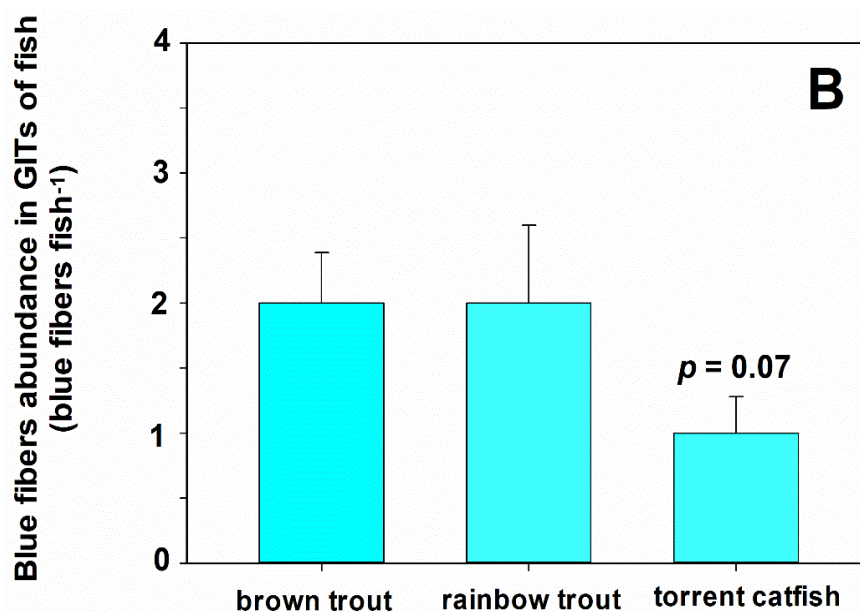


Figure 3. Total fiber abundance (A) and blue-fiber abundance (B) in fish GITs from Mendoza River in the Central Andes range. Bars represent medians and the whiskers standard errors. Different lower-case letters indicate significant differences in the fiber abundance among fish species (Kruskal–Wallis H test (KW), followed by a posteriori multiple comparisons of mean ranks for all groups).

Table 3. Morphometric description of fish collected from Mendoza River.

Species	n	Total Weight (g)	Standard Length (cm)
Rainbow trout (<i>Oncorhynchus mykiss</i>)	12	474 ± 43.8 (250–765)	34.9 ± 1.51 (25–41.5)
Brown trout (<i>Salmo trutta</i>)	21	492 ± 39.9 (210–800)	36.8 ± 1.22 (27.9–49)
Torrent catfish (<i>Hatcheria macraei</i>)	13	9.21 ± 1.65 (3.3–19.3)	9.87 ± 0.49 (7.5–12.2)

Note: Morphometric values are mean ± standard error (range in brackets).

4. Discussion

Regarding the occurrence of MPs in the fish analyzed here, the observed differences between the trout species and the torrent catfish for the total fiber abundance in GITs (Figure 3) could be attributed to species-specific feeding ecology, as suggested in previous reports on other freshwater fish species [40–42]. This scenario is discussed as follows. The feeding ecology of the fish species studied in the Mendoza River in the Central Andes region has been scarcely studied. Of the non-native salmonids here considered, both trout species are generally regarded as opportunistic top-predator carnivores feeding primarily on fish and macroinvertebrates from the benthos and drift [16,28]. The food habits of the native torrent catfish agree with the bentophagous type, and the dominant preys are chironomid larvae (Chironomidae family, Diptera), caddisflies larvae (Or. Trichoptera), and cladocerans in the freshwater environments of the southern Andes [43].

In the present study, fibers (85%) were the main MPs type recovered from the fish analyzed here, followed by fragments (15%). Blue fibers were the main type of MPs in the analyzed specimens: brown trout (50%), rainbow trout (71%), and torrent catfish (63%). The predominance of blue fibers is consistent with previous reports in the region and with the world trends reported for marine [44], estuarine [45], and freshwater fish species [31,46–48]. The prevalence of blue-colored fibers could be associated with the resistance of this color to UV-radiation degradation [49].

Worldwide, there seems to be a trend that blue fibers are the most frequently found plastic items in the GITs of wild fish from freshwater systems, including in Southeast Asia [50], Africa [46], North America [22,51], South America [31,48], and Europe [23]. Factors such as the shape and size [52] as well as the color [53,54] influence MPs' intake for both marine and freshwater fish species. A literature survey for freshwater environments in South America gives an account of the dominance pattern of blue fibers in the stomachs of fish analyzed and is outlined next. For example, in Brazil, the incidence of MPs in the diet of two freshwater fish species (*Iheringthys labrosus* and *Astyanax lacustris*) from the middle section of the Uruguay River was reported. The authors found that the predominant color was blue at 74% (white 17%, red 4%, and black 4%), and the most frequent synthetic materials were fibers, representing 88% of MPs [48]. In Uruguay, it was reported that the most frequent MPs were blue fibers (67%) in the analyzed GITs of 29 freshwater fish species from urban and rural streams [31]. In Argentina, the presence of MPs was reported to be 100% in the gut content of 11 species of fish analyzed from the Río de la Plata estuary. In this study, the authors found that 96% of MPs in the GITs of fish correspond to fibers of the colors red, green, yellow, white, black, and blue, the latter being the dominant color [45]. However, to date, it is not clear how shape, density, and coloration of the plastic debris relates to selectivity of different species of fish [12,55]

Microplastic fibers mainly originate from the textile industry [10] and items such as clothing, fishing lines and nets, and plastic bags [56]. Fibers are the main MPs found in the GITs of freshwater fish species [48]. The predominance of blue over the other colors found in several studies may be due to a greater abundance of blue MPs in aquatic systems, significant contamination of fish prey with blue MPs, and/or the preferential active ingestion of blue MPs by fish when confused with their natural food [44,53,54,57,58]. If there were a greater abundance of blue MPs in the water than other colors, then the blue MPs would have a higher probability of being consumed by fish and their prey. For example, Alfonso et al. [59] reported the first evidence of MPs in the surface water from nine Patagonian lakes in the Southern Andes region. The authors reported that the predominant MPs found were blue fibers (42%), followed by black fibers (37%). Former results agree with our findings for fish of the Central Andes region and with other studies where blue and black colors prevailed in the targeted samples [12,59]. However, it remains an open question whether the strong predominance of blue particles in fish guts is because the color blue is attractive to fish. Testing this hypothesis would require laboratory experiments performed with different fish species to understand the mechanisms underlying the consumption patterns of MPs according to color [53,54].

Regarding the intraspecific correlational approach, the Kendall's tau rank correlation revealed no relationship between MPs abundance in fish GITs and fish body size (weight and length). Previous fish studies have reported positive correlations between MPs content in fish and body size [22,33], which could be attributed to greater feeding rates, habitat preferences, or trophic positions. However, the lack of correlations between MPs in GITs and fish size in the present study is consistent with previous reports conducted with brown trout in an Irish riverine system [16] and estuarine fish from Argentina [45] and Brazil [34]. Nonetheless, it is worth noting that more field studies are needed to increase the number of samples and thus address this issue with even modest certainty.

5. Conclusions

In this study, the presence of MPs in specimens of the non-native brown trout and rainbow trout and the native torrent catfish, collected from the middle section of Mendoza River in the Central Andes range, is reported for the first time. In this mountainous river, which is of key importance for the socio-productive activity of the province of Mendoza, blue fibers were the main type of MPs in the fish analyzed here, and this finding is consistent with the global pattern. According to patterns found in this study, both salmonids analyzed here are suitable bioindicator species for future MPs' assessments in this mountainous region. Seasonal assessment of MPs occurrence in fish, coupled with

analysis of environmental compartments (e.g., water, snow, sediment), would further inform us about the plastic intake of Andean freshwater fish. The evidence provided through such studies could be useful in articulating policies aimed at mitigating MPs pollution as well as their impacts on mountain-dwelling fish and man. It is important to highlight the joint efforts of numerous scientific and governmental entities focused on mitigating the environmental problem generated by plastic pollution. The possible solutions to counteract the plastic dilemma can be summarized as follows: (i) implement a circular economy which encourages more responsible and sustainable consumption; (ii) implement the use of effective waste-collection systems; (iii) reduce waste generation; (iv) educate and sensitize consumers; and (v) ensure that waste is properly managed and disposed of.

Author Contributions: J.M.R.: sample collection and processing, data organization and analysis, field and lab work, writing—manuscript; F.T.d.M.: conceptualization, writing—review and editing; B.D.F., E.K., C.V. and V.A.L.-A.: processing samples; E.E.S.: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: Financial support was supplied by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT PICT 2021:00077), Programa de Desarrollo de las Ciencias Básicas-PEDECIBA (Geociencias y Biología), and Sistema Nacional de Investigaciones (SIN-ANIII).

Institutional Review Board Statement: To carry out field studies on wild animals, we have a permit from the Government of Mendoza to capture fish and invertebrates for scientific purposes. The capture methodology, number of specimens proposed, and the transfer of the specimens to the laboratory are correspondingly authorized by a permit to carry out scientific fishing (Scientific Fishing Resolution #420-21, granted by the Directorate of Renewable Natural Resources DRNR belonging to the Ministry of Environment and Sustainable Development of the Government of Mendoza). This capture permit complies with all current government regulations on wildlife management.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during this study are included in this manuscript.

Acknowledgments: This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT PICT 2021-00077). F.T.d.M. thanks Programa de Desarrollo de las Ciencias Básicas-PEDECIBA (Geociencias y Biología) and Sistema Nacional de Investigaciones (SIN-ANIII). Although InfoStat professional software is not free, Monica Balzarini, who led the design of the software, kindly gave J.M.R. a free license. J.M.R. thanks J.G. Ríos and J.M. Sarmiento for help with the trout capture. Dedicated to the lovely memory of R.A. Ríos.

Conflicts of Interest: The authors declare no conflict of interest.

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