

Original Article

Quantifying microplastics in native and farmed fish: Evidence from *Orestias agassii* and *Oncorhynchus mykiss* in Lake Titicaca

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Abstract: *Orestias agassii* (black carachi) and *Oncorhynchus mykiss* (rainbow trout) are key fish species in the diet of local populations surrounding Lake Titicaca. However, recent studies have raised concern about the presence of microplastics (MPs) in this high-altitude aquatic ecosystem. Hence, this study aimed to quantify MPs in the gastrointestinal tracts of black carachi and rainbow trout from the Chucuito and Uros Chulluni areas of Lake Titicaca. The research was conducted between May and July 2024. A total of 100 *O. agassii* and 50 *O. mykiss* individuals were dissected, and gastrointestinal contents were digested using 10% KOH to isolate MPs. The results showed an average of 6.02 MPs/individual in black carachi from Uros Chulluni and 5.34 MPs/individual in Chucuito, providing the first documented evidence of MP ingestion in this native species in Lake Titicaca. In rainbow trout, MP concentrations were significantly higher, with averages of 21.1 MPs in Capachica and 44.6 MPs in Chucuito, and maximum values of 71 and 135 MPs/individual, respectively. A positive relationship was found between MP ingestion and digestive tract morphometry in both native and farmed species.

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Introduction

The presence of microplastics (MPs), defined as particles smaller than 5 mm, has become a global environmental problem (Bollaín and Vicente, 2019). Each year, 6-8 million tons of solid waste enter the oceans, with more than 80% being plastics (Martínez et al., 2023), which eventually fragment into MPs and are found in both Aquatic environments (Iannacone et al., 2021; Ríos, 2022). The drastic increase in plastic waste worldwide is mainly due to domestic and industrial activities (Li et al., 2016). These wastes subsequently enter freshwater aquatic ecosystems (Aker et al., 2024) and marine environments (Andrady, 2011).

The accumulation of MPs has also reached the fishing industry (Venkatesh et al., 2024), where MPs have been detected in 100% of trout feed due to the degradation of plastic packaging, shipping materials, and additives containing MPs, with up to 2200 MPs/kg of feed being recorded (Siddique et al., 2023).

MPs pose a significant threat to aquatic ecosystems, reducing the diversity of flora and fauna (Castro and Rajadel, 2021), including lakes such as Titicaca (Sierralta, 2020), where concentrations of up to 30 MPs/L have been reported. This pollution directly affects the reproduction, health, and endemic biota of species. MPs in aquatic environments, such as lakes (Paredes-Zavala et al., 2019), have been documented to contribute to considerable ecological deterioration. Moreover, their presence in benthic areas facilitates their entry into the gastrointestinal tract of aquatic organisms (O'Connor et al., 2022).

MP ingestion in fish is a widely reported phenomenon. In the first record, 644 MPs were found in more than 45% of individuals from 15 species, with the most common types being fibers (82%), followed by fragments (12%) and films (6%) (Mazariegos et al., 2021). These MPs affect locomotion and metabolism, and cause developmental delays (Hawke et al., 2024), as well as tissue damage that increases enzymatic

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activity (Banaee et al., 2023) and generates potentially toxic effects (Simionov et al., 2023). The highest concentration of MPs has been recorded in freshwater fish, with a probability of ingestion every 300 hours, while in marine fish this probability is ten times higher (Castañeta et al., 2020). Negative effects have also been observed in the gastrointestinal tract and fish metabolism (Alak et al., 2023), generating serious repercussions on aquatic life and the overall health of biomes (Castañeta et al., 2020).

The black carachi (*Orestias agassii*), a native species of Lake Titicaca and important for human consumption (Rojas et al., 2024), is among the species most affected by MPs (Ordoñez and Sanchez, 2018). The presence of MPs in this species is attributed to industrial waste, urban residues, and the fragmentation of larger plastic products (Ochochoque and Ancca, 2016). Rainbow trout (*Oncorhynchus mykiss*), a species introduced to Lake Titicaca (Aguilera et al., 2006), has also been impacted by this pollution. Trout is valued for its high nutritional content in lipids and proteins (Wind et al., 2022; Bao et al., 2023), but exposure to MPs such as high-density polyethylene (HDPE) can cause adverse effects on reproductive activity, reduce immune response, and induce malformations (Sánchez, 2022; Escobar et al., 2020). MPs in rainbow trout have been documented to range from 63 to 106.6 μm (Jakubowska et al., 2020; O'Connor et al., 2020), with 3 to 92 particles per individual being recorded (Alak et al., 2023; Bilal et al., 2023).

Currently, plastic pollution is affecting Lake Titicaca's ecosystems and biodiversity (Godoy et al., 2022; Pastorino et al., 2023), posing an increasing threat to local species and to human food security. Therefore, this study aimed to quantify microplastic abundance in the gastrointestinal tracts of *O. agassii* and *O. mykiss* from the Capachica and Chucuito peninsulas, and the Uros Chulluni area of Lake Titicaca.

Materials and Methods

Study area: The research was conducted in Lake Titicaca, located in the Andean highlands of southern

Peru, at an altitude of 3,812 meters above sea level. The lake has an average depth of 107 meters and a maximum depth of 281 meters, with a cold, semi-arid climate, temperatures ranging from 5 to 15°C, and seasonal rainfall. The areas selected for the study were the Chucuito and Capachica peninsulas, as well as the Uros Chulluni community, chosen for their ecological representativeness and varying levels of anthropogenic influence.

Sampling design and sample collection: A stratified sampling design by zone was applied, with a total sample of 150 fish specimens: 100 individuals of *O. agassii* (50 per zone: Chucuito and Uros Chulluni) and 50 individuals of *O. mykiss* (25 per area: Capachica and Chucuito). Samples were obtained directly from local fishermen during May to July 2024 and transported under controlled conditions to the Ecology Laboratory of the Faculty of Biological Sciences at UNA Puno.

Analysis procedure: The fish were dissected with sterile instruments, and the entire digestive system was extracted. The gastrointestinal contents were subjected to alkaline digestion with 10% KOH for seven days at 26°C, following standardized protocols. Subsequently, filtration was performed using Whatman No. 40 filter paper (retention $\geq 8 \mu\text{m}$) to recover the MPs, which were morphologically identified by stereomicroscopy (SZX16, Olympus Scientific) at 3x magnification.

Evaluated parameters and statistical analysis: MPs were quantified per individual and classified into fibers, fragments, and films. Additionally, morphometric variables of the digestive tract (length and weight) were recorded to explore potential relationships with the number of ingested MPs. Non-parametric statistical tests, i.e., the Wilcoxon test and Spearman's correlation, were applied, with a significance level of $P < 0.05$. Analyses were performed using Infostat software.

Results

MPs in *O. agassii*: The analysis of *O. agassii* specimens revealed the presence of MPs in the gastrointestinal tract across all sampling areas. The

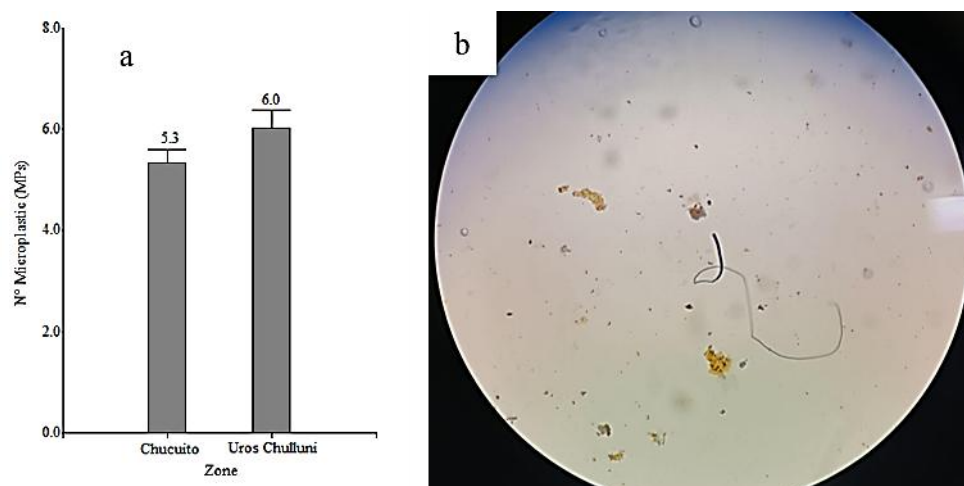


Figure 1. (a) Mean quantity and standard error of microplastics in the gastrointestinal tract of *Orestias agassii* from Lake Titicaca, Peru ($n = 50$; $P=0.009$), and (b) microscopic observation of microplastics retrieved from the gastrointestinal tract of *O. agassii*.

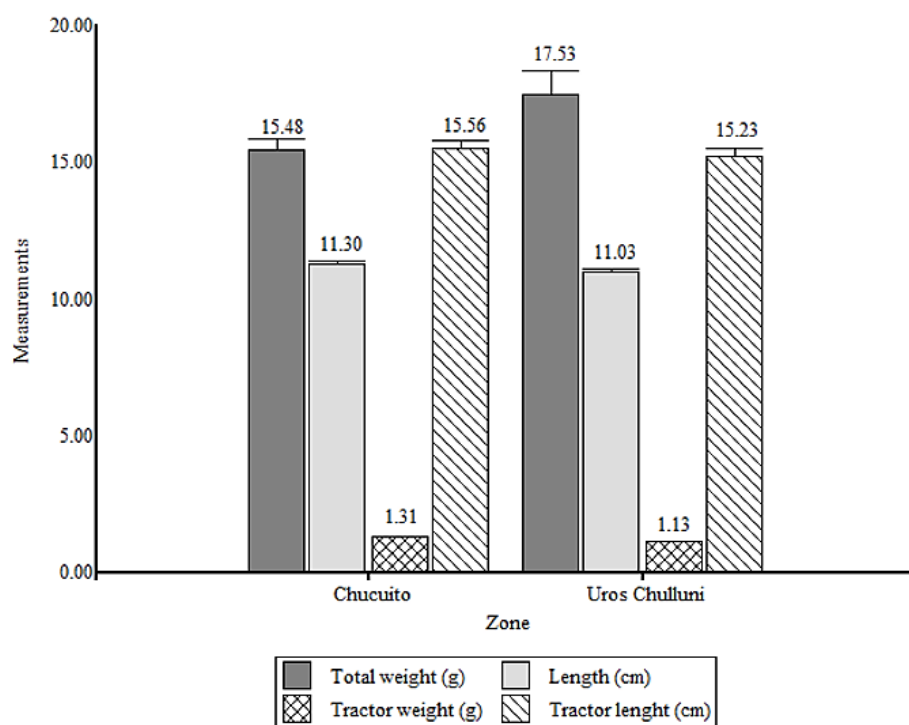


Figure 2. Measurements of weight and length of the digestive tract of *Orestias agassii*.

average number of MPs per individual was lower on the Chucuito peninsula (mean = 5.3 MPs/individual) than in the Uros Chulluni area (mean = 6.0 MPs/individual). Despite this difference, the variation was not significant ($P = 0.27$). In terms of distribution, specimens from the Uros Chulluni exhibited a broader range of MP ingestion, with values ranging from 3 to 14 MPs per individual. Specimens from the Chucuito peninsula showed a narrower range, with 3 to 9 MPs per individual (Fig. 1). The results suggest a slightly

higher exposure to MPs in the Uros Chulluni area, possibly linked to greater anthropogenic pressure and proximity to urban activities. However, the absence of significant differences indicates that both areas are impacted by microplastic contamination, highlighting a pervasive presence of MPs in the lake ecosystem. No significant difference was observed in the number of microplastics (MPs) in *O. agassii* from the Chucuito peninsula and the Uros Chulluni area ($P = 0.27$). However, a higher numerical average was recorded in

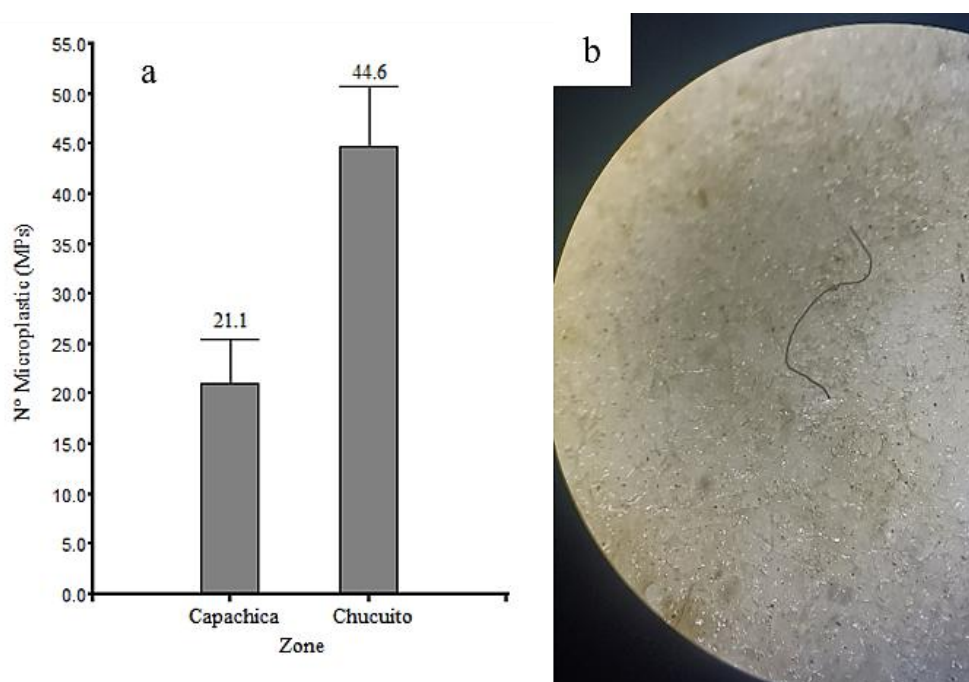


Figure 3. (a) Mean quantity and standard error of microplastics in the gastrointestinal tract of rainbow trout (*Oncorhynchus mykiss*) from Lake Titicaca, Peru ($n = 50$; $P = 0.009$), and (b) microscopic visualization of microplastics extracted from the gastrointestinal tract of *O. mykiss*.

specimens from Uros Chulluni, suggesting a possible trend toward greater microplastic exposure in this zone.

The average length of the digestive tract in *O. agassii* was 15.56 cm in individuals from Chucuito and 15.23 cm in those from Uros Chulluni, showing a slight variation between the two sampling areas. In terms of digestive tract weight, fish from Chucuito exhibited a higher average weight (1.31 g) compared to those from Uros Chulluni (1.13 g). Although these differences were not statistically tested, the observed variations in digestive tract morphometrics could be influenced by environmental factors such as food availability and quality, which play a crucial role in the development and physiological condition of *O. agassii*. Additionally, variations in digestive tract size of *O. agassii* may affect the dynamics of microplastic ingestion and accumulation rates in these native fish populations (Fig. 2).

MPs in *O. mykiss*: In rainbow trout, a notable variation was observed in the quantity of MPs in different sites. The highest number of MPs per individual was recorded in the Chucuito area, reaching 135 MPs/individual, whereas on the Capachica peninsula the maximum was 71 MPs/individual. The results revealed a significant difference between both

areas ($P = 0.0019$), indicating a higher degree of microplastic contamination in rainbow trout from Chucuito. The average number of MPs per individual was 44.6 in specimens from Chucuito, nearly double the average in Capachica (21.1 MPs/individual). The distribution range in Capachica samples varied from 1 to 71 MPs per individual, whereas in Chucuito it ranged from 7 to 135 MPs per individual, suggesting greater variability and accumulation in this area. These findings highlight spatial differences in microplastic exposure, likely associated with differing levels of anthropogenic activity and environmental pollution between the two locations. The variation in standard error also reflects greater dispersion in MP ingestion among individuals from Chucuito, pointing to heterogeneous contamination sources and differential feeding patterns. Such differences may also be influenced by factors including habitat characteristics, trophic behavior, and bioaccumulation dynamics over time (Fig. 3).

The average length of the gastrointestinal tract of *O. mykiss* varied slightly between the two sampling areas. On the Capachica peninsula, the average length was 19.18 cm, while in Chucuito it was 20.88 cm, suggesting possible morphological adaptations

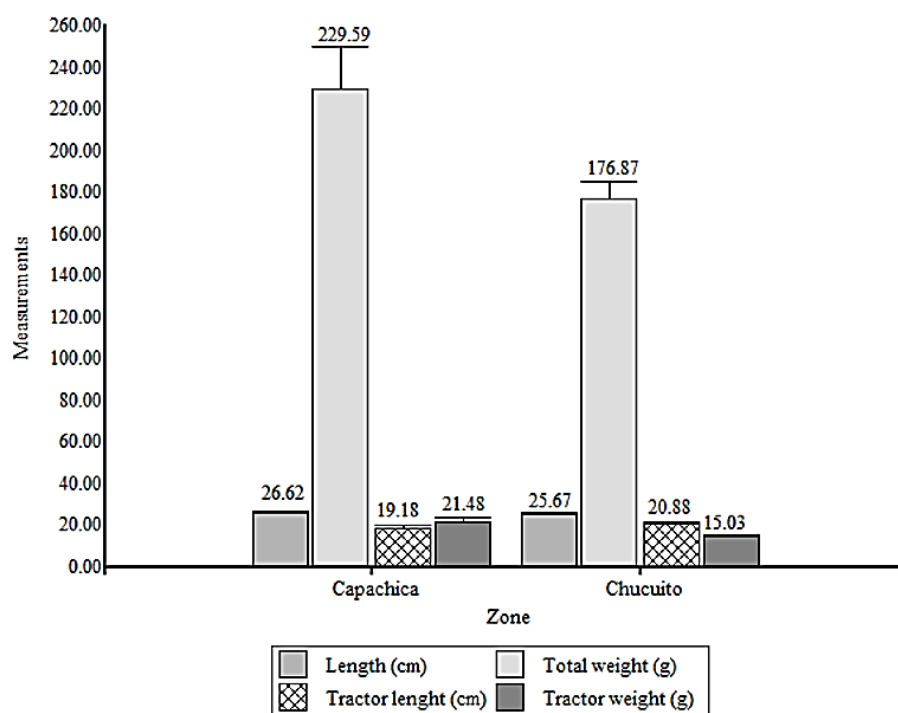


Figure 4. Weight and length of the digestive tract of *Oncorhynchus mykiss*.

influenced by local environmental conditions. In terms of weight, the digestive tract of specimens from Capachica exhibited a higher average value (21.48 g) compared to those from Chucuito (15.03 g). This notable disparity may be associated with differences in food availability, nutritional quality, and habitat characteristics between the two regions, which could directly influence the trout's physiological development and digestive capacity. Environmental factors, such as water quality, resource abundance, and ecological interactions, play a key role in shaping the growth patterns and organ development of fish species. Moreover, anthropogenic pressure appears to differ between the two sites: Capachica is characterized by relatively low human activity and minimal urban impact, whereas Chucuito is subject to higher population density and greater anthropogenic influence. These conditions may alter the ecological balance and the nutritional dynamics of the aquatic environment, potentially affecting the foraging behavior and overall health of *O. mykiss* populations in each area (Fig. 4).

Discussions

MPs are a global environmental concern due to their

physical and chemical impacts on aquatic organisms (Ríos, 2022). According to Morillo (2021), the presence of MPs in Lake Titicaca has been confirmed both in surface water—where concentrations reached an average of $175,916.67 \pm 68,136.00$ MPs/m³—and in the water column, with an average of $22,583.34 \pm 10,220$ MPs/m³, with surface waters showing the highest abundance. Based on their results, MPs were detected in 100% of the specimens analyzed, with transparent fibers being the predominant type. The Uros Chulluni sampling station showed the highest concentration, with 34.82 ± 14.97 MPs per individual. Loayza (2022) reported the presence of MPs in the beach sands of Lake Titicaca, with 76 particles (48%) found in sand samples, followed by sediments (52 units, 33%) and surface waters (30 units, 19%). The highest concentrations were recorded at Chatuma Beach (65 particles, 41%), Chifrón Beach (51 particles, 37%), and Juli Beach (35 particles, 22%), accounting for 100% of the identified MPs. These findings are consistent with our study, which also observed a higher concentration of MPs in *O. agassii* individuals from the Uros Chulluni area. Our findings also corroborate the presence of MPs in the Chucuito-Puno district, with *O. agassii* specimens

averaging 5.3 MPs per individual. Although lower than previously reported levels. Mio and Alvarez (2023) reported significantly higher concentrations in *Orestias* species, with an average of 82.40 ± 79.09 MPs per individual, surpassing the values obtained in this study. This difference may be attributed to localized contamination sources or variations in sampling methodology. In our study, although *O. agassii* showed variable MP ingestion, no significant impact on fish size was observed, whereas rainbow trout, farmed in floating cages in Lake Titicaca and of high commercial value (Aguilera et al., 2006; Bao et al., 2023), their MPs range in size from 63 to 106.6 μm (Jakubowska et al., 2020).

The size of ingested MPs is often related to the fish's body length—smaller individuals tend to ingest smaller particles (Platónov, 2002). In our study, a strong correlation was observed between the number of ingested MPs and digestive tract weight in *O. agassii*, suggesting that MP accumulation may influence organ morphology. Although the trend indicates a relationship, the effect was not significant, implying that other physiological or ecological factors may also contribute. Martínez et al. (2023) highlighted that MPs can have both physical (e.g., digestive blockage) and chemical effects through the transport of pollutants, thereby posing a high risk of infections and toxicological effects. This study also evaluated the relationship between the number of MPs and stomach length in *O. agassii*, finding a moderate positive correlation, suggesting that longer digestive tracts may allow for greater MP ingestion. Escobar et al. (2020) noted that fish size influences the size of ingested MPs, although the specific MP size in our samples was not determined.

Alak et al. (2023) reported that rainbow trout fed contaminated feed contained up to 92 MPs per individual, while only 14 MPs were found in unfed specimens. Bilal (2023) reported up to 3 MPs per trout in Lake Mahodand. In our study, the median MP count was 9 per trout in Capachica and 47 in Chucuito, with the latter likely influenced by proximity to Puno city. Li (2016) attributes this phenomenon to urban and industrial sources (Aguilera et al., 2006; Godoy et al.,

2022; Pastorino et al., 2023). Additionally, 100% of the rainbow trout sampled from both Capachica and Chucuito contained MPs, with red-colored MPs being predominant in their gastrointestinal tracts. Given that the rainbow trout are farmed in floating cages, MP contamination may also originate from their feed. In some cases, 100% of the rainbow trout feed has been shown to contain MPs (Siddique et al., 2023).

MP concentrations in *O. agassii* varied by location, with higher values in Uros Chulluni than in Chucuito. Although no significant difference was found, this is the first report documenting MP ingestion in *O. agassii* in the Chucuito area. A strong positive correlation was found between MP count and fish weight, suggesting possible impacts on growth and health. The MPs identified in our study consisted mainly of fibers, fragments, and films, consistent with patterns reported in other high-Andean aquatic ecosystems, such as Lake Titicaca (Sierralta, 2020). This type of contamination affects reproduction, health, and biodiversity (Paredes-Zavala et al., 2019), particularly in endemic species like *O. agassii*. Overall, the presence of MPs varied across sampling sites, affecting both *O. agassii* and *O. mykiss*. Areas closer to urban centers, such as Chucuito, exhibited higher contamination levels.

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