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Using historical fish collections to track microplastic contamination in Costa Rican freshwater ecosystems

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ABSTRACT

Introduction: Microplastics are now pervasive in aquatic ecosystems and represent a growing environmental concern. Their persistence and biological uptake threaten aquatic biodiversity and may affect food webs. In Costa Rica, research on microplastics has mainly focused on coastal and marine systems, leaving freshwater environments less explored. Museum specimens offer a unique opportunity to reconstruct historical patterns of microplastic contamination in these inland waters.

Objectives: To examine temporal and spatial variation in microplastic ingestion in *Astyanax aeneus*, an abundant and widely distributed freshwater fish in Costa Rica. To (1) evaluate long-term changes, (2) compare basins with contrasting levels of human disturbance, and (3) test whether body size influences microplastic ingestion rates.

Methods: We analyzed 180 specimens from a national collection spanning seven decades (1960–2025) across three river basins (San Carlos, Tárcoles and Térraba). Gastrointestinal tracts were digested with KOH and examined under stereomicroscopy to identify microplastic particles. Temporal and spatial variations were tested using

ANOVAs, and relationships between fish size and microplastic load were assessed with correlation analyses. All procedures included strict contamination control.
Results: All specimens contained microplastics, mainly fibers. Microplastic loads increased markedly over time in the San Carlos basin and were substantially higher in the heavily impacted Tárcoles basin. No relationship was detected between fish body size and the number of ingested particles.
Conclusions: Freshwater fishes in tropical ecosystems are experiencing increasing microplastic exposure, primarily driven by watershed-level disturbance. The absence of a size-dependent pattern suggests that environmental availability and ecological context play a central role in shaping exposure. Moreover, this study supports the use of museum collections for retrospective monitoring of microplastic pollution and underscores the need for management strategies targeting plastic inputs and wastewater contamination.

Key words: *Astyanax aeneus*; freshwater fish; microplastic ingestion; temporal variation; watershed disturbance.

RESUMEN

Uso de colecciones ictiológicas históricas para evaluar la contaminación por microplásticos en ecosistemas dulceacuícolas de Costa Rica

Introducción: Los microplásticos se han vuelto contaminantes comunes en los ecosistemas acuáticos y representan una creciente amenaza ambiental. Su persistencia y bioacumulación pueden afectar la biodiversidad y las redes tróficas. En Costa Rica, la investigación sobre microplásticos se ha centrado principalmente en ambientes marinos, relegando los sistemas de agua dulce. Las colecciones científicas ofrecen además una oportunidad única para reconstruir patrones históricos de contaminación en estos ecosistemas.
Objetivos: Analizar la variación temporal y espacial en la ingestión de microplásticos en *Astyanax aeneus*, un pez de agua dulce abundante y ampliamente distribuido en Costa Rica. (1) Evaluar los cambios a lo largo de siete décadas, (2) comparar cuencas con distintos niveles de impacto antrópico y (3) examinar la posible relación entre el tamaño corporal y la carga de microplásticos.
Métodos: Se analizaron 180 ejemplares, recolectados entre 1960 y 2025, de tres diferentes cuencas hidrográficas: San Carlos, Tárcoles y Térraba. Los tractos gastrointestinales fueron digeridos con KOH y examinados bajo el estereomicroscopio para la identificación de microplásticos. Las variaciones temporales y espaciales se evaluaron mediante ANOVAs y las correlaciones entre tamaño y partículas ingeridas se determinaron con pruebas paramétricas y no paramétricas. Todos los procedimientos incluyeron estrictos controles de contaminación.
Resultados: Todos los especímenes examinados presentaron microplásticos, principalmente fibras. Las cargas de microplásticos aumentaron de manera marcada a lo largo del tiempo en la cuenca de San Carlos y fueron sustancialmente mayores en la cuenca del Tárcoles, una cuenca fuertemente impactada. No se detectó una relación entre el tamaño corporal de los peces y la cantidad de partículas ingeridas.
Conclusiones: Los peces de agua dulce en ecosistemas tropicales están experimentando una exposición creciente a microplásticos, impulsada principalmente por perturbaciones a nivel de cuenca. La ausencia de un patrón dependiente del tamaño sugiere que la disponibilidad ambiental y el contexto ecológico desempeñan un papel central en la configuración de la exposición. Por otro lado, este estudio respalda el uso de colecciones museográficas para el monitoreo retrospectivo de la contaminación por microplásticos y subraya la necesidad de estrategias de gestión orientadas a reducir los aportes de plásticos y la contaminación por aguas residuales.

Palabras clave: *Astyanax aeneus*; ingestión de microplásticos; peces de agua dulce; perturbación de cuencas; variación temporal.

INTRODUCTION

Plastics have become ubiquitous contaminants in aquatic ecosystems worldwide, with global production and environmental inputs increasing sharply since the mid-20th century (Agbekpornu & Kevudo, 2023; Ahmad et al., 2020; Zuñiga-Umaña et al., 2025). Once released, larger plastic items fragment into microplastics (< 5 mm in length), which are persistent, widely distributed, and bioavailable across different environmental compartments (Agbekpornu & Kevudo, 2023; Hermsen et al., 2018; Ilechukwu et al., 2023). Their physical persistence and capacity to absorb and concentrate other chemical pollutants make microplastics a particular concern for both ecosystem integrity and human uses of aquatic resources (Agbekpornu & Kevudo, 2023; Sharma & Chatterjee, 2017).

The ingestion of microplastics by fishes is now relatively well documented in both marine and freshwater ecosystems (Agbekpornu & Kevudo, 2023; Bermúdez-Guzmán et al., 2020; Sequeira et al., 2020; Zazouli et al., 2022; Zuñiga-Umaña et al., 2025), and mounting evidence indicates a range of potential biological effects, from physical injury and reduced feeding efficiency (Bermúdez-Guzmán et al., 2020; Sharma & Chatterjee, 2017; Zazouli et al., 2022) to sublethal physiological impacts and altered energy budgets (Agbekpornu & Kevudo, 2023; Sequeira et al., 2020; Zuñiga-Umaña et al., 2025). Since fish occupy central positions in many aquatic food webs and are important sources of animal protein for human populations, microplastic contamination carries potential implications for trophic transfer and food safety (Agbekpornu & Kevudo, 2023; Bermúdez-Guzmán et al., 2020; Sequeira et al., 2020). These dual ecological and public-health dimensions make quantitative assessments of plastic ingestion in wild fish essential for understanding both environmental change and human exposure pathways (Agbekpornu & Kevudo, 2023; Bermúdez-Guzmán et al., 2020; Sequeira et al., 2020; Zazouli et al., 2022).

Longitudinal assessments of contaminant trends in aquatic systems are often constrained by the lack of historical baseline data (Hou et al., 2021; Ilechukwu et al., 2023; Modica et al., 2020). In this sense, natural history and ichthyological collections provide an under-utilized resource for retrospective studies (Boisen et al., 2024; Hou et al., 2021; Ilechukwu et al., 2023; Modica et al., 2020); e.g., preserved specimens (stomach content archives, ethanol- or formalin-fixed whole specimens, an/or frozen tissues) permit the detection and characterization of microplastics across decades, enabling temporal reconstructions that complement synchronic field surveys (Hou et al., 2021; Ilechukwu et al., 2023). In fact, recent syntheses (e.g., Ilechukwu et al., 2023; Zazouli et al., 2022) highlight both the feasibility and value of museum-based approaches to reconstruct the "Plasticene" and to establish historical baselines for microplastic pollution (Hou et al., 2021; Ilechukwu et al., 2023).

In Costa Rica, as in most countries around the world, research on plastic pollution has grown in recent years, reporting microplastics in coastal and aquatic sediments (Monzó-López, 2022; Zuñiga-Umaña et al., 2025), sand beaches (Loureiro-Madureira et al., 2025; Sagot-Valverde, 2022), bivalves (Rojas-Jiménez et al., 2022) and crustaceans (Astorga-Pérez et al., 2022; Guillén-Watson et al., 2023) across both Pacific and Caribbean coasts. Most local (and regional) studies have focused on water, sediment or invertebrate matrices (Astorga-Pérez et al., 2022; Guillén-Watson et al., 2023; Zuñiga-Umaña et al., 2025), while fishes in general, freshwater fishes in particular, and especially historical specimens from scientific collections, remain proportionally understudied (Astorga et al., 2022; Bermúdez-Guzmán et al., 2020; Monzó-López, 2022; Sagot-Valverde, 2022; Zuñiga-Umaña et al., 2025). This last point, despite the existence of well-curated ichthyological holdings in national collections (Angulo et al., 2016). Leveraging collection material therefore offers a strategic route to investigate long-term trends and basin-scale differences in contaminant load, relevant to both biodiversity conservation and human uses of aquatic resources (Agbekpornu & Kevudo, 2023; Hou et al., 2021; Zuñiga-Umaña et al., 2025).

Here we use specimens of *Astyanax aeneus* (Günther, 1860), sensu Bussing (1998), a relatively abundant and widely distributed freshwater tetra fish in Costa Rica, as a model to examine long-term and spatial patterns of microplastic occurrence in stomach contents from museum collections. Specifically, we (1) quantify and characterize microplastic presence in *A. aeneus* specimens from the San Carlos basin over six decades (1965-2025) to test for temporal increases consistent with regional demographic and plastic-use trends, (2) compare microplastic burdens in specimens collected from three basins with contrasting anthropogenic pressure [i.e., Tárcoles, heavily anthropized, vs. San Carlos and Térraba, historically less impacted (Bussing, 1998; Pérez-Gómez et al., 2021; Portuguez et al., 2003; Schröter et al., 2023; Umaña-Villalobos & Springer, 2006)] to test whether basin-scale differences in human disturbance are reflected in contaminant loads, and (3) evaluate the relationship between body size and microplastic ingestion to explore whether individual morphological traits influence ingestion rates. We hypothesize that (1) microplastic ingestion has increased through time; (2) fish from more anthropogenically impacted basins exhibit higher microplastic incidence and abundance than contemporaneous specimens from historically less impacted basins; and (3) larger individuals ingest a greater number of microplastic particles.

MATERIALS AND METHODS

We analyzed preserved material of *A. aeneus* (Characiformes: Acestrorhamphidae; Fig. 1) deposited in the Ichthyological Collection of the Museo de Zoología of the Universidad de Costa Rica (UCR) (Angulo et al., 2016). The dataset comprised 140 specimens from the San Carlos basin (SC) (Fig. 2) sampled at a rate of 20 per decade, between 1962 and 2025, allowing a reconstruction of temporal trends for the last six decades. To assess spatial variation under contrasting levels of human disturbance, we examined 40 additional specimens collected between 1966 and 1968, with 20 originating from the Tárcoles basin (Ta) (Fig. 2) and 20 from the Térraba basin (Te) (Fig. 2). Species and basin selection were based on the availability of sufficient museum specimens for consistent

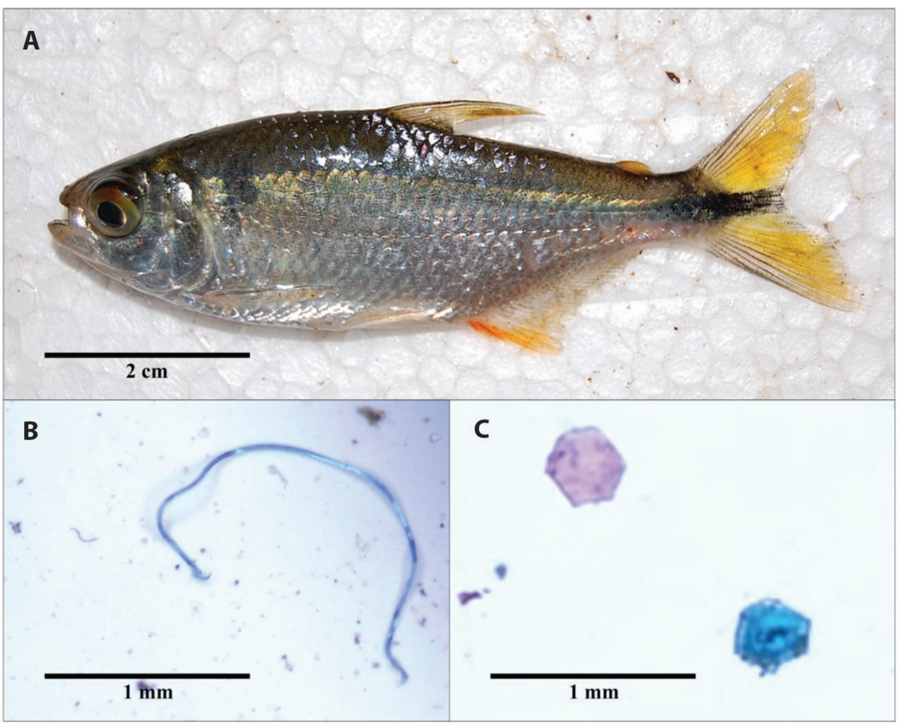

Fig. 1. Model species and microplastic particle types identified in this study. **A.** Live specimen of *Astyanax aeneus*. **B.** Fibers. **C.** Fragments. Photographs in B and C illustrate the morphological characteristics used for particle classification under stereomicroscopy.

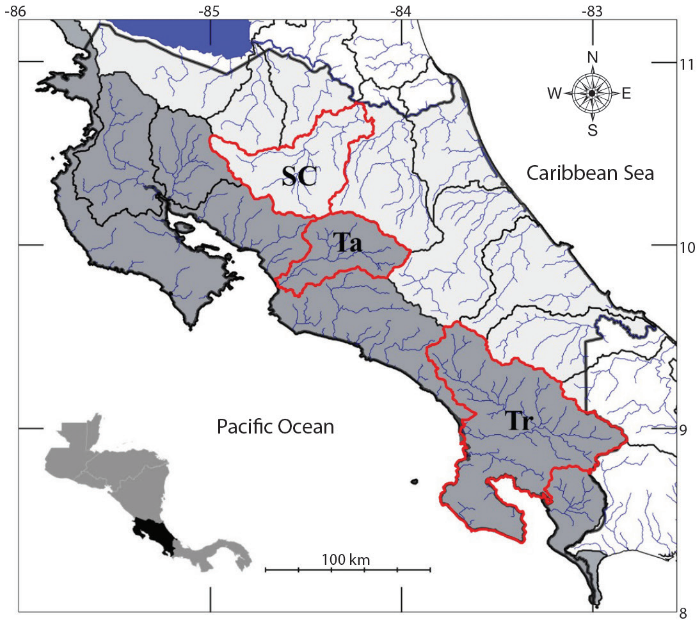

Fig. 2. Geographic location of the study area in Costa Rica showing the main hydrographic basins, sensu Angulo (2021). The San Carlos (SC), Tárcoles (Ta), and Térraba (Te) basins are highlighted in red. The map also depicts the national drainage network and major watershed boundaries, providing spatial context for the sampled basins within the Pacific and Caribbean slopes.

temporal and spatial assessment. Basin boundaries and geographic assignments of specimens were defined according to the hydrographic framework followed by Angulo (2021).

The three basins included in this study (Fig. 2) represent contrasting environmental and socio-economic contexts within Costa Rica, although it is important to note that the spatial comparison is based on specimens collected during the 1960s (1962–1968). During this period, the Ta basin, situated in the central Pacific region, had begun to experience increasing urbanization and industrial development associated with the growth of the Greater Metropolitan Area, although at lower intensities than observed today (Pérez-Gómez et al., 2021; Schröter et al., 2023). The SC basin, located in the Northern region of the country, was already characterized by expanding agricultural activities, particularly cattle ranching and crop production, reflecting moderate levels of human disturbance (Rodríguez-Barrientos, 2007). In contrast, the Te basin, located in the Southern Pacific region, remained comparatively less developed, with extensive forest cover, low population density, and limited infrastructure (Portuguez et al., 2003; Umaña-Villalobos & Springer, 2006). While all three basins have undergone substantial environmental and socio-economic changes since that time, these historical conditions provide a relevant baseline for evaluating spatial differences in microplastic ingestion under differing levels of anthropogenic influence.

All specimens were originally fixed in 10 % formalin and subsequently preserved in 70 % ethanol. Standard length (mm) and body mass (g) were recorded prior to dissection. Gastrointestinal tracts, from the esophagus to the anus, were removed, following Hou et al. (2021), under sterile laboratory conditions. Then they were digested in a 10 % KOH solution at a ratio of at least three parts solution to one part of biological material and left at room temperature

(21-23 °C) for 10-14 days until organic matter was fully dissolved. All dissections and processing were carried out under strict contamination control, following Lasdin et al. (2023) and Boisen et al. (2024), including: (1) the use of cotton lab coats and nitrile gloves; (2) the use of new utensils (i.e., scalpel blades, vials, and plastic Petri dishes) that were freshly unsealed from their original packaging; (3) the systematic cleaning of working surfaces and utensils with 70 % ethanol; and (4) the filtration of the KOH solution and cleaning ethanol through a 1.0 µm filter before use. Plastic equipment was avoided whenever possible, and glass or metal tools were used instead, except when plastic materials were essential for sample containment.

After digestion, the residual material was visually examined, following Rivas-Mena et al. (2024), in new plastic Petri dishes under an Olympus SZX16 stereomicroscope equipped with a DP22 digital camera. Microplastic particles were identified based on the presence of unambiguous synthetic characteristics, and all identified particles were classified according to type (fibers, fragments, or pellets) following Lusher et al. (2020) and Rivas-Mena et al. (2024). Although particle attributes such as color, shape, and surface texture were considered during identification, detailed records of these characteristics (e.g., color) were not consistently documented for all particles and were only systematically recorded for a subset of samples. Representative particles from this subset were photographed and archived for reference (Fig. 1).

To assess background contamination, procedural blanks were collected after/during (1) the filtration of both KOH and cleaning ethanol solutions; (2) the cleaning and disinfection of laboratory materials; (3) the dissection of specimens; and (4) the digestion of gastrointestinal tracts (Boisen et al., 2024). A total of ten blanks were prepared, handled, and analyzed following the same procedures as the experimental samples. After "digestion", blank KOH solutions were transferred to new Petri dishes and examined under a stereomicroscope to establish baseline levels of environmental and procedural contamination. The number of microplastic particles detected in the blanks was averaged to determine the background contamination level, which was subsequently used as a reference for data correction.

For each specimen, the number of ingested microplastic particles was recorded together with body length (i.e., standard length (SL) in mm). Measurements of body length were taken using a Mitutoyo digital caliper (0-150 mm range), which has a manufacturer-specified measurement uncertainty of ± 0.02 mm (k = 2) for this range. In the results section, mean values of both variables are reported by basin and/or decade, accompanied by their corresponding standard deviations (mean ± SD).

Temporal variation within the SC basin was analyzed using a one-way Analysis of Variance (ANOVA) (Sokal & Rohlf, 2012) to test for differences in microplastic abundance among decades. Spatial variation between contemporaneous (1966-1968) samples of the SC, Te and Ta basins was evaluated using another one-way ANOVA to assess differences in microplastic loads between basins that differ in the degree of anthropogenic impact. Boxplots were constructed to visualize the distribution of microplastic counts per individual across decades and basins. Statistical analyses were conducted in PAST version 4.13 (Hammer et al., 2001), with statistical significance set at $\alpha = 0.05$.

Finally, relationships between body size and microplastic ingestion were examined using correlation analyses that included all specimens ($n = 180$). Normality of quantitative variables (i.e., body size and microplastic counts) was tested using the Shapiro-Wilk (Shapiro & Wilk, 1965) and Anderson-Darling tests (Anderson & Darling, 1952). Both variables significantly deviated from normality ($p < 0.05$ for all tests). Consequently, non-parametric Spearman's rank correlation (r_s) was used; while Pearson's correlation coefficient (r) was calculated for comparative purposes (Hauke & Kossowski, 2011). Normality tests and correlation analyses were conducted in PAST version 4.13 (Hammer et al., 2001), with statistical significance set at $\alpha = 0.05$.

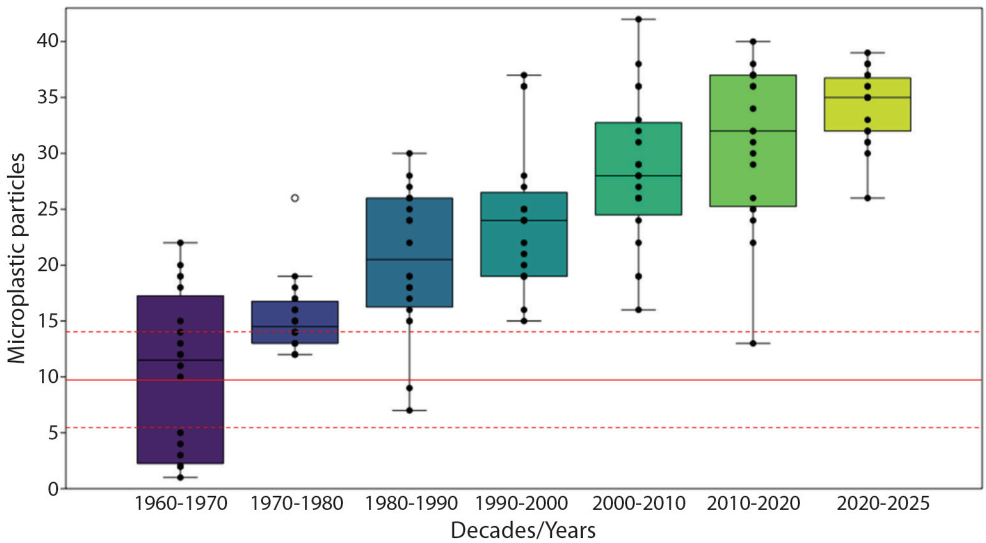

Fig. 3. Temporal variation in the number of ingested microplastic particles in specimens of *A. aeneus* from the San Carlos River basin between 1960 and 2025. Boxplots show decade-wise distributions, including means, interquartile ranges, and outliers (unfilled circles), revealing a significant long-term increase followed by stabilization in recent decades. Red solid and dashed lines indicate the mean (9.7 particles) and standard deviation range (± 4.2) derived from blank controls, respectively.

RESULTS

Microplastic particles were detected in all analyzed specimens (n = 180, SL = 52.90 ± 9.37 mm). A total of 3 804 particles were identified, the vast majority (99.4 %) were classified as fibers, while only 21 fragments were recorded (Fig. 1). Within a subsample of fibers (n = 692), color distribution was dominated by blue (52.0 %), followed by black (27.3 %) and white or transparent particles (11.0 %), whereas red and yellow accounted for 6.5 % and other colors comprised 3.2 % of the total. Microplastic loads varied considerably among individuals, decades, and river basins. The number of particles per specimen ranged from 1 to 42 in the SC basin (23.4 ± 9.8, n = 140), from 8 to 36 in the Ta basin (17.7 ± 7.3, n = 20), and from 3 to 21 in the Te basin (8.7 ± 4.7, n = 20). Notably, fragments were detected exclusively in specimens collected after 2010.

Blank controls yielded an average of 9.7 ± 4.2 particles per sample (n = 10), indicating a substantial background contribution likely associated with airborne or external contamination. This baseline represented approximately 45 % of the average particle load per individual and would be considered when interpreting total loads.

A marked temporal increase in microplastic ingestion was observed in the San Carlos basin (Fig. 3). Specimens collected between 1960 and 1980 consistently contained fewer particles than those collected after 2000, with mean loads increasing from 22.5 ± 7.6 to 38.2 ± 6.2 particles per individual. However, no clear differences were detected among the three most recent periods (2000-2010, 2010-2020, and 2020-2025; Table 1), suggesting a possible stabilization of microplastic loads in recent decades. These temporal differences were statistically significant (Welch ANOVA: F = 71.44, df = 58.18, $p = 5.31 \times 10^{-25}$ (Welch, 1947), with decade explaining a substantial proportion of the observed variance (ω^2 = 0.64) (Kroes & Finley, 2023).

Spatial comparisons also revealed differences among river basins. Individuals from the Te and SC basins contained fewer microplastic

Table 1
Results of the Tukey post hoc pairwise comparisons (Tukey, 1949) testing for differences in the number of ingested microplastic particles in specimens of *A. aeneus* among decades in the San Carlos River basin

Decades	1960-1970	1980-1990	1970-1980	1990-2000	2000-2010	2010-2020	2020-2025
1960-1970		3.84×10^{-3}	0.12	3.21×10^{-7}	0	0	0
1970-1980	3.76	0.07		0	2.02×10^{-6}	7.18×10^{-10}	0
1980-1990	7.78		4.02	0.50	0	2.06×10^{-3}	4.93×10^{-7}
1990-2000	10.44	2.66	6.68		0.25	0	5.55×10^{-3}
2000-2010	13.70	5.92	9.95	3.26		0.77	0.04
2010-2020	15.75	7.97	11.99	5.31	2.05		0.64
2020-2025	18.11	10.32	14.35	7.67	4.40	2.35	

Values represent mean differences (below) and p-values (above), with significant differences ($p < 0.05$) in gray, indicating temporal changes in microplastic ingestion levels.

particles than those from the Ta basin, whereas no apparent differences were observed between Te and SC (Fig. 4). Basin-related differences were significant (Welch ANOVA: $F = 10.80$, $df = 36.20$, $p = 2.09 \times 10^{-4}$), and basin identity explained a relatively large proportion of the variance ($\omega^2 = 0.25$). These results are consistent with the contrasting levels of anthropogenic pressure characterizing the studied watersheds.

Finally, no meaningful relationship was detected between body size and the number of ingested microplastics, regardless of decade or basin. Correlation analyses indicated a negligible association between both variables, with coefficients close to zero for both non-parametric and parametric tests (Spearman's rs = -0.05, p= 0.71; Pearson's $r = 4.28 \times 10^{-3}$, p = 0.97). These results indicate that microplastic

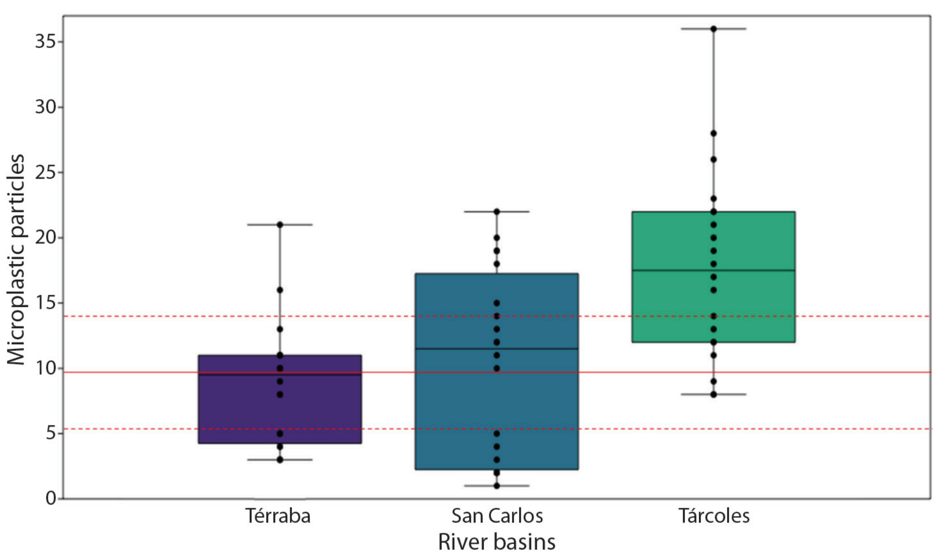

Fig. 4. Comparison of microplastic ingestion between the Te, SC and Ta basins. Boxplots represent basin-wise distributions of the number of ingested microplastic particles in specimens of *A. aeneus*, including means and interquartile ranges, showing higher loads in specimens from the more anthropogenically impacted Ta basin. Red solid and dashed lines indicate the mean (9.7 particles) and standard deviation range (± 4.2) derived from blank controls, respectively.

ingestion does not vary systematically with body size in the analyzed specimens.

DISCUSSION

The results presented here underscore the value of museum-held specimens to reconstruct long-term and basin-scale patterns of microplastic ingestion in tropical freshwater ecosystems. We documented persistent ingestion of microplastics in *A. aeneus* sensu lato (Bussing, 1998), an abundant Neotropical tetra fish, which makes it an efficient species for monitoring contamination in low- and mid-elevation water bodies. This species is widely distributed in Costa Rica and Central America, occupies a broad range of habitats including small headwater streams, large rivers and lakes (Bussing, 1998), and has been employed, as other congeneric species, in ecotoxicological studies (Hinojosa-Garro et al., 2020; Zebral et al., 2024). Its ecological traits, including an omnivorous diet, benthic and limnetic foraging, and tolerance to a wide range of environmental conditions (Bussing, 1998; Small et al., 2011), support its use as a model for contaminant exposure across several geographical, physical and/or ecological gradients.

The predominance of fibers observed in this study is consistent with previous reports in freshwater systems, where microfibers are frequently identified as the dominant form of microplastic ingestion (Conowall et al., 2024; Hou et al., 2021; Munno et al., 2022). The predominance of blue and black fibers in the analyzed subsample also is consistent with patterns reported in freshwater fish from diverse geographic regions, where these colors are frequently dominant (Conowall et al., 2024; Hou et al., 2021; Munno et al., 2022). This pattern is commonly attributed to the widespread release of synthetic textile fibers, particularly from domestic wastewater, which are often blue or dark-colored due to common dyeing processes. Although experimental studies have suggested that fish may exhibit color-based selectivity when ingesting particles (Okamoto et al., 2022), field observations generally reflect environmental availability rather than active preference (Azizi et al., 2021; Conowall et al., 2024; Munno et al., 2022). It should be noted, however, that color was only systematically recorded for a subset of fibers in this study, which limits the extent to which color-related ingestion patterns can be evaluated. Nonetheless, the observed distribution aligns with broader evidence indicating that textile-derived microfibers are a major component of microplastic pollution in freshwater ecosystems (Azizi et al., 2021; Hou et al., 2021).

The strong temporal signal found in microplastic loads in the SC basin is consistent with our first hypothesis and mirrors socio-environmental changes in Costa Rica over the past six decades. National data show that Costa Rica's population grew from approximately 2.9 million in 1960 to over 5 million by 2000, along with the expansion of metropolitan areas and increased plastic production and waste generation (Holl et al, 1995; Schröter et al., 2023; Vadera & Khan, 2021; Zuñiga-Umaña et al., 2025). The relative stabilization in particle loads in recent years may suggest, as discussed by previous authors (e.g., Bermúdez-Guzmán et al., 2020; Hou et al., 2021; Sequeira et al., 2020; Zazouli et al., 2022), either a saturation of ingestion pathways, an equilibrium between exposure and egestion, or early signs of behavioural shifts reducing incremental loads. These interpretations point to the need for further temporal extensions and fine-scale analyses of exposure mechanisms.

In the spatial contrast between basins, our results validated the second hypothesis. The higher loads observed in the Ta basin align with its strong urban and industrial influence, as this watershed drains the Costa Rican Greater Metropolitan Area, i.e., the most densely populated and economically active region of the country (Pérez-Gómez et al., 2021; Schröter et al., 2023). Conversely, the lower counts of ingested microplastic particles in the Te and SC basins likely reflect its comparatively limited urban development and greater forest cover (Portuguez et al., 2003; Umaña-Villalobos & Springer, 2006), which can mitigate

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contaminant inputs through natural filtration and sediment retention. Previous studies have linked urban-industrial watershed disturbance with elevated microplastic ingestion in freshwater fishes (Bermúdez-Guzmán et al., 2020; Hou et al., 2021; Munno et al., 2022; Sequeira et al., 2020; Zazouli et al., 2022). Our findings thus reinforce that basin-scale anthropogenic pressure is a reliable predictor of fish microplastic loads in tropical systems. Moreover, the consistent detection of microplastics across all examined specimens of *A. aeneus* highlights the pervasive nature of this pollutant in Costa Rican freshwater systems, even in areas with lower anthropogenic pressures.

Contrary to our third hypothesis, no meaningful relationship was detected between body size and microplastic ingestion, indicating that microplastic loads in *A. aeneus* are largely independent of organismal size. This pattern is consistent with other freshwater studies showing that size-microplastic relationships are weak, variable and/or strongly context-dependent (Conowall et al., 2024; Sequeira et al., 2020; Zazouli et al., 2022). In such systems, microplastic exposure is likely driven more by environmental availability and foraging context than by body size per se, with widespread contamination across habitats and trophic pathways potentially obscuring ontogenetic effects related to diet or habitat use (Sequeira et al., 2020; Small et al., 2011; Zazouli et al., 2022). Overall, these results suggest that body size alone is a poor predictor of microplastic ingestion in this species, highlighting the need to integrate trophic ecology, habitat use, and local contamination patterns when interpreting microplastic exposure in freshwater fishes (Conowall et al., 2024).

From a management and monitoring perspective, our results carry several implications. First, the temporal increase of microplastic ingestion in a widespread freshwater fish signals the need for regular contaminant surveillance in tropical continental waters, beyond the predominantly marine focus (Hou et al., 2021; Ilechukwu et al., 2023; Sequeira et al., 2020; Zazouli et al., 2022). Second, the large basin effect implies that watershed-scale management of plastic waste and wastewater inputs is critical (Conowall et al., 2024; Munno et al., 2022). In the Ta basin, upstream interventions such as improved wastewater treatment and watershed-scale management of solid waste and runoff inputs may help reduce the overall load of plastics entering the system, thereby contributing to lower microplastic exposure. These measures are most effective when combined with strategies that target diffuse sources and pathways, which are key drivers of microplastic contamination (Conowall et al., 2024; Munno et al., 2022). Finally, museum-based approaches as applied here should be integrated into monitoring protocols to provide historical baselines and to detect long-term change, a point especially relevant for tropical regions lacking long-term field data (Hou et al., 2021; Ilechukwu et al., 2023).

In conclusion, our study (1) demonstrates the utility of *A. aeneus* as a sentinel species for microplastic research in Costa Rican and Neotropical freshwater ecosystems, and (2) reveals pronounced temporal and spatial patterns in microplastic ingestion. In contrast, microplastic loads were largely independent of body size, indicating that organismal size alone is a poor predictor of exposure in this species. Together, these findings highlight that freshwater biota in tropical systems are not exempt from the global microplastic challenge and suggest that management and monitoring strategies should prioritize environmental contamination patterns and ecological context, rather than relying solely on biological traits such as body size (Hou et al., 2021; Ilechukwu et al., 2023; Sequeira et al., 2020; Zazouli et al., 2022). Future research should expand basin coverage, refine contamination-correction protocols, investigate dietary pathways of particle ingestion and explore ecosystem-level consequences of fish microplastic loads (e.g., trophic transfer or human exposure via fisheries).

Ethical statement: The authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgments section. A signed document has been filed in the journal archives.

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