

Evidence of microplastics in water and commercial fish from a high-altitude mountain lake (Lake Titicaca)

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Plastic pollution has attracted worldwide attention and studies on the occurrence of microplastics (0.1 μm to 5 mm) in aquatic ecosystems are increasing. This study collected water samples during the dry season (July and August 2021) in the Lago Menor sub-basin of the emblematic Lake Titicaca and biological samples of the main commercial fish (May and June 2021). Biological samples consisted of stomach contents of four fishery species (*Orestias luteus*, *Orestias agassizii*, *Trichomycterus dispar*, and *Odonthestes bonariensis*; N=594). Water analysis shows a high abundance of microplastics in water ($52 \pm 38 \text{ L}^{-1}$), while the frequency of occurrence is low in the fish studied ($<5\%$ for each fish species). In both cases, the most frequently found microplastic was fibers. These results suggest that the Lago Menor of Lake Titicaca shows microplastic pollution. However, because of Lake Titicaca's high UV radiation characteristics, the plastics present in the environment could be fragmented even into smaller pieces (nanoplastics or smaller). Further research is required to understand better the source of microplastic pollution and its impacts on the food web of this high Andean ecosystem.

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Abstract

Plastic pollution has attracted worldwide attention and studies on the occurrence of microplastics (0.1 μm to 5 mm) in aquatic ecosystems are increasing. This study collected water samples during the dry season (July and August 2021) in the Lago Menor sub-basin of the emblematic Lake Titicaca and biological samples of the main commercial fish (May and June 2021). Biological samples consisted of stomach contents of four fishery species (*Orestias luteus*, *Orestias agassizii*, *Trichomycterus dispar*, and *Odonthestes bonariensis*; N=594). Water analysis shows a high abundance of microplastics in water ($52 \pm 38 \text{ L}^{-1}$), while the frequency of occurrence is low in the fish studied ($<5\%$ for each fish species). In both cases, the most frequently found microplastic was fibers. These results suggest that the Lago Menor of Lake Titicaca shows microplastic pollution. However, because of Lake Titicaca's high UV radiation characteristics, the plastics present in the environment could be fragmented even into smaller pieces (nanoplastics or smaller). Further research is required to understand better the source of microplastic pollution and its impacts on the food web of this high Andean ecosystem.

Introduction

Plastic pollution has attracted worldwide attention. As plastic breaks into smaller pieces (microplastics: size between 0.1 μm to 5 mm), this pollutant has become a global concern due to its impact on human health and the environment (Pervez et al., 2020). Due to the material properties, microplastics (and indeed plastics of all sizes) hardly decompose and remain for a long time in the ecosystems, so the presence of microplastics in terrestrial and marine environments are well documented (Hidalgo-Ruz et al., 2012; Van Cauwenberghe et al., 2013; Wang & Wang, 2018). The release of plastics into the marine environment comes from the terrestrial environment and occurs through different pathways, including rivers and atmospheric transport (Jambeck et al., 2015; Lebreton et al., 2017). Although about 80% of microplastics in marine environments come from fluvial sources, their study for freshwater systems is still limited (Mani et al., 2016; Blettler et al., 2017; Akdogan & Guven, 2019; Dusaucy et al., 2021).

In aquatic environments, microplastics can harm wildlife by obstructing the gastrointestinal tract and decreasing nutrition when ingested (Derraik, 2002). However, the effects on fish species are at least controversial and depend on different extrinsic (particle size, type of plastic, presence of chemical contaminants) and intrinsic factors (fish species, life stage of fish, feeding behavior) (Rainieri et al., 2018). Ingestion of plastics by fish is not new (Carpenter et al., 1972; Hoss & Settle, 1990). However, incidental ingestion is probably the most common impact associated with plastic debris in different taxa and trophic levels (Laist, 1997; Cole et al., 2011). Incidental ingestion occurs when items are swallowed along with typical food items (Peters & Bratton, 2016) or through trophic transfer when fish consume prey that has ingested plastic debris (Cedervall et al., 2012). One of the relevant factors of plastic ingestion by fish relates to using these organisms as a food resource by humans (Rochman et al., 2015), as plastics and their associated contaminants can accumulate in tissues. Humans are thus potentially exposed to these

contaminants through bioaccumulation and biomagnification in the food web. Certainly, plastics in fish required more research, particularly because most of the available information is for marine species (Silva-Cavalcanti et al., 2017).

In addition, studies highlighted the problem of microplastics in the aquatic environment. Its biota results from inadequate disposal of solid waste and usually comes from cities, wastewater treatment discharge, domestic sewage, which contain clothing fibers and plastic fragments (Alomar, Estarellas & Deudero, 2016; Horton et al., 2017; Blettler et al., 2017). Likewise, another source is the degradation of macroplastics in aquatic environments (fishing nets, discarded plastics debris, among others) through fragmentation by mechanical abrasion and exposure to ultraviolet radiation (UVR) (Andrady, 2011; Pervez et al., 2020). This type of degradation becomes relevant for high-altitude freshwater systems. For example, Lake Titicaca, one of the highest ancient great lakes (3809 m a.s.l.), is located in the high plateaus in the Altiplano. Because of its geographical location and high altitude, Lake Titicaca is subjected to high UVR, that generates a strong surface photo-inhibition of phytoplankton photosynthesis (Lazzaro & Gamarra, 2014). Lake Titicaca, shared between Bolivia and Peru, is the largest lake in South America and is part of the endorheic system, consisting of the Titicaca-Desaguadero-Poopo-Coipasa Salt Lake (Revollo, 2001) and is the most important water resource of the Altiplano. However, the main problem on the Bolivian side of Lake Titicaca (Lago Menor, shallow sub-basin) is the rapid pollution from Lago Menor's human activities, flushed into the Katari River due to poor waste control and poor land management. The main source of anthropogenic pollution reaches the lake in Cohana Bay, through the Katari River, which drains the densely populated area of El Alto, with more than 1.2 million inhabitants, industries, and a poor water waste treatment (Molina et al., 2017).

Lake Titicaca also is the major source of fish for ~3 million people in the region (De Sostoa et al., 2011; Ibañez et al., 2014) and has been an important source of animal protein since pre-Inca times (Lauzanne, 1992; Miller, Capriles & Hastorf, 2010). Despite the value of fish for the subsistence for rural families and commercial fishery networks, their population, particularly of native fish, has been declining (Ibañez et al., 2014). Regulation and management programs for fisheries are not enforced in Lake Titicaca. In both Peru and Bolivia, management measures (reproductive closures, minimum size catch, and mesh sizes) are not complied with, regardless of the Bolivian sector of Lake Titicaca was incorporated into the wetlands convention (RAMSAR 1971) in 1998 (Flores, 2003) because of its important ecological position. Therefore, there is an important open question about the status and influence of plastic in the lake ecosystem. This study examined microplastics pollution in surface water and the gut content of the most important fishery resources in Lago Menor of Lake Titicaca. This study seeks to provide general information about the status of microplastic pollution in Lago Menor. This study seeks to provide general information about the status of microplastic pollution in Lago Menor, which will provide a baseline for the assessment of sources and potential risks of microplastics in Lake Titicaca.

Materials & Methods

Study area

Lake Titicaca (Fig. 1) is divided into two sub-basins, the Lago Mayor (7131 km²; mean depth = 100 m; max depth = 285 m) and the smaller and shallow lake, Lago Menor (1428 km²; mean depth = 9 m; max depth = 40 m) (Dejoux & Iltis, 1992). The Strait of Tiquina and a single outlet for the lake, the Rio Desaguadero, which drains out the southern end of Lago Menor to the central Altiplano, particularly to the Uru-Uru, and Poopo lakes, which together form the Titicaca-Desaguadero, Poopo and Salares System (TDPS) (Dejoux & Iltis, 1992; Cross et al., 2000). Because of its tropical location and high altitude (3809 m a.s.l.), Lake Titicaca's temperature and light conditions remain almost constant, with altitude-related intense UVR. Lake Titicaca is subject to the tropical zone climate, a dry season (April-November) and a rainy season (November-March) (Myers et al., 2000; Vila, Pardo & Scott, 2007). In addition, its hydrological regime is dominated by evaporation (~95%), whereas rivers outflow represents ~5% (Dejoux & Iltis, 1992).

In general, Lake Titicaca is covered by totoras (*Schoenoplectus californicus*) on the inland margin (0-2 m depth) and by macrophytes (mainly Characeae spp.) between 4.5 and 7.5 m. However, Lago Menor is a mainly shallow ecosystem where large areas encompass depth between 5 to 10 m (Dejoux & Iltis, 1992; Lazzaro & Gamarra, 2014), and macrophytes constitute more than 60% of the total biomass and colonize one-third of its bottom (~436 km²) between 2 and 15 m depth (Collot, Koriyama & Garcia, 1983). In Bolivia, Lago Menor is mesotrophic to eutrophic in the shallowest littoral areas. The main source of anthropogenic pollution reaches the lake through the highly polluted Cohana Bay (Molina et al., 2017).

The fish community of Lake Titicaca is mainly composed of species of the native genera *Trichomycterus* (two species) and *Orestias* (more than 20 species) (Parenti, 1984; Lauzanne, 1992; Ibañez et al., 2014). In addition, two *Orestias* species known as carachis have relevant importance for traditional fisheries in Lago Menor (*O. agassizii* and *O. luteus*). However, since the 1940s, Lake Titicaca native fish has been perturbed repeatedly by the introduction of exotic piscivores (rainbow trout: *Oncorhynchus mykiss*, and the “pejerrey” silverside: *Odontesthes bonariensis*) (Loubens, 1992; Loubens & Osorio, 1992), which now belong to the lake's fishery resources. Nonetheless, rainbow trout is mainly produced by aquaculture in Lago Mayor and was not contemplated for this study.

Fish sampling

In Lago Menor of Lake Titicaca, three important landing zones were selected (Huarina, Cachilaya, and Desaguadero; Fig. 1). A sample of each species from the fishermen's catches during June 2021 was purchased. The sample selection was carried out following a simple random sampling, which consisted of the selection of a sample size between 60 and 100

specimens, when possible per species. All specimens were euthanized in ice and transported in coolers to the laboratory. The specimens of each fish species were measured (TL, mm; digital Vernier CD-20CP Mitutoyo), weighed (W, g) (PT 120 Sartorius Laboratory precision balance, 0.01g), and their guts were extracted and evaluated under a stereomicroscope (WILD M3, Heerbrugg, 50X). Previously, to prevent sample contamination, petri dishes used to observe gut content samples were cleaned with alcohol (96%) and distilled water. The presence of microplastics in each specimen was registered and classified by type (McCormick et al., 2016).

The quantification of microplastics ingested was based on the frequency of occurrence (FO) (Hyslop, 1980), calculated from the equation: $FO (\%) = Fi/Ft*100$; where Fi is the number of guts containing microplastics and Ft in the total number of gut contents examined.

Water sampling

Water samples were collected in five stations in the Lago Menor of Lake Titicaca during July and August 2021 (Fig. 1). Three replicates of water samples were obtained at 0.5 to 1 m depth using a 1L Van Dorn bottle per station. Five L per replicate was collected and filtered with a 20 μ m mesh and then preserved in 50 mL falcon tubes.

Detection of microplastics in water samples

Each sample was filtered through 10 μ m sieves to isolate the solid material. Then the sieved material was transferred to a Sedgwick-Rafter counting cell and observed under an Olympus LC30 microscope. First, the microplastics were identified and counted via visual examination. Next, each microplastic was categorized by its color and whether it was a fiber or fragment. Finally, 10-15 microplastics in each category were measured using the microscope's camera software (LCmicro, Olympus Image Analysis Software).

Data analysis

The microplastics data corresponded to counts and were not normally distributed as well as it did not meet the assumptions required to conduct a one-way ANOVA. Therefore, the mean number of microplastics per fish species and sites were analyzed by a non-parametric test (Kruskal-Wallis). Infostat software was used to perform statistical analysis and drawing.

Results

During the sampling period, 1283 fish were evaluated, belonging to four species characterized by being mainly omnivorous and carnivorous (Table 1). Body length of the native fish *O. agassizii*, size ranged between 62 to 203 mm, and the body weight ranged between 5 to 116 g. Likewise, *O. luteus*, a benthic native fish, size range between 66 to 172 mm, and the body weight ranged between 4 to 92 g. As the latter, *T. dispar*, a benthic species, showed a size range between 109 to 193 mm, and the weight ranged between 12 to 80 g. The piscivorous *O. bonariensis* showed a size range between 136 to 370 mm, and the weight ranged between 14 to 33 g.

The gut content analysis showed only microplastics fibers, mostly dark-colored (blue and black). Coiled filaments (Fig. 2a) and individual ones (Fig. 2b) characterized the observed fibers. However, the occurrence of fibers was low in all species ($FO < 5\%$; Fig. 3), and the mean number of microplastics per species and sites were similar ($p=0.528$ and $p = 0.119$, respectively). Microplastics were detected in all water samples. A total of 3395 pieces of microplastics were counted, with 45.8 % to fibers (Fig. 4a) and 54.2 % to fragments (Fig. 4b). Only three colors of fibers were observed (blue: 69.4%; red: 29.4%; and black: 1.2%). Water analysis showed the presence of microplastics ($52\text{ L}^{-1} \pm 38\text{ L}^{-1}$), and the mean number and abundance were similar between types (fibers and fragments; Table 2) regardless of the sampling site ($p = 0.148$). However, the mean number of fibers (Fig. 5a), as well as the mean sizes of them (Fig. 5b), was higher in Central Islands ($41\text{ L}^{-1} \pm 34\text{ L}^{-1}$; and $645\text{ }\mu\text{m} \pm 585\text{ }\mu\text{m}$). For the fragments, the mean number was higher in Desaguadero ($75\text{ L}^{-1} \pm 19\text{ L}^{-1}$), whereas the larger was observed in Central islands ($117\text{ }\mu\text{m} \pm 184\text{ }\mu\text{m}$).

Discussion

South America is the third region after Asia and Africa in terms of plastics transported by freshwater systems, despite being the region with the lowest production of plastics (Gourmelon, 2015). To date, in Bolivia there are no data or studies on microplastics pollution (Castañeta et al., 2020). Thus, the present study provides the first report on the presence of this pollutant in the emblematic Lake Titicaca in this country, and that fish fauna ingest them.

The present study registered a low occurrence of microplastics in gut content of fish ($FO < 5\%$ in all species). Nevertheless, microplastics were observed in all species, hence demonstrating the presence of this pollutant in these fish populations. A remarkable result is the high percentage of fibers in guts, although the abundance of this kind of microplastics showed no difference with fragments in water samples. We could not identify the reason for this higher ingestion of fibrous plastics in fish, but if this can be connected with the feeding behavior of the fish, this aspect may be worth further investigation as it may provide insights on how to reduce microplastics ingestion. Overall, no relationship could be observed between feeding habit and the occurrence of microplastics, but detailed nutritional ecology studies may facilitate further insights. For the water samples, the largest fibers and fragments were found in the Central Islands, which is close to the area of influence of Cohana Bay. However, the largest number of microplastics fragments were observed in Desaguadero (373 ± 95) which could reflect entrainment by the flow of the lake itself.

The comparisons of the abundance of microplastics among different publications must be performed with caution, because of considerable methodological differences among them (Hidalgo-Ruz et al., 2012; Wang & Wang, 2018). However, we could mention that based on our

results, Lago Menor of Lake Titicaca had a higher pollution load than other freshwater lakes from Europe (Lake Chiusi, Italy: 2.7-3.4 L⁻¹) (Fischer et al., 2016), Asia (Lake Taihu, China 3.4-25.8 L⁻¹) (Su et al., 2016) and South America (Lagos de la Patagonia, Argentina: 0.9 L⁻¹ ± 0.6 L⁻¹) (Alfonso et al., 2020). The origins for this higher pollution load may include waste management policies and differences in abiotic factors such as effluent rivers, but it does point to the necessity to monitor this pollution load over time to identify the rate of the increase in microplastic pollution.

The exposure to environmental conditions will produce different wear effects on the plastics. For example, mechanical abrasion produced by wind and wave can fragment plastic, but coupled with UV exposure, it can produce photoaging that fragments the plastic particles into increasingly smaller pieces (Hebner 2020; Song 2017; Castañeta 2020; Sonrensen 2021). Therefore, photodegradation is a major source of microplastics in the ecosystem, and photochemically induced embrittlement can lead to the formation and release of even undetectable plastic particles such as sub-microns (Song 2017). This may be particularly relevant due to the high dose of UVR received at the surface water of Lake Titicaca (793 kJ m⁻²) (Helbling et al., 2002). As the lake exists at a high altitude, it is possible that the microplastics present in the water fragment into even smaller particles, which reduces the chances of their detection. It is for this reason that the analysis of nanoplastics in the environment must be taken into account, in addition to the use of chemical detection techniques. Likewise, plastic photoaging has been studied mainly under laboratory conditions (Cheng et al., 2021), so studying it under the particular conditions of Lake Titicaca could provide relevant information on plastic contamination in high altitude ecosystems, as well as its permanence in the ecosystem.

Conclusions

The scarce information on plastic pollution in freshwater systems shows the need for a greater effort to understand this problem in inland waters. This study represents a first report on the presence of plastics in water and biota of a tropical high-altitude lake shows that microplastics can be consumed by the fish community, although still at a low occurrence. Compared to other lakes in Europe, Asia and South America for which information is available, the microplastic load in the water of Lago Menor of Lake Titicaca is high. Furthermore, the high UVR of Lake Titicaca could fragment the plastics present in the habitat to even smaller fragments (nanoplastics or smaller), so their presence in primary consumers (i.e. zooplankton) is still unknown. Moreover, the physiological and toxicological consequences of plastics in aquatic biota is still poorly understood, so more research could contribute to improve the environmental management of waters, particularly in high Andean ecosystems.

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Figure 1

Location map of sampling station in Lago Menor of Lake Titicaca.

Blue dots show water-sampling sites. Red dots show fish sampling sites. The star reflects the area of influence of Cohana Bay and the green areas correspond to the extent of cattails in Lake Titicaca Minor.

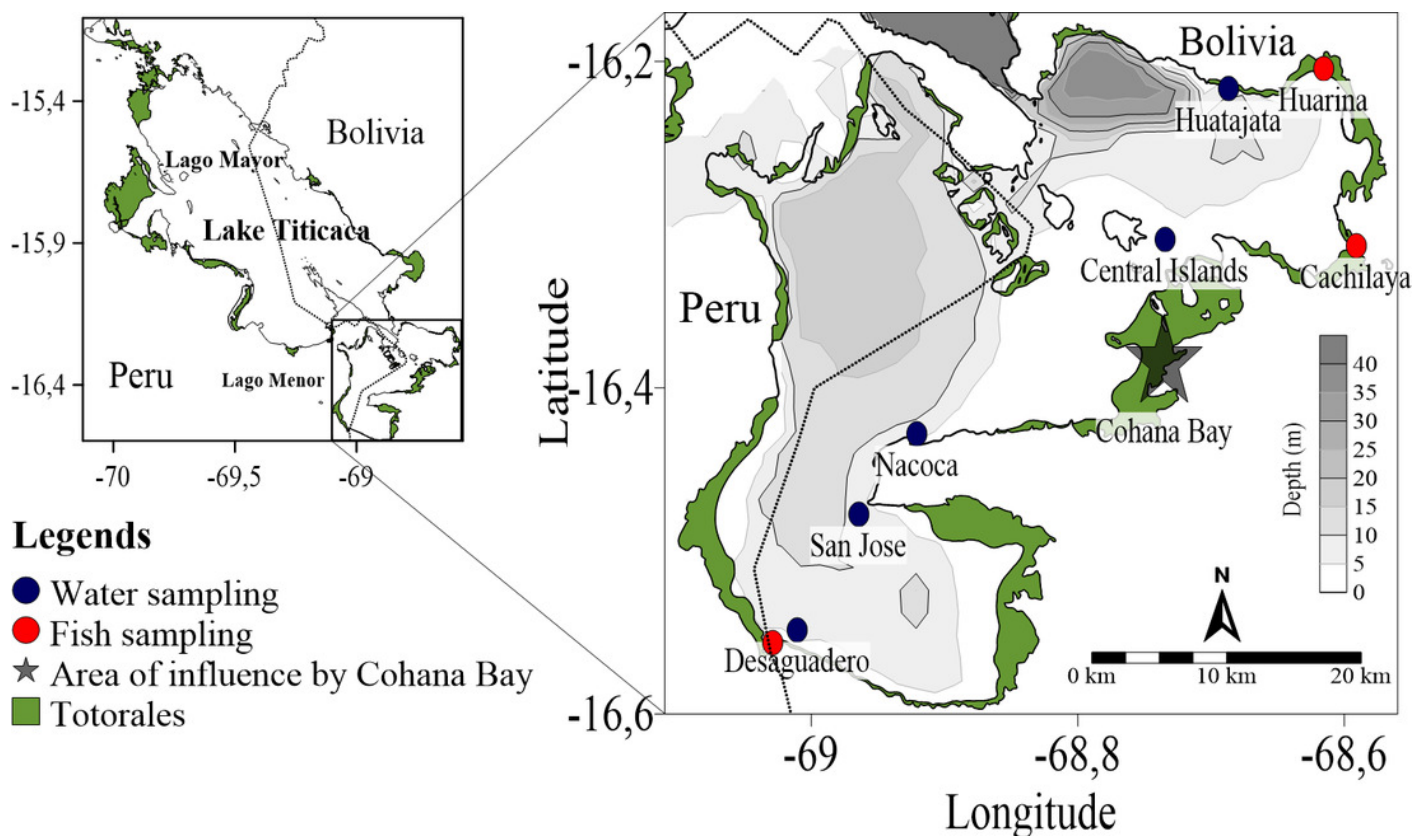


Figure 2

Examples of fibers of microplastics found in gut content.

Fibers of microplastics found in gut content found in (A) *O. luteus* and (B) *O. agassizii* of Lago Menor of Lake Titicaca. Scale bar represents 1 mm.

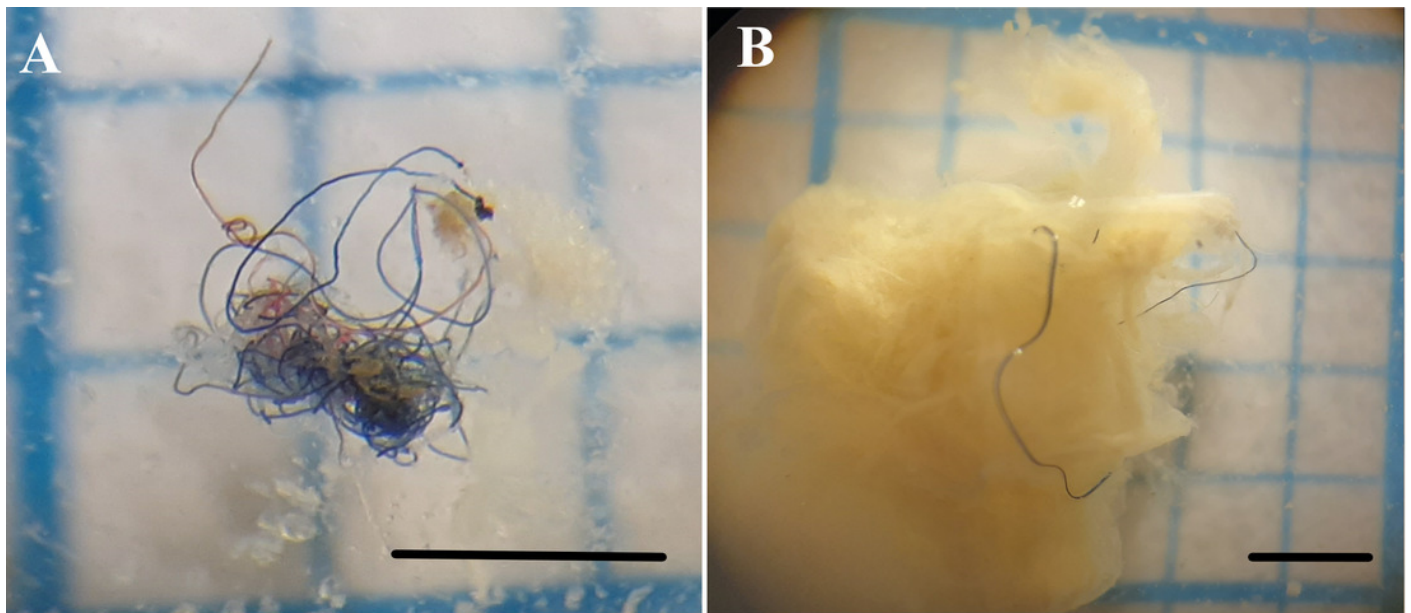


Figure 3

Frequency of occurrence (FO) percentage of microplastics found in gut content of the fish studied in Lago Menor of Lake Titicaca.

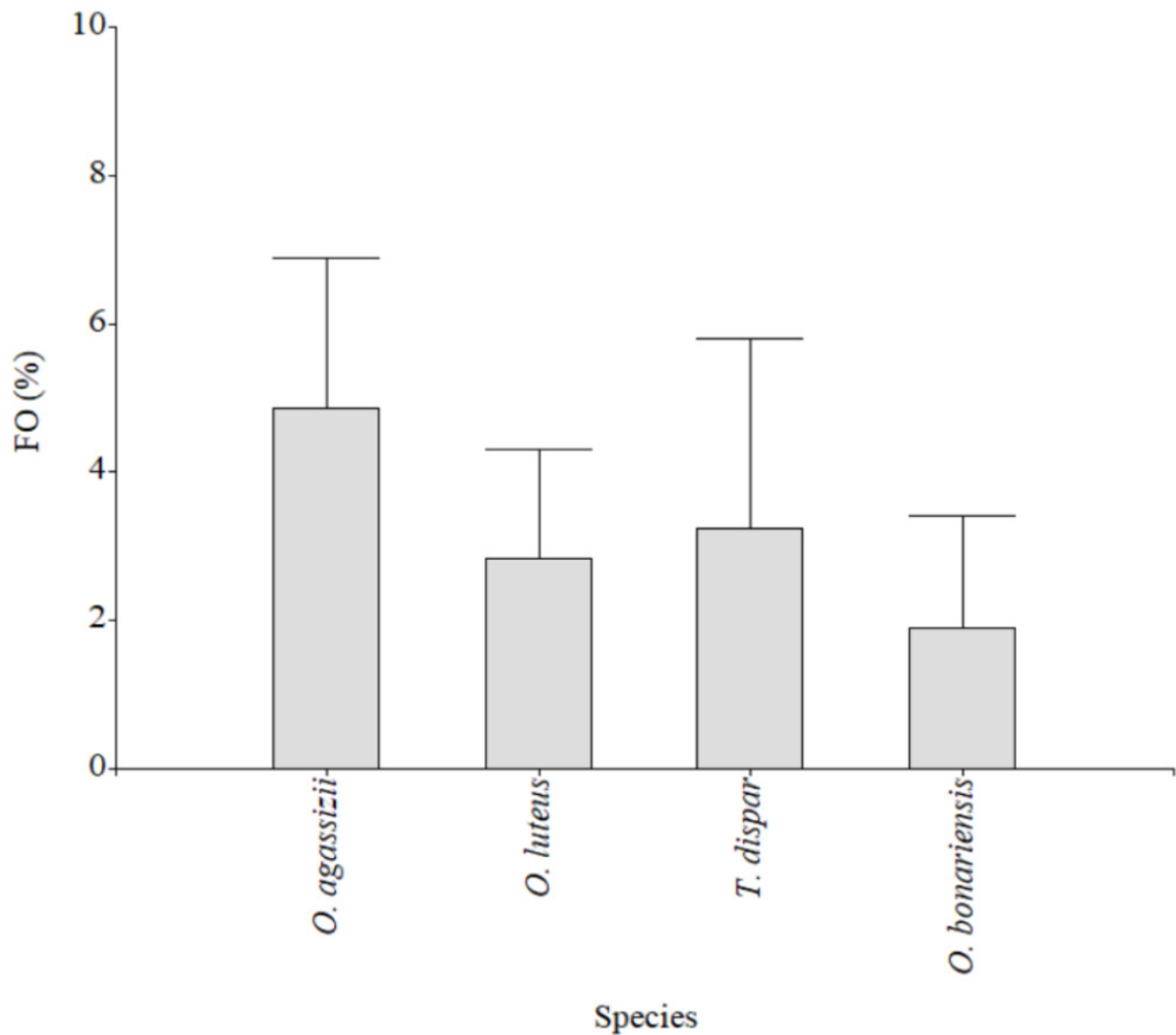


Figure 4

Examples of two types of microplastics found in water samples in Lago Menor of Lake Titicaca.

A) Example of fibers and (B) fragments of Lago Menor of Lake Titicaca.

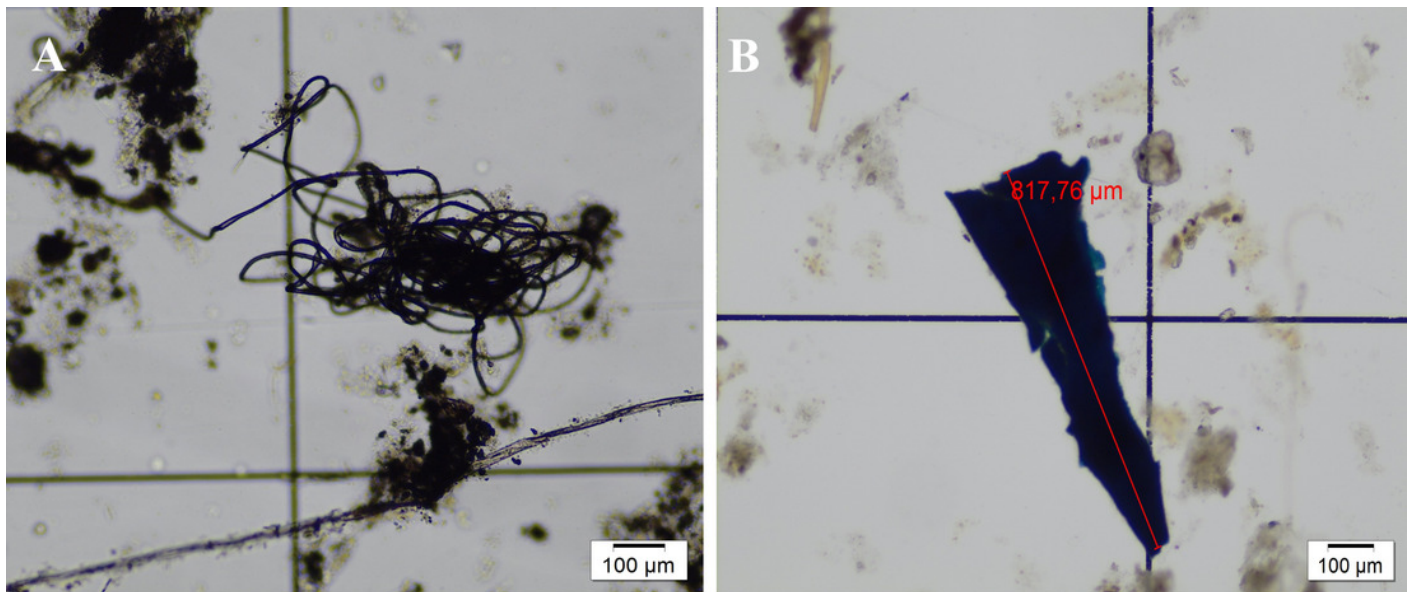


Figure 5

Summary of microplastics found in water samples in Lago Menor of Lake Titicaca.

A) Number of microplastics particles per L per site and (B) size of microplastics (μm) found in Lago Menor of Lake Titicaca.

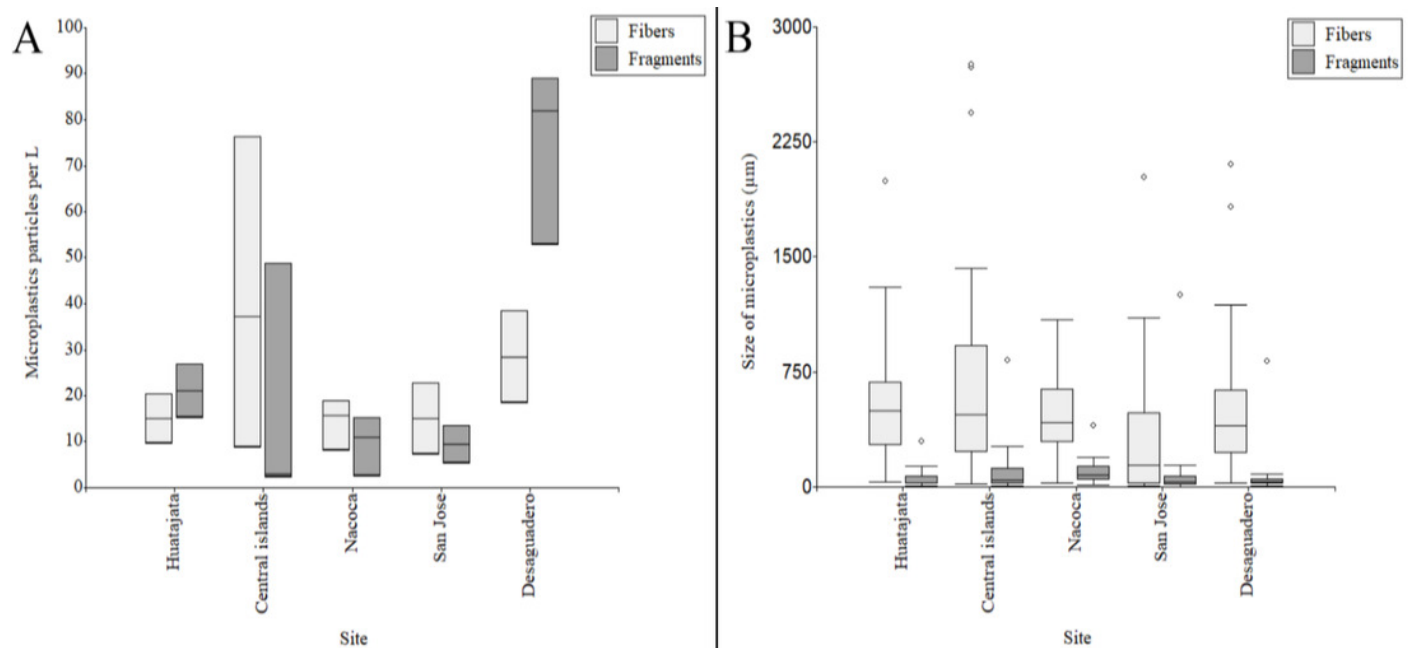


Table 1 (on next page)

Number of specimens analyzed, habitat, feeding habits and diet, body length and weight ranges of the sampled fish from Lago Menor in Lago Menor of Lake Titicaca

Species	Landing zones	N	Habitat	Diet	TL (mm) ± SD; range (mm)	W (g) ± SD; range (g)	Source for habitat, feeding habits and diet
<i>Orestias agassizii</i>	Cachilaya, Huarina and Desaguadero	407	Ubiquitous (littoral, benthic and pelagic)	Omnivorous: Amphipods, mollusks, insects and plankton	114 ± 25; 62-203	27 ± 18; 5-116	(Lauzanne, 1982, 1992; Parenti, 1984; Vila, Pardo & Scott, 2007; Maldonado et al., 2009; Ibañez et al., 2014; Monroy et al., 2014; Loayza et al., 2020)
<i>Orestias luteus</i>	Cachilaya, Huarina and Desaguadero	347	Benthic	Omnivorous-Microcarnivorous : Amphipods, mollusks, insects, eggs	108 ± 18; 66-172	30 ± 16; 4-92	(Lauzanne, 1982, 1992; Parenti, 1984; Vila, Pardo & Scott, 2007; Maldonado et al., 2009; Ibañez et al., 2014; Monroy et al., 2014; Loayza et al., 2020)
<i>Trichomycterus dispar</i>	Cachilaya and Huarina	287	littoral, benthic	Microcarnivorous : Amphipods, mollusks, insects, and eggs	139 ± 15; 109-193	26 ± 10; 12-80	(ALT, 2003; Ibañez et al., 2014)
<i>Odontesthes bonariensis</i>	Cachilaya, Huarina and Desaguadero	242	Pelagic, littoral	Carnivorous-Piscivorous: Amphipods, zooplankton, insects, fish and frogs	213 ± 55; 136-370	70 ± 63; 14-330	(Loubens, 1989; Vila, Pardo & Scott, 2007; Monroy et al., 2014)

Table 2(on next page)

Characteristics and abundance of microplastics found in water of Lago Menor of Lake Titicaca

Type of microplastic	Microplastics (mean particles number \pm SD)	Abundance (item/L) \pm SD
Fiber	120 \pm 94	24 \pm 19
Fragment	141 \pm 151	28 \pm 30
TOTAL	278 \pm 190	52 \pm 38

1