



Presence of anthropogenic fibers in gills and digestive tract of the silverside fish *Odontesthes bonariensis* from the lower basin of the Tunuyán River in the Central Andes

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Abstract. Anthropogenic pollution has become a threat to the freshwater environments on a global scale. Like other contaminants, plastic particles can reach mountain ecosystems through atmospheric transport and regional transport. Nowadays, there are few reports on anthropogenic particles such as microplastics in mountain ecosystems. Here, we measured the abundance of anthropogenic particles in gastrointestinal tracts (GIT) and gills of silverside fish specimens captured during three consecutive years (2013, 2014, and 2015) at the end of the spring season (November-December) from Carrizal dam in the Central Andes region of Argentina. In this study only fibers were detected. The abundance of anthropogenic fibers in the GIT of silverside fish specimens was significantly higher in the year 2013 (5 ± 0.8 fibers fish⁻¹) than in the years 2014 and 2015 (2 ± 0.4 fibers fish⁻¹; 1.3 ± 0.3 fibers fish⁻¹, respectively). These findings suggest the need to update and design monitoring programs that include environmental (surface water) and other biological matrices (prey items) to enhance our understanding on the sources of pollution in this Andean region.

Key words: Central Andes; biomonitoring; anthropogenic fibers; silverside fish, gills, gut.

Fibras antropogénicas en branquias y tracto digestivo del pejerrey *Odontesthes bonariensis* de la cuenca baja del Río Tunuyán en los Andes Centrales. Resumen. La contaminación antropogénica se ha convertido en una amenaza para los ambientes de agua dulce a escala global. Al igual que otros contaminantes, los desechos plásticos pueden llegar a los ecosistemas de montaña a través del transporte atmosférico y el transporte regional. Actualmente, existen pocos informes sobre partículas antropogénicas tales como los microplásticos en ecosistemas de montaña. Aquí, medimos la abundancia de partículas antropogénicas en tractos gastrointestinales (TGI) y branquias de especímenes de pejerrey

capturados al final de la temporada de primavera (noviembre-diciembre) durante tres años consecutivos (2013, 2014 y 2015) en la represa el Carrizal ubicada en la región de los Andes Centrales de Argentina. En este estudio sólo se detectaron fibras. La abundancia de fibras antropogénicas en el tracto gastrointestinal de los ejemplares de pejerrey fue significativamente mayor en el año 2013 ($5 \pm 0,8$ fibras pez⁻¹) que en los años 2014 y 2015 ($2 \pm 0,4$ fibras pez⁻¹; $1,3 \pm 0,3$ fibras pez⁻¹, respectivamente). Estos hallazgos sugieren la necesidad de actualizar y diseñar programas de monitoreo que incluyan matrices ambientales (aguas superficiales) y otras matrices biológicas (presas) para mejorar nuestro conocimiento sobre las fuentes de contaminación en esta región andina.

Palabras clave: Andes Centrales; biomonitoreo; fibras antropogénicas; pejerrey, branquias, tracto digestivo.

Introduction

Anthropogenic particles are infiltrating ecosystems at an alarming rate, transforming them into a global environmental threat. Despite their societal benefits, plastic products have raised concerns as contaminants due to its persistence, widespread use and associated consequences in mountain ecosystems (Liro *et al.* 2023a, b). Currently, microplastics (MPs) are of great interest in environmental pollution studies, due to their ubiquity, which has been reported even in the most remote places on the planet. Examples include the depths of the ocean in the Mariana trench (Weston *et al.* 2020) and the Himalayan mountain system (Talukdar *et al.* 2023) including the summit of Mount Everest (Napper *et al.* 2020). However, there are few reports on anthropogenic particles in freshwater fish inhabiting the Andean ecosystems in South America.

Microplastics are particles less than 5 mm in size and have become a focal point in recent environmental pollution research. These particles, which can vary widely in shape and color, often originate from the degradation of plastic waste (Hartmann *et al.* 2019). Concerns about rapid accumulation, atmospheric transport, and harmful health implications associated with MPs have led to an exponential increase in research into this phenomenon over the last two decades (PlasticsEurope 2022). Microplastics pose a threat to freshwater biota as they can cause intestinal obstruction (Ríos *et al.* 2022a,b; Horie *et al.* 2024) and be transferred between trophic levels (Hasegawa & Nakaoka 2021, Horie *et al.* 2024). It has been reported that exposure to MPs negatively affects the physiology and feeding behaviour of tadpoles, fish, and crustaceans (Ríos *et al.* 2022a, Attademo *et al.* 2023, Ríos *et al.* 2023, Horie *et al.* 2024).

Fish species adapted to mountain aquatic systems have biological characteristics that make

them suitable for studies of plastic pollution in these environments (Driscoll *et al.* 2021, Galafassi *et al.* 2021, Loayza *et al.* 2022, Ríos *et al.* 2022b, Bilal *et al.* 2023). The silverside fish (hereinafter, silverside: *Odontesthes bonariensis*, Atherinopsidae, Valenciennes, 1835) is native to the southern area of the Río de la Plata basin (Eastern Argentina) but was initially introduced in 1960 in the Central Andes region due to its sporting and gastronomic importance (Villanueva & Roig 1995). According its feeding ecology, silverside is a piscivore fish that feeds in the surface and water column (Loayza *et al.* 2022). In the present study, the abundance of anthropogenic particles in different organs of the silverside was assessed over three consecutive years: 2013, 2014, and 2015. Here, we postulated that there will be an increase in the abundance of anthropogenic particles in the fish from the Carrizal Dam over the years. Additionally, the viability of this species as a sentinel of anthropogenic pollution in the Central Andes region is explored.

Materials and methods

Fish sampling and initial processing: The fish used in this study were captured in the Carrizal dam (latitude: -33.3402; longitude: 68.7321; altitude: 788 meters above sea level) located in the lower basin of the Tunuyán River in the central Andes, Argentina. The Carrizal dam (360 hm³) was built for multiple uses, including hydroelectric power generation, crop irrigation, and recreational and aquatic sports (Clavijo *et al.* 2016). Recreational clubs and the surrounding town have a population of ca. 5000 inhabitants, where agricultural activities are also carried out. Additionally, the Carrizal Dam, located 77 km downstream from the Uco Valley (a relevant wine region in Argentina), could potentially receive the runoff of particles and plastic waste from vineyards and wineries. The location of the dam where the fish specimens were captured to carry out

this study is shown in Figure 1. All fish specimens collected ($n = 29$) were adults during the post-reproductive period and were caught by conventional hook and live bait fishing (*Psalidodon eigenmanniorum*) in accordance our scientific fishing authorization. It is noteworthy that out of the 29 specimens dissected, seven were specifically utilized to extract their gastrointestinal tracts (GIT) for the study of diet composition (unpublished data). Consequently, this study focused on analysing the gills of all 29 specimens and GIT of 22 specimens.

Immediately after capture, the fish specimens were sacrificed using the "stunning and killing" method, based on euthanized by a blow to the head. This method is frequently recommended to induce loss of consciousness and insensitivity in fish, making this method the most popular and most used (Robb *et al.* 2022). Before dissection, the specimens were transported to the laboratory in cold storage with ice, and once there they were weighed (total fresh weight; to 0.01 g precision) and measured (standard length; with a precision of 0.1 cm). After dissection, GIT and gills of the fish were carefully removed with the help of surgical forceps and

scissors previously washed with Milli-Q water, weighed and stored at -18°C until analysis. The capture dates, the number of individuals, the fishing methodology, and the slaughter method were in accordance with the research permit granted by the Directorate of Renewable Natural Resources (see resolution #420 in the section on Ethical approval and governmental permission). This species is not threatened according to the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN 2024).

Analysis of anthropogenic particles present in silverside: Currently, there is no consensus on a single classification for the study of plastics and their sub-fractions, which creates difficulties when comparing data (Vidal *et al.* 2021). In the present study, we use a generalized classification, where anthropogenic particles are defined as possible plastic items less than 5 mm in their longest axis (Hartmann *et al.* 2019, Vidal *et al.* 2021). Considering their shape, anthropogenic particles can be classified into two large categories: fibers (when one of the dimensions predominates over the others)

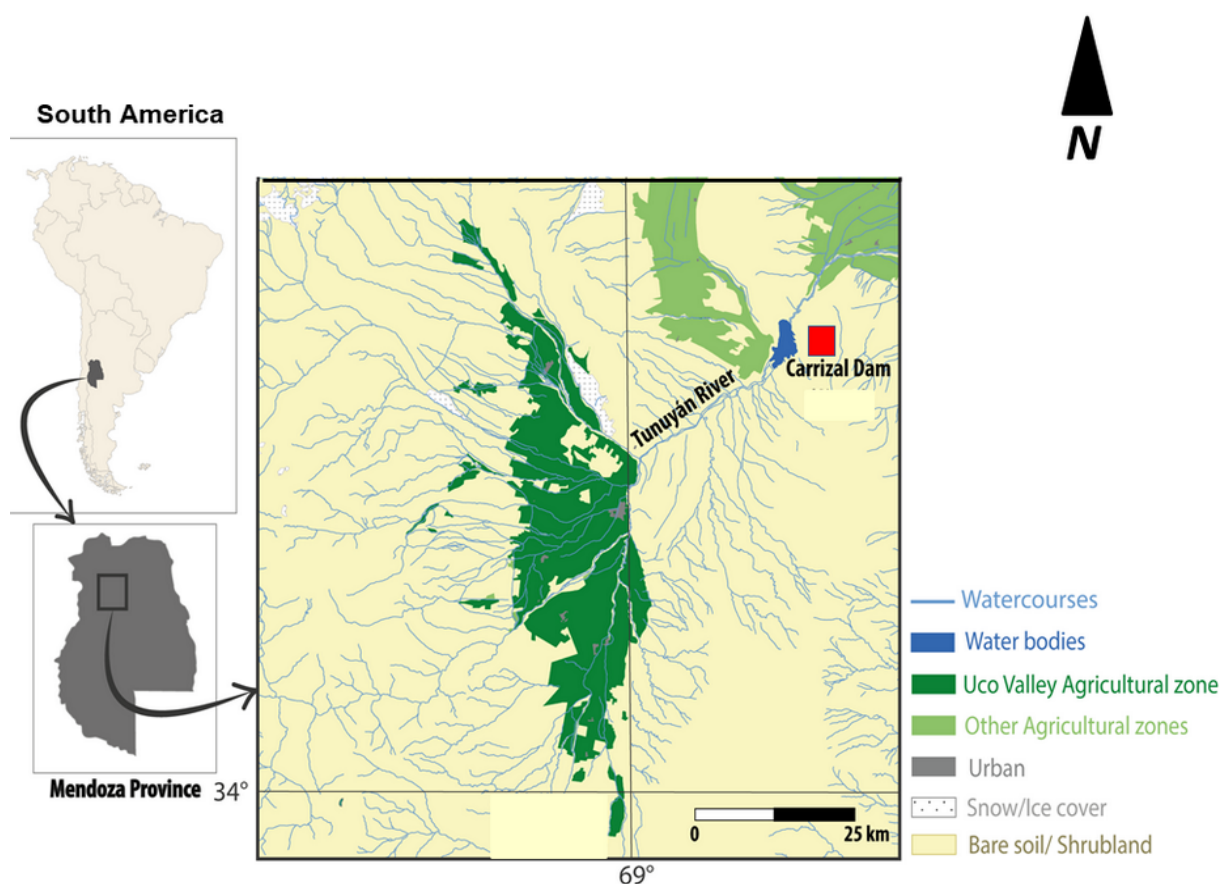


Figure 1. Map showing the Carrizal dam (red square), located at the lower basin of the Tunuyán River in the Central Andes range, where silverside fish specimens were collected during three consecutive years.

and fragments (when two dimensions predominate over the third) (Dris *et al.* 2015). Information on the color of anthropogenic particles is also often documented. Fibers constitute one of the most abundant groups of anthropogenic particles (i.e., relation of 50-90% in surface water and biota samples, Dris *et al.* 2015, Hartmann *et al.* 2019).

Gastrointestinal tracts and gills were digested using a modified alkaline digestion process from Dehaut *et al.* (2016). To carry out the digestion, samples were placed separately in Erlenmeyers of 250 ml previously rinsed with Milli-Q water and covered with aluminium foil. Then, 50 ml of KOH 10% solution was added to each (the sample was completely submerged) and placed in an oven at 60° for 20 hours (Vidal *et al.* 2021). Subsequently, the contents of the Erlenmeyers were filtered using a 100 µm mesh and the retained material was taken to a glass Petri dish (with Milli-Q water) to be observed under a stereoscopic magnifying glass (SMZ745, 2.5X, Nikon, Tokio, Japan). The Petri dish remains always closed except when a possible plastic particle was found, and it was examined under polarized light, measured, and photographed. Particles were characterized using an Up-right Nikon 80i microscope (Nikon, Japan) equipped with a polarized light filter to confirm that it was indeed a particle of anthropogenic origin. To identify the plastic, exposure to polarized light was taken as a reference (Sierra *et al.* 2019). Polarized light allows us to identify several polymers, such as polyethylene, polypropylene, and polyethylene terephthalate, as they are anisotropic materials and glow when exposed to polarized light (i.e., optically active).

Precautions to avoid sample contamination (quality controls and assurance): Since anthropogenic particles are also present in the indoor air, precautions must be adopted to guarantee good quality results preventing the contamination of samples which leads to the overestimations of environmental concentrations (Vidal *et al.* 2021; Prata *et al.* 2021). Fibers primarily originate from textile wear, laundry, and tearing. These fibers can become incorporated into paper products and water systems, necessitating stringent handling precautions in the laboratory space to ensure non-contamination of the samples throughout their processing and observation (Dris *et al.* 2015). Given this consideration, all laboratory work surfaces and materials were cleansed with pre-filtered ethanol:milli-Q water (7:3) before the initiation of experiments. This cleaning solution was used

because helps to prevent cross-contamination (Oliveira-Paiva *et al.* 2022). Following the recommendations of the bibliography, we worked with the air conditioning equipment turned off to avoid the movement of particles present in the air, and with all materials covered with aluminium foil to avoid the deposition of free particles.

To control for possible cross-contamination in our laboratory, a procedure blank was carried out in the same conditions as the samples. The observation and identification process were carried out with a Petri dish containing Milli-Q water ('control dish'), opened simultaneously with the samples. Both the blank and the 'control dish' were observed under a magnifying glass at the end of processing each sample and if particles were found, they were counted, photographed, measured, and classified (according to shape and color). When anthropogenic particles in control samples exhibited matching morphology and coloration to those in the fish samples analyzed during the same session, such particles were excluded from the final particle counts.

Statistical analysis: Non-parametric statistics were used, as the data did not fit a normal distribution (Shapiro-Wilk test). To test differences in fish morphometry as well as anthropogenic particles abundance between the years considered for each tissue, the Kruskal-Wallis (KW) *H* test was used followed by multiple comparisons for mean ranges as a *posteriori* test. Wilcoxon Matched Pairs Test was used to explore differences between organs analyzed within each year of monitoring as well as to compare fiber size between organs. Spearman correlations were used to explore possible association between fish morphometry and anthropogenic particles load in organs. Statistical analysis were performed using InfoStat Software, version 2011 (Di Rienzo *et al.* 2011). For all statistical comparisons, a *p*-value < 0.05 was considered significant.

Results

Fish morphometry of the specimens analyzed in this study are shown in Table I. A significant interannual difference was observed only for standard length, with 2013 measurements being significantly higher than in both following years (Table I, Fig S1). Total weight remained consistent throughout the study period, with no significant interannual differences detected (Table I, Fig. S2).

The analysis of anthropogenic particles in GIT and gills of silverside allowed the identification of

fibers of anthropogenic origin (Fig. 2). No fragments, foam, rubber, granules, beads, or films were found. The percentage of contribution, relative abundance, and color types of fibers detected in GIT and gills are shown in Table II and Table III, respectively. Overall, the analysis found that fibers were present in 82% of the GIT ($n = 22$) and in 65% of the gills ($n = 29$) of the specimens analyzed. Regardless of color, the total number of fibers found in the GIT of the silverside specimens was 49, 10, and 4 for the years 2013, 2014, and 2015, respectively (Table II). Likewise, the total number of fibers found in the gills of silverside specimens was 7, 9 and 8 for the years 2013, 2014, and 2015, respectively (Table III). The sizes of the fibers in the GIT ranged between 0.4 and 12 mm, while in the gills they ranged between 0.8 and 4.7 mm (Fig. 2). When comparing the median, the size of the fibers found in the GIT were significantly larger than in gills ($Z = 4.285$, $p < 0.01$).

A significant difference was found in the fiber content (the abundance of fibers per fish) in the GIT of silverside between the years of testing ($KW H = 9.163$; $p = 0.01$). The *a posteriori* analysis indicates that this difference is due to a significantly greater abundance of fibers in the GIT during 2013 (5 ± 0.8 fibers fish⁻¹) compared to the years 2014 (2 ± 0.4 fibers fish⁻¹), and 2015 (1.3 ± 0.3 fibers fish⁻¹), respectively (Fig. 3). However, in the gills, no significant differences were detected according to the years of sampling ($KW H = 0.244$; $p = 0.88$). Statistical comparisons revealed no significant differences between GIT and gill tissues across all study years.

Discussion

The present work provides the first record of anthropogenic fiber uptake by silverside specimens from the Carrizal dam located in the lower basin of the Tunuyán River in the Central Andes, Argentina. Our findings show that 82% of the GIT and 65% of the gills contained anthropogenic fibers. Similar studies in the Northern Andes have also focused on the silverside. For example, Loayza *et al.* (2022) reported for the first time the presence of anthropogenic fibers in the gut contents of silverside from Lake Titicaca (between Bolivia and Peru, Northern Andes), one of the great ancient mountain lakes of South America (3809 meters above sea level). This study highlighted contamination by anthropogenic fibers, raising concerns about the unknown consequences for the ecosystem and human health, as silverside is an edible species utilised by local fisheries and gastronomy throughout the Andes range (Loayza *et al.* 2022).

Fibers are ubiquitous particles, originating from different sources, primarily associated with anthropogenic activities such as textile industry and washing clothes. For instance, the mechanical action of washing releases large quantities of synthetic fibers, which eventually end up in rivers and are carried to dams (Kelly *et al.* 2019, Emmerik & Schwarz, 2020). Additionally, fishing gear is also a source of fibers in ecosystems, as many of them are made of synthetic materials that shed fibers during use, or when abandoned (Jonathan *et al.* 2021). Although the standard length of the specimens

Table I. Morphometric description of silverside fish collected from Carrizal dam. Morphometric values are mean, median (in brackets) $\pm$ standard error. Different capital letters indicate significant differences among year for the length or weight of specimens here analyzed (Kruskal–Wallis H test, followed by *a posteriori* multiple comparisons of mean ranks for all groups).

Catching date	Number of specimens	Standard length (cm)	Total weight (g)
07/11/2013	10	23.1 (22.5) ^A ± 0.53	66.7 (64.5) ^A ± 9.51
08/12/2014	11	21.1 (21.0) ^B ± 0.28	60.1 (60.6) ^A ± 1.45
21/11/2015	8	20.5 (20.5) ^B ± 0.64	58.4 (54.6) ^A ± 5.99

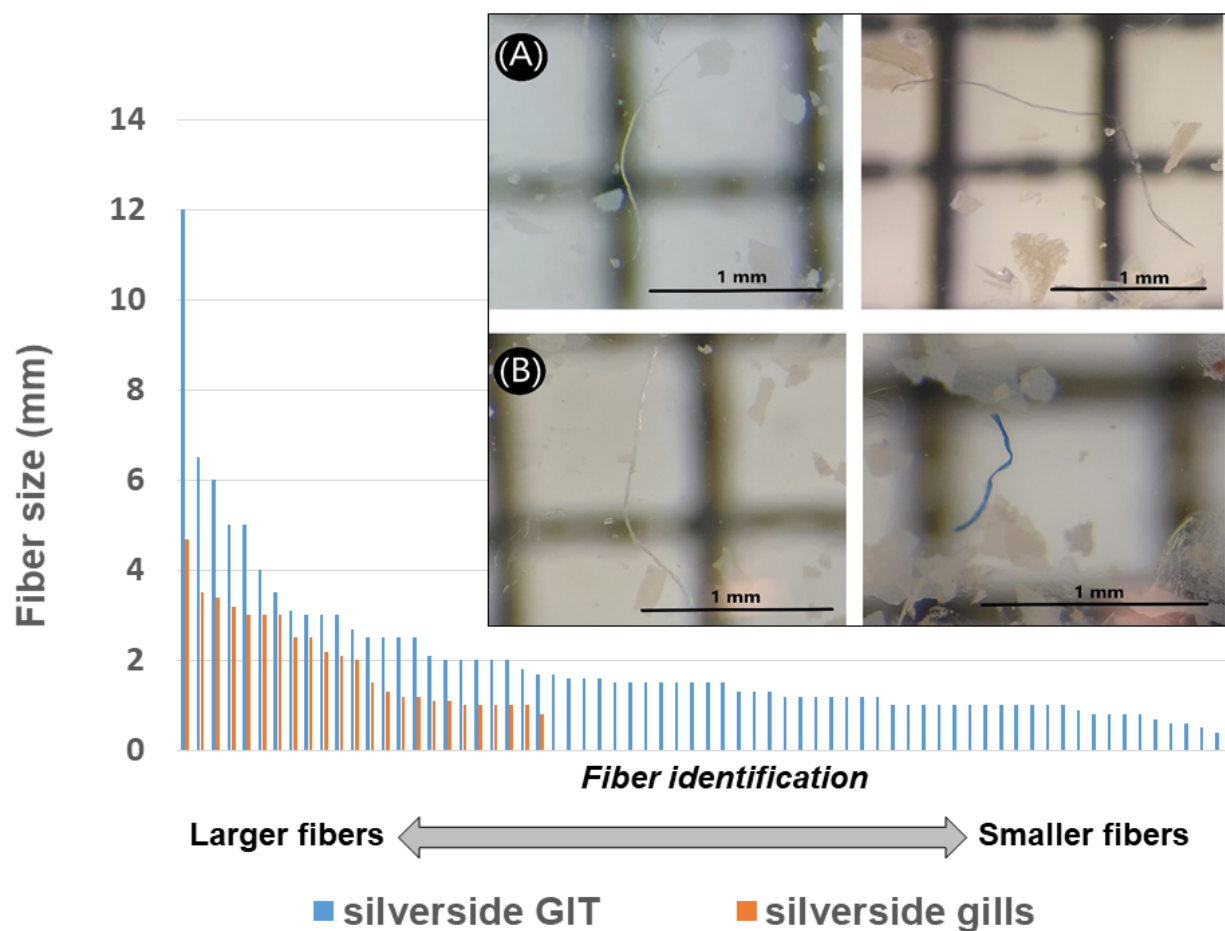


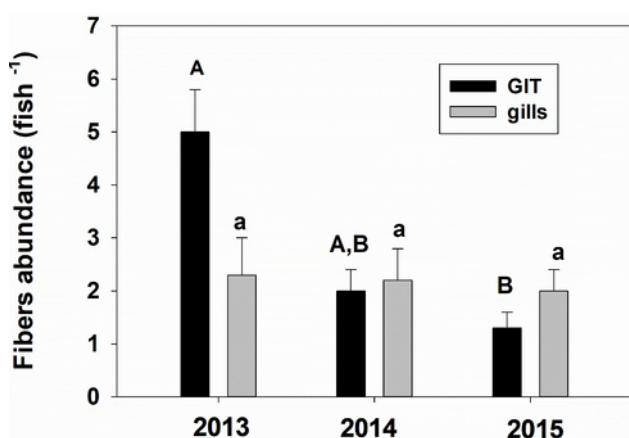
Figure 2. Distribution of fiber size expressed in millimeters found in GIT and gills of the silverside specimens analyzed. Example of anthropogenic fibers (upper right side of figure) recovered from digestions carried out in A) gills and B) GIT of the silverside specimens analyzed.

Table II. Percentage of contribution and total number of anthropogenic fibers in GIT of the silverside fish from the Carrizal dam through three consecutive years of the past decade.

Color	2013 (n = 10)		2014 (n = 7)		2015 (n = 5)	
	Contribution (%)	Fibers (number)	Contribution (%)	Fibers (number)	Contribution (%)	Fibers (number)
transparent	70	34	50	5	50	2
blue	20	10	20	2	50	2
black	8	4	20	2	-	-
red	2	1	10	1	-	-
Average		5		2	-	1.3
Total		49		10		4

Table III. Percentage of contribution and total number of anthropogenic fibers in gills of the silverside fish from the Carrizal dam through three consecutive years of the past decade.

Color	2013 (n = 10)		2014 (n = 11)		2015 (n = 8)	
	Contribution (%)	Fibers (number)	Contribution (%)	Fibers (number)	Contribution (%)	Fibers (number)
transparent	71	5	78	7	25	2
blue	-	-	-	-	50	4
black	14	1	11	1	12	1
red	-	-	11	1	12	1
yellow	14	1	-	-	-	-
average		2.3		2.3		2
Total		7		9		8

**Figure 3.** Total abundance of anthropogenic fibers in GIT and gills of the silverside fish from Carrizal dam through three consecutive years of the previous decade. The bars represent the mean with its standard error. Different capital letters indicate significant differences in fibers content between years (Kruskal-Wallis (KW) H test, followed by post hoc multiple comparisons of mean ranks for all groups).

captured in 2013 was greater than for 2014 and 2015, Spearman correlations did not detect significant associations between the size of the fish and the fiber load present in GIT nor in the gills (Table SI in Supplementary material). Therefore, a plausible explanation for our findings on fish caught in 2013 is proposed next.

During the year 2013, there was a high input of river water (DGI 2015), which caused the dam to fill to its maximum level (Table S2 in Supplementary material). Increased river flows are usually associated with an increase in particles transport (Liro *et al.* 2023a, b) due to diffuse inputs from the basin washout and runoff from cities (Campanale *et al.* 2022), soils (Rillig *et al.* 2017), and agricultural (Ouyang *et al.* 2020) and livestock land uses (Ramachandraiah *et al.* 2022). Therefore,

this likely resulted in a greater availability of anthropogenic particles in the water, and consequently, a higher abundance of fibers in the silverside specimens collected in 2013. These findings suggest the need to implement an updated monitoring program that includes not only biological matrices (e.g., prey items) but also environmental matrices (for example, surface water, sediment). Such a program would provide a comprehensive understanding of the possible sources and amplification pathways of anthropogenic pollution in fish in this Andean region. Although greater food availability in the spring of 2013 could explain the larger size of the specimens analyzed in that year than in the following two years, no studies have been conducted to support this hypothesis.

Nowadays there are few reports regarding the presence of anthropogenic fibers in mountain fish species in the Andes mountain range. Recently, our research group reported for the first time the abundance of MPs in non-native (salmonids) and native fish species (torrent catfish) from the Mendoza River in the Central Andes range, highlighting the plastic pollution issue in this region (Ríos *et al.* 2022b). In this previous study, we found that blue fibers were the most abundant type of MPs, a strong pattern already reported for other South American freshwater fish species (Vidal *et al.* 2021, Loayza *et al.* 2022). However, in the present study, the fiber colors detected in the GIT of fish collected in 2013 were mostly transparent, followed by blue, black, and red. This pattern was consistent across the years 2014 and 2015 as well. In addition, the fiber colors detected in the gills of fish collected in 2013 and 2014 were predominantly transparent, followed by black, and red. However, in 2015, the predominant fiber color detected in the gills was blue followed by transparent, black, and red. This latter pattern aligns with findings from previous

studies on mountain fish in the Andean region (Ríos *et al.* 2022, Loayza *et al.* 2022).

Conclusions

This study reports the presence of anthropogenic fibers in silverside fish specimens collected during 2013, 2014, and 2015 from a dam located in the lower section of the Tunuyán River basin in the Central Andes range. The Carrizal dam is of key importance for the socio-economic activities of the province of Mendoza, particularly its vineyard production. Fibers were the only anthropogenic particles detected in fish organs. As silversides represent an edible species and are widely consumed, the evidence presented here could be instrumental in shaping policies aimed at mitigating anthropogenic pollution and its potential impacts on the humans who consume this wild fish. Our findings suggest the importance of updating and designing monitoring programs that include surface water and prey items to deepen our understanding of anthropogenic pollution in this Andean region.

Ethical statement

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 #1148-22, granted by the Directorate of Renewable Natural Resources DRNR belonging to the Ministry of Environment and Sustainable Development of the Government of Mendoza, Argentina). This capture permit complies with all current government regulations on wildlife management.

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Supplementary material:

Rios JM et al. Presence of anthropogenic fibers in gills and digestive tract of the silverside fish *Odontesthes bonariensis* from the lower basin of the Tunuyán River in the Central Andes

Table S1. Output of the Spearman's correlations used to explore associations between fish morphometric characteristics (size) and total fiber load in GIT as well as gills. Correlations were considered significant for $p < 0.05$.

Variables involved	2013		2014		2015	
	<i>Spearman r</i>	<i>p-value</i>	<i>Spearman r</i>	<i>p-value</i>	<i>Spearman r</i>	<i>p-value</i>
Standard length & GIT total fiber load	0.27	0.43	0.16	0.79	0.00	1.00
Standard length & gills total fiber load	-	-	0.21	0.78	0.00	1.00
Total weight & GIT total fiber load	0.63	0.17	0.31	0.60	0.00	1.00
Total weight & gills total fiber load	0.86	0.33	-0.31	0.68	0.00	1.00

Table S2. Average, maximum and minimum levels (masl) of the El Carrizal reservoir along hydrological years. n.a. = not available.

years	Average (masl)	Maximum (masl)	Minimum (masl)
2012	781.85	785.50	777.50
2013	783.01	785.58	779.70
2014	780.89	785.37	776.14
2015	n.a.	n.a.	n.a.

Data taken of Dirección General de Irrigación (DGI 2015): Water balance of the lower Tunuyán.

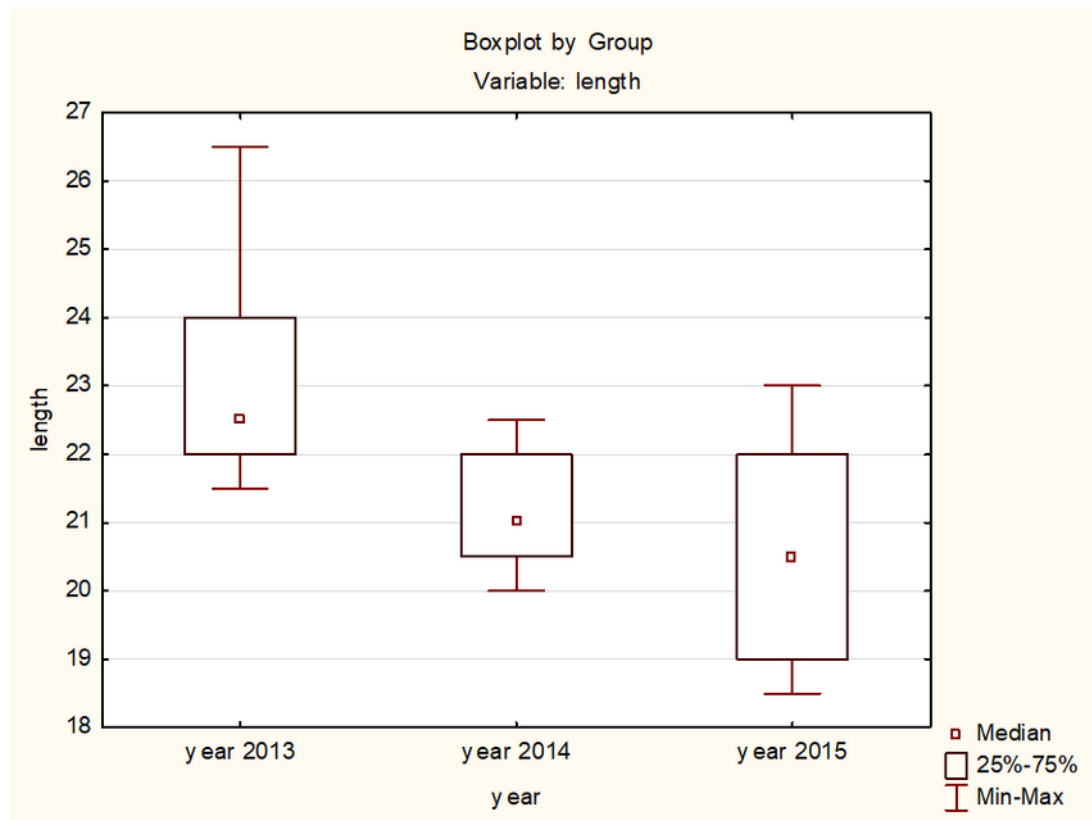


Fig. S1. Blox-plots of fish standard length comparisons among years. Kruskal-Wallis (Chi square = 10.650; $p = 0.0049$).

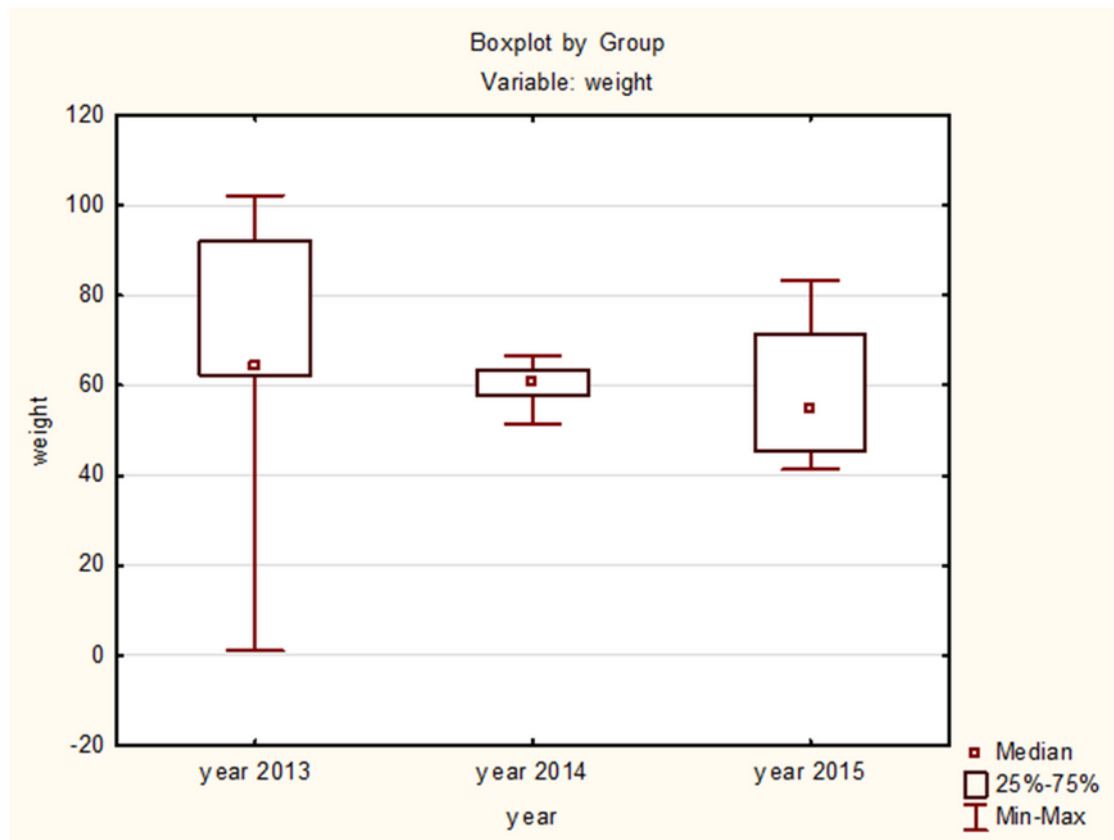


Fig. S2. Blox-plots of fish total weight comparisons among years. Kruskal-Wallis (Chi square = 2.887; $p = 0.236$).