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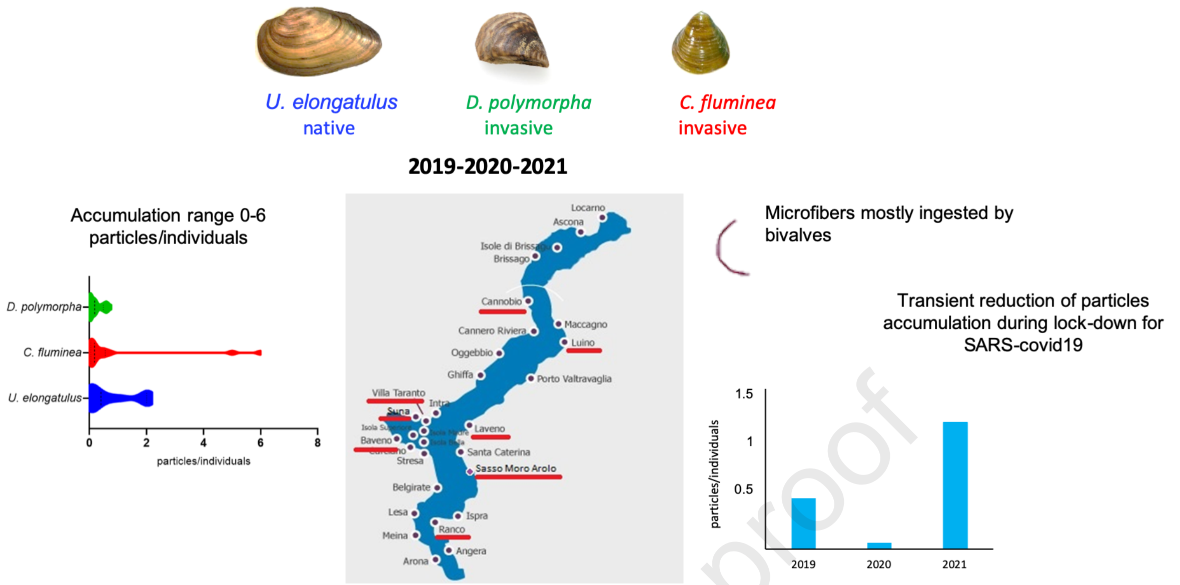
U. elongatulus
native
D. polymorpha
invasive
C. fluminea
invasive
2019-2020-2021
Accumulation range 0-6 particles/individuals
D. polymorpha
C. fluminea
U. elongatulus
0
2
4
6
8
particles/individuals
Locarno
Ascona
Brissago
Cannobio
Cannero Riviera
Maccagno
Luino
Oggebbio
Ghiffa
Porto Valtravaglia
Villa Taranto
Intra
Laveno
Baveno
Stresa
Santa Caterina
Sasso Moro Arolo
Belgirate
Lesa
Ispra
Meina
Ranco
Angera
Arona
Microfibers mostly ingested by bivalves
Transient reduction of particles accumulation during lock-down for SARS-covid19
particles/individuals
1.5
1
0.5
2019
2020
2021

First comparative assessment of contamination by plastics and *non*-synthetic particles in three bivalve species from an Italian sub-alpine lake

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Abstract

This study aimed to compare the contamination from plastics and *non*-synthetic particles in the three freshwater bivalve mollusks *Unio elongatulus*, (native) and *Corbicula fluminea* and *Dreissena polymorpha* (invasive), collected in Lake Maggiore, the second greatest Italian lake. Organisms were collected from eight sites located throughout the lake, during three years (2019-2021). The quali-quantitative characterization of particles has been carried out using a Fourier Transform Infrared Microscope System (µFT-IR). Results showed that both plastics and *non*-synthetic particles released in the water are taken up by bivalves, even though low intake-up to 6 particles/individuals-were measured for all the three species. Microfibers of both synthetic (polyester, polyamide) and natural (cellulose) origin represented the particles mostly ingested by bivalves. A significant decrease of particle loads was observed in 2020 with respect to 2019 and 2021, significantly different for *D. polymorpha* and *U. elongatulus*, suggesting a transient reduction of the particle release in the lake in this year. Our findings highlight the need to improve the understanding of the mechanisms of uptake and clearance of these contaminants by filter feeding organisms, and their adverse consequences in realistic environmental conditions.

Keywords: freshwater bivalves, anthropogenic particles, plastics, fibers, ecotoxicology

1. Introduction

Plastic contamination represents one of the most worrisome environmental issues of the last decades. The huge demand of plastic-based products in several applications as packaging, building and constructions, automotive, electronics etc. (Plastics Europe, 2022) results in a voluntary or accidental release of plastics into the environment, with negative impacts for natural ecosystems (Barnes et al., 2009). Among aquatic environments, lentic ecosystems are highly susceptible to this contamination due to the high residence time of water and the release of plastic debris from soil leaching, road runoff, household waste, wastewater treatment plants or industrial discharges and by the atmospheric transport (Dusauchi et al., 2021), to the point that plastics have been detected also in remote lake basins (Free et al., 2014; Negrete Velasco et al., 2020).

Once released in the aquatic environment, it is difficult to predict the adverse outcomes of plastics to biota, since plastics may have a different fate in relation to chemical-physical characteristics (floating or sinking) and trigger different negative effects in relation to size, shape, polymer composition, color and the capability to transport other environmental contaminants (Paul-Pont et al., 2018).

In general, the adverse consequences of large plastic items and mesoplastics (items in the range from 1 to <10 mm according to Hartmann et al., 2019) are entanglement, wounds, suffocation, blockage of digestive tract, starvation and death (Gregory, 2009). Moreover, plastics fragmentation into smaller pieces - microplastics (MPs) for items in the range from 1µm to < 1mm- and nanoplastics for items < 1 µm (NPs) (Hartmann et al., 2019) - elicit a huge release in the environment of plastic particles that can be ingested by a broad range of aquatic taxa (from zooplankton to birds), can be transferred among different trophic levels and can trigger different adverse consequences (Nelms et al., 2018). The effects of MPs span from molecular and cellular effects to systemic effects up to the impairment of feeding, growth, locomotion and reproduction (Franzellitti et al., 2019 and citations therein). A further concern is also related to the release of harmful chemicals (additives and plasticizers) from plastics (Franzellitti et al., 2019) and the capability of plastics to host microbial communities, the so-called ‘Plastisphere’, potentially increasing the spread of pathogens (Gregory, 2009; Zettler et al., 2013).

In addition to plastics *sensu stricto*, recently emerged as potentially harmful the presence in the environment of natural origin textile fibers (e.g. cotton), regenerated cellulose fibers (e.g. rayon) and artificial fibers (e.g. viscose), since microfibers represent the dominant form in the aquatic environment (De Falco et al., 2020). Their adverse effects, still largely understudied, include physical damages and toxicity related to chemical additives and dyes (Acharia et al., 2021 and citations therein; Athey and Erdle 2022 and citations therein). Therefore, it is important to assess the risk related to the presence of these anthropogenic particles by increasing current knowledge about the levels of these debris in the environment, as well as their bioavailability for biota and the resulting toxicity.

Except for a few invasive species, freshwater bivalves are experiencing a pronounced global decline due to their susceptibility to environmental and anthropogenic disturbances, including pollution (Lopes-Lima et al., 2018). Bivalves are recognized as a useful bioindicator of environmental quality due to their high filtration rate and accumulation of chemical pollutants (Zieritz et al., 2022). Instead, the use of bivalves as bioindicators of plastic contamination is currently a matter of debate (e.g., Zhao et al., 2018; Miller et al., 2019; Ward et al., 2019; Multisanti et al., 2022). Several studies showed that bivalves can take up and retain small plastic particles in their tissues (Ringwood, 2021; Martyniuk, 2022). Ingested plastics are able to translocate through the hemolymph (Browne et al., 2008; Magni et al., 2018; Wang et al., 2021) and reach internal tissues as gonads, mantle, foot (Kolandhasamy et al., 2018) and byssus (Li et al., 2019a). In line with these evidences, Su and collaborators (2018) measured plastic level in the freshwater bivalve *Corbicula fluminea* from Taihu Lake, China, reporting values from 0.3 to 5 particles/individuals. The authors highlighted a positive correlation between plastics in clams with respect to sediment levels, thus suggesting that the species can be considered a good bioindicator of plastic contamination. Nevertheless, several studies showed that bivalves have a species-specific selective uptake mechanism and that ingested plastics have a limited retention time, conditions that limit their suitability as biomonitors of the contamination in aquatic environments (Ward et al., 2019a, b).

Lake Maggiore is a pre-alpine lake of fluvioglacial origin with the largest perimeter among the Italian lakes. The lake houses 4 native Unionid mussels (*Anodonta exulcerata*, *A. cygnea*, *A. anatina*, *Unio elongatulus*) and 3 invasive bivalves (*Corbicula fluminea, Dreissena polymorpha*, *Sinanodonta woodiana*).

Previous studies reported the presence of plastic in surface water of Lake Maggiore up to 100,000 items/km^2 (Sighicelli et al., 2018; Binelli et al., 2020), whereas the levels of plastics in biota inhabiting the lake are limited to a study on perch (*Perca fluviatilis*; Galafassi et al., 2021). By contrast, unknown is the level of accumulation of MPs in the lower trophic levels and their distribution in the different lake compartments.

Therefore, our study aimed to characterize the contamination of Lake Maggiore from anthropogenic particles using filter feeding organisms, namely the bivalve species *U. elongatulus, C. fluminea* and *D. polymorpha* that for their benthic habit and limited mobility can be used to map the distribution of such contaminants in the environment. *Unio elongatulus* (Pfeiffer, 1825) is a generalist native freshwater mussel species, common in both lotic and lentic habitats of northern Italy (Froufe et al., 2017). Although no IUCN conservation status assessment exists for this species in Italy (Prié and Puillandre, 2014), it is protected under the Habitats Directive (Annex V) and the Bern Convention (Annex III). The Asian clam *Corbicula fluminea* (Müller, 1774) original from Southeast Asia, and *Dreissena polymorpha* (Pallas, 1771) native from the Ponto-Caspian region, are extremely versatile invaders in freshwater habitats worldwide (Ilarri and Sousa, 2012; Sousa et al., 2014; Crespo et al., 2015). In recent decades, these both invasive bivalves colonized lotic and lentic habitats in several continents, where attained huge densities and wide spatial distribution (Sousa et al., 2008; Strayer, 2009).

Our monitoring considered not only plastics debris, but also artificial particles manufactured from natural material (mostly fibers of textile origin), since they represent a large portion of anthropogenic debris released in aquatic systems, and are able of exerting adverse effects to biota (Lusher et al., 2013; Remì et al., 2015; Compa et al., 2018; Gago et al., 2018; Acharia et al., 2021; Athey and Erdle, 2022; Muraro et al., 2022).

Given the know species-specific mechanisms of particle uptake and disposition in bivalves, a first aim was to evaluate potential differences in contamination level among the three species. Our hypothesis is that the species might accumulate particles differently, in relation to their ecological and physiological features. Since Lake Maggiore is characterized by different local anthropogenic pressures and level of chemical contamination (Guzzella et al., 2018), bivalves were collected in several sampling sites located throughout the lake, to point out the potential presence of local point-source of particle contamination. Furthermore, the bivalve sampling was carried out in the three-year period 2019-2021, to evaluate potential fluctuation in the accumulation over time.

2. Material and Methods

2.1 Sampling

Specimens of the three species were collected in May-June from 2019 to 2021 by scuba divers at 4–6 m of depth at different sampling sites selected according to environmental conditions, morphometric characteristics, and anthropic impact (Fig. 1). Specimens were washed with lake water, transported to the laboratory in aluminium sheets in refrigerated bags, and frozen at -20 °C prior to processing for plastic characterization. An overview of number and size of collected bivalves is shown in table 1.

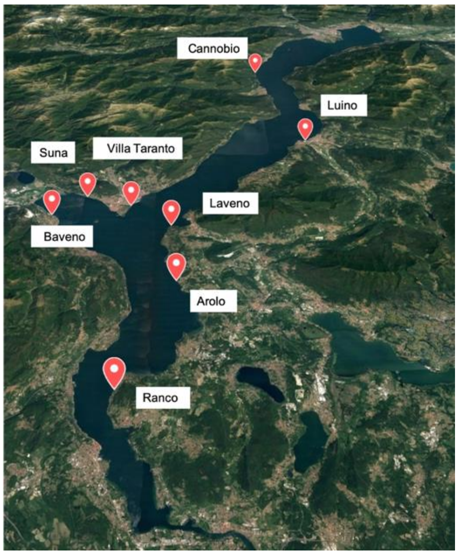

Fig. 1 Map showing sampling sites

Table 1. Number of individuals of the three species collected, size, total particles identified and the number of particles/individuals in 2019, 2020 and 2021.

Year	Species	Individuals collected	Mean size (cm) ± s.d.	Total plastics	Total natural particles	% plastics	particles/individuals ± s.d.
2019	*C. fluminea*	7	1.1 ± 0.2	0	5	0	0.71
	D. polymorpha	236	1.3 ± 0.3	7	41	17	0.21 ± 0.16
	U. elongatulus	45	7.4 ± 3.1	4	17	23	0.74 ± 0.77
2020	*C. fluminea*	67	2.9 ± 1.5	5	3	63	0.17 ± 0.18
	D. polymorpha	288	1.3 ± 0.1	1	2	33	0.02 ± 0.01
	U. elongatulus	86	5.7 ± 0.6	3	0	100	0.04 ± 0.06
2021	*C. fluminea*	77	2.5 ± 0.5	5	34	13	1.78 ± 2.47
	D. polymorpha	240	1.7 ± 0.2	16	89	18	0.44 ± 0.20
	U. elongatulus	43	5.8 ± 0.6	9	51	15	1.39 ± 0.62

2.2 Extraction of plastics from soft tissues

The separation of plastics from the collected samples was carried out using a sodium chloride (NaCl) hypersaline solution, which allows the separation of plastics from the organic matter by exploiting the generated density gradient (Binelli et al., 2020). All individuals were pooled and homogenized, using a Potter-Elvehjem Tissue Homogenizer in 50 mL of NaCl hypersaline solution (1.2 g/cm^3).

Homogenates were then transferred to 50 mL glass bottles, subsequentially filled with hypersaline solution.

The glass bottles were left for 3-4 days at 4 °C to allow an optimal separation of plastics from the organic matter. The supernatants were then filtered using a vacuum pump and 8 μm cellulose nitrate membrane filters (Sartorius™ 50 mm). The filters were then treated with 15 % hydrogen peroxide (H_2O_2) to complete the digestion of the organic residues left, keeping the filters under a laminar flow hood to avoid the potential contamination of the samples by microfibers.

To monitor the eventual atmospheric contamination by plastics (especially fibers), nitrate cellulose membrane filters were also processed in parallel to samples during homogenization and filtration process and debris detected in this filter were subtracted from the final count (Binelli et al., 2020; Magni et al., 2021, 2022). The particles eventually identified in blanks are provided in table S1.

2.3 μFT-IR analysis

The digested filters were visually analysed using a stereomicroscope to select the suspected plastic particles. These particles were transferred to a clean filter in order to carry out a chemical characterization that confirmed their plastic nature. All particles (natural and synthetic) extracted by the bivalves were quantified and their size, shape, color and polymer composition were characterized using a Fourier Transform Infrared Microscope System (μFT-IR; Spotlight 200i equipped with Spectrum Two, PerkinElmer). Spectra of particles was acquired in attenuated total reflectance (ATR) mode, compared with library standard spectra and accepted only if matching factor ≥ 0.70 (Binelli et al., 2020).

2.4 Statistical analysis

To evaluate statistically significant differences in plastics accumulation, data were statistically analyzed using GraphPad Prism 8.0.2 software package. One-way analysis of variance (ANOVA) was applied to verify the presence of statistically significant differences in the extent of particles accumulation/individuals, comparing the years of sampling and the different species. The same analysis was applied to assess the amounts of particles/individuals measured at each site integrating the three years of sampling, for each species. The significant differences were identified by the Tukey post-hoc test, considering $p \leq 0.05$ as significant cut-off. Due to the fact that only one sample was available for C. *fluminea* in 2019, the comparison among years was made excluding 2019 (through T-test), while comparison among sites was not carried out for this species.

3. Results

A total of 292 particles were analyzed from 1050 individuals sampled in the lake during the three-year period. Among them, 50 debris were identified as plastics, while the remaining were particles of natural origin (mostly cellulose) (Detailed information in SI).

Comparing the uptake of particles in the three different species, fewer particles were found in *D. polymorpha*, with a range from 0 to 0.77 particles/individual, while for the other two species a wider range was observed: from 0 to 6 particles/individual in *C. fluminea* and from 0 to 2.2 particles/individual in *U. elongatulus* (Fig. 2A). Anyway, due to the wide variability in uptake, the differences between the species were not statistically significant (ANOVA: $p = 0.1924$ $F_{(2,55)} = 1.698$). The size distribution of the particles accumulated was also similar, even though particles of bigger dimensions were found in *C. fluminea* (up to 10.7 mm) (Fig. 2B).

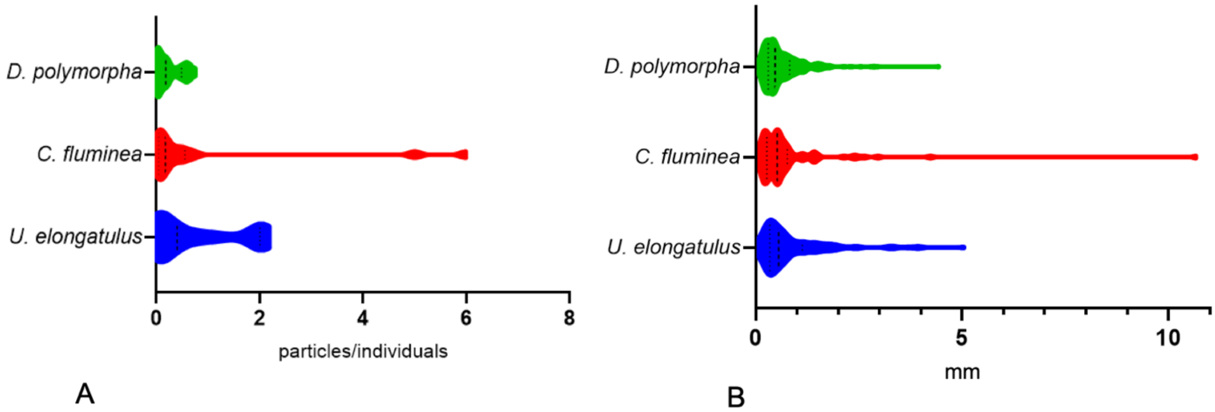

Fig. 2. Distribution of the number of particles accumulated (A) and particle size (B) in the three species

Comparing the extent of accumulation in the different sites during the three years of monitoring, no significant difference was observed for *D. polymorpha* (ANOVA: $p = 0.8663$ $F_{(7,14)} = 0.432$) and for *U. elongatulus* (ANOVA: $p = 0.9647$ $F_{(7,14)} = 0.2476$) (Fig. 3)

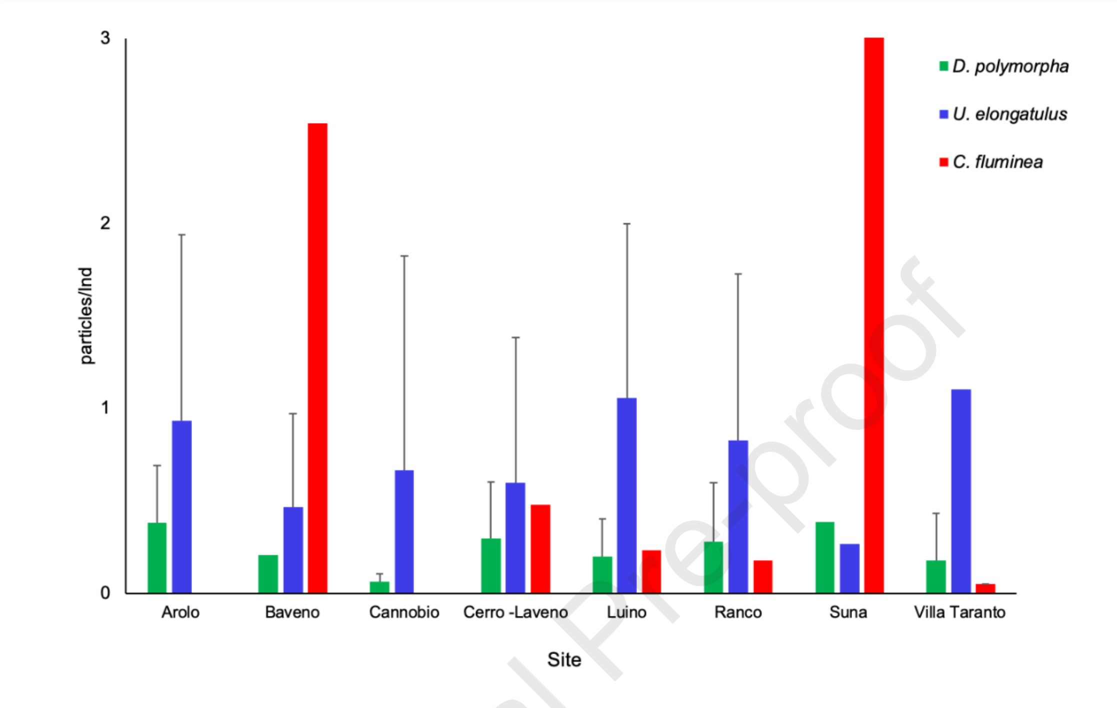

Fig. 3. Particles identified in the three bivalve species collected in the different sites of Lake Maggiore. Data are expressed as mean + s.d. of particles/individuals collected in 2019, 2020 and 2021.

Taking in consideration the trend of uptake over the three years for the three species, the amounts of particles measured in 2019 was 0.71 particles/individual in *C. fluminea*, which was collected only at Villa Taranto (Table S2). In *D. polymorpha* the amount ranged from 0.06 to 0.55 particles/individual, while in *U. elongatulus* the range was from 0.2 to 2 particles/individual (Table S2). The particle uptake decreased clearly in 2020. Indeed, the number of particles measured in *C. fluminea* ranged from 0 to 0.5 particles/individual, in *D. polymorpha* from 0 to 0.03 particles/individual, while in *U. elongatulus* from 0 to 0.13 particles/individual. In 2021 the accumulation raised to values similar to those measured in 2019, ranging in *C. fluminea* from 0 to 6 particles/individual, in *D. polymorpha* from 0.07 to 0.77 particles/individual and in *U. elongatulus* from 0.4 to 2.2 particles/individual (Table S2).

The temporal trend in the uptake is shown in Figure 4. A significant difference in the extent of particle accumulation/individual among different years was observed in *D. polymorpha* (ANOVA: $p = 0.0002$ $F_{2,19} = 14.37$) and in *U. elongatulus* (ANOVA: $p = 0.00102$ $F_{2,19} = 13.67$). A similar temporal profile of uptake was observed also in *C. fluminea*, albeit not statistically significant (T test: $p = 1.681$ df 6.066).

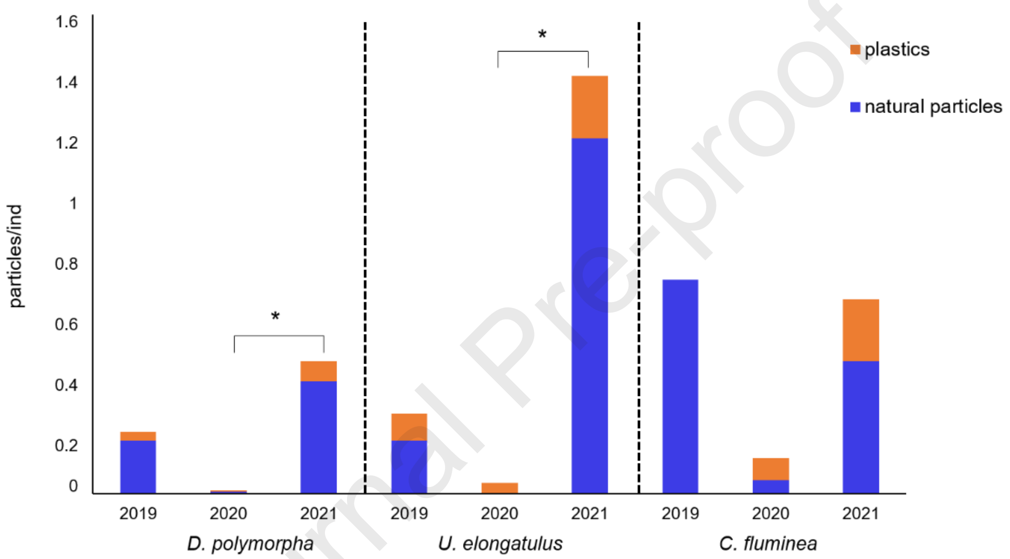

Fig. 4. Particles identified in the three bivalve species collected in the Lake Maggiore in 2019, 2020 and 2021. Asterisk indicates statistically significant differences in particles accumulation among different years in each species $p \leq 0.05$ (ANOVA followed by Tuckey post-hoc test; for C. fluminea only 2020 and 221 were compared).

The percentage of plastic particles with respect to natural ones was quite similar in the three species in 2019 and 2021, whereas the percentage of plastics was higher and more variable among species in 2020 (Table 1).

Regarding the qualitative characterization of the observed plastics (Fig. 5), the size was defined according to the classification recommended by Hartmann et al. (2019): macroplastics (≥ 1 cm), mesoplastics (from 1 mm to 10 mm), MPs (from 1 µm to 1 mm) and NPs (< 1 µm). In all the three years, the largest fraction corresponded to microplastics, ranging from 67 to 78 %, while the remnants were mesoplastics. As for the shape, in 2019 and 2021 most of the plastics observed were made of fibers, accounting for 100 % and 97 % in the two years, respectively, whereas 67 % was represented by fragments and 33 % by fibers in 2020. The polymer composition of the plastics found was in line with the shape, in fact in 2019 and 2021 the most abundant polymers were polyester (PEST), presented a percentage of 67 and 70 % in the two years, respectively, and polyacrylate (PAK) with a percentage of 25 and 20 % in the two years, respectively. Furthermore, polyamide (PA) was identified in 8 % in 2019, while in 2021 the 3 % of plastics was made up of PA, polypropylene (PP) and polystyrene (PS). In 2020, the percentage of PEST reached 56 %, PA and PP accounted for 11 % and only in this year were identified plastics made of polyethylene (PE), accounting for 22 % of the total. The color also showed similarities between 2019 and 2020, where black and transparent were the predominant colors, followed by blue and red (Fig. 5). Conversely, the most found color in 2020 was orange (33 %), followed by red and transparent (22 %) and to a lesser extent black and purple (11 %).

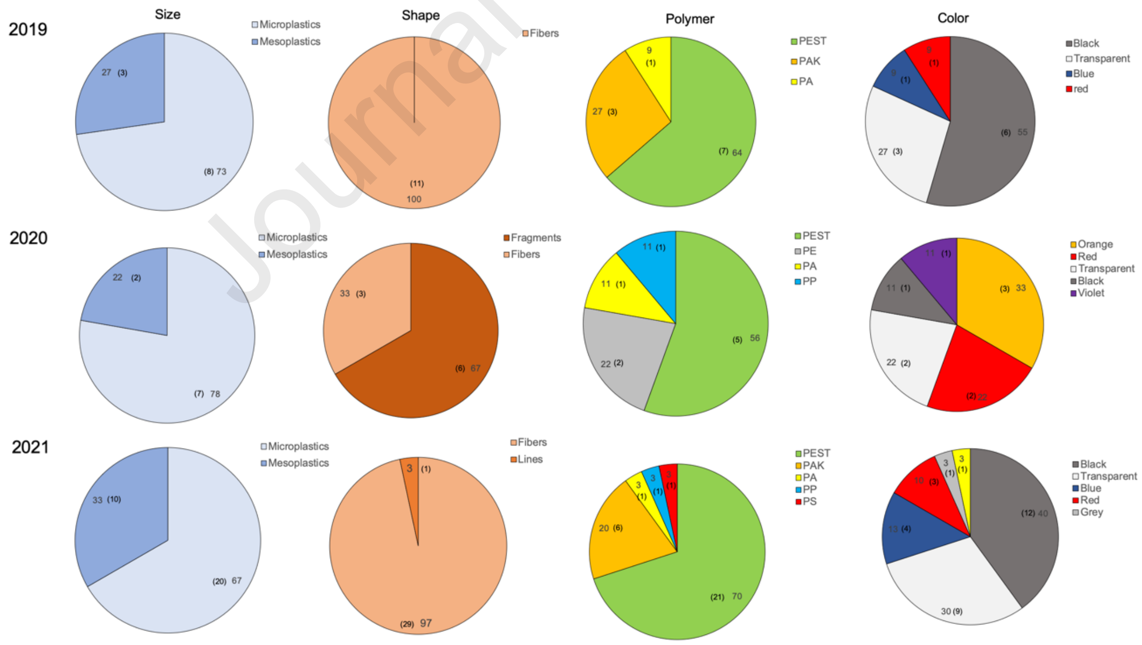

Fig. 5. Size, shape, polymer composition and color of plastics identified in the three species in 2019, 2020 and 2021. The percentage is shown together with the number of items considered, showed in brackets.

4. Discussion

The objective of this study was to improve the current knowledge regarding the contamination by plastics and *non*-synthetic particles in the biota of lentic environments. Bivalves were chosen as sentinel species, given their ecological role within freshwater ecosystems, as well as their global vulnerability to anthropogenic and environmental pressures (Zieritz et al., 2022).

Our first aim was to evaluate potential species-specific differences in particle accumulation. Despite the extremely wide variability range in number and size of ingested particles, *D. polymorpha* appears to accumulate fewer particles and of smaller size compared to the other two species. Filter-feeding bivalves are selective feeders, and a given species will only filter and remove particulate matter of an appropriate size which is dependent on the size of the species and on the structure of its filtration apparatus. Therefore, differences in size (5-22 mm for *D. polymorpha*, 5-35 mm for *C. fluminea*, 42-81 mm for *U. elongatulus*) can justify the observed differences in accumulation. The variability in the accumulation capacity can be also justified by the different filtration rates: up to 4 L/ind/h in *C. fluminea*, up to 8 L/ind/h in *U. elongatulus* (Riccardi personal communication) and up to 0.5 L/ind/h in *D. polymorpha* (Binelli et al., 2014). Also, different habitat preferences can be expected to influence the uptake: while *D. polymorpha* use byssal threads to attach to hard substrates, *C. fluminea* and *U. elongatulus* live buried in sediments, and therefore, besides particles filtered from the water column, these species can ingest also particles resuspended from sediments. *Corbicula* further differs from *Unio* in its tendency to be more sunken in the sediment and in its pedal feeding capability (Hakenkamp and Palmer, 1999).

Overall, our results showed a low level of plastic contamination for all the three species regardless of the sampling site. The combined data of the three years for all species did not show significant differences in the levels of particle accumulation for the 8 sampling sites. This result suggests that organisms living close to more urbanized sites were not subjected to higher particle contamination. This result seems to confirm the plastic load measured in the surface waters of the lake in previous studies, which ranged from 39,000 plastics km^{-2} to 100,036 plastics km^{-2}, corresponding to 0.039 plastics/m^2-0.1 plastics/m^2, respectively (Sighicelli et al., 2018; Binelli et al., 2020). Our recent data on the surface waters of the Lake Maggiore measured in 2022 reported about 0.4 particles/m^3 (data non shown) therefore, based on the filtration rates reported above, *U. elongatulus* would assume a mean of 0.08 particles per day, *C. fluminea* 0.04 particles per day and *D. polymorpha* 0.005 particles per day. The occurrence of particles in mussels from the Lake Maggiore measured in the present study is in line with what has been reported by similar studies carried out in other lakes worldwide. A monitoring using the freshwater mussel *Lasmigona costata* collected in the Grand River Watershed, Canada, reported up to 7 MPs/individual (Wardlaw and Prosser, 2020). A very low number of MPs has been reported in *Unio pictorum* sampled downstream of a sewage treatment plant (Domogalla-Urbansky et al., 2019). In another monitoring study carried out on Lake Iseo, Northern Italy, *D. polymorpha* was used as bioindicator species and the items/individual ranged from 0.07 to 0.23 (Pastorino et al., 2021). The same species was also used in a monitoring of plastic contamination in some Danish Lakes and a very low accumulation (0.067 particles/10 individuals) has been reported (Kallenbach et al., 2022). Up to 1 MP/individual has been measured in the soft tissue of *D. polymorpha* from Turkish lakes (Gedik and Atasaral, 2022). A similarly low level of particle uptake (0.6 ± 1.3 s.e. particles/individuals) has been reported for this species after 60 days translocation in the Milwakee River, USA (Hoellein et al., 2021). It should be noted that, although the analytical procedure through μFT-IR analysis is one of the most sensitive methods for the characterization of such contamination, it does not allow to characterize the particles below 10 μm of size, thus providing a possible underestimation of the actual load of smallest particles inside the organisms.

Unlike chemical pollutants, the analysis of particle content in mussel soft tissues provides only a snapshot of items that are passing through the organism, and not of the material actually retained and accumulated over time. Indeed, despite the high filtration rate and efficiency to capture suspended particles of these organisms (Ringwood et al., 2021), anthropogenic particles seem to have a very low residence time in bivalves (hours to days), since these organisms are able to activate mechanisms of excretion and ejection through faeces and pseudofaeces (Ward et al., 2019a). In particular, bivalves are able to make a selection based on the chemical and physical features of the particles (i.e., shape, dimension, surface charge), being able to distinguish between high quality material, which will be internalized and poor debris, that will undergo rapid ejection through pseudofaeces (Ward et al., 2019b; Magni et al., 2020). Therefore, this might entail an underestimation of the effective environmental load of anthropogenic debris in the water system that are bioavailable and can interact with biota. However, the description of these processes has been carried out only on a limited number of species, mainly marine (Rosa et al., 2018 and citations therein). It is therefore fundamental to obtain more information to clarify species-specific mechanisms of uptake-distribution and extrusion/excretion of particles in bivalves, focusing especially on microfibers, which represent the majority of anthropogenic particles identified in aquatic organisms.

Despite the low levels detected in organisms, our results showed that bivalves can suffer the possible adverse effects of plastics and *non*-synthetic particles. This could interfere with the ecological functions provided by bivalves such as nutrient cycles, bioturbation and water filtering (Green et al., 2016; Christoforou et al., 2020; Pedersen et al., 2020). On the other hand, plastic incorporation into biodeposits generated by bivalves might modify the environmental fate and partitioning of particles in the aquatic environment (Van Colen et al., 2021). This might affect the integrity of freshwater ecosystems and the ecosystem services provided by bivalves. In this perspective, future investigations focusing on the potential impact of anthropogenic particles on the bivalve ecological functions are warranted, to achieve a broader evaluation of the environmental risk related to the presence of these contaminants in the aquatic ecosystems.

A further aim of our study was to evaluate if the extent of particle accumulation could change on a multi-year basis. The temporal trend of the particle levels in all the three species showed a clear decrease of particle loads of about ten-fold in 2020 with respect to 2019 and 2021. The sampling of organisms in 2020 took place immediately after a very strict lockdown imposed in Italy to contain the spread of SARS-CoV-2 pandemic. From March 2020 until May 2020, most of the population has been banned from traveling, ferry trips across the lake have been reduced and recreational boating has been forbidden. Industrial anthropogenic activities have also undergone a reduction. These limitations have reduced the anthropic load on Lake Maggiore which is located in a highly anthropized and touristic area. A recent study showed an increase of anthropogenic pressure on the lake during weekend and summer season, likely related to tourism and recreational activities (Vavassori et al., 2022). Since a positive correlation between MP contamination and population density has been documented (Yonkos et al., 2014; Eerkes_Medrano et al., 2015; Lebreton et al., 2017), we can assume that the abatement of the load of particles to the lake was caused by the anthropopause due to the lockdown. In fact, tourist presences decreased during 2020 by 53-57% in the Italian basin and by 59% in the Swiss basin (Istituto di Ricerche Economiche – Osservatorio del Turismo 2020; Osservatorio delò Turismo Verbano Cusio Ossola 2020; Bregonzo, 2020; Cavedo, 2021). However, the effects of the lockdown were transient and short-lived as particle levels in 2021 recovered to values similar to 2019. Anthropogenic particles can reach the lentic environments through riverine transport, urban and agricultural runoff, wastewaters, and atmospheric deposition (Dusaucy et al., 2021). Therefore, since the lake has a large catchment area (6,599 km^2) and several tributaries, it must be considered that other factors such as the fluctuations of the meteorological and hydrological conditions may have influenced the levels of contamination. Indeed, heavy annual fluctuations in the levels of plastics have been detected in the lake in previous years: 39,000 plastics/km^2 in 2016 (Sighicelli et al., 2018) against 100,036 plastics/km^2 in 2018 (Binelli et al., 2020). To confirm the impact of the SARS-CoV-2 pandemic restrictions on the levels of MP contamination, data on their water concentrations would be needed, and, possibly, in a long-term time interval to verify if 2020 levels represented actually an anomaly or whether such kind of fluctuations can occur under normal conditions.

Finally, the qualitative characterization of the particles highlighted that most of the plastics taken up by the organisms are MPs of secondary origin, linked to the fragmentation of woven clothes because most of them are made up of fibers of textile origin, both made of synthetic polymers such as PEST, PA and PAK. The input of anthropogenic fibers into aquatic systems is currently huge and mainly linked to wastewater treatment plants and household wastes (Gavigan et al., 2020). The evidence concerning the uptake of microfibers in aquatic organisms showed that it is necessary to increase current knowledge on the ecotoxicity of this specific MP shape, which is currently understudied with respect to pellets and fragments.

The different fingerprint of plastic contamination corroborates the hypothesis that SARS-CoV-2 pandemic may have influenced the plastic contamination of the lake, as already indicated by the evidence of a recent monitoring study carried out on some watercourses of Milan (Northern Italy, Binelli et al., 2022). In particular, PE fragments were detected only in 2019, while PAK fibers were identified in 2019 and 2021, but not in 2020. On the contrary, PP was detected only starting from 2020. This polymer is one of the main constituents of face masks, since PP-based microfibers are non-allergenic and hydrophobic (Czigány and Ronkay, 2020; Rathinamoorthy and Balasaraswathi, 2022). The massive use during the pandemic and thereafter throughout 2021 likely contributed to the introduction of PP microfibers into the lake. Indeed, if unproperly disposed, such litters can persist in the environment, potentially fragmenting into MPs thus representing a source of plastic contamination for aquatic ecosystems (Allison et al., 2020).

The plastics that are most detected through the analysis of surface water and sediments in lakes are fibers and fragments of PS and PP (Dusaucy et al., 2021). At the surface water level, these types of low-density polymers tend to remain floating and available for pelagic organisms, while the biota residing in the benthic compartment has greater availability of particles with higher density (Paul-Pont et al., 2018). Moreover, once in the environment, anthropic particles undergo a series of environmental modifications such as weathering and (bio)fouling, that influence the fate and bioavailability of plastics (Galloway et al., 2017). Finally, physical chemical properties of particles emerged as a key factor that influence the uptake/release by bivalves (Ward et al., 2019 a; b). For instance, PEST fibers were more prone to be accumulated by *C. fluminea* with respect to other microfibers under laboratory conditions (Li ei al., 2019b).

As final remark, we can point out that the choice of the environmental matrix to be monitored for plastic contamination is based on the information to be obtained, since while water and sediments allow the evaluation of the water system pollution, the identification and characterization of plastics present in the organisms instead allows to highlight the different bioavailability of these physical contaminants and the possibility of their transport along the food web.

5. Conclusions

Our study provided first comparison in the contamination levels by plastics and *non*-synthetic particles in native and invasive species of bivalve mollusks. Results pointed out that particles released in the water ecosystems can be taken up by bivalves and might impacts on the ecological functions provided by these organisms. Microfibers represent the anthropogenic particles mostly ingested by bivalves and more studies are warranted to increase knowledge on their toxicity on aquatic organisms. The load of particles measured in bivalve soft tissues seems in line with the levels observed in other studies and indicates the absence of point-source release of particles in the lake. The transient reduction in the amount of particles detected in all species in 2020 might be related to the restrictions to counteract the spread of SARS-CoV-2 pandemic that may have reduced temporarily the release of anthropogenic particles in Lake Maggiore, but other environmental factors might be responsible of such decline. On the contrary, the widespread use of medical mask and their improper dispersion can generate a long-term impact on the quantity/quality of particles load to aquatic environments. Our study underlines the need to increase the current knowledge on the presence of anthropogenic particles in freshwater ecosystems, as well as to improve the understanding of the mechanisms of uptake and clearance of these contaminants by filter-feeding organisms and their adverse consequences in realistic environmental conditions.

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Highlights

Pollution by plastics and non-synthetic particles in freshwater bivalves
Microfibers represent the anthropogenic particles mostly ingested by bivalves
Low accumulation of particles for all the three species regardless the sampling site
Transient reduction of particles accumulation during lock-down for SARS-covid19

Author's contribution:

CDT, NR, AB conceptualization; NR, VM performed the field samplings; CDT, MF performed the experimental work; SM, data elaboration; AB and NR provided resources; all Authors contributed to writing and revision of the text and figures.

Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: