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Microplastics in biotic and abiotic compartments of high-mountain lakes from Alps

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ABSTRACT

Microplastic (MP) pollution is a major environmental concern for mountain ecosystem and for high-mountain lakes in particular, which are recognized indicators of global change. In this study, the presence of MPs was assessed in abiotic (water and sediment) and biotic (zooplankton, tadpoles, fish) compartments of two high-mountain lakes (Upper Lake Balma and Lower Lake Balma) in the Cottian Alps (northwest Italy). No MPs were found in water and zooplankton samples, whereas the mean MPs in sediment samples was 1.33 ± 0.67 items/m^3 and 1.75 ± 0.62 items/m^3 in Lower and Upper Lake Balma, respectively. The mean MPs in tadpoles of *Rana temporaria* was 0.33 ± 0.58 items/individual and 0.66 ± 0.58 items/individual in Lower and Upper Lake Balma, respectively. The mean number of MPs items found in the gastrointestinal tract (GIT) of fish (*Salvelinus fontinalis*) was considerably higher in specimens from the Lower (0.45 items/g GIT) than in those from the Upper Lake (0.20 items/g GIT). There was a negative relationship between fish size (weight and age) and MPs abundance in the GIT of fish, indicating that young fish accumulated more MP items probably due to the high prey ingestion rate compared to adults. The same MPs color (blue, white, black), shape (fibers and fragments), and chemical type (polypropylene and polyethylene) were found in the compartments of both lakes. Our findings suggest the use of *S. fontinalis* as an indicator of MP pollution in high-mountain lakes. Further studies are needed to better understand the sources and the effects of MPs in these remote ecosystems.

1. Introduction

Plastics are non-biodegradable organic materials composed of macromolecules formed by the polymerization of single molecules organized into very long and complex polymeric chains (Andrady, 2017). Plastic has become a ubiquitous feature of daily life and modern lifestyle in the Plastic Age (Thompson et al., 2009). Yet this irreplaceable ally has become a global environmental and health emergency (Burgos-Aceves, et al., 2021; Yang et al., 2022). Approximately 400 million tons of plastic are produced every year; this figure is expected to double by 2050 (PlasticsEurope, 2022). Because of its strength, flexibility, low cost, and waterproofness, global plastic production has increased in tandem with its ever-expanding range of use (medical, health, technological, food) and application (Anagnosti et al., 2021). Plastics production increased from 1.7 million tons in the 1950s to 390.7 million tons in 2021, with Europe alone producing about 57.2 million tons (14.64%) (PlasticsEurope, 2022). Only 10.1% of plastic waste is recycled, 12% is incinerated, and 79% is disposed of in landfills (PlasticsEurope, 2022). Degradation occurs by photooxidation induced by sunlight exposure and other natural processes (i.e., temperature variation, ionization, hydrolysis, solubilization) and by interaction with microorganisms (Arpia et al., 2021).

Plastic degraded into smaller items is called microplastics (MPs; 1–5000 μm) (Ivleva et al., 2017). MPs refers to solid plastic items of various size, structure, density, color, and chemical type released into the environment (Frias and Nash, 2019). Two main types are distinguished: primary and secondary MPs (Issac and Kandasubramanian,

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2021). Primary MPs derive from products for human use variously contained in cosmetics, scrubs, and abrasives (Issac and Kandasubramanian, 2021). The environmental fate and effects of MPs are not well known (Blackburn and Green, 2022); attempts are being made to evaluate the associated potential risks (Prokić et al., 2021; Li et al., 2022; Doyle et al., 2022; Koelmans, et al., 2022; Wang et al., 2022). Secondary MPs result from the fragmentation of larger into smaller plastics that originate during use and from degradation (Issac and Kandasubramanian, 2021). Both types enter the terrestrial and the aquatic environment via drainage. Because of their small size, MPs may be caught in wastewater treatment plants or enter freshwater lakes and rivers and ultimately the oceans (Bhatt et al., 2021; Ateia et al., 2022; Jiang et al., 2022; Li et al., 2023).

MPs transported by natural and artificial vectors contaminate the water and the atmosphere distant from areas of industrial production (Petersen and Hubbart, 2021; Zhang et al., 2021; Abbasi et al., 2023). This form of anthropic contamination has no physical and/or geographical barriers and is found also in remote areas (Multisanti et al., 2022; Citterich et al., 2023; Forster et al., 2023). Wind can transport MP particles over distances, then deposited dry or wet on land or in water (Allen et al., 2019; Bullard et al., 2021).

Microplastics can reach remote and pristine ecosystems even when there are no local point sources of plastic production (Zhang et al., 2019; Mishra et al., 2021; Stefánsson et al., 2021; Padha et al., 2022). For example, MPs have been found in remote ecosystems such as the Arctic polar regions, the deep-sea environment, high-mountain lakes, and glaciers via atmospheric transport and deposition (Fang et al., 2018; Ambrosini et al., 2019; Esposito et al., 2022; Pastorino et al., 2021, Pastorino et al., 2022a). Due to their low density, MPs are lifted by wind currents into the upper layers of the atmosphere and then deposited by snowfall or rainfall in high-mountain ecosystems where they may pose environmental risks (Allen et al., 2019). Pollutants and particulate materials carried by air (wind, storm, and/or rain) and then deposed on the ground are captured by snow, which acts as a scavenger (Xie et al., 2020; Fu et al., 2021).

High-mountain lakes are, by definition, located above the tree line at an altitude above 1500 m a.s.l., generally between 1800 m and 2500 m a.s.l., and in a context of highly natural apparitions (Catalan et al., 2006). These lakes hold interest for ecologists owing to the variety in chemical, physical, and biological features closely linked to lake basin morphology, lithology, and hydrology (Pastorino and Prearo, 2020; Alcocer et al., 2021). By the virtue of their remote location, high-mountain lakes are believed to be less influenced by local anthropogenic impact such as direct pressure from agriculture and wastewater, which typically affect lakes at lower altitudes (Moser et al., 2019; Ebner et al., 2022; Pastorino et al., 2022b). However, despite their apparent inaccessibility, the influence of humans can be seen even in these environments, for example, signs of physicochemical, biological, and morphological change to these ecosystems (Pastorino and Prearo, 2020). High-mountain lakes are indicators of problems of global concern. They are extremely sensitive to the deposition of acidic substances from the atmosphere, medium-long range transport of contaminants, and climate change (Moser et al., 2019; Pastorino et al., 2022b; Schirpke and Ebner, 2022).

These ecosystems are considered “ecosystem sensors” of entire mountain environments. They are ideal sites (natural laboratories) for monitoring the effects of large-scale human pressure and global change on water quality and biodiversity (Moser et al., 2019; Medina-Sánchez et al., 2022). In their recent study, Pastorino et al. (2022b) proposed the use of high-mountain lakes as indicators of MPs pollution. Most of the few studies published to date have been conducted in the Tibetan Plateau (China) (Zhang et al., 2016; Dong et al., 2021; Liang et al., 2022).

Moreover, abiotic samples (mainly sediment and water) are the most frequently studied matrices, but there is little information about MPs in the aquatic biota of high-mountain lakes. With this study we wanted to determine whether MPs can be found only in abiotic compartments (water and sediment) or also in aquatic organisms typical to high-mountain lake, such as zooplankton, tadpoles, and fish. The study site was two high-mountain lakes (Upper and Lower Lake Balma) located in the western Alps (Italy); the lakes were selected because of their use as indicators of environmental changes in previous studies (Perilli et al., 2020; Cantonati et al., 2021; Pastorino et al., 2022c; Salvi et al., 2022).

2. Material and methods

2.1. Study area

Both lakes (Upper and Lower Balma) (Fig. 1) are located in the Cottian Alps at 2101 m a.s.l. (Lower Lake Balma; 45°02′13.799″ N; 07°10′52″ E) and 2213 m a.s.l. (Upper Lake Balma; 45°02′15.055″N; 07°10′27.724″ E). The lakes fall within the SAC IT1110006 Orsiera Rocciavré (Municipality of Coazze, Province of Turin, northwest Italy).

The Upper Lake forms an S-shape with two sub-basins separated by a shallow midsection. The lake is 774 m in perimeter, 1.82 ha in surface area, and 2.7 m in maximum depth. It is situated in a catchment core of ophiolite metamorphic bedrock. The immediate surrounding is dominated by rocky outcrops, ridges, mountain walls, and a meadow. The outlet originates in the lake’s northeastern corner and flows downstream toward the Lower Lake’s inlet (Bertoli et al., 2023).

The Lower Lake is circular in shape, 414 m in perimeter, 1.21 ha in surface area, and 6.4 m in maximum depth. The composition of the main catchment core is the same as the Upper Lake, and the landscape is similar to that of the other lake (Bertoli et al., 2023). The small inlet is located on the lake’s western shore, where it divides into three small branches before entering the lake. Although no true outlet is visible, Balma Creek is formed by water filtrating through the sediments on the basin’s eastern side. The major anthropogenic impact is the medium-long transport of pollutants from urban areas, grazing, and fishing. Although the lakes were originally devoid of fish, brook trout (*Salvelinus fontinalis* Mitchill, 1814) was introduced for recreational fishing (Pastorino et al., 2020a).

The study area has 14 habitats of community interest and at least 850 plant species have been identified, 20 of which are on the National Red List. The flora consists of numerous species endemic to the Central Alps: *Campanula alpestris*, *Campanula elatines*, *Veronica allionii*, and *Viola cenisia*, in addition to several rare species in Piedmont (*Cortusa matthioli*, *Cerastium linear*, *Aconitum anthora*, *Cardamine plumieri*). Two species are listed in the Habitat Directive (92/43/EEC): *Aquilegia alpina* and *Saxifraga valdensis*, endemic to the Cottian and Graian Alps. There are 73 nesting bird species, including *Accipiter gentilis*, *Carduelis spinus*, *Loxia curvirostra*, *Turdus torquatus*, *Nucifraga caryocatactes*, *Tichodroma muraria*, *Montifringilla nivalis*, and about 30 mammalian species, including *Rupicapra rupicapra*, *Capra ibex*, *Cervus elaphus*, and *Capreolus capreolus*. Of note is the return of the wolf (*Canis lupus*) in the 1990s by way of expansion of its distribution in the Alps. Finally, the study area is an important breeding site for amphibians, particularly for the European common frog (*Rana temporaria*).

2.2. Water and sediment sampling

On 18 August 2021 water and sediment samples were collected to determine and quantify the MPs in the abiotic compartments of the lakes. Sediment samples (n = 12 per lake; n = 3 replicates per site) were collected at four sampling stations by means of a manual corer (250 cm^2 sampling surface). The samples were collected from the lakebed, a few meters from the shore, depending on accessibility. Each sediment replicate was then stored separately in a stainless-steel container, covered with aluminum foil to prevent external particle contamination, and frozen at −20 °C until analysis (Pastorino et al., 2021; Bertoli et al., 2022). MPs analysis was performed on the samples following the methodology reported in section 2.6.

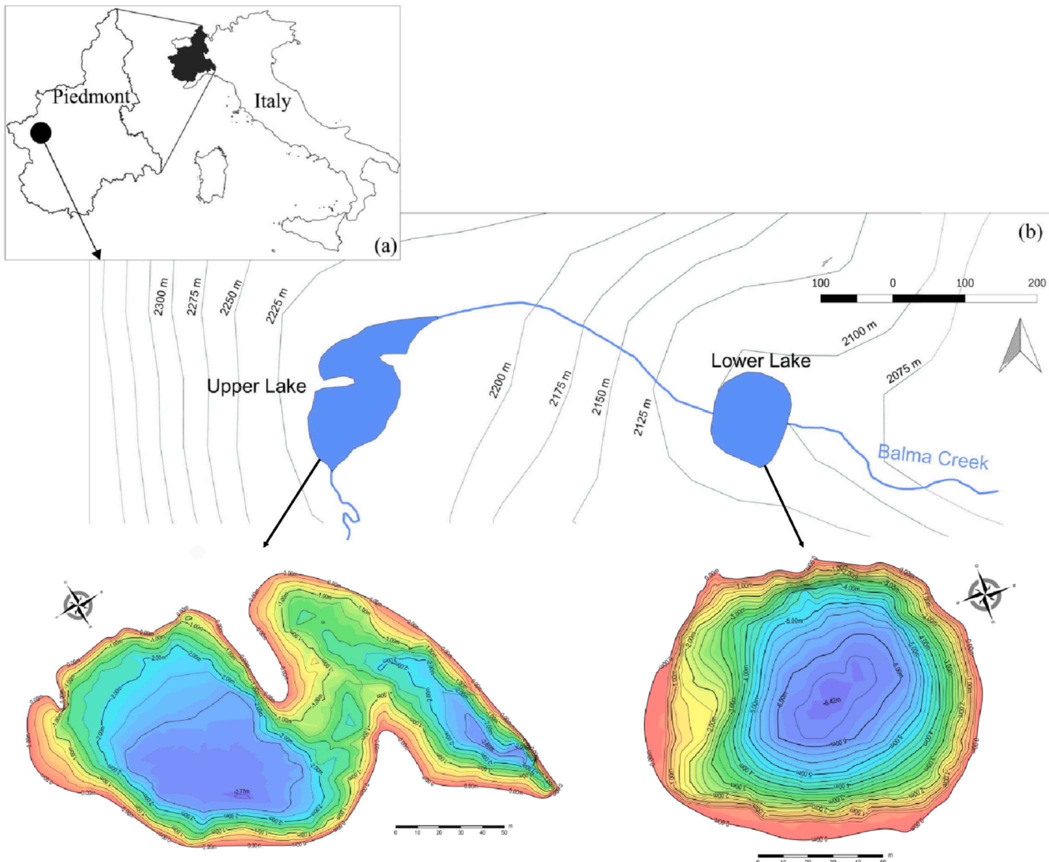

Fig. 1. Study area. (a) Location of Balma Lakes in Piedmont, northwest Italy; b) details of Upper (left) and Lower (right) Balma Lake (Bertoli et al., 2023, modified).

Water samples were collected at the same time as the sediment samples from the entire water column (surface down to the maximum depth). A low-speed dinghy hauled an Apstein plankton net (opening 400×1000 mm; mesh size 50 μm) to determine the horizontal and vertical distribution of MPs (Prata et al., 2019). The volume (V) of filtered water was calculated using the formula: $V = \pi(d/2)^2 \times h$, where "d" is the diameter of the plankton net opening and "h" is the depth (6.4 m and 2.4 m for the Lower and the Upper Lake, respectively) (Cera et al., 2022). Water samples (803 dm^3 and 301 dm^3 for the Lower and the Upper Lake, respectively; three replicates) were collected in glass jars (1 l), brought to the laboratory, and kept at +4 °C until analysis. The net was cleaned with ultrapure water between each replicate to prevent contamination (Prata et al., 2019).

2.3. Zooplankton sampling

Zooplankton samples were collected from both lakes with a Van Dorn Bottle (8 l; 610x250 mm) at −1 m (n = 3 samples per lake) and at maximum depth (6.4 m and 2.4 m for the Lower and the Upper Lake, respectively) (n = 3 samples per lake). The samples were transferred to glass bottles and transported refrigerated (+4 °C) to the laboratory within hours by helicopter. The samples were sorted with stainless steel pincers under a stereomicroscope and transferred to a glass Petri dish to separate zooplanktonic organisms by taxonomic group. Copepods (n = 250 per lake), the most abundant taxonomic group in both lakes with the species *Arctodiaptomus alpinus*, *A. bacillifer,* and *A. wierzejiskii* (Calanoida, Diaptomidae), were placed in glass vials and stored until MPs analysis (section 2.6).

2.4. Tadpole sampling

Rana temporaria tadpoles were collected by means of a net at three stations (n = 10 per station) along the shoreline (n = 30 per lake) (Pastorino et al., 2022a). The tadpoles were immediately suppressed with MS-222 (30 mg/L; tricaine methanesulfonate, Sigma–Aldrich, St Louis, MO, USA), placed in aluminium foil, and then stored until analysis (section 2.6). Authorization for tadpole sampling was obtained from the "Ente di Gestione delle Aree Protette delle Alpi Cozie" (Determination no. 106/2021).

2.5. Fish sampling and processing

Fish were captured using the standardized method for fish sampling in European lakes, in which a single sampling session is carried out using both benthic and mesopelagic nets in relation to lake type, depth, and surface (Pastorino et al., 2020b). Six nets (2 mesopelagic and 4 benthic) were used for fish sampling in Lower Lake, whereas six benthic nets were used in the Upper Lake. The benthic nets (30 m long, 1.5 m high) were composed of 12 panels (2.5 m long) ranging in mesh size from 5 to 55 mm. The mesopelagic nets (27.5 m long, 6 m high) were composed of 12 panels (2.5 m long) ranging in mesh size from 5 to 55 mm. The nets were positioned according to the lake's bathymetric profile. The nets were placed at approximately 6p.m. and recovered 12 h later. The fish (*Salvelinus fontinalis*) were transported refrigerated (+4 °C) within hours by helicopter to the laboratory, where they were measured for weight (g) (technical balance Radwag, PS06.R2, Poland) and total length (cm), and 5 scales (dorsal region) from each specimen were removed for age determination by scalimetry (Pastorino et al., 2020b). The fish were dissected with a stainless-steel dissecting kit (scalpel, forceps, scissors) and the entire gastrointestinal tract was removed, weighted, wrapped in aluminum foil, and stored at −20 °C until analysis (section 2.6). Authorization for fish sampling was obtained from Città Metropolitana di Torino (authorization no. 4025/2021).

2.6. Microplastics determination

Mechanical agitation (20 min, 100 rpm) was used to extract sediment samples from a prefiltered saturated NaCl solution; the supernatant was filtered through 6-μm pore paper disk filters (Whatman®, Sigma-Aldrich, USA). To prevent contamination during oven drying (35 °C), the filters were placed on a glass Petri disk. Stereomicroscopy (Nikon, P-DSL32, 10-80x, Japan) was used to examine the dried samples, and MP particles were collected for chemical analysis. Tadpoles and the gastrointestinal tracts from the fish were digested in a saturated solution of KOH + NaOH sonicated at 40 Hz for 20 min (30 °C), then vacuum filtered through a paper fiber filter disk (6 μm; Millipore®, Sigma-Aldrich) and oven dried at 35 °C until complete exsiccation. Zooplankton were digested using the digestion protocol described in Zavala-Alarcón et al. (2023). Stereomicroscopy (Nikon, P-DSL32, 10-80x) connected to a webcam was used to sort the filters (Nikon; P-DSL32, NS-Elements D.4.60 64-bit). Targeted items were collected and analyzed in reflection mode using the FT-IR technique (Nicolet iN10, ThermoFisher Scientific, USA). The target-item spectral match (%) was calculated and compared to spectral libraries of normal and aged microplastics (OMNICTM PictaTM software libraries, ThermoFisher Scientific) integrated with BsRC laboratory spectral libraries. The particle size had a limit of detection (LOD) of 10 μm. The MP particles were classified by shape and color according to Joint Research Centre criteria (2014).

2.7. Quality assurance/quality control

The QA/QC approach was used to ensure that the data met standard quality criteria (sample processing and storage, laboratory preparation, clean air conditions, target component, sample treatment, polymer identification) (Hermsen et al., 2018). To avoid sample contamination, samples and sorting activities were carried out while wearing a box-glove (Iteco Engineering, mod. SGS20-13599, serial number 103421, Italy). Positive and negative controls (n = 5) were created to prevent recoveries, airborne pollution, and sample cross contamination. Negative controls were unpolluted, and the particle recovery rate of the spiked MP items was nearly 100%. Standard reference materials (ThermoFisher®, Instrument Qualification Kit) were used to verify instrument qualification.

2.8. Statistical analysis

Data were initially checked for normality and homoscedasticity of variance using the Shapiro-Wilk test and Levene's test, respectively. As the data were not normally distributed, the Mann-Whitney *U* test was used to check for differences in MPs abundance in the biotic and the abiotic compartment of the two Lakes. Spearman's rank correlation analysis was performed to test for a correlation between the biometric features of the fish (total length and weight) and the features of the MPs accumulated in in their gastrointestinal tract (data from the two lakes merged). Simple linear regression analysis was used to check the strength of the correlation between MPs abundance in the gastrointestinal tract of the fish (dependent variables) and their weight (independent variable). Statistical significance was set at $p < 0.05$. Statistical analysis was performed using RStudio® version 1.1.463.

3. Results

3.1. Microplastics occurrence in abiotic samples

Water samples tested negative at all sampling stations of both lakes, whereas MPs were detected in the sediment samples from both lakes (cumulatively 18 and 21 items in the Lower and the Upper Lake, respectively) (Fig. 2). The mean MP items (counts merged from the four sampling sites) was 1.33 ± 0.67 items/m^3 in the Lower and 1.75 ± 0.62 items/m^3 in the Upper Lake. There was no statistically significant difference in MPs count in the sediment samples between the lakes (Mann-Whitney *U* test, $U = 1.5$; $p = 0.41$). The mean size of MP items was 116.6 ± 15.9 μm (range 101.1–138.6 μm) and 118.6 ± 19.9 μm (range 100.2–142.1 μm) in the Lower and the Upper Lake, respectively. Polypropylene (PP) and polyethylene terephthalate (PET) were the only two chemical types of MPs in the sediment samples (Lower Lake Balma 88.9% PE, 11.1% PP; Upper Lake Balma 90.5% PE, 9.5% PP). Fibers (Lower Lake Balma 50%; Upper Lake Balma 57%) and fragments (Lower Lake Balma 50%; Upper Lake Balma 43%) were recorded, whereas blue (55.5% and 66.7% in Lower Lake Balma and Upper Lake Balma, respectively), white (27.8% and 19% in Lower Lake Balma and Upper Lake Balma, respectively), and black colored items (16.7% and 14.3% in Lower Lake Balma and Upper Lake Balma, respectively) were recorded in the sediment samples.

3.2. Microplastics occurrence in biotic samples

All copepod species from both lakes tested negative for MPs, whereas MP items were found in the tadpoles from both lakes (cumulatively 1 and 2 items in the Lower and the Upper Lake, respectively). The mean MP items (counts merged from the three sites) was 0.33 ± 0.58 items/individual and 0.66 ± 0.58 items/individual in the Lower and the Upper Lake, respectively. The mean size of MP items was 116.6 ± 15.9 μm (range 101.1–138.6 μm) and 102.6 ± 10.9 μm (range 98.7–121 μm) in the Lower and the Upper Lake, respectively. Blue fragments made from PE (n = 2) and PP (n = 1) were found in both lakes.

The mean fish weight (n = 40 per lake) was 88.3 ± 72.1 g and 101.2 ± 87.4 g in the Lower and the Upper Lake, respectively. Table 1 reports total length, weight, age, and occurrence (no. items/individual, no.

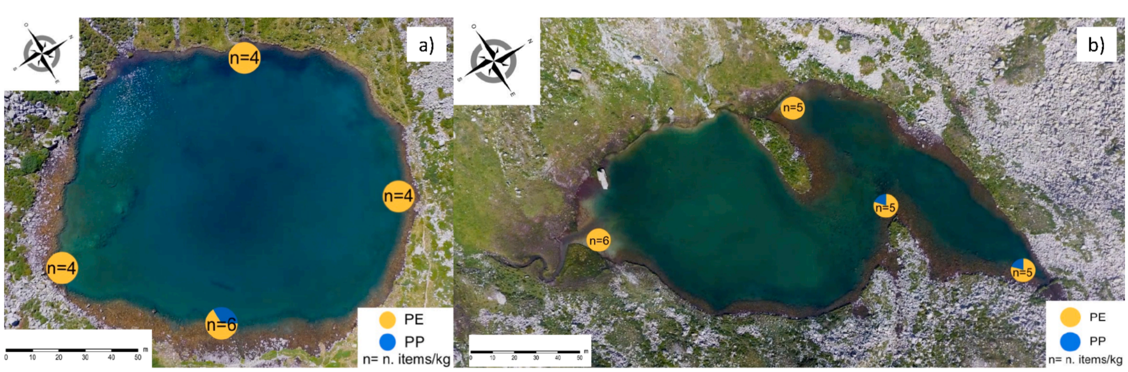

Fig. 2. Location of sampling sites for sediment samples in Lower (a) and Upper (b) Balma Lake. Colors (yellow and light blue) represent a different chemical type of microplastics (PE = polyethylene; pp = polypropylene). It is also reported the number of items (n) recorded at each site. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Sites of sampling (Lower and Upper Balma Lake), weight (g), total length (cm) and age (year) of *Salvelinus fontinalis*. It is also reported the occurrence (n. items/individual, n. items/g of gastrointestinal tract-GIT) and characteristics of microplastics (size in μm, shape, color, and chemical type) recorded in the fish samples (n = 9 and n = 11 for Lower and Upper Balma Lake, respectively).

Site	Weight (g)	Total length (cm)	Age	Item(s)/individual	Items/g GIT	Size (μm)	Shape	Color	Chemical
Lower	113.44	22.3	2+	1	0.12	97.8	Fragment	white	PE
Lower	77.91	18.1	2+	2	0.35	112; 118.2	Fiber; Fragment	black; blue	PE; PP
Lower	89.88	19.7	2+	2	0.30	110.2; 119	Fragment; Fragment	black; white	PP; PE
Lower	69.64	19.2	2+	3	0.58	88.7; 103; 100.1	Fiber; Fiber; Fragment	blue; black; white	PE; PE; PP
Lower	47.85	14.8	0+	3	0.85	104.5; 101; 97.8	Fiber; Fiber; Fragment	blue; black; white	PP; PE; PE
Lower	82.21	18.7	2+	1	0.16	112.9	Fiber	blue	PE
Lower	67.53	18.2	2+	2	0.40	99.8; 103	Fiber; Fragment	white; black	PP; PE
Lower	79.55	18.5	1+	3	0.51	108.8; 101; 99.2	Fiber; Fiber; Fiber	blue; black; white	PP; PE; PP
Lower	53.16	17.8	1+	3	0.76	102.8; 105; 113.5	Fragment; Fiber; Fiber	black; black; white	PP; PE; PE
Upper	104.17	20.4	2+	1	0.13	93.4	Fiber	black	PE
Upper	78.26	18.3	1+	2	0.35	127; 114	Fragment; Fiber	white; black	PP; PE
Upper	146.86	23.8	3+	1	0.09	104	Fragment	white; black	PP; PP
Upper	120.16	22.1	2+	1	0.11	136.6	Fragment	blue	PP
Upper	92.8	20.1	2+	1	0.15	115.5	Fiber	blue	PE
Upper	114.85	24.4	3+	1	0.12	131.4	Fragment	white	PP
Upper	109.4	21.3	2+	1	0.12	134.5	Fiber	blue	PE
Upper	93.45	21.8	2+	1	0.14	102.9	Fiber	black	PP
Upper	97.53	20.7	2+	1	0.14	110.8	Fragment	blue	PE
Upper	73.02	18.8	2+	2	0.37	126.6; 118.7	Fiber; Fragment	black; black	PE; PE
Upper	52.64	16.8	1+	2	0.51	110; 107.2	Fiber	blue; black	PP; PE

items/ g GIT), and characteristics (size, shape, color, chemical type) of MP items recorded in the fish samples. The mean fish length (n = 40 per lake) was 18.4 ± 3.1 g and 20.8 ± 2.2 cm in the Lower and the Upper Lake, respectively. Age ranged from 0+ to 3+ in the Lower and from 1+ to 3+ in the Upper Lake, respectively. MPs (cumulatively 20 and 15 items in the Lower and the Upper Lake, respectively) were detected in 9/40 (22.5%) and 11/40 (27.5%) fish samples in the Lower and the Upper Lake, respectively. The mean size of the MP items was 106.29 ± 6.75 μm (range 93.4–136.6 μm) and 114 ± 16 μm (range 88.7–119 μm) in the Lower and the Upper Lake, respectively. Polypropylene (PP) and polyethylene terephthalate (PET) MPs were the only two chemical types of MPs found in the gastrointestinal tract (GIT) of fish (Lower Lake Balma 60% PE, 40% PP; Upper Lake Balma 53% PE, 47% PP). Fibers (Lower Lake Balma 50%; Upper Lake Balma 57%) and fragments (Lower Lake Balma 50%; Upper Lake Balma 43%) were recorded. Blue (25% and 33% in Lower Lake Balma and Upper Lake Balma, respectively), white (35% and 20% in Lower Lake Balma and Upper Lake Balma, respectively), and black (40% and 47% in Lower Lake Balma and Upper Lake Balma, respectively) were recorded in the fish samples.

Fig. 3a presents the boxplots of the weight of the fish in which MP items were detected. Weight was significantly higher in the fish from Upper Lake Balma compared to those of Lower Lake Balma (Mann-Whitney *U* test, $U = 22$; $p = 0.03$). Fig. 3b presents the boxplots of the number of items/g in the GIT, which was significantly higher in the fish from Lower Lake Balma (mean 0.45 items/g GIT) compared to Upper Lake Balma (mean 0.20 items/g GIT) (Mann-Whitney *U* test, $U = 18$; $p = 0.01$).

Spearman's rank correlation analysis revealed a significant positive correlation between items/individual and items/g GIT ($\rho = 0.916$; $p < 0.01$), age and weight ($\rho = 0.713$; $p < 0.01$), age and total length ($\rho = 0.787$; $p < 0.01$), total length and weight ($\rho = 0.940$; $p < 0.01$) (Fig. 4). A significant negative correlation was found between age and items/g GIT ($\rho = -0.740$; $p < 0.01$), weight and items/g GIT ($\rho = -0.967$; $p < 0.01$), total length and items/g GIT ($\rho = -0.899$; $p < 0.01$), age and items/individual ($\rho = -0.677$; $p < 0.01$), weight and items/individual ($\rho = -0.839$; $p < 0.01$), total length and items/individual ($\rho = -0.788$; $p < 0.01$) (Fig. 4).

Regression analysis showed a significant negative linear relationship between fish weight and number of items/g GIT ($F = 47.11$; $DFn = 1$; $DFd = 18$; $p < 0.001$) (Fig. 5a). The same finding was observed for the relationship between age and number of items/g GIT ($F = 28.33$; $DFn = 1$; $DFd = 18$; $p < 0.001$) (Fig. 5b).

4. Discussion

Our findings suggest that sediment was the only abiotic compartment polluted by MP items. The literature reports a wide range of MPs abundance in the water samples from high-mountain lakes. For example, high levels of MPs can be found in tourist sites while Free et al. (2014) found about 20,264 items/km^2 in the remote Lake Hovsgol (Mongolia). Higher numbers (180,900 items/km^2) were also reported by Xiong et al. (2018) in remote Qinghai Lake (China). In contrast,

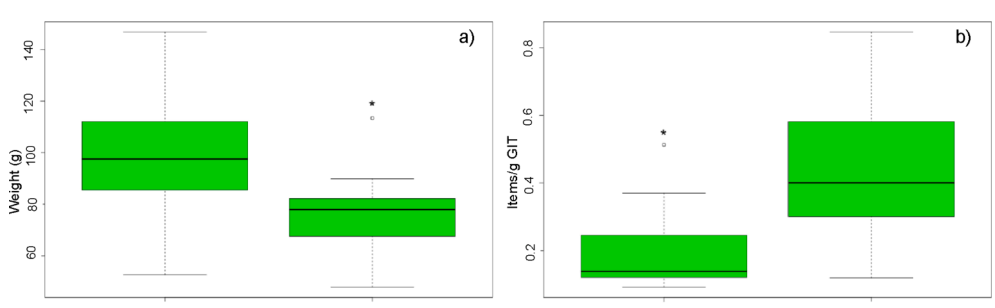

Fig. 3. (a) Boxplots of weight of *Salvelinus fontinalis* in both Upper (n = 11) and Lower (n = 9) Balma Lake in which microplastics items were recorded; (b) Boxplots of the number of microplastic items/g of gastrointestinal tract (GIT) recorded in *S. fontinalis* captured in Bama Lakes. Asterisk (*) denotes significant differences revealed by the Mann-Whitney *U* test.

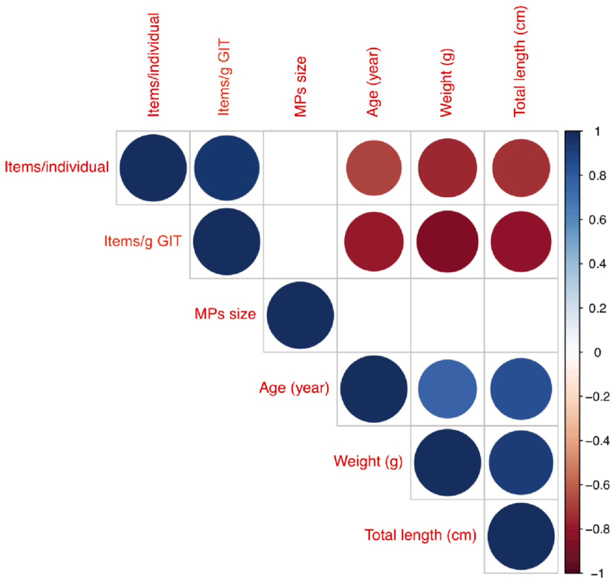

Fig. 4. Spearman's rank correlation analysis between biometrical features (weight, total length, and age) and abundance of microplastic items (number of items/g gastrointestinal tract-GIT and number of items/individual) in *Salvelinus fontinalis* (n = 9 and n = 11 for Lower and Upper Balma Lake, respectively). Larger dots indicate high correlation (positive in blue; negative in red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Malygina et al. (2021) found very few MP particles (5–8 particles/L) in two high-remote lakes (Lake Dzhulukul and Lake Talmetn) in Siberia (Russia). The absence of MP particles in the water samples of Dimon Lake (Carnic Alps) (Pastorino et al., 2021) was in line with the present study.

No MP particles were found in sediment samples from a high-mountain lake in the Carnic Alps (northeast Italy) (Pastorino et al., 2021). A range of 8–563 items/m^2 was found in six Tibetan Plateau high-mountain lakes (China) (Zhang et al., 2016). Xiong et al. (2018) reported a similar range (5.4–1292 items/m^2) for Lake Qinghai (Tibetan Plateau, China). A mean of 33 items/kg was found in a high-mountain lake (Sassolo Lake) in Switzerland (Negrete Velasco et al., 2020). A mean of 606 items/kg was recorded for Lake Anchar (Himalaya, India) (Neelavannan et al., 2021). Liang et al. (2022) reported higher values (mean 2644 items/kg) compared to the lakes mentioned above.

MPs in the biotic samples were noted for tadpoles and fish, whereas the zooplankton (copepods) tested negative. This finding could be explained by the fact that we found no MP items in the water samples and by their feeding behavior. Experimental and field studies have shown that zooplankton, copepods included, can ingest MP particles (Coppock et al., 2019). In addition, copepods are noted to discriminate between their natural prey (phytoplankton) and MP items. Xu et al. (2022) demonstrated that *Temora longicornis* (Copepoda) reject about 80% of the MP particles after putting their mouths on them. MPs rejection rates were high regardless of polymer type, shape, presence of biofilm or adsorbed in a pollutant (pyrene), indicating that MPs are unpalatable to feeding-current copepods and that post-capture taste discrimination is a major sensorial mechanism in MP rejection (Xu et al., 2022).

The tadpoles (*Rana temporaria*) from both lakes were found to contain MPs. In their previous study, Pastorino et al. (2022a) reported MP items (1 item/individual; PA 60%, 20% PP, 20% PET) in adult frogs of this species captured from Selleries Pond (Cottian Alps) but no items in tadpoles, suggesting that the MPs source might be related to the terrestrial environment. There exist no other studies for direct comparison with other mountain lakes, though previous studies on species in lowland environments reported a higher number of MP items compared to our study. For example, Karaoğlu and Gül (2020) recorded about 302.62–306.69 items/g in tadpoles (*Pelophylax ridibundus* and *Rana macrocnemis*) inhabiting small waterbodies in Rize (northeast Turkey). MPs in tadpoles can have negative effects on their growth and development. Boyero et al. (2020) reported negative effects of polystyrene MPs on the survival and the body condition of *Alytes obstetricans*. A future area of focus is to assess the negative effects of MPs exposure in tadpoles (*R. temporaria*), given the importance of this species for mountain ecosystems.

The gastrointestinal tracts (GIT) of *Salvelinus fontinalis* from both lakes contained MPs. The MP items recorded in *S. fontinalis* had the same shape, color, size, and chemical type as those recorded in the abiotic compartments of the lakes, which suggests this species as a good candidate sentinel for MPs pollution in high-mountain lakes. There are few studies on MPs in salmonids in mountain areas, however, the number of MP items/individual we found was similar to that reported by previous studies. Driscoll et al. (2021) reported 1 to 3 MP items/individual in *Oncorhynchus clarkii bouvieri* and *Salvelinus namaycush* from the high-mountain Lake Yellowstone (North America). In their prevalence study on MPs in *Salmo trutta* from a mountain river catchment (River Slaney) in south-east Ireland, O'Connor et al. (2020) reported 1.88 ± 1.53 MPs/individual.

Analysis of the relationship between MP content in the GIT and the biometrical features of *S. fontinalis* showed a negative correlation between fish size (weight and total length) and MPs content in the GIT. This observation was confirmed by linear regression analysis, which demonstrated a clear negative trend for these two variables.

Linear regression model has been used to determine the relationship between MP content in fish and their size (Parvin et al., 2021; Sultan et al., 2023). For example, Sultan et al. (2023) investigated MPs

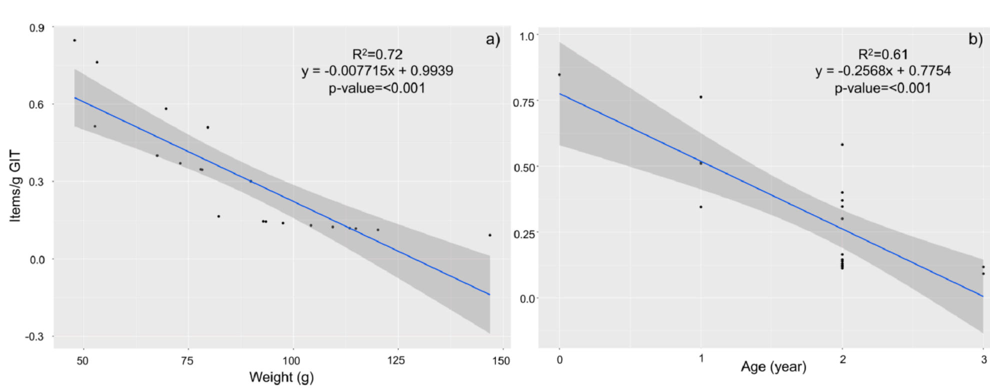

Fig. 5. Simple linear regression model between weight (a) or age (b) and microplastic abundance (n. items/g of gastrointestinal tract-GIT) in *Salvelinus fontinalis*. The blue line represents the regression line; the grey zone represents the 95% confidence interval. It is also reported the coefficient of determinations (R^2), the equations, and the p-values of the two models. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

abundance in the GIT of 20 estuarine species and found a negative relationship between MPs abundance in the GIT (MP items/g) and fish weight. This could be due to feed ingestion rate (metabolic rate) and prey selectivity, which depend largely on fish age (Lawson et al., 2018). Juvenile fish generally consume more food than adults since they feed on less energy-dense prey but have higher energy needs to meet rapid growth rates (Lawson et al., 2018). For example, *S. fontinalis* feed on terrestrial insects (>80% of food intake) (Pastorino et al., 2020b), however, we have no information about MPs abundance in such organisms to date.

Polypropylene (PP) and polyethylene (PE) were the two main chemical types of MPs found in the biotic and the abiotic compartment of the lakes. This finding is shared by previous studies on water and sediment samples from high-mountain lakes (Zhang et al., 2016; Xiong et al., 2018; Çomaklı et al., 2020; Negrete Velasco et al., 2020; Bulbul et al., 2023). Polypropylene is one of the most used materials in making plastics for a wide range of applications (packaging, equipment parts, textiles). Owing to its low density (0.90 g/cm^3), winds carry particles far from the source of production. Together with PE, it accounts for the largest share of MPs pollutants in lakes worldwide (Cera et al., 2020).

Only white, blue, and black colored MPs items were found in the biotic and the abiotic compartment of the lakes. These findings are in line with the colored items recorded for other high- mountain lakes. For example, Liang et al. (2022) studied MPs in sediment samples from 12 remote high-altitude lakes on the Tibetan Plateau (China) and found that black and blue particles accounted for more than 60% of all items recoded. Color is a useful criterion to pinpoint potential sources of MP items (Huang and Xu, 2022). For example, white MPs derive from packaging or shopping bags, while blue or other colored items derive from clothes or fish nets. Black MP items may derive from a variety of sources (packaging, cooking tools, toys) and constitute about 15% of total domestic waste (Turner, 2018).

The MP items we recorded in the two compartments were <150 μm. Particles 1000–5000 μm in size were common in sediment samples from seven remote Tibetan Plateau lakes (China) (Zhang et al., 2016). MP particles (50–500 μm) were recorded in sediment samples from twelve remote Tibetan Plateau lakes (Liang et al., 2022). Similar findings were shared by Xiong et al. (2018) who reported MP items 100–500 μm in size in water and sediment samples from high-altitude Lake Qinghai (China) and by Negrete Velasco et al. (2020) who reported particles 125–500 μm in size from Lake Sassolo (Switzerland). These sizes are similar to those we recorded for the two lakes. An item measuring 220 μm was found in the snow samples collected from the shore of a remote lake (Dimon Lake) in the Carnic Alps (northeast Italy) (Pastorino et al., 2021). Smaller MP particles (8–15 μm) were found in sediment samples from Crater Lake (Turkey) (Çomaklı et al., 2020).

The MP items recorded in the biotic and the abiotic compartment of the two lakes were usually fibers and fragments. Although the exact source of the fragments is not clear, our hypothesis is that they derived from degradation of larger particles and that their surface (rough or smooth) resulted from the effects of exposure to UV radiation (Luo et al., 2022). High-mountain lakes are an oligotrophic or ultraoligotrophic environment with high UV radiation penetration that can accelerate plastic degradation and fragmentation (Sommaruga, 2001). But because high-mountain lakes are covered by snow from November to May, degradation is very slow, so larger items tend to persist in the environment.

MP fibers can derive from recreational activities such as fishing and trekking. The two lakes under study are a popular destination for hikers and fishermen. The polyethylene fibers from tourist apparel can be easily released by abrasion into the environment and transported by wind into the lake catchment. This hypothesis is shared by Xiong et al. (2018) who found a high MP fiber concentration in the remote Lake Qinghai (China), which is popular with tourists and acts as a sink of MP particles due to the lack of lake water outflow. In addition, fishing, and waste disposal were the main sources of MPs pollution in two remote Siberian lakes (Malygina et al., 2021) with no permanent population. Finally, Neelavannan et al. (2021) reported that the high-altitude Himalayan Lake Anchar has a complex source of MPs derived from the automotive, the textile, and the packaging industry.

Although recreational trekking and fishing could be a source of MPs in the two lakes, it is more likely that atmospheric deposition (rain, snow) or wind is the major source of MP items Because of their diverse sources (urban, rural), atmospheric MPs vary greatly in size and chemical composition (Allen et al., 2019). Liang et al. (2022) suggested that long-range transport is the main source of MPs in remote lakes in Tibet. This observation was shared by Çomaklı et al. (2020) who found MP particles in Lake Crater (Turkey), located far from local settlements. Since the particles have a low specific density, wind currents transporting MPs cause mechanical erosion in the Tibetan Plateau (Zhang et al., 2016). In brief, MPs can be easy transported far from the source of origin via wind currents and then released in mountain areas during precipitation events.

5. Conclusions

This study reports for the first time a complete assessment of the MPs presence in the biotic and the abiotic compartment of two high-mountain lakes. Although the number of MP items in the samples (sediment, tadpoles, fish) was low, evidence suggests long-range transport, wind, and tourism as potential sources of MPs contamination in these remote ecosystems. Furthermore, our findings suggest that MPs contamination was greater in young fish due to the higher ingestion rate compared to older fish. *Salvelius fontinalis* may be considered a good bioindicator of MPs pollution in such environments. Since most high-mountain lakes were originally fishless, fish could be captured for monitoring MPs in high-mountain environments. Further studies are needed to investigate the effects of MPs on the development and growth of tadpoles, which could influence frog population structure and dynamics and their place in the trophic chain of high-mountain ecosystems. Finally, further studies are desirable to better understand the source and the dynamics of MPs in high-mountain lakes.

CRediT authorship contribution statement

Paolo Pastorino: Writing – original draft, Investigation, Conceptualization, Methodology, Data curation, Writing – review & editing, Funding acquisition. **Serena Anselmi:** Investigation, Conceptualization, Methodology, Writing – review & editing. **Giuseppe Esposito:** Investigation, Methodology, Writing – review & editing. **Marco Bertoli:** Investigation, Methodology, Writing – review & editing. **Elisabetta Pizzul:** Investigation, Methodology, Writing – review & editing. **Damià Barceló:** Conceptualization, Methodology, Writing – review & editing. **Antonia Concetta Elia:** Investigation, Methodology, Writing – review & editing. **Alessandro Dondo:** Investigation, Methodology, Writing – review & editing. **Marino Prearo:** Investigation, Methodology, Writing – review & editing, Supervision. **Monia Renzi:** Investigation, Conceptualization, Methodology, Writing – review & editing, Supervision.

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.

Data availability

Data will be made available on request.

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