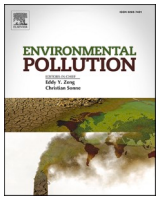
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Plastic microfibers in water, macroinvertebrates and Chilean pencil catfish across the Mapocho River basin (Chile): Occurrence, characteristics, exposure and trophic transfer trends

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ABSTRACT

Microplastics (MPs) are omnipresent particles that can cause diverse perturbation in aquatic biota. In freshwater systems, their presence and accumulation in aquatic fauna depends on a combination of abiotic and biotic factors that are specific to each basin, with Wastewater Treatment Plant (WWTP) effluent being a significant source. In this study, we determined the abundance and characteristics of Microfibers (MFs) in water samples, as well as in different feeding guilds of benthic macroinvertebrates, and Chilean pencil catfish upstream and downstream of discharges from five WWTPs in Santiago. Additionally, an evaluation of bioaccumulation and/or trophic transfer trends of MFs in biotic matrices was conducted. The highest and lowest MF abundances in water samples were found at RS1 and RS2 locations, respectively (reference sites upstream any WWTPs). In macroinvertebrates, the highest and lowest MF intake was in RS1 and DS1, respectively. In catfish, the highest and lowest MF intake was in DS2 and DS3, respectively (both downstream sites). A certain relationship was evident: the larger catfish had a higher abundance of MFs, but only in the sites DS2 and DS3 located downstream of the WWTPs. However, this relationship was not statistically significant (p-value >0.05). Filter-feeders and collectors (scrapers and shredders) ingested the highest amounts of MFs at sites upstream and downstream of the WWTPs, respectively. A trophic transfer of MFs was evident when considering the total macroinvertebrate group and the pencil catfish. This process was enhanced at RS2, DS1, and DS2, but not at the three downstream sites. This study provides the first assessment of MPs in multiple environmental matrices for rivers in Chile. These findings highlight the necessity for continuous monitoring and long-term studies to understand the site-specific processes and factors that may influence the presence and movement of MPs within river systems, beyond the mere proximity of WWTPs.

1. Introduction

Plastics are synthetic organic polymers, derived from petroleum that are non-biodegradable and highly persistent in the environment (Rios et al., 2007; Andrady and Neal, 2009; Thompson et al., 2009). Due to their widespread use and low production cost, they are considered ubiquitous in ecosystems (Rios et al., 2007; Andrady and Neal, 2009; Xia et al., 2021), with rivers acting as major transport routes of plastic from land to ocean (Oswald et al., 2025). These synthetic materials can be classified according to size (e.g., macroplastics), shape (e.g., spheres), and polymer type (e.g., thermoplastics) (Van Cauwenberghe et al., 2015; Jiang et al., 2020; Gan et al., 2023). Plastics are considered an

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emerging pollutant because they contain additives and plasticizers that give them physicochemical properties such as thermal stability and durability (Thompson et al., 2009; Jiang et al., 2020). Furthermore, due to their large specific surface area, organic composition, and hydrophobic components, plastics can adsorb potential contaminants in aquatic environments, such as Polychlorinated Biphenyls (PCBs), Dichloro-Diphenyl-Trichloroethane (DDT), and trace elements (TEs), which may generate an additive or synergistic toxic effect in aquatic systems (Engler, 2012; Lin et al., 2018; Yu et al., 2020; Wei et al., 2025).

Large plastics do not biodegrade, they fragment into smaller particles, increasing their dispersal capacity, bioavailability, and potential incorporation into food webs (Sulaiman et al., 2023). Microplastics (MP) are defined as plastic particles smaller than 5 mm and are classified by their origin as primary (manufactured at microscopic sizes) or secondary (resulting from the fragmentation of larger plastic debris) (Cole et al., 2011; Ngo et al., 2019). Among them, Microfibers (MF) originate mainly from the washing of synthetic textiles and enter the wastewater flow, eventually reaching aquatic systems through the effluents of Wastewater Treatment Plants (WWTP) (Freeman et al., 2020; Tserendorj et al., 2024).

Therefore, WWTPs are considered a major source of MPs in freshwater systems through effluents, sewage sludge, and the degradation of plastic materials within the treatment facilities themselves (Freeman et al., 2020). Although WWTPs can retain large proportions of MPs (>90%), current technologies are not designed for their complete removal or retention (Talvitie et al., 2017; Koelmans et al., 2019; Freeman et al., 2020). For example, washing a synthetic garment can release more than 1,900 MFs (Browne et al., 2011), up to 1,500,000 (De Falco et al., 2019) or release up to 2,820,000/kg of washed textile, depending on washing conditions and garment type (Dreillard et al., 2022). Consequently, a WWTP can release more than 100 billion MPs annually into receiving water (Freeman et al., 2020). Additional sources of MPs in continental waters include landfills and atmospheric deposition (Geyer et al., 2017; Leslie et al., 2017; Lacerot et al., 2020; Huang et al., 2021).

The availability of MPs in environmental matrices such as water, macroinvertebrates, and fish, is determined by multiple abiotic and biotic factors within each watershed (Horton et al., 2018; Windsor et al., 2019; García et al., 2021; Jurina et al., 2025). In macroinvertebrates, MP ingestion and elimination varies between taxa depending on biological and morphological characteristics, trophic positions, and feeding strategies (Windsor et al., 2019; García et al., 2021; Bertoli et al., 2022). In fish, MP ingestion is dependent on feeding habits and vertical distribution within the water column (Mazariegos-Ortíz et al., 2020; Merga et al., 2020; Savoca et al., 2021). Furthermore, larger fish and higher trophic levels of species may show higher probability of ingestion, suggesting potential for bioaccumulation and biomagnification processes (Horton et al., 2018; McNeish et al., 2018; García et al., 2021). In addition, hydrodynamics processes such as water circulation, flow, and wind also influence the transport and distribution of MP in freshwater systems (Bellasi et al., 2020; Chauhan and Banerjee, 2025), and recent studies show that hydrodynamic conditions can influence MP bioaccumulation, tissue distribution, and toxicological responses in fish (Jo et al., 2025; Rasta et al., 2025; Rasta et al., 2026). Other factors affecting MP presence include the proximity of anthropogenic sources such as WWTP effluents (Leslie et al., 2017; Talvitie et al., 2017; Freeman et al., 2020) and seasonal environmental processes such as rainfall or runoff (Xia et al., 2021; Moses et al., 2023). Consequently, MPs pose a threat to aquatic organisms through direct ingestion, bioaccumulation, and trophic transfer, potentially causing tissue damage, digestive tract obstruction, false satiation, physiological stress, among others (Li et al., 2018; Du et al., 2025; Jo et al., 2025).

The Mapocho River, located in Santiago, Chile, is a highly anthropized lotic system that provides multiple ecosystem services such as climate regulation and flood mitigation for more than eight million inhabitants (Vásquez, 2016). The river crosses sixteen municipalities and functions as a biological corridor for wildlife. However, it is exposed to multiple pollution pressures associated with urban development, illegal dumping, landfills, and WWTP effluents (Katz et al., 2009; Ferrando, 2010). Currently, some WWTPs discharge treated effluents directly into the Mapocho River, using activated sludge technology (SiSS, 2024). Although Chilean regulations governing WWTP discharges (Supreme Decree N°. 90/2000 and Supreme Decree N°. 53/2013) establish maximum range concentrations for several pollutants; they do not include MPs as a regulated parameter (Higuera, 2022).

The environmental legal framework explicitly promotes the use and analysis of physical and chemical variables for assessment purposes, but MPs are not included among these factors. Various initiatives have sought to strengthen this framework by incorporating biological responses to stressors in aquatic ecosystems as key monitoring and assessment tools (Munkittrick and Dixon, 1989; Gibbons and Munkittrick, 1994). In Chile, this approach has also been applied through studies that explored contaminant effects and biological responses in local aquatic environments (Orrego et al., 2006, 2009; Chiang et al., 2010, 2011; 2014). *Trichomycterus areolatus* (Valenciennes, 1846) or "Chilean pencil catfish", is a native benthic fish species commonly found among rocks and currents in central-southern Chilean rivers (Arratia, 1983), and it is a model organism in ecotoxicological studies (e.g., Chiang et al., 2011; Ali et al., 2020; Bertin et al., 2020). It is the only native fish species distributed throughout the Mapocho River basin, making it a suitable bioindicator for pollution in this system. Furthermore, the pencil catfish is an active hunter of benthic macroinvertebrates (Duarte et al., 1971; Arratia, 1983). However, this may facilitate the biomagnification of contaminants through the food web. In this regard, Functional Feeding Groups (FFGs) of macroinvertebrates (Cummins, 1974; Merritt and Cummins, 2006) can serve as indirect indicators of trophic position, providing insights into the potential biomagnification of contaminants, such as MPs. Despite this, to date, no study has comprehensively assessed the transfer of MPs from WWTPs into abiotic and biotic compartments of a highly urbanized South American river. Furthermore, the role of macroinvertebrate FFGs as mediators of MF transfer to native benthic fish like *Trichomycterus areolatus* remains unexplored in this region.

Therefore, this study aims to fill this gap by evaluating MF pollution in the Mapocho River, using a multi-compartment approach that links environmental contamination with biological uptake, both upstream and downstream of effluent discharges from five WWTPs along this river. We hypothesized that: (i) MF concentrations in water, macroinvertebrates, and fish would be significantly higher at sites downstream of WWTP effluent discharges compared to upstream sites, confirming WWTPs as point sources of these pollutants. (ii) Due to their feeding strategies, macroinvertebrates from different FFGs (e.g., collectors vs. predators) would exhibit different MF loads, with predators potentially showing higher levels if trophic transfer occurs. (iii) *Trichomycterus areolatus*, as a benthic predator, would present evidence of MF dietary exposure (e.g., a positive relationship between fish size and MF load) and trophic transfer (e.g., higher MF loads than their macroinvertebrate prey), and these effects would be more pronounced downstream of WWTP discharges, indicating an amplification of these processes due to the additional pollution source.

2. Methodology

2.1. Study area

The study area considered five sampling sites distributed from the upper to the lower reaches of the Mapocho River. We collected samples and data from two reference sites: Arrayán (RS1) and Clarillo (RS2) (the last located within the Clarillo River sub-basin); and three sites downstream WWTPs discharges: Hualtatas (DS1), Trapiche (DS2), and Monte (DS3) (Fig. 1). RS1 and RS2 were considered reference sites, as they are located upstream of the discharges and are located within legally

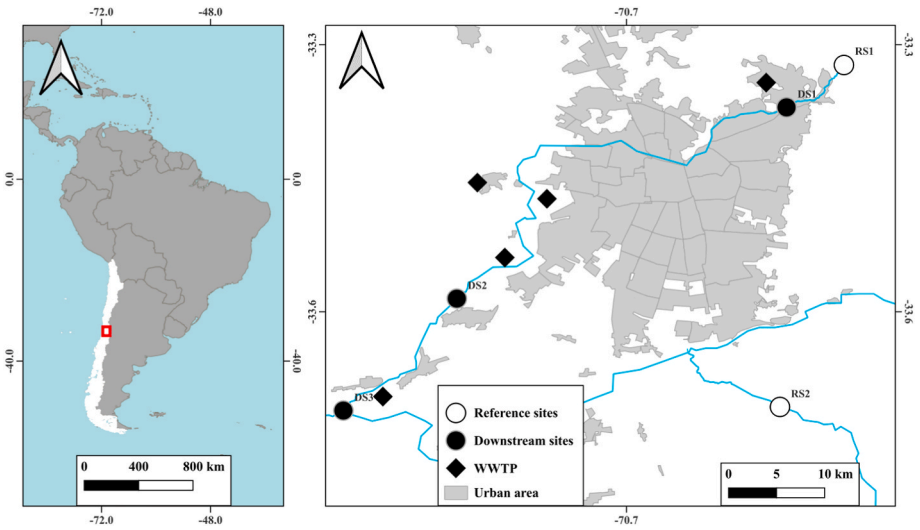


Fig. 1. Map of the study area.
Description: Map of the study area showing the five sampling sites, reference sites (RS) as white circles, and downstream sites (DS) as black circles. WWTPs that influence the sites are represented as black diamonds.

protected areas in Chile (a nature sanctuary and a national park, respectively).

2.2. Sample collection

Fieldwork was conducted during the austral spring of 2023 (September and October), considered the melting season in the Southern Hemisphere. Water samples, benthic macroinvertebrates, and fish specimens (specifically *T. areolatus*) were collected. At each sampling site, water samples were collected with a zooplankton net submerged for approximately 30 s, positioned upstream at three points along the channel, and their contents were deposited in 100 ml glass jars. Filtered water volume was calculated based on the speed flow of each site and the diameter of the net (50 cm), which will allow the calculation of the number of MFs per m^3. Benthic macroinvertebrate samples were collected with a Surber net in characteristic microhabitats (gravel, cobble, and boulders), at three points along the sampling site, and deposited into 100 ml glass jars. Finally, fish were captured during the pre-spawning season, following the experimental guidelines of Munkittrick (2009) and Chiang et al. (2011). Electrofishing backpack (HT-2000, Halltech Aquatics) and nets were used, capturing up to 32 juveniles/adults per sampling site. Individuals over 4 cm in size were caught only at DS2 site and over 6 cm at the other sampling sites, since the latter size ensures a reproductive cycle for the species (Chiang et al., 2011). Fishing was carried out under Exempt Resolution N°. E-2023-154 granted by the Subsecretaría de Pesca y Acuicultura (SUBPESCA) and in compliance with all bioethical guidelines established for research with aquatic vertebrates. Fishes were later transferred in coolers to the university facilities.

2.3. Laboratory procedures

Laboratory analyses were conducted at the Center for Sustainability Development (Andres Bello University, Chile) and filter examination was carried out at the Microplastic Research Center (Fairfield University, United States). Water samples (n = 15, three per site) were stored at room temperature. Macroinvertebrate samples (n = 15, three per site) and fish (previously euthanized via cervical cut) samples were frozen at −20 °C (n = 120, 20 to 32 per site). Macroinvertebrates were examined with a stereomicroscope for counting individuals (abundance) and were classified according to their morphology for subsequent taxonomic identification at the Family or Order level and stored in labeled glass vials (102 vials were obtained from 15 samples). FFGs were assigned based on standard identification guides and keys: phytophagus, nectarivore, collector-filter feeders, collector-scrapers, collector-shredders, parasites, predators, or "varied diet" in the case of having more than one feeding habit among species of the same family or at different stages of the life cycle (Cummins, 1974; Roldán-Pérez, 1996; Merritt and Cummins, 2006; Resh and Cardé, 2009; Barrios et al., 2012; Palma, 2013; Rincon et al., 2016). Finally, the length of the fish was measured post-mortem using an ichthyometer (±0.1 cm) and weighed (body and stomach) (±0.1 g). The stomachs were preserved in labeled vials at −20 °C for their subsequent degradation.

2.4. Microplastic extraction

MP isolation was carried out using the method recommended by Pérez-Guevara et al. (2021). The extraction process was consistent across all environmental matrices, with minor adjustments depending on the sample type: Samples were thawed at room temperature without applying heat to avoid altering the MP integrity. Organic material was digested with 35% hydrogen peroxide (H_2O_2), with a duration of up to three days for water samples and up to three months for biological samples. After digestion, samples were sieved using a 500 μm mesh to separate coarse sediment and undigested biological material. A saturated sodium chloride (NaCl) solution was added to the remaining liquid to increase its density and allow MPs to float; the mixture was then left for at least 2 h. The supernatant was filtered using a vacuum filtration system with a Buchner funnel, Kitasato flask, and 47 mm diameter, 0.45 μm pore size glass fiber filters (GC-50). Filtration was conducted slowly to avoid resuspension of particles. Filters were placed in sterile, pre-labeled plastic petri dishes lined with aluminum foil on both the base and lid. Three filters were obtained in each water sample, two filters in each catfish stomach sample, and one filter in each macroinvertebrate vial.

2.5. Quality control

Strict contamination control measures were implemented: All solutions (H_2O, H_2O_2, and saturated NaCl) were double-filtered and stored in glass bottles. All procedures were conducted under a fume hood to minimize airborne contamination. Only glass, aluminum, stainless steel, and cotton materials were used, including gloves. If plastic materials were necessary (e.g., petri dishes), they were pre-lined with aluminum.

An assessment of exogenous contamination was performed as a result of the methodological procedures using air control blanks and solutions prior to analysis, resulting in 18 control filters. The control assessments revealed a low number of particles, only three possible particles, which were therefore excluded from subsequent analyses. To subtract them from the final quantification of MFs in the environmental matrices, it is necessary to confirm the polymeric nature of these three particles using the FTIR spectrometer, which will be carried out subsequently.

2.6. Microplastic analysis

The filters were analyzed under stereoscopic microscopes by dividing each filter into 12 quadrants to reduce overestimation of particle counts. All potential MFs were photographed. Fiber length (±0.01 mm) was measured using ImageJ software, based on standardized images captured at 3.3X magnification. This will subsequently guide us to review the potential MFs identified under infrared microscopes and confirm the nature of the particles. Therefore, in this work we focus on potential MFs of synthetic origin. The classification of the MFs was carried out according to their color and size, in the latter, categorizing the fibers between 0 and 1, 1-2, 2-3, 3-4, and 4-5 mm, depending on their length calculated through ImageJ.

2.7. Statistical analyses

We calculated the relative abundance of MFs either by color or by size (mm). For the bioaccumulation analysis, a linear regression was performed to evaluate the relationship between fish size (used as a proxy for age) and abundance of log-transformed MFs. To identify biomagnification patterns, a Kruskal-Wallis test was performed to compare MF abundance between FFG or biotic matrices from the different sites. In case of finding significant differences (p-value <0.05), a post-hoc test of Dunn with a Bonferroni correction was used due to the multiple comparisons between groups (FFG). Additionally, a Trophic Transfer Index (TTI) was calculated to assess whether the relationships were higher than one between macroinvertebrates and fish, and to evaluate the differences by site: TTI = $[MP_{fish}]/[MP_{inverterbrates}]$, were $[MP_{fish}]$ means MP load in fish gastrointestinal tract and $[MP_{inverterbrates}]$ means MP load in whole invertebrate body. Values > 1 indicate that fish gastrointestinal loads exceed the whole-body burdens of invertebrates at the same site, suggesting effective trophic transfer of MPs via predation. TTI was calculated separately for each site to examine spatial variability in transfer efficiency.

It is worth noting that fish size and FFGs were treated as indirect indicators of bioaccumulation and trophic transfer of MP, respectively, providing preliminary insights into these processes within the biotic matrices of the Mapocho River.

3. Results

3.1. Abundance, sizes and colors of microfibers

3.1.1. Water samples

Across all water samples, a total of 561 potential MFs were identified. The average MF count ranged from 0.25 to 21.36 total MFs per cubic meter (TMF/m^3) (Table SM1), corresponding to the RS2 (0.25 ± 0.18 TMF/m^3) and RS1 (21.36 ± 1.73 TMF/m^3) sampling sites, respectively, both considered reference sites, where the lowest

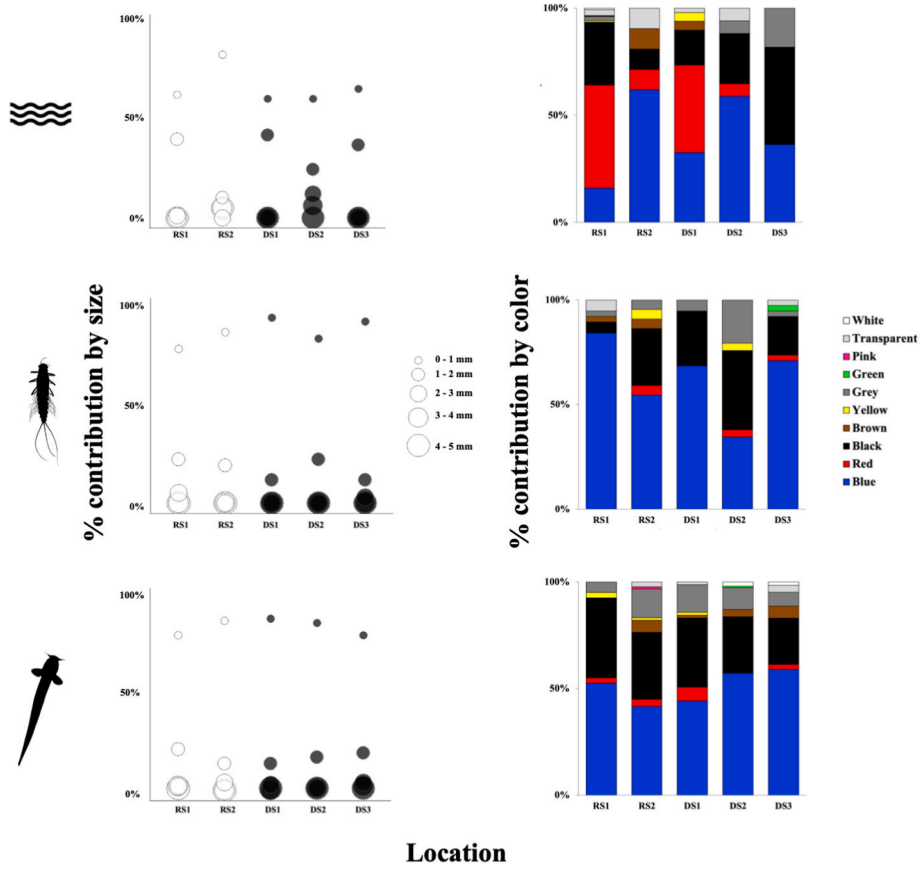


Fig. 2. Relative abundance (%) of microfibers by color and size (mm) in the three environmental matrices.
Description: Relative abundance (%) of size (mm) (left column) and color (right column) of MFs in water samples (left symbol water), macroinvertebrates (left symbol mayfly), and Chilean pencil catfish (left symbol catfish), respectively. Reference sites (RS) are represented by white circles, and downstream sites (DS) are represented by black circles. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

abundance of MFs/m^3 was expected. Sites with indirect influence from WWTPs, in this case, DS1 (2.37 ± 0.83 TMF/m^3), showed higher abundance than sites with direct influence from WWTPs (DS2, 0.42 ± 0.04 TMF/m^3 and DS3, 0.33 ± 0.19 TMF/m^3).

MF lengths ranged from 0.051 to 4.926 mm (±0.01 mm), and ten colors were identified across all sites: red, black, blue, gray, yellow, brown, green, pink, transparent, and white (Fig. 2). However, the most common colors were blue and black, present at all sampling sites, and red fibers were present at all sites except DS3. Further details of the number of MFs/individual or m^3 of water individualizing the colors are provided in the Supplementary Material (Table SM2; Table SM3).

3.1.2. Macroinvertebrates

Macroinvertebrate taxa were identified for each site and each taxon was assigned to a FFG (Table SM3). Among all the macroinvertebrates sampled, 146 potential MFs were found, identified in 81% of the macroinvertebrate samples. MF counts ranged from 0.00 to 4.00 fibers per individual (Table SM1; Table SM3), depending on the FFG and sampling site. DS1 individuals showed the lowest average consumption of MFs (0.60 ± 0.81 TMF/individual) and the RS1 individuals followed the same trend as in the water samples, showing the highest average concentration of MFs (1.13 ± 1.09 TMF/individual) (Table SM1).

MF lengths ranged from 0.036 to 2.198 mm (±0.01 mm), and eight colors were identified across all sites: blue, black, gray, red, brown, yellow, transparent, and green (Fig. 2; Table SM3). Blue and black fibers were observed in individuals from all sampling areas.

3.1.3. Chilean pencil catfish

Finally, among all the stomachs of *Trichomycterus areolatus*, 527 potential MFs were found, identified in 117 of the 120 individuals (97.5%). The number of fibers ranged between 0 and 19 MFs per individual, depending on the sampling site. The highest and lowest occurrences of MFs per individual were recorded at DS2 (6.39 ± 2.87 TMF) and DS3 (3.69 ± 2.53 TMF), respectively.

MF lengths ranged from 0.060 to 4.475 mm (±0.01 mm), and ten colors were identified across all sites: blue, black, gray, brown, red, transparent, yellow, green, pink, and white. As with macroinvertebrates, the most common colors were blue and black (Fig. 2; Table SM3).

3.2. Microplastic burdens in Chilean pencil catfish

Although statistically significant differences were only found at the RS1 (p-value <0.05), a pattern emerged related to the presence or absence of WWTP influence: At the RS1, RS2 and DS1 locations, larger individuals tended to have lower MF loadings, whereas in DS2 and DS3 catfish, larger individuals showed higher MF abundances in their stomachs (Fig. 3). These last two sites are directly influenced by WWTP effluent. Furthermore, the low R^2 values suggest that only a small proportion of the variance in MFs abundance is explained by fish size, indicating *a priori* that other factors may also play a role in the presence of MFs in these fish and the dietary exposure process, beyond the size by itself.

3.3. Trophic Transfer Index in biotic matrices

Higher concentrations of MF (p-value <0.05) were found between the biotic matrices at all sites, showing biomagnification trends from macroinvertebrates (without discriminating FFG) to catfish (Fig. 4). Additionally, the Trophic Transfer Index (TTI) was calculated between both biotic matrices of each sampling site, obtaining at all sites a ratio higher than 1 (RS1 = 3.88, RS2 = 6.24, DS1 = 6.42, DS2 = 6.09, DS3 = 4.39) (Table SM4), which does reflect trophic transfer trends, indicating that the MF load would be higher in Chilean pencil catfish compared to their prey (macroinvertebrates).

When assessing MFs abundance among macroinvertebrate FFG (Fig. 5), the initial test indicated significant results in RS2 and DS1 ($p \leq 0.05$). However, no significant differences were found between pairs of FFG after performing Dunn's post-hoc test with Bonferroni correction (p.

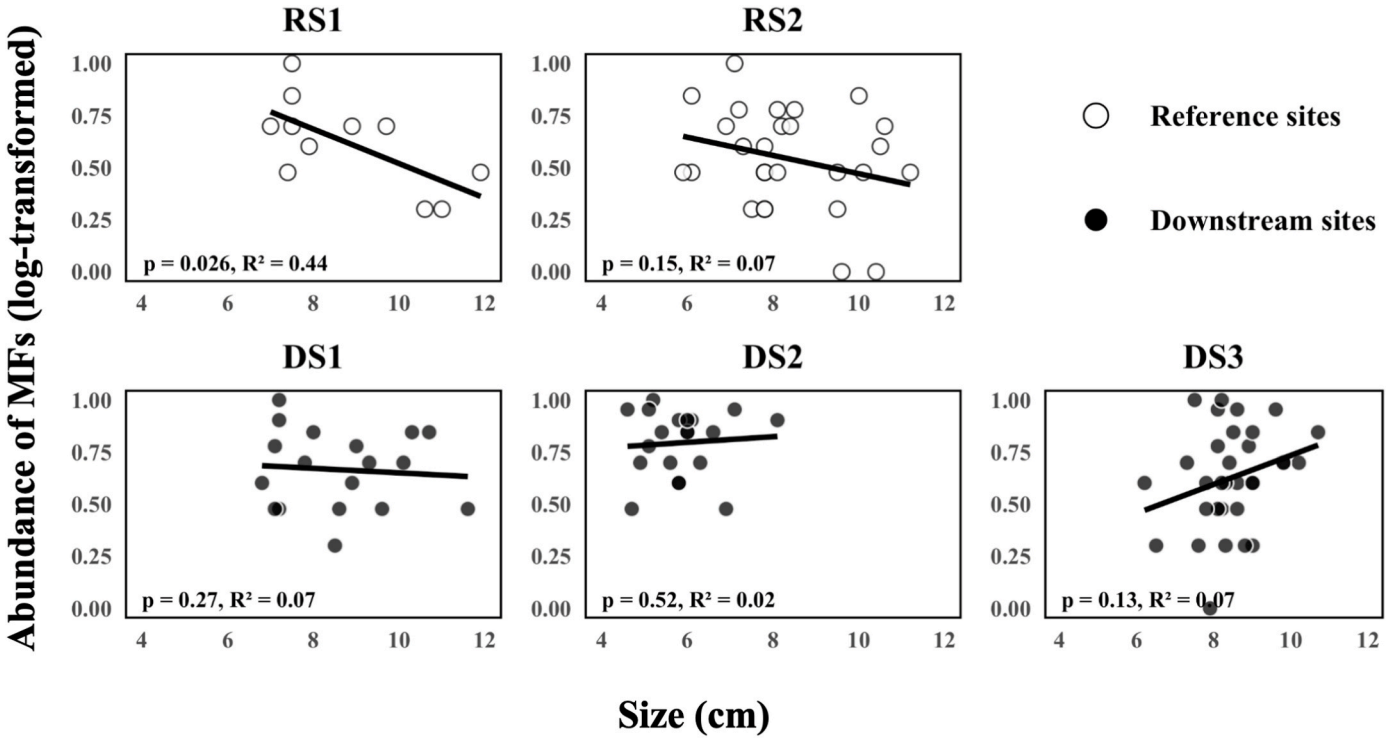


Fig. 3. Dietary exposure trends in the Chilean pencil catfish.
Description: Linear regression between the length (cm) of catfish at each site and the total microfiber abundance (log-transformed). Reference sites (RS) are represented with white circles and downstream sites (DS) with black circles.

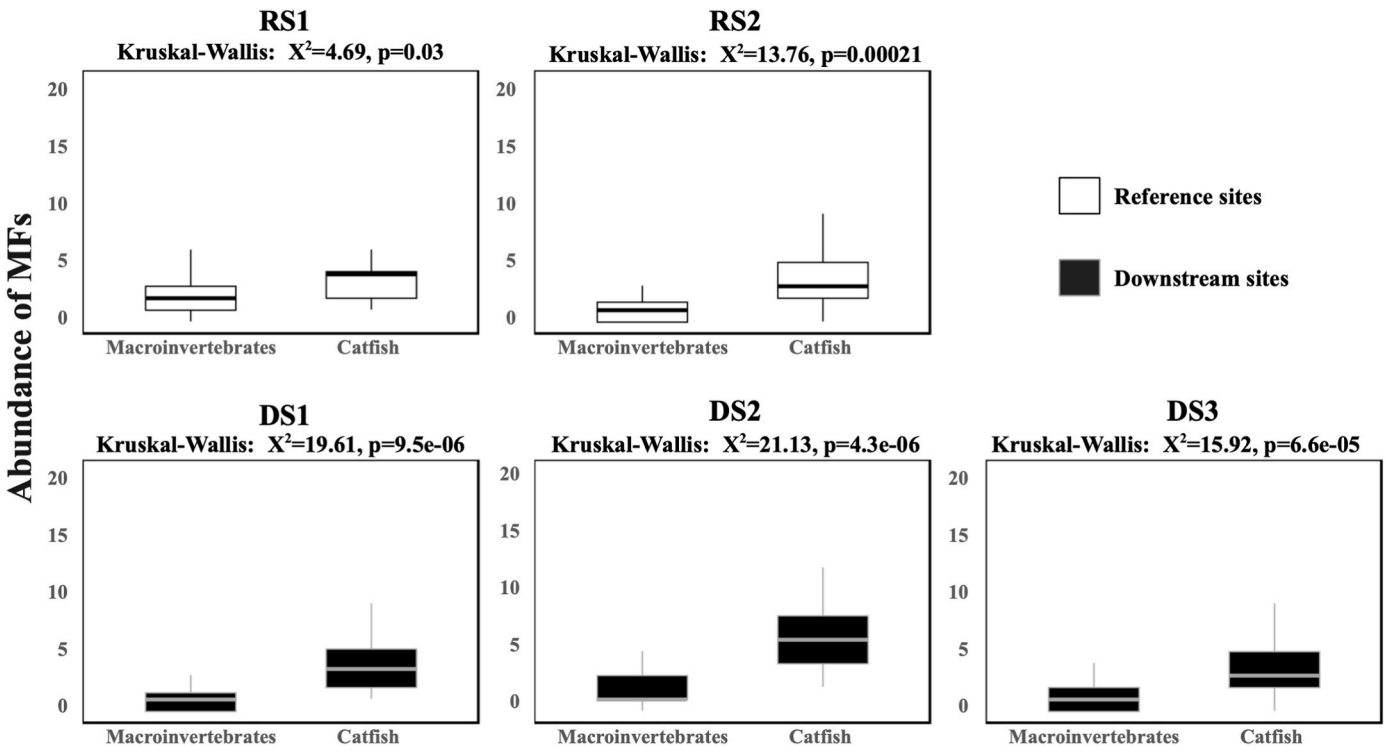


Fig. 4. Trophic transfer trends among biotic matrices.
Description: Kruskal-Wallis box plot. Indicates the total abundance of microfibers in catfish and macroinvertebrates. Reference sites (RS) are represented by white boxes and downstream sites (DS) by black boxes. The chi-square (χ^2) value and p-value are shown at the top of each plot.

adj >0.05). The filter-feeder group was the group with the highest MFs abundance in RS1 and RS2. At downstream sites, the highest abundance of MFs was found in collector-scrapers (DS1 and DS2) or collector-shredders (DS3) individuals. Therefore, no higher MF loads were found in individuals at higher trophic levels, such as predators.

4. Discussion

The likely presence of plastic MFs was demonstrated in all three environmental matrices evaluated across the Mapocho River. However, contrary to expectations, the highest abundance of MF/m^3 was found in Arrayán (a protected and upstream site), and *a priori* no MP ingestion in fish was associated with their size or trophic transfer trends among macroinvertebrate FFG. However, a high predator-prey exposure ratio trend was demonstrated from macroinvertebrates to catfish, with RS2, DS1, and DS2 being the sites with the most pronounced TTI, compared to RS1 which was the lowest. This is in line with our hypothesis, at least for DS, which indicated that this process of accumulation of MPs in the trophic web should be enhanced downstream of the discharges.

4.1. Abundance of microfibers in water

The lack of correlation between the proximity of WWTP effluents and MF abundance in water could be explained by environmental factors that affect transport patterns, such as the low influence of rainfall during the southern spring in rivers with mediterranean climates, which could lead to higher concentrations of MPs in sediments, but not in water in places far from mountain ranges (Xia et al., 2021; Moses et al., 2023). The wind direction from the northwest to the southeast during the spring season could also be a factor, as it can transport MPs in the water from the west to pre-mountain sites such as RS1, a factor that affects MP transport patterns within riparian systems (Bellasi et al., 2020). This would explain the high concentration of fibers on this site. On the other hand, the increase in temperature also causes melting of ice from the mountain range, increasing the flow rate, and resuspending particles from the sediment (Moses et al., 2023). Some seasonal comparison studies have shown that during dry seasons, there is a higher abundance and diversity of MPs in rivers with a nivo-pluvial regime such as the Garona River in Spain-France (de Carvalho et al., 2021) and the Clackamas River in the United States (Talbot et al., 2022), probably associated with some of the environmental factors mentioned above (e.g. precipitation, run off, among others). This seasonal comparison should be addressed in future studies for the Mapocho River, considering seasonal hydrological data, to confirm the role of certain factors in the movement of MPs within the river. All these environmental factors, even when combined, could explain why at the RS1 site a concentration of MF/m^3 of water was found to be 9 to 85 times higher compared to the other sites. Although this site is located upstream and is located within a nature sanctuary, it remains a highly touristic and urbanized area which would explain the presence of MPs, as demonstrated in other studies (Bian et al., 2022; Wang et al., 2023), although this needs to be corroborated. These factors should be considered in future studies, as this reflects the uncertainty in predicting the occurrence of MP in environmental samples. These factors can arise even at a local scale, where the availability of MPs in water will depend on the prevailing environmental conditions, characteristics of the sampling site, proximity to anthropogenic sources, among others.

Previous studies have reported a wide range of MF values in water samples associated with WWTP effluents, ranging from 0 to 750.000 MP/m^3 (Menéndez-Manjón et al., 2022). This considerable variation may depend on the type and number of treatment stages and the MP removal technologies used, which may vary by country or even by individual facility. The WWTPs potentially influencing the environmental matrices in this study are: *Los Trapenses–Hualtatas* (discharging into *Estero Hualtatas* before it converges with the Mapocho River, upstream of DS1), *Lomas de Lo Aguirre* (upstream of DS2), *La Farfana* (upstream of DS2), *El Trebal* (upstream of DS2), and *Talagante* (upstream of DS3). These five WWTPs operate using "activated sludge" technology (SiSS,

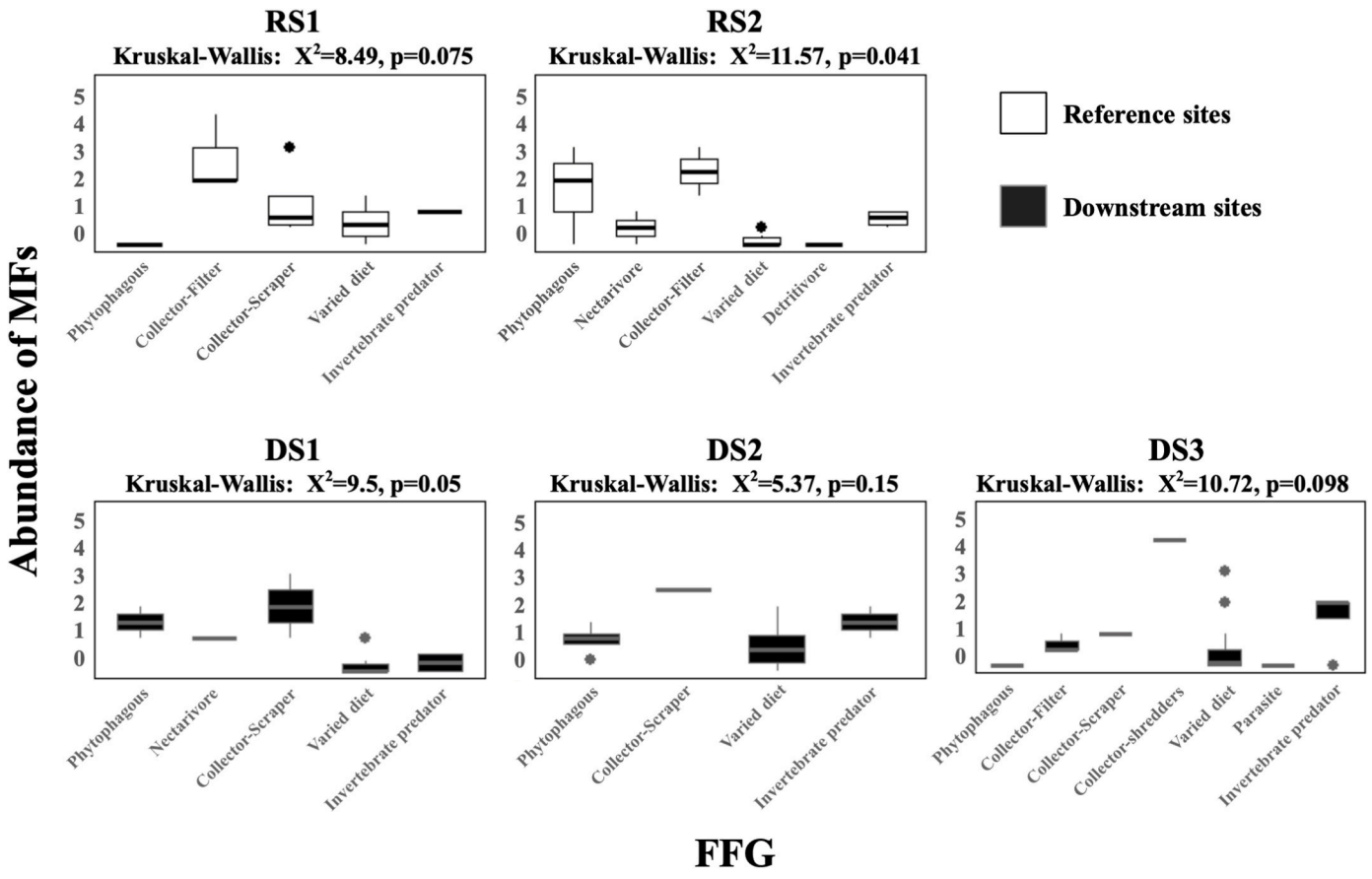


Fig. 5. Trophic transfer trends among macroinvertebrates FFGs.
Description: Kruskal-Wallis box plot. The total abundance of MFs in each Functional Feeding Group (FFG) in macroinvertebrates is shown. Reference sites (RS) are represented by white boxes and downstream sites (DS) by black boxes. The chi-square (χ^2) value and p-value are shown at the top of each plot.

2024). This biological treatment process involves microorganisms to remove non-settleable solids and stabilize organic matter in the wastewater (Tyagi and Lo, 2013). However, this treatment does not remove non-biodegradable compounds such as MPs. As a result, these particles often accumulate in the residual sludge, sometimes unintentionally ending up in nearby agricultural soils (Tyagi and Lo, 2013; Talvitie et al., 2017; Corradini et al., 2019; Freeman et al., 2020), while the remaining MP are transported in the effluent discharged into the Mapocho River. This could explain the relatively low TMF/m^3 values found in the water samples, compared to other studies.

4.2. *Trophic transfer of microfibers in macroinvertebrates*

Contrary to expectations, our results revealed that filter-feeding individuals had a higher average concentration of MFs in both RS and collector individuals in all three DS, but not invertebrate predators, therefore, no evidence of trophic transfer was found. This may be explained by the ease with which filter-feeders capture suspended particles in the water, which in turn can be associated with the season they were collected in. As mentioned above, the southern spring is considered a melting season, as rising temperatures cause melting of, in this case, the Andes mountain range. This results in an increase in water flow and resuspension of plastic particles from the sediment (Moses et al., 2023), making them available for filter-feeding organisms at sites located upstream or near the mountain range like RS1 and RS2. However, this should be addressed in subsequent studies of seasonal variation. Studies by Winkler et al. (2022) and Drummond et al. (2025) showed that filter-feeding individuals such as hydropsychid larvae (Trichoptera) or culicid larvae (Diptera) had the highest MP load, which aligns with the results of this work in the RS, since these particles can be collected directly from the environment by being confused with their natural prey or ingested indirectly through feeding (Winkler et al., 2022; Bertoli et al., 2025).

Several studies indicate that MP ingestion and/or elimination in macroinvertebrates is strongly influenced by a combination of species-specific biological and morphological traits (e.g., life stage, body size, mandible adaptations), FFG, and abiotic factors (e.g., flow conditions) (Windsor et al., 2019; Bertoli et al., 2022; Parker et al., 2022; Haque et al., 2025). Therefore, there is a discrepancy in literature as to which groups would tend to have the highest concentrations of MPs. Khedre et al. (2023) found that the highest MP load per gram of wet weight of macroinvertebrates was recorded in collector-gatherers such as beetles and chironomids species. According to García et al. (2021), macroinvertebrates at higher trophic levels may accumulate higher MPs loads. On the contrary, other studies such as Parker et al. (2022) reported higher MP occurrence in lower trophic level groups such as collector and scraper organisms (e.g., mayfly larvae). This is consistent with the results found for the three DS (sites far from the mountain range and downstream of WWTP), where the collector-scraper or collector-shredder individuals had the highest intake of MFs. The fact that collectors have a higher MP load is probably due to organic matter accumulation influenced by abiotic conditions, encounter rates with MP, or particle similarity to food items, particularly blue MF (the most frequently found color in this study too), which may be mistaken for blue-green algae (Nel et al., 2018; Bertoli et al., 2022). Additionally, benthic taxa are generally more likely to ingest MP compared to insects in the upper water column, regardless of FFG (Besseling et al., 2017; Windsor et al., 2019), although this depends on the sedimentation rate

of particles, which in turn is influenced by external factors like water flow.

In summary, the variability in results can be attributed to the heterogeneity of rivers, which form complex food webs and result in diverse ingestion scenarios. Thus, MF abundance is likely influenced by the characteristics of macroinvertebrate groups, external sources (e.g., WWTPs), prevailing environmental conditions (e.g., precipitation), and the physical characteristics of the MPs themselves (e.g., density). This may help explain the lack of significant results in this study and support the need to consider more explanatory variables, including the ultimate identification of fibers, which presents higher complexity in real environmental samples (Tian et al., 2022).

4.3. Trophic transfer of microfibers between biotic matrices

While no evidence of trophic transfer was found among macroinvertebrates (when discriminating between FFG), evidence of trophic transfer of MF from the entire macroinvertebrate group towards catfish was found at all sites. The higher abundance of MFs in catfish could be explained by their higher trophic position (compared to macroinvertebrates) (McNeish et al., 2018; García et al., 2021), which implies the transfer of MPs in the trophic web through the consumption of prey that have previously ingested the contaminant, as well as the consumption of many more prey (which increases the MP load). This could also be attributed to the color of the MFs, considering fish tend to consume blue and black fibers (which aligns with our results and foraging habitat of *T. areolatus*) (Enamul-Kabir et al., 2025; Ševove et al., 2025; Michon et al., 2025), since a large proportion of MPs end up deposited in the benthos, causing accidental ingestion (e.g., swimming with their mouths open) and direct consumption when confused with macroinvertebrates due to their color (McGoran et al., 2017; Parvin et al., 2021; Parker et al., 2022).

MF abundance was higher in macroinvertebrates and fish at RS1 and DS2, respectively. This could be explained by the proximity (or lack thereof) to the mountain range and, therefore, its influence on the resuspension rate of MPs. The TTI in RS2, DS1, and DS2 showed that the stomach contents of the fish contained up to six times more MF than the macroinvertebrates, meaning that this process was amplified, at least in two out of three downstream sites. Therefore, dietary exposure and trophic transfer patterns are site-specific, with different conditions and factors that affect MF availability (Windsor et al., 2019). This reinforces what was discussed in the previous point: the need to include more factors and long-term monitoring and not just consider the influence (or lack thereof) of the WWTPs.

4.4. Microfibers ingestion in Chilean pencil catfish

Some studies have reported a correlation between body size and MP load in fish, suggesting that larger fish consume more prey and higher diversity of prey, and are therefore more likely to ingest MP (Horton et al., 2018; Nath et al., 2024), a trend well documented in marine fish. However, others argue that this relationship is not necessarily the main factor influencing MP loads in freshwater fish. In the Chilean pencil catfish, although our results did not show a statistically significant relationship between body size and MF ingestion—and thus provided no firm evidence of bioaccumulation—individuals from DS2 and DS3 did show a trend of increased MF ingestion with larger size, particularly for black and blue fibers. In contrast, in the RS1, RS2, and DS1, larger individuals tended to have lower MF gut content. While DS1 receives indirect WWTP input (*Los Trapenses* WWTP), the fish were captured at the confluence with the Mapocho River, which may have diluted MF concentrations and influenced the absence of a size-abundance pattern. In this aspect, Pazos et al. (2017), who found mainly MFs in the intestinal contents of all sampled fish from the Río de la Plata estuary, showed a relationship only between the MP count per individual and the proximity to effluent discharges, not size, weight, or feeding habits. Reinforcing the above, Parker et al. (2022) found no evidence of bioaccumulation, which may be explained by similar encounter rates (Sun et al., 2021) and high MP elimination rates, possibly due to specific gastrointestinal features. These features have been widely documented (Jabeen et al., 2017; Bosshart et al., 2020; Roch et al., 2021) and suggest that retention rates may be ecologically or physiologically unpredictable (Parker et al., 2022). The measurements in this study were based exclusively on the gastrointestinal contents of the catfish, reflecting recent ingestion and not the particles internalized in the animal's different tissues. Some studies have shown that hydrodynamic conditions such as water velocity, turbulence, and current direction, as well as physicochemical parameters such as water salinity, influence the environmental fate of MP within a watershed (Yuan et al., 2022; Mendrik et al., 2023), and therefore, influence MP bioaccumulation in different fish tissues, as well as the associated toxicological responses (Rasta et al., 2025; Rasta et al., 2026). Therefore, the lack of relationship between MP in stomach contents and the size of individuals does not necessarily indicate a lack of exposure, as MP may accumulate in other organs such as the brain, liver, or gills (Ding et al., 2018), and there may be a correlation between the growth and dilution of MPs (Collins et al., 2025) throughout the life of the fish, factors that should be considered in future studies with Chilean pencil catfish to obtain concrete evidence regarding bioaccumulation patterns.

The lack of correlation could result from other factors influencing MP ingestion, similar to those affecting macroinvertebrates. The difference in MP burdens among sites also may be due to the difference in the size of the fish or the number of individuals caught per site, since both depended on the individuals that were able to catch fish in the wild. Altogether, the various influences on the presence of MP in both abiotic and biotic matrices indicate that rivers are highly dynamic systems, subject to multiple factors that influence the presence and bioavailability of MPs (Horton et al., 2018). These influences could be not only watershed-specific, but site-specific within each river stretch as well, meaning that observed values will always depend on local environmental context and sampling limitations. Therefore, comparisons of freshwater faunal MP ingestion across studies must be made with caution, as space-time distribution, species traits, and even sampling and laboratory methods significantly affect the results (Sánchez et al., 2014; Pazos et al., 2017), due to the complexity of identifying true MPs in real environmental samples (Tian et al., 2022).

4.5. WWTPs as a source

To date, MP research has focused primarily on freshwater systems in North America, Europe, and Asia (e.g., Jabeen et al., 2017; Horton et al., 2018; Parker et al., 2022). However, plastic pollution is a global issue that affects all ecosystems, and there is an urgent need to conduct studies in South American rivers, particularly in Chile. To date, only four studies on MPs in the country's rivers have been published in journals (Correa-Araneda et al., 2022; Perez et al., 2024; Nacaratte et al., 2025; Recabarren et al., 2025), the remaining published studies have focused on coastal/oceanic systems, estuarine zones and Patagonian channels/fjords. Moreover, there is currently no national legislation in Chile regulating MP. Countries like Argentina and Canada have implemented short-term measures to reduce MP release from WWTPs, such as banning microbeads in personal care and hygiene products, types of MPs that have been the focus of international regulations (Higuera, 2022; Narciso-Ortiz et al., 2025). Yet, regulating emissions from the textile industry is more complex, as synthetic garments are mass-produced due to their low cost and accessibility, often tied to socioeconomic factors. Reducing the use, washing, and subsequent release of MFs from synthetic clothing may not be a viable solution. Thus, improving WWTP technology, filters, and treatment processes, which aim explicitly at retaining MP alongside standardized protocols for separation and quantification, may be the most effective approach for reducing MP contamination in freshwater systems (Higuera, 2022). This highlights

the importance and urgency of establishing a robust scientific foundation to support effective management and mitigation strategies for MP discharge from WWTPs.

Although this study did not find a clear relationship between MF abundance and proximity to WWTPs, this does not imply that these facilities are not sources of MPs. WWTPs contribute MPs, but their availability downstream of the discharge can be affected by environmental factors and river flow. This highlights the need to not only quantify MPs, but also to understand the factors that affect their long-term movement patterns in rivers with Mediterranean climates of Southern Cone and Chile.

5. Conclusion

The presence of MFs in water, macroinvertebrates, and fish in the Mapocho River basin was evident. The presence and ingestion of these particles is not solely due to the presence of WWTPs, reflecting the complexity of studying this global pollutant, as well as the complex mix of biological and abiotic factors that must be considered when assessing the presence of MFs in environmental matrices. Furthermore, the mere presence of MPs in water, invertebrates, and fish highlights the need for and importance of conducting studies in continental waters within the country, due to the limited information and the difficulty in comparing results with studies from other countries and/or continents. Certain bioaccumulation and/or trophic transfer trends among biotic matrices reinforce the possibility that other sources or attributes may exist and be affected, such as hydrodynamic processes or seasonal changes and their related factors. This demonstrates that exposure, ingestion, and the environmental fate of these synthetic particles may have site-specific or local responses, something that should be considered in further studies.

CRediT authorship contribution statement

Isidora Catalán-Muñoz: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Brian G. Walker:** Resources, Methodology, Funding acquisition. **Andrew White:** Methodology. **Mauricio J. Cárter:** Writing – original draft, Supervision. **Mariana Gajardo-Gamargo:** Writing – original draft, Methodology. **Blanca E. Molina-Burgos:** Writing – original draft, Methodology. **Gustavo Chiang:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2026.128034.

Data availability

Data will be made available on request.

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