Microplastic pollution in perch (*Perca fluviatilis,* Linnaeus 1758) from Italian south-alpine lakes

Silvia Galafassi[1], Maria Sighicelli[2], Antonio Pusceddu[3], Roberta Bettinetti[4], Alessandro Cau[3], Maria Eleonora Temperini[5], Raymond Gillibert[5], Michele Ortolani [5], Loris Pietrelli[6], Silvia Zaupa[1], Pietro Volta[1]

[1]CNR Water Research Institute, L.go Tonolli 50, 28922 Verbania Pallanza

[2]ENEA, Department for Sustainability (SSPT), C.R. Casaccia-Via Anguillarese 301, 00123 Rome

[3]University of Cagliari, Department of Life and Environmental Sciences, via T. Fiorelli 1, 09126 Cagliari, Italy

[4]University of Insubria, Dep. of Human and Innovation for the Territory, Via Valleggio 11, 22100 Como, Italy

[5]Sapienza University of Rome, Department of Physics, P.le A. Moro 5, 00185 Rome, Italy

[6]Sapienza University of Rome, Department of Chemistry, P.le A. Moro, 5, 00185 Rome, Italy

Corresponding author:

Silvia Galafassi, silvia.galafassi@cnr.it, CNR Water Research Institute, L.go Tonolli 50, 28922 Verbania Pallanza

Abstract:

Microplastic particles (MPs) contamination of aquatic environments has raised a growing concern in recent decades because of their numerous potential toxicological effects. Although fish are among the most studied aquatic organisms, reports on MPs ingestion in freshwater environments are still scarce. Thus, there is still much to study to understand the uptake mechanisms, their potential accumulation among the food webs and their ecotoxicological effects. Here, MPs presence in the digestive system of one of the most widespread and commercially exploited freshwater fish, the perch (*Perca fluviatilis*, Linnaeus 1758), was investigated in four different south-alpine lakes, to assess the extent of ingestion and evaluate its relation to the body health condition. A total of 80 perch specimen have been sampled from the Italian lakes Como, Garda, Maggiore and Orta. Microplastic particles occurred in 86% of the analysed specimens, with average values ranging from 1.24 ± 1.04 MPs fish^{-1} in L. Como to 5.59 ± 2.61 MPs fish^{-1} in L. Garda. The isolated particles were mainly fragments, except in L. Como where films were more abundant. The most common polymers were polyethylene, polyethylene terephthalate, polyamide, and polycarbonate, although a high degree of degradation was found in 43% of synthetic particles, not allowing their recognition up to a single polymer. Despite the high number of ingested MPs, fish health (evaluated by means of Fulton's body condition and hepatosomatic index) was not affected. Instead, fullness index showed an inverse linear relationship with the number of ingested particles, which suggests that also in perch MPs presence could interfere with feeding activity, as already described for other taxa.

Keywords: emerging contaminants, plastic polymers, microplastic ingestion, freshwater fish, plastic litter, uptake, exposure

Introduction

Microplastics (MPs), defined as fragments of plastic litter of dimensions within the range 1 μm – 5 mm (Frias and Nash, 2019), are among the emerging contaminants raising more concern both in the scientific community and in public opinion. The massive use of plastic polymers in everyday life, that in 2019 in Europe reached a total annual production of 57.9 million tonnes (PlasticEurope, 2020), had led to the presence of a very high number of possible sources (Galafassi et al., 2019) and pathways of dispersions into the environment (Allen et al., 2019; Bergmann et al., 2019; Brahney et al., 2020; Gündoğdu et al., 2018; Kane et al., 2020; Liu et al., 2019). As a consequence, MPs contamination is ubiquitary in water, soil, sediments and air (Bellasi et al., 2020; Mbachu et al., 2020; Wang et al., 2019), in the most polluted areas of the world (Han et al., 2020) as well as in the most remote ones (González-Pleiter et al., 2020; Tan et al., 2020).

Evidence of ingestion by animals at all levels of marine food webs is documented by the scientific literature (Tosetto et al., 2017). Effects of MPs on biota start from the physical damage of their passage through the gastrointestinal tract (GIT), which can result in lacerations, suffocation and the development of altered behaviours (de Sá et al., 2015; Lusher et al., 2013; Miranda et al., 2019; Yin et al., 2018). Their permanence in the GIT can result in the leakage of the chemicals present in the particles: these include both the residues of the production process and the pollutants adsorbed during weathering in the environment (Bucci et al., 2020). These chemicals can be absorbed by the tissues, accumulate, and induce toxicological effects. Furthermore, the bacteria forming a biofilm on MPs' surface can be a source of pathogenic and antibiotic-resistant microorganisms (J. Wang et al., 2020; Xue et al., 2020). However, the assessment of the different effects induced by MPs exposition still needs research efforts, since discrepancies exist between characteristics of the particles used in *in-vitro* exposure and those found in the environment (Bucci et al., 2020; de Sá et al., 2018).

MPs pollution has been firstly surveyed in the sea since the '70s of the last century (Colton et al., 1974) and only later in freshwaters, being the North American Great Lakes the first target of these investigations (Zbyszewski and Corcoran, 2011). Since then, however, seawater has received much more attention than freshwater (Blettler et al., 2018; Lusher et al., 2017). Among aquatic organisms, fishes have been targeted more frequently in surveys for MPs detection compared to other taxa (23% of the total of the articles published before November 2017, de Sá et al., 2018) although the attention has always been skewed towards seawater species (W. Wang et al., 2020). First evidence indicated that colour, size, shape and floating depth could be drivers of possible ingestion, since both the intentional and the accidental ingestion are considered among the most probable mechanisms of exposure (de Sá et al., 2018, 2015; Ivar Do Sul and Costa, 2014).

Recent investigations revealed the massive presence of MPs in rivers, lakes and reservoirs (Koelmans et al., 2019; Lambert and Wagner, 2018; Li et al., 2018). Growing interest is especially directed to lentic environments, where accumulated MPs may persist for decades due to long water residence times (Di Pippo et al., 2020). Deep south-alpine lakes are important environments not only because they represent a strategic water source for industry and agriculture, but also because they provide a direct source for human nutrition through drinking water and food production. Despite plastic pollution has been already documented in the water of Italian south-alpine lakes (Binelli et al., 2020; Sighicelli et al., 2018), to date, no investigations on the ingestion of MPs by freshwater fishes is yet available, thus preventing the assessment of any possible relation between plastic concentrations in waters and their putative effects on the biota.

The Eurasian perch (hereafter perch), *Perca fluviatilis* (Linnaeus 1758) is one of the most ubiquitous freshwater fish species in Europe (Arranz et al., 2016) and is abundant also in south-alpine lakes (Volta et al., 2018). Furthermore, it has been introduced in the southern hemisphere (South Africa, Australia, New Zealand) and it has a biologically equivalent (Thorpe, 1977), the *Perca flavescens* Mitchill 1814, in North America. It is a carnivorous species, ontogenetically shifting from zoobenthivory in young specimens to piscivory in adult specimens as revealed by stomach content (Horppila et al., 2000; Persson and Greenberg, 1990) and stable isotopes analyses (Cicala et al., 2020), mostly inhabiting inshore areas of lakes (e.g. Volta et al., 2018; Cicala et al., 2020), rivers and brackish waters at highest latitudes. Perch is one of the most appreciated commercial and game freshwater fish. The global capture production is 28984 tons (year 2018, FAO, 2020) steadily increasing in the last 50 years, being Finland and the Russian federation the countries with the major catches. In central and south Europe perch capture production is limited mainly to large lakes (e.g. Rösch R, 2014; Volta et al., 2018) but can be very important for local economies and tourism. All these characteristics make it a potential target species to monitor the presence of pollutants, such as MPs, in freshwaters on a wide geographical scale, to assess the potential risks for fish species health and, through their consumption, for human health.

In this study, we investigated the presence, type, colour, size, and chemical composition of MPs in the gastrointestinal tract of the perch in four major south-alpine lakes in Italy: Lake Garda, Lake Como, Lake Maggiore and Lake Orta. In particular, our aims were to (i) quantify MPs ingestion by perch in the studied environments, (ii) investigate the possible effect exerted by MPs ingestion on fish health by evaluating structural descriptors like Fulton's body condition, hepatosomatic index and fullness index.

2. Material and Methods

2.1 Study area

The lakes included in the present study are located south of the Alps barrier in the Po river basin (Figure S1) and all have a fluvioglacial origin (Bini et al., 1978). Lakes Maggiore, Como, Garda flow directly into the Po river basin which enters the Adriatic Sea, whereas Lake Orta is connected to Lake Maggiore by the Strona River. The maximum depth of the studied lakes ranges from 143 m in Lake Orta to 425 m in Lake Como, and their surface areas range from 18.2 km^2 of Lake Orta to 370 km^2 of Lake Garda. All lakes are naturally oligomictic and turnover generally takes place in late February–early March. Mixing depth is very variable from lake to lake and in the last three decades the number of complete overturns and the thickness of the mixing layer at winter overturn have decreased markedly (Mosello et al., 2010). All lakes have experienced warming in recent decades due to ongoing climatic changes (Rogora et al., 2018). The trophic status of the lakes ranges from ultraoligotrophic to eutrophic and their main morphometric and limnological characteristics are shown in Table 1, together with MPs abundance values in each lake (extrapolated from the literature). Population density of Orta lake basin, not available in the literature, has been calculated as the total population resident in the area divided by the total extension of the catchment (data from ISTAT, http://dati.istat.it/).

2.2 Sample collection

At each lake, 15 to 28 specimens of perch were collected from professional fishermen in October 2018. Gillnets of 25 mm mesh size were used. Nets were set on the lake bottom at a depth comprised between 8 and 15 m at dusk (7 p.m.) and retrieved after ca. 12 hours (ca. 7 a.m.). The nets were set close to the following cities (Figure S1): Como city (Lake Como), Salò (Lake Garda), Verbania (Lake Maggiore), Omegna (Lake Orta). Once taken out from the nets, six to ten scales were taken from each specimen, stored in Eppendorf tubes with formaldehyde (1%) and used later for age determination. Fish were then stored on ice and, later, at -20 °C until analysed for MPs presence.

After defrosting fish at room temperature overnight, the total body length (centimetres, TL) of each fish and the total body mass (grams, BW) were measured. After that, each individual was dissected in a clean metal tray: liver was excised and weighted (grams, LW), the sex was determined (female, male, not detectable) by gonadal inspection, and the GIT, from the oesophagus to the anal sphincter, was placed in a clean glass jar with clean metal lid, weighted (grams, GITW) and frozen. The age of the fish was determined by counting the number of annuli on scales.

The analysis of MPs in the GIT has been preferred over that on muscles because of the higher number of already published reports in the literature, allowing comparisons with different environments and species. The complete dissolution of the entire GIT was achieved with the addition of KOH 10% in the proportion of 10 mL per gram of wet biomass, followed by incubation at 60°C for 24/48 hours. Once the tissues were completely dissolved, MPs were extracted applying a density separation protocol in supra-saturated NaCl successfully tested in marine and invertebrate marine fauna (Avio et al., 2015; Cau et al., 2019). A slight modification of the protocol was introduced: the retrieved solution was filtered on custom-made 25 µm polyester net filters to avoid the rapid filter occlusion that happened with the 8 µm nitrocellulose filters indicated in the protocol, because of the elevated presence of fat and chitin only partially dissolved by the KOH treatment. Finally, filters were incubated overnight at 60 °C with 1 mL of H_2O_2 15%. To avoid the accidental release of synthetic particles during the use of custom-made filters, the net was cut as a circle with 6 cm of diameter, borders were hot sealed with a flame and visually inspected just before use.

Putative MPs counts were done through the examination of the filtrate under a stereomicroscope (Zeiss model 47 50 53, equipped with 8X, 12X, 20X and 50X lenses) with the help of a clean stainless steel needle. Plastic particles were identified according to the following general rules: homogeneity of colour, absence of cellular or other organic structure, resistance to rupture, and unnatural colouration (Nor and Obbard, 2014). Criteria for shape classification were the following: fragments were thick particles with irregular dimensions and with edges of various nature, even jagged; films were irregular in shape but thin and flexibles; beads were round MPs with a spherical shape. Fibres were not considered in this study. Suspicious particles were tested with a hot needle. A sub-sample of putative MPs corresponding to at least 19% of the particles retrieved from each lake was analysed by FT-IR spectroscopy to assess their synthetic nature, as recommended by the European Commission guidelines (Galgani et al., 2013) and as already done for other studies (Feng et al., 2019; Garcia-Garin et al., 2019; Horton et al., 2018; Jabeen et al., 2017; Li et al., 2020; Su et al., 2019). MPs size was determined on a sub-sample of the particle analysed by FT-IR, corresponding to the 38%, 23%, 29%, and 10% of the total of MPs retrieved from fishes of the L. Como, L. Garda, L. Maggiore, and L. Orta respectively (see Supplementary Table S1 for details). Since MPs measurement was done on a sub-sample of particles, and thus characterized in a reduced number of specimens, MPs sizes were not utilised further for statistical analysis of fish health to avoid the introduction of a bias due to the small sample number.

Due to the method adopted in this study, namely the manual sorting of putative MPs, the results could be an underestimation of the real MPs content.

2.3 FT-IR analysis, Polymer identification and measurement

Fourier-transform Infrared (FT-IR) spectroscopy with a quasi-confocal microscopy arrangement (Hyperion microscope with liquid-nitrogen-cooled HgCdTe detector by Bruker, Germany) was employed to identify the vibrational fingerprints of particles isolated from fish samples. A spectral library of the FT-IR absorption spectra of most common plastic polymers was formed using the data in the NIST Chemistry WebBook (https://webbook.nist.gov/chemistry). Spectra of sand (limestone) and biopolymers (chitin) were also added to the library to help discard non-plastic particles from the analysis (Figure S2). The particles were dispersed on a CaF_2 microscopy slide (EKSMA Optics, Lithuania). Their absolute reflectance in the 400-4000 cm^{-1} range (spectral resolution of 4 cm^{-1}) was measured by adapting the microscope knife-edge aperture to the shape of each particle and measuring two reference spectra, one on the clean CaF_2 slide and one on a gold-coated flat surface, with the same aperture shape. Vibrational fingerprints were identified by visual inspection of the reflectance spectra: frequency, linewidth and intensity relative to the strongest feature were all evaluated for each detected spectral feature. The list of fingerprint frequencies was compared to the spectral library considering that a fingerprint produces an absorption peak frequency that corresponds to an inflexion point frequency in the reflectance spectrum. The identification was accepted when 90% of the peak frequencies corresponded in frequency and intensity within the range of the linewidth.

2.4 Contamination control

Clean cotton laboratory coats and nitrile gloves were worn during all the steps of the procedure. All the solution used were filtered with glass fibre filters (GF/C, pore size 1.2 µm, Whatman) to avoid any possible contamination. Equipment was washed three times with filtered distilled water and the 25 µm nets filters were visually checked at the stereomicroscope for MPs presence before the utilization. During the observation at the stereomicroscope, a clean petri dish with a clean filter was kept open close to the operator to detect the possibility of atmospheric deposition and procedural blank controls were performed regularly on the extraction procedure and no beads, fragments or films were found (only fibres have occasionally been found, a category for this reason not considered in this work).

2.5 Fish health condition

Fish health condition was assessed calculating (i) the hepatosomatic index (HIS), as LW/(TW-LW) ×100; (ii) Fulton's body condition factor (K; Ricker, 1975), as $TW / TL^3 * 100$; and (iii) fullness index (FI), as GITW/TW*100 (Sbrana et al., 2020).

2.6 Data analysis

From the FT-IR spectroscopic analysis, a correction coefficient was calculated for each lake, as the ratio of real synthetic particles (particles identified to a single polymer plus particles of polymeric origin but too degraded to be uniquely identified) respect to the total putative MPs analysed by FT-IR (supplementary Table S1). Real MPs contamination in fish GITs was calculated by applying the calculated correction coefficient to the putative MPs count done through stereomicroscopic observation, as already done in other studies (Feng et al., 2019; Su et al., 2019), as described in eq. 1 and 2.

$$Correction\ coefficient = \frac{number\ of\ plastic\ particles}{number\ of\ particles\ analysed\ by\ FT\!-\!IR} \qquad (eq.\ 1)$$

$$MPs\ content = putative\ MPs\ counting \times correction\ coefficient \qquad (eq.\ 2)$$

Data analysis was conducted in R (v 3.6.2). Figures were made in Excel or R and processed in InkScape (v 1.0). Weight and length were highly correlated (R^2= 0.885, $p < 0.001$) so only the latter was used in the dataset. Two different sets of statistical analysis were performed. The first set of tests (ANOVA and Kruskal Wallis) were used to assess differences between populations, like MPs content in the GIT of fish sampled in different lakes or of different sex. Differences in MPs presence in fish GIT among lakes and sex, and MPs size among lakes (not-normally distributed) were assessed with the Kruskal Wallis test followed by Pairwise comparisons using Wilcoxon rank sum test. Variations in length and weight among lakes (normally distributed) were assessed with ANOVA followed by Tukey's post hoc test. The D'Agostino-Pearson test was used before the analysis to assess the normality of variables. The second set of tests (Principal Component Analysis and Linear Mixed Model selection based on Akaike's information criteria) was instead used to find a relationship between MPs content and fish health descriptors (K, HIS, FI and TL). Principal component analysis (PCA) was made to have a first visualization of the data and was done with the `ggfortify` package. To assess the Linear Mixed Model that best describes the relationship between MPs content (response variable) and fish condition, we compared models done with all the possible combination of explanatory variables (K, HIS, FI and TL) using (R package `lme4`) and the selection was based on Akaike's information criteria (AIC) with small sample bias adjustment, AICc (`R` package `MuMln`). To avoid any possible bias, lake and sex were set as nested random effects.

3. Results and Discussion

3.1 Fish biometry, age, and MPs contamination

A total of 80 perch specimens were analysed (Table 2). The total length of the fish was on average 14.4 ± 1.81 cm and the total body mass was 30.78 ± 13.93 g. Fish from L. Garda were larger and heavier than those of L. Maggiore and Orta, whereas specimens from L. Orta were smaller than those of Como and Maggiore ($p < 0.001$, Table S2). All fishes belonged to the age class 1^+ (i.e. at the end of their second summer of growth). According to the literature, perch of this size are mostly zoobenthivory (Horppila et al., 2000; Persson and Greenberg, 1990).

Analysis of the GIT revealed that fish are exposed to MP pollution in all the studied lakes. MPs occurrence (*i.e.*, the number of fish showing at least one piece of plastic in GIT) ranged from 75% in fish from L. Maggiore to 94% in those from L. Garda (Table 2). The number of ingested particles was on average 2.90 ± 2.61 fish^{-1} (range = 0 - 11). Significant differences were found in MPs abundance in fish from the four lakes (Chi-square = 25.675, df = 3, $p < 0.001$; Table S3) being the number of MPs in fish from L. Garda (5.59 ± 2.61 MPs fish^{-1}) significantly higher than those from all the other lakes (Table S3, Figure 1). MPs occurrences reported in this study are similar in magnitude to those reported for other species retrieved in highly anthropized freshwater environments (Table 3). For example in the Brazos River, MPs ingestion by sunfish was higher in urban areas and positively correlated to the presence of roadways (Peters and Bratton, 2016; Table 3). Occurrence similar to those found in Lake Garda was also found in Taihu Lake, China (95.7% of fish contained MPs, with an average up to 3.8 fragment fish^{-1}), in some sampling point of the River Thame (occurrence in the 90% of the specimen analysed) and in Rio de la Plata, where all the fishes contained at least one MPs, although mainly fibres (see Table 3 for an overview of the recently published literature, with references). Despite this, there are also reports of lower MPs presence. Su and colleagues (Su et al., 2019), for instance, found MPs in only the 19.4% of *Gambusia holbrooki* specimen analysed, with an average of 0.6 particles per individuals, even though samplings were performed in the highly anthropized urban wetlands surrounding Melbourne (Australia). Also, data from Lake Constance (Switzerland) showed lower values, with MPs presence in only 16.5% of the analysed fishes and a mean of 0.2 ± 0.5 fragments fish^{-1} (Roch et al., 2019).

Our data showed a significantly higher MPs abundance in fish from L. Garda compared to those from other lakes, which is inconsistent with the relative abundance of MPs in water samples from the four investigated lakes (see Table 1). However, we have to underline that the possible correlation between MPs concentration in water and MPs occurrence in fish stomach is still unclear and overlooked. In fact, although there are reports of an increased MPs ingestion in the proximity of MP sources (Browne et al., 2011; Mcgoran et al., 2017; Peters and Bratton, 2016; Phillips and Bonner, 2015; Silva-Cavalcanti et al., 2017), laboratory investigations suggested higher importance of prey availability respect to the MPs concentration in the surrounding water (Critchell and Hoogenboom, 2018; Kim et al., 2018). Moreover, considering the number and heterogeneity of MP sources (Galafassi et al., 2019), it can be difficult to obtain a reliable estimate of the MP load in each of the lakes studied. Previously published MPs concentrations in lake water vary greatly among sampling years and transects (Table 1), leading to differences among lakes that are not always remarkable. However, MPs presence in surface water highlights the extent and abundance of plastic contamination in Italian south-alpine lakes and explains the high rate of MP ingestion by the resident biota. Nonetheless, since the high level of ingestion found in L. Garda could not be directly explained by MPs concentration in its waters, we remark here that further studies are needed to understand the relationship between MPs distribution in water and MPs ingestion by fish. A possible but rough estimation of anthropogenic impact and, thus, of MPs sources, can derive from the evaluation of the resident population around the lake (Table 1). These data suggest a similar level of urbanization of L. Como, Maggiore, and Garda catchments, whereas L. Orta has an almost double value because of its small catchment area. However, this information is again not exhaustive since the resident population can be a misleading parameter to assess human impact, especially when highly touristic areas (such as those under investigation) are considered. Among the investigated lakes, Garda is an internationally renowned tourist destination, whose influx of visitors is significantly higher than that of the other lakes considered in this study. The Italian National Institute of Statistics has compiled a ranking of the 50 Italian cities most frequented by tourists and among these, 7 belong to Lake Garda catchment, while cities around the other lakes are not included (National Institute of Statistics, 2017). As already reported, a high touristic flow not balanced by an adequate sewerage system can impact water quality due to uncontrolled sewage outflow (Moncheva et al., 2012). This can be reflected in a higher concentration of MPs, especially at the end of the summer when our study was conducted, whereas previous sampling campaigns for surface water MPs presence were done in late spring (Binelli et al., 2020). Also, it must be noted that L. Garda is the water body with the longest renewal time among the four studied lakes (Table 1). Consequently, floating plastic particles entering the lake could likely remain for a longer time in the environment. Therefore the encounter rate of MPs with fish is likely higher than in other lakes, increasing the probability to enter the food webs. Moreover, though having a similar age, fishes from L. Garda revealed a size larger than that of fishes from the other lakes: this allows the speculations of a higher feeding rate (and, thus, a higher presence of MPs) of the former respect to the other lakes.

Overall, our data suggest that the MPs ingestion by fish could not be directly correlated with MPs presence only, but could be the result of other concurring factors including lake limnological characteristics or the feeding rate.

3.2 MPs characteristics

Fragments were the most abundant type of MPs in all lakes except in L. Como, where films were the most retrieved type (Figure 2A). Considering that fibres have not been quantified in this work, these results were consistent with results published in the literature. For instance, Roch and colleagues (2019) found that fragments and fibres are ingested with higher proportion respect to other MPs types by fishes from both German lakes and rivers, and Lusher and colleagues (2013) found that, besides fibres and fragments, beads were more frequent than films in GIT of fishes from the English Channel.

Isolated MPs were on average smaller than 400 µm, but with significant differences between the investigated environments (Chi-square =16.33, df = 3, $p < 0.001$). In particular, sizes ranged from 159 µm (± 106) in L. Maggiore to 386 µm (± 157) in L. Como (Table 2), with Como statistically bigger than those from lake Garda and Maggiore, whereas lake Orta showed differences only with lake Garda (Table S4). Although some differences exist among particles isolated from the investigated lakes, measurements have been done on a small pool of particles (Table S1), thus any speculation about a correlation with frequencies of MPs ingestion would be inaccurate. Nevertheless, considering all the analysed MPs together, we did not found any correlation between MPs size and fish length ($F_{(1,34)} = 0.57$, $p = 0.454$, Adjusted $R^2 = -0.012$).

The great majority of MPs isolated from the four investigated lakes showed dark colours, like black/grey or blue, with the only exception for the samples from L. Maggiore in which transparent and white MPs are predominant (Figure 2B). The colour of MPs can be an indicator of the origin or/and of typology and can represent different levels of risk for organisms. Particles' colour influences the predatory activity (de Sá et al., 2015; Mizraji et al., 2017; Roch et al., 2020) and, thus, could enhance the probability of MPs accidental ingestion by predators, who tend to confounds plastic particles with their prey. In fact, in contrast to filter feeders which passively filter water (like mussels) allowing uniquely for particle size selection (Walkinshaw et al., 2020), particulate feeders select their prey on a visual basis (Lazzaro, 1987) as happens also for fish (de Sá et al., 2015; Mizraji et al., 2017; Roch et al., 2020). Among published studies, a great variety of colours have been indicated as the favourite ones: transparent colours were more abundant in 27 coastal and freshwater fish species in China (Jabeen et al., 2017) and white has been preferred by common goldfish *Carassius auratus* in Poyang Lake, China, probably mistaking it for plankton (Yuan et al., 2019). Dark colours can also be preferred. For instance, the reared Palm ruff *Seriolella violacea* juveniles prefer them because of their similarity to feed pellets (Ory et al., 2018). Also, blue MPs were actively predated by Amberstripe scad *Decapterus muroadsi* along the coast of Rapa Nui (Easter Island), likely because resembling copepods (Ory et al., 2017). Shreds of evidence of a colour preference do exist but are highly susceptible to variations in relation to different environmental factors, such as feeding condition and prey availability (as reported in de Sá et al., 2015 and Kim et al., 2019), and to environmental conditions occurred during previous developmental life stages (de Sá et al., 2015), thus it is difficult to speculate about the different colour preferences showed in the lakes analysed in this work.

3.3 MPs chemical composition

Spectrophotometric analysis of the particles retrieved from GITs revealed that 39% of them were not MPs. Common non-plastic materials were sand, glass, chitin and other inorganic material not further classified. Among the 61% of synthetic particles, 43% were highly degraded (Table S1) making their identification up to the polymer type impossible. Instead, their assignment has been based on the identification of narrow and intense IR peaks in the aromatic and aliphatic C-H stretching region (2800-3150 cm^{-1}), because plastics generally feature a much higher density, and level of local ordering of C-H bonds than e.g. proteins or cellulose. Among the identified polymers, polyethylene (PE) and polyethylene terephthalate (PET) were the two most frequently polymers found, although both of them were not present in all of the studied environments. Composition in terms of polymers seems to be slightly different in each lake being Garda the one characterized by the most abundant diversity (PE, PET, polystyrene, PS, polyamide, PA, and polycarbonate, PC) and Como in which only PE and PET were present. Polymers found in our sample are among the most common retrieved either in samples from those environments (Faure et al., 2015; Sighicelli et al., 2018) and other fish guts (Rummel et al., 2016). The presence of highly degraded MPs found in this work could be due to prolonged action of weathering agent, that could derive from a longer presence in the environment subjected to light and oxygen (UV photo-oxidation combined with mechanical abrasion; Dong et al., 2020) or in the gastrointestinal tract of fish (or other biotas) in contact with digestive enzymes and gastric juices that, for example in perch, could have a pH of 3.5 (Solovyev et al., 2015). According to recent studies, it can be speculated that plastic fragmentation and degradation is associated with exposure to mechanical forces, gut enzymatic processes, or a combination of the two (Mateos-Cárdenas et al., 2020; Cau et al., 2020).

3.4 MPs ingestion and fish body condition

The possibility of MPs accumulation and biomagnification within the food web and its potential consequences on fish health are still unclear and under debate (Bucci et al., 2020). Body growth and condition, physiology, and metabolism are aspects that, potentially, can be affected by the presence of MPs and their interaction with fish (Wang W. et al., 2019). A metadata analysis of *in vitro* studies using virgin MPs showed that, although fitness (e.g. growth, mortality, and reproductive success) was one of the most studied aspects, it was one of the less affected (Jacob et al., 2020). Nevertheless, the analysis of chemical pollutants and microbial pools that are brought by MPs suggest a much more deeper effect of the environmental MPs. To date, however, to our knowledge, only a few studies have analysed the growth performance of wild freshwater fish (Horton et al., 2018) and not merely their MPs ingestion (Campbell et al., 2017; Kasamesiri et al., 2020; Kuśmierek and Popiołek, 2020).

In our study, Fulton's body condition factor and the hepatosomatic index were calculated to describe the health status of the sampled fish (Table 2), and the fullness index was used as a possible indicator of the feeding activity. To explore the relationship between MPs presence and fish health status we ran a PCA with all the specimens sampled in the four different lakes investigated (Figure 4). Results showed that the greater part of variability (grouped in principal component 1, PC1, which accounted for 62.74% of the total variance) was driven by the MPs content and FI, whereas the principal component 2 (PC2, 27.72%) was mainly due to differences in fish length and, only marginally, to MPs and FI variations. Individuals were grouped according to their respective lake, indicating the importance of the environment as a determining factor in both the health of the fish and the MPs ingestion. Within the fish health status indexes, K and HIS did not show any importance whereas FI seemed to be inversely correlated to MPs ingestion, meaning that an empty stomach had more probability to be associated with a higher MPs content.

The fullness index FI was also the parameter selected when linear mixed models (LMM) with all the combination of fish health status indexes and length were generated and selected for the lowest AICc (Table S5). To better describe the correlation between MPs and FI we report their linear model in Figure 5 ($F(1,78) = 25.07$, $p < 0.001$, Adjusted $R^2 = 0.233$), a correlation that suggests that fish with an empty stomach have a higher probability to have ingested MPs. A possible explanation for this correlation could be the alteration in feeding habits that have already been described during in vivo laboratory experiments both with fish (de Sá et al., 2015; Miranda et al., 2019; Yin et al., 2018) and other marine organisms (Hämer et al., 2014; Watts et al., 2015), but the presence of other causal factors cannot be ruled out. However, this alteration does not seem to affect fish health status, here evaluated as K and HIS, since these parameters are only weakly associated with MPs presence as showed by PCA (Figure 4) and LMM picking (Table S5).

Presented data do not support the existence of a correlation between length and probability of MPs ingestion, contrary to what has been found in freshwater environments for roach (*Rutilus rutilus*, Horton et al., 2018), eastern mosquitofish (*Gambusia holbrooki,* Su et al., 2019) and in largemouth bass (*Micropterus salmoides,* Hurt et al., 2020). In fact, although L. Garda and L. Orta fish where respectively longer (and heavier) and shorter (and lighter) than those from other lakes, no correlation between fish length and MPs presence in GIT was observed ($F(1,78) = 0.94$, $p = 0.335$, Adjusted $R^2 = -0.00037$), even when considering only specimens from L. Garda ($F(1,17) = 0.08$, $p = 0.781$, Adjusted $R^2 = -0.054$) or Orta ($F(1,24) = 2.72$, $p = 0.112$, Adjusted $R^2 = 0.065$). However, the absence of a correlation between MPs ingestion and fish length in the presented data could also be due to the narrow length range in the specimen analysed in this work.

A sex-related tendency to ingest more MPs has been shown by fish both in freshwater (Su et al., 2019) and in marine (Sbrana et al., 2020) environments, but this was not confirmed by our data (Chi-square = 0.0456, df = 2, $p = 0.977$; Table S3) and the same tendency was not confirmed by other authors, i.e. as shown for five different sea species in the work by Campbell and colleagues (2017).

Conclusions

We reported the first assessment of MPs presence in GITs of perch from four south-alpine lakes. Our data confirmed that plastic contamination, already reported for surface waters of these lakes, affects also an iconic representative of their biota. Our data are consistent with those registered for environments with high urbanization and/or high touristic pressure indicating that the presence of MPs in the biota may reflect, with a certain bias, the MPs loads from the catchment, but also that it is most likely mediated also by the limnological characteristics of the recipient environment or the fish feeding activity.

Fish health was not affected by MPs presence, although an inverse relation was found between the presence of food in the gut (evaluated through the fullness index) and the number of ingested MPs. The possibility that an empty stomach has more MPs could be due to partial retention of particles that cannot be excreted through digestion, which in turn can interfere with feeding, well-documented side effects of MPs ingestion in several organisms (de Sá et al., 2015; Hämer et al., 2014; Miranda et al., 2019; Watts et al., 2015; Yin et al., 2018). Instead, we did not find a relationship with fish sex or length.

This study contributes to the knowledge of MPs pollution of the freshwater biota and is a further shred to understand the behaviour and effect that MPs have in natural environments. Moreover, it can lead the way in the use of perch to biomonitoring MPs concentration in freshwater environments. Indeed, fishes are currently used in many European countries as indicator taxon for the assessment of the Ecological Status of water bodies according to the Water Framework Directive 2000/60/EC (Birk et al., 2012; Poikane et al., 2017). Potentially, fishes can also be used as an indicator of MPs pollution in freshwaters since their capability to ingest and eventually accumulate MPs in their gut. Perch meet many of the criteria that are recommended for indicator species (GESAMP, 2019): (i) is one of the most widespread freshwater fish species in Europe; (ii) is subjected to both intense recreational and commercial fishing; (iii) it is a carnivorous species and, depending on size, it can be representative of zoobenthivorous or piscivorous feeding guild; (iv) it can be easily captured during routine surveys that are carried on by many EU member states for the Water Framework Directive (2000/60/EC) monitoring programs.

However, the present research should be considered only a preliminary study. A deeper understanding of the mechanisms that drive MPs ingestion is necessary for the correct choice of a bioindicator. Future research should consider more accurately the characteristics and concentrations of MPs and natural perch's prey and both in the environment and in perch's stomach, to possibly evaluate the existence of a direct predation mechanism. Moreover, due to the typical ontogenetic diet shift of perch at the increasing size (from zoobentivory to piscivory), a careful investigation on size-dependent MPs ingestion should be done to avoid misinterpretation of data.

Acknowledgements

This work was carried on within the project "Ecological restoration of Lake Orta – ITTIORTA", funded by "Demanio Idrico Lacuale del Lago d'Orta". Special thanks to Francesca Polli and Marina Ferrara who helped in processing fish samples.

Table 1. Characteristics of the studied lakes. Total phosphorus (TP) and oxygen (O) values are mean values across the water column at winter turnover. The population density is calculated as the total resident population in the whole catchment area whereas MPs concentration in the lake water is given as the average value found during the annual monitoring campaign of the "Goletta dei Laghi"..

	Como	**Garda**	**Maggiore**	**Orta**
Area (km^2)	146[1]	368[1]	213[1]	18[2]
Catchment area (km^2)	4508[1]	2290[1]	6599[1]	116[2]
Max depth (m)	425[3]	350[1]	370[1]	116[2]
TP ($\mu g\ L^{-1}$)	35[1]	18[1]	13[1]	5[2]
O_2 ($mg\ L^{-1}$)	8.4[1]	9.6[1]	8.3[1]	8.9[2]
Renewal time (years)	4.5[1]	26.6[1]	4.1[1]	10.7[2]
Population density (inhabitant km^{-2})	95[4]	94[4]	114[4]	173[5]
MPs abundance (MPs km^{-2})				
2016	na	25000 ± 16000[6]	39000 ± 18000[6]	na
2017	53000 ± 19000[7]	8600 ± 6700[7]	9800 ± 1600[7]	na
2018	29000 ± 14000[8]	36000 ± 28000[8]	100000 ± 35000[8]	63000 ± 25000[7]

[1]Rogora et al., 2018; [2]Rogora et al., 2016; [3]Fanetti et al., 2008; [4]Rogora et al., 2015; [5]This study; [6]Sighicelli et al., 2018; [7] Unpublished data ("Goletta dei Laghi" campaigns of Legambiente in 2017, for methodology see Sighicelli et al., 2018); [8]Binelli et al., 2020; na: not available.

Table 2. Number and characteristics of fish sampled in the studied lakes. TL: total length; TW: total weight; HSI: hepatosomatic index; K: Fulton's body condition factor; FI: fullness index.

		Como	Garda	Maggiore	Orta	Overall
Fish sampled		15	19	20	26	80
Sex (female/male/undetermined)		10/5/0	13/6/0	15/5/0	14/5/7	52/21/7
Fish without MPs	(num.)	2	1	5	3	11
Fish with MPs	(%)	87	95	75	88	86
MPs content	Mean ± st. dev (n. MPs $fish^{-1}$)	1.24 ± 1.04	5.59 ± 2.61	1.73 ± 1.83	2.75 ± 2.29	2.90 ± 2.61
MPs size	Mean ± st. dev (μm)	386 ±157	283 ± 797	159 ± 106	310 ± 292	277 ± 572
TL	Mean ± st. dev (cm)	14.80 ± 0.75	16.01 ± 1.76	14.73 ± 0.95	12.79 ± 1.53	14.41 ± 1.81
TW	Mean ± st. dev (g)	36.74 ± 5.54	43.38 ± 16.1	30.99 ± 6.08	17.96 ± 8.33	30.78 ± 13.93
HSI		1.07 ± 0.25	0.81 ± 0.32	0.94 ± 0.19	0.86 ± 0.30	0.91 ± 0.28
K		1.13 ± 0.09	1.02 ± 0.13	0.96 ± 0.06	0.82 ± 0.05	0.96 ± 0.14
FI	(%)	6.09 ± 0.72	3.55 ± 1.45	5.03 ± 0.53	4.88 ± 0.77	4.83 ± 1.24

Table 3. Summary of recent literature reporting MPs ingestion by freshwater fish species. When available, ranges of mean values are reported, to underline the variety of results obtained between different species or different sampling points.

Environment	Specie	MPs occurrence (%)	MPs presence (MPs fish^{-1})	Most aboundant polymers	Reference
Lakes (Como, Garda, Maggiore, and Orta, Italy)	*Perca fluviatilis* (perch)	75 - 95	1.24 ± 1.04 – 5.59 ± 2.61	PE, PET, PA	This study
Lakes (Evergreen and Bloomington, Illinois, USA)	*Micropterus salmoides* (largemouth bass)	100	15.5 ± 1.86 - 15.67 ± 2.00	na	Hurt et al., 2020
	Dorosoma cepedianum (gizzard shad)	100	1.11 ± 0.23 - 2.81 ± 0.55	na	
Lake (Taihu, China)	Several different species	95.7	1.8 ± 1.7 – 3.8 ±2.0	cellophane, PET, PL	Jabeen et al., 2017
Lake (Poyang, China)	Carassius auratus (wild crucian)	90.9[a]	9.27 ± 5.12[a]	PP, PE, nylon, PVC	Yuan et al., 2019
Lakes (Huron, Ontario and Eire, USA)	*Salvelinus fontinalis* (lake trout), *Oncorhynchus mykiss* (rainbow trout), *Micropterus dolomieu* (smallmouth bass)	30 – 50	0.40 ± 0.70 – 0.70 ± 0.82[a]	PE, styrene acrylonitrile, PS, nylon, PET	Wagner et al., 2019
Lakes (Constance, Germany)	Several different species	12.5 – 20	0.1 ± 0.3 – 0.3 ± 0.6	na	Roch et al., 2019
Lakes (Mead and Mohave, USA)	*Morone saxatilis* (striped bass)	-	4.2	na	Baldwin et al., 2020
Rivers and lake (Brazos, USA)	*Lepomis megalotis* (longear sunfish), *Lepomis macrochirus*	45 (19 - 75)	1.63 - 0.19	na	Peters and Bratton, 2016

	(bluegill sunfish)				
River (Río de la Plata, Argentina)	Several different species	100	18.5 ± 18.9 (fibres) 0.7 ± 1.7 (other MPs)	na	Pazos et al., 2017
Rivers (Lake Michigan tributaries, USA)	Several different species	85	10 ±2.3 – 13 ±1.6	na	McNeish et al., 2018
River (Pajeú, Brazil)	*Hoplosternum littorale*	83	3.6 (1 – 8.8)	na	Silva-Cavalcanti et al., 2017
River (Wascana Creek, Canada)	*Esox lucius* (northern pike), *Catostomus commersoni* (white sucker), *Notropis atherinoides* (emerald shiner), *Pimephales promelas* (fathead minnow)	73.5	2.36 ± 2.66[a]	na	Campbell et al., 2017
River (Chi, Thailand)	Several different species	72.9 (50 – 86.7)	1.76 ± 0.97	na	Kasamesiri and Thaimuangpho, 2020
River (Widawa, Poland)	*Gobio gobio* (gudgeon)	54.5	1.15 ±1.65	na	Kuśmierek and Popiołek, 2020
	Rutilus rutilus (roach)	53.9	1.18 ±1.89	na	
	Cyprinus carpio (common carp)	-	6.3 – 1.2	na	
River (Thames, UK)	*Rutilus rutilus* (roach)	33	0.69 ± 1.25	PP, PE, PL	Horton et al., 2018
River (Xiangxi, China)	Several different species	25.7	0 - 1.5 ± 1.38	PE	Zhang et al., 2017
River (Thames, UK)	*Platichthys flesus* (European flounder), *Osmerus eperlanus* (European smelt)	20 - 90	0.2 ± 0.42 - 0.85 ±1.17 (fibres)	PA, nylon, PE, PET	Mcgoran et al., 2016
Rivers	Several different	18.8	0.2 ± 0.5	na	Roch et al.,

(Germany)	species	(7.5 – 42.9)			2019
Rivers (Greater Melbourne Area, Australia)	*Gambusia holbrooki* (eastern mosquitofish)	3.3 – 38.3	0.18 ± 0.84 – 1.13 ± 1.57[b]	PL, nayon	Su et al., 2019
Rivers (surrounding of plastic production plant, China)	*Hemiculter leucisculus* (wild carp)	-	1.9 – 6.1	PL, PP	Li et al., 2020
Agricultural ponds (Rice-fish co-culture plants, China)	*Monopterus albus* (eel), *Misgurnus anguillicaudatus* (loach)	-	0.0 ± 0.0 - 4.7 ±0.9	PE, PP	Lv et al., 2019

[a]: calculated from data provided in the supplementary material.

[b]: digestion of the whole body (without the head).

Abbreviations: PET, polyethylene terephthalate; PA, polyamide; PS, polystyrene; PE, polyethylene; PP, polypropylene; PL, polyester; na: not available.

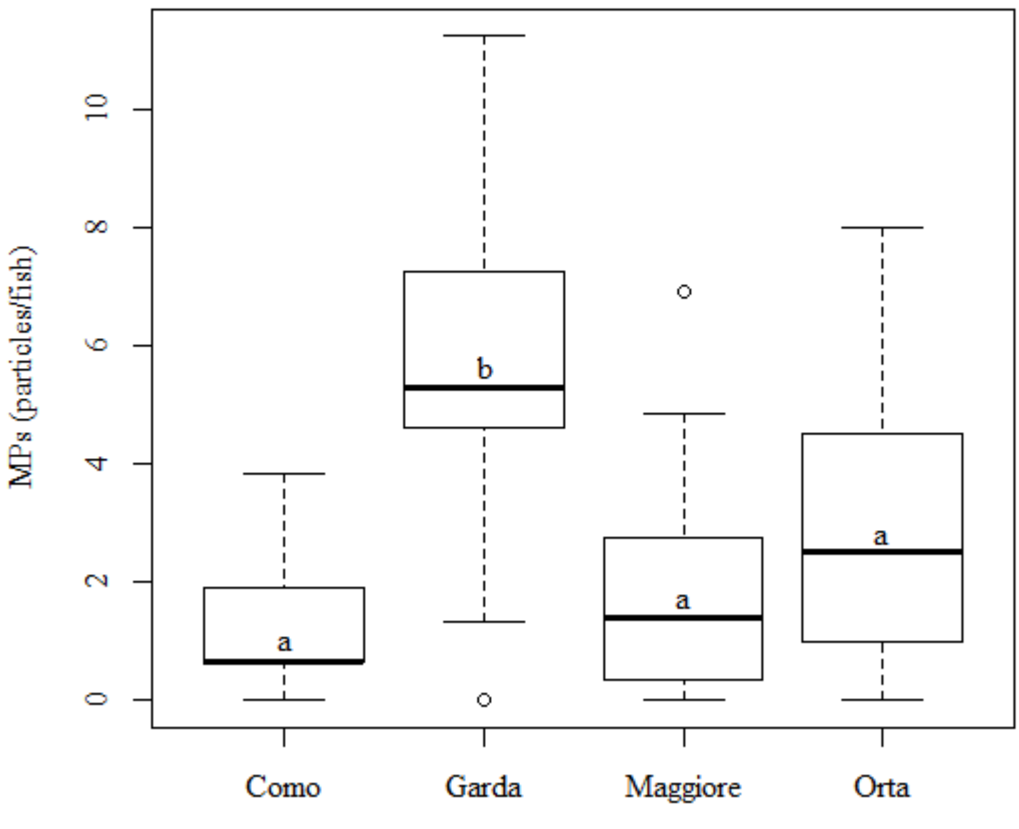


Figure 1. MPs presence in fish GITs. Letters represent the significance groups (see Table S3 for statistical details).

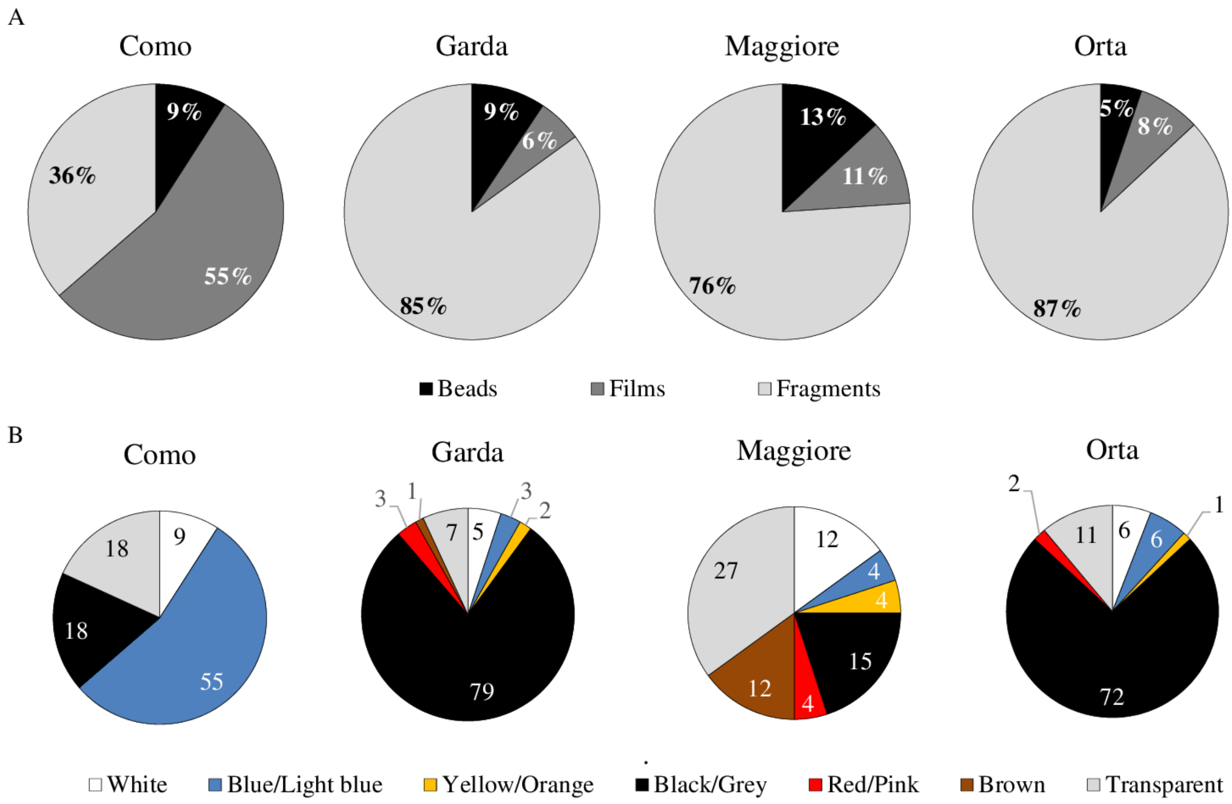


Figure 2. Classification of MPs found in fish stomachs according to type (A) and colour (B), expressed as a percentage of the total amount of particles isolated.

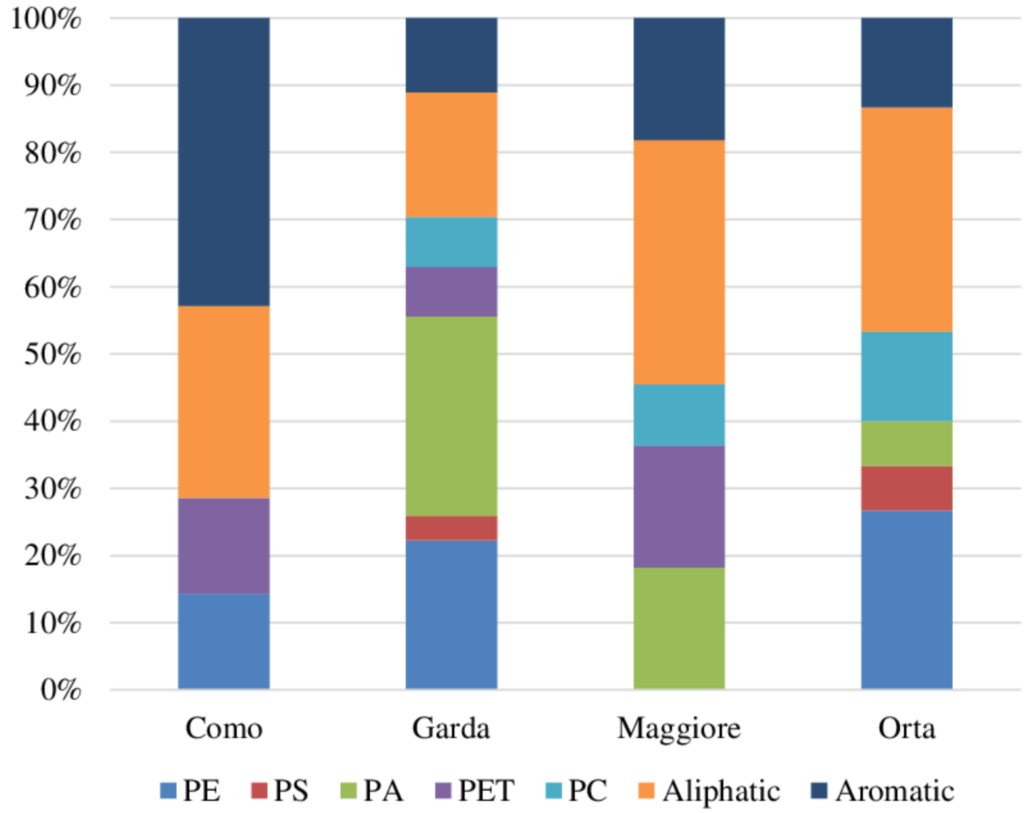
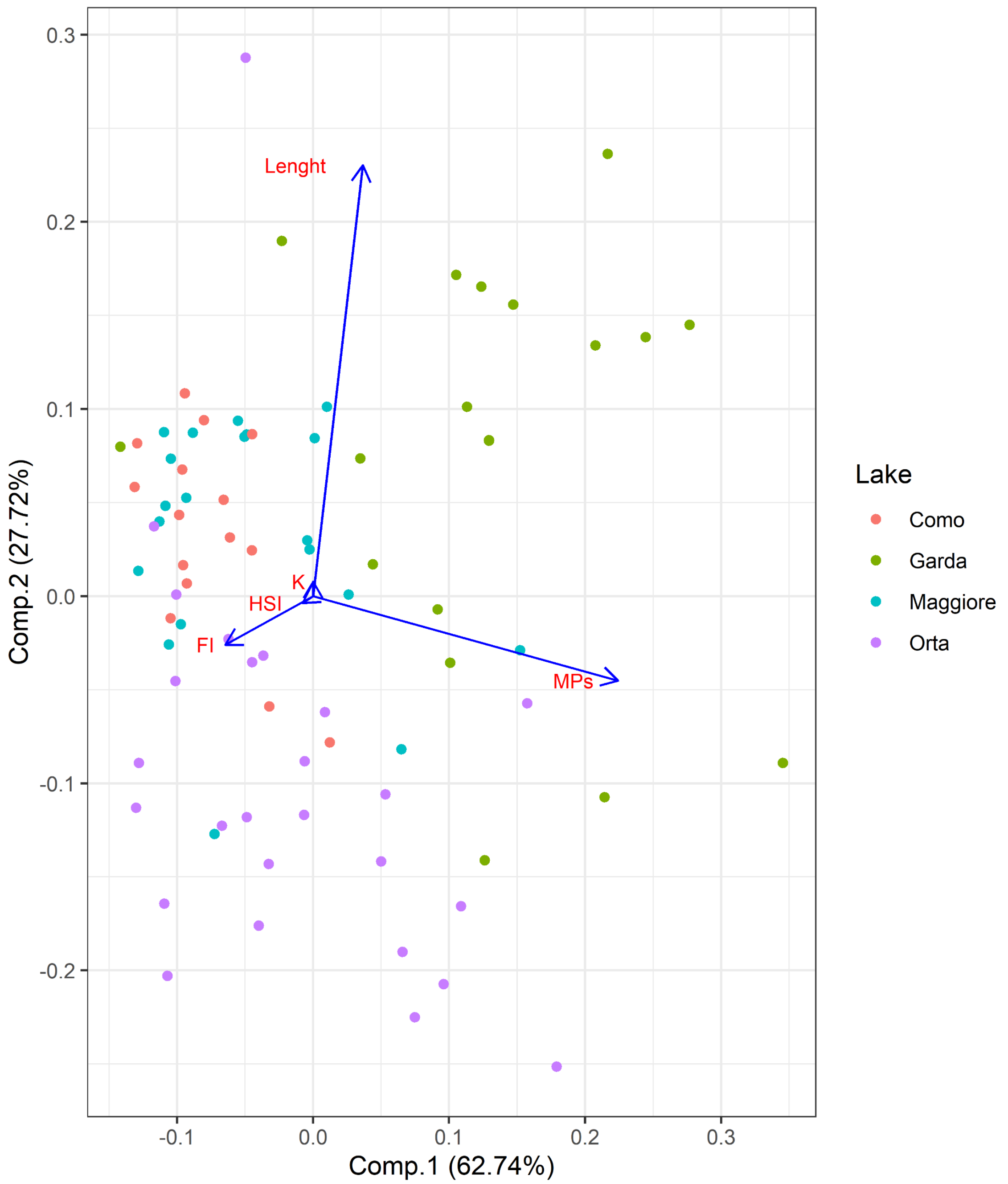


Figure 3. Chemical characterization of the isolated MPs. PE: polyethylene; PS: polystyrene; PET: polyethylene terephthalate; PC: polycarbonate; PA: polyamide. Highly degraded polymers have been generically classified within the aromatic and aliphatic categories.



460

461 **Figure 4.** Principal component analysis of MPs presence together with fish body condition describers as

462 length, fullness index (FI), hepatosomatic index (HIS) and Fulton's condition factor.

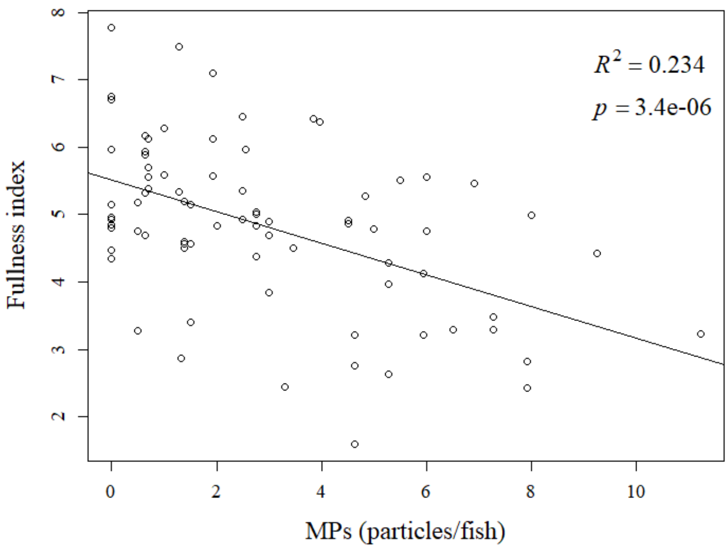


Figure 5. Correlation between the fullness index and MPs ingestion analysed as a linear model.

References

Allen, S., Allen, D., Phoenix, V.R., Le Roux, G., Durántez Jiménez, P., Simonneau, A., Binet, S., Galop, D., 2019. Atmospheric transport and deposition of microplastics in a remote mountain catchment. Nat. Geosci. 12, 339–344. https://doi.org/10.1038/s41561-019-0335-5

Arranz, I., Mehner, T., Benejam, L., Argillier, C., Holmgren, K., Jeppesen, E., Lauridsen, T.L., Volta, P., Winfield, I.J., Brucet, S., 2016. Density-dependent effects as key drivers of intraspecific size structure of six abundant fish species in lakes across Europe. Can. J. Fish. Aquat. Sci. 73, 519–534. https://doi.org/10.1139/cjfas-2014-0508

Avio, C.G., Gorbi, S., Regoli, F., 2015. Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: First observations in commercial species from Adriatic Sea. Mar. Environ. Res. 111, 18–26. https://doi.org/10.1016/j.marenvres.2015.06.014

Baldwin, A.K., Spanjer, A.R., Rosen, M.R., Thom, T., 2020. Microplastics in Lake Mead National Recreation Area, USA: Occurrence and biological uptake. PLoS One 15, e0228896. https://doi.org/10.1371/journal.pone.0228896

Bellasi, A., Binda, G., Pozzi, A., Galafassi, S., Volta, P., Bettinetti, R., 2020. Microplastic Contamination in Freshwater Environments: A Review, Focusing on Interactions with Sediments and Benthic Organisms.

Environments 7, 30. https://doi.org/10.3390/environments7040030

Bergmann, M., Mützel, S., Primpke, S., Tekman, M.B., Trachsel, J., Gerdts, G., 2019. White and wonderful? Microplastics prevail in snow from the Alps to the Arctic. Sci. Adv. 5, eaax1157. https://doi.org/10.1126/sciadv.aax1157

Binelli, A., Pietrelli, L., Di Vito, S., Coscia, L., Sighicelli, M., Torre, C. Della, Parenti, C.C., Magni, S., 2020. Hazard evaluation of plastic mixtures from four Italian subalpine great lakes on the basis of laboratory exposures of zebra mussels. Sci. Total Environ. 699, 134366. https://doi.org/10.1016/j.scitotenv.2019.134366

Bini, A., Cita, M.B., Gaetani, M., 1978. Southern Alpine lakes — Hypothesis of an erosional origin related to the Messinian entrenchment. Mar. Geol. 27, 271–288. https://doi.org/10.1016/0025-3227(78)90035-X

Birk, S., Bonne, W., Borja, A., Brucet, S., Courrat, A., Poikane, S., Solimini, A., Van De Bund, W., Zampoukas, N., Hering, D., 2012. Three hundred ways to assess Europe's surface waters: An almost complete overview of biological methods to implement the Water Framework Directive. Ecol. Indic. 18, 31–41. https://doi.org/10.1016/j.ecolind.2011.10.009

Blettler, M.C.M., Abrial, E., Khan, F.R., Sivri, N., Espinola, L.A., 2018. Freshwater plastic pollution: Recognizing research biases and identifying knowledge gaps. Water Res. https://doi.org/10.1016/j.watres.2018.06.015

Brahney, J., Hallerud, M., Heim, E., Hahnenberger, M., Sukumaran, S., 2020. Plastic rain in protected areas of the United States. Science (80-.). 368, 1257–1260. https://doi.org/10.1126/science.aaz5819

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines woldwide: Sources and sinks. Environ. Sci. Technol. 45, 9175–9179. https://doi.org/10.1021/es201811s

Bucci, K., Tulio, M., Rochman, C.M., 2020. What is known and unknown about the effects of plastic pollution: A meta-analysis and systematic review. Ecol. Appl. 30. https://doi.org/10.1002/eap.2044

Campbell, S.H., Williamson, P.R., Hall, B.D., 2017. Microplastics in the gastrointestinal tracts of fish and the water from an urban prairie creek. FACETS 2, 395–409. https://doi.org/10.1139/facets-2017-0008

Cau, A., Avio, C.G., Dessì, C., Follesa, M.C., Moccia, D., Regoli, F., Pusceddu, A., 2019. Microplastics in the crustaceans Nephrops norvegicus and Aristeus antennatus: Flagship species for deep-sea environments? Environ. Pollut. 255, 113107. https://doi.org/10.1016/J.ENVPOL.2019.113107

Cicala, D., Polgar, G., Mor, J.R., Piscia, R., Brignone, S., Zaupa, S., Volta, P., 2020. Trophic Niches, Trophic Positions, and Niche Overlaps between Non-Native and Native Fish Species in a Subalpine Lake. Water 12, 3475. https://doi.org/10.3390/w12123475

Colton, J.B., Knapp, F.D., Burns, B.R., 1974. Plastic particles in surface waters of the Northwestern Atlantic. Science (80-.). https://doi.org/10.1126/science.185.4150.491

Critchell, K., Hoogenboom, M.O., 2018. Effects of microplastic exposure on the body condition and behaviour of planktivorous reef fish (Acanthochromis polyacanthus). PLoS One 13, 1–19. https://doi.org/10.1371/journal.pone.0193308

de Sá, L.C., Luís, L.G., Guilhermino, L., 2015. Effects of microplastics on juveniles of the common goby (Pomatoschistus microps): Confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. Environ. Pollut. 196, 359–362. https://doi.org/10.1016/j.envpol.2014.10.026

de Sá, L.C., Oliveira, M., Ribeiro, F., Rocha, T.L., Futter, M.N., 2018. Studies of the effects of microplastics on aquatic organisms: What do we know and where should we focus our efforts in the future? Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2018.07.207

Di Pippo, F., Venezia, C., Sighicelli, M., Pietrelli, L., Di Vito, S., Nuglio, S., Rossetti, S., 2020. Microplastic-associated biofilms in lentic Italian ecosystems. Water Res. 187, 116429. https://doi.org/10.1016/j.watres.2020.116429

Dong, M., Zhang, Q., Xing, X., Chen, W., She, Z., Luo, Z., 2020. Raman spectra and surface changes of microplastics weathered under natural environments. Sci. Total Environ. 739, 139990. https://doi.org/10.1016/j.scitotenv.2020.139990

Fanetti, D., Anselmetti, F.S., Chapron, E., Sturm, M., Vezzoli, L., 2008. Megaturbidite deposits in the Holocene basin fill of Lake Como (Southern Alps, Italy). Palaeogeogr. Palaeoclimatol. Palaeoecol. 259, 323–340. https://doi.org/10.1016/j.palaeo.2007.10.014

FAO, 2020. FAO Yearbook. Fishery and Aquaculture Statistics 2018.

Faure, F., Demars, C., Wieser, O., Kunz, M., De Alencastro, L.F., 2015. Plastic pollution in Swiss surface waters: Nature and concentrations, interaction with pollutants. Environ. Chem. 12, 582–591. https://doi.org/10.1071/EN14218

Feng, Z., Zhang, T., Li, Y., He, X., Wang, R., Xu, J., Gao, G., 2019. The accumulation of microplastics in fish from an important fish farm and mariculture area, Haizhou Bay, China. Sci. Total Environ. 696, 133948. https://doi.org/10.1016/j.scitotenv.2019.133948

Frias, J.P.G.L., Nash, R., 2019. Microplastics: Finding a consensus on the definition. Mar. Pollut. Bull. 138, 145–147. https://doi.org/10.1016/j.marpolbul.2018.11.022

Galafassi, S., Nizzetto, L., Volta, P., 2019. Plastic sources: A survey across scientific and grey literature for their inventory and relative contribution to microplastics pollution in natural environments, with an emphasis on surface water. Sci. Total Environ. 693, 133499. https://doi.org/10.1016/J.SCITOTENV.2019.07.305

Galgani, F., Hanke, G., Werner, S., Oosterbaan, L., 2013. Guidance on Monitoring of Marine Litter in European Seas. MSFD Technical Subgroup on Marine Litter (TSG-ML).

Garcia-Garin, O., Vighi, M., Aguilar, A., Tsangaris, C., Digka, N., Kaberi, H., Borrell, A., 2019. Boops boops as a bioindicator of microplastic pollution along the Spanish Catalan coast. Mar. Pollut. Bull. 149, 110648. https://doi.org/10.1016/j.marpolbul.2019.110648

GESAMP, 2019. Guidelines for the monitoring and assessment of plastic litter in the ocean (Kershaw P.J., Turra A. and Galgani F. editors), (IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP/ISA Joint Group of Experts on the Scientific Aspects of Marine Environmental Prote. Rep. Stud. GESAMP no 99, 130p.

González-Pleiter, M., Velázquez, D., Edo, C., Carretero, O., Gago, J., Barón-Sola, Á., Hernández, L.E., Yousef, I., Quesada, A., Leganés, F., Rosal, R., Fernández-Piñas, F., 2020. Fibers spreading worldwide: Microplastics and other anthropogenic litter in an Arctic freshwater lake. Sci. Total Environ. 722. https://doi.org/10.1016/j.scitotenv.2020.137904

Gündoğdu, S., Çevik, C., Ayat, B., Aydoğan, B., Karaca, S., 2018. How microplastics quantities increase with flood events? An example from Mersin Bay NE Levantine coast of Turkey. Environ. Pollut. 239, 342–350. https://doi.org/10.1016/j.envpol.2018.04.042

Hämer, J., Gutow, L., Köhler, A., Saborowski, R., 2014. Fate of microplastics in the marine isopod idotea emarginata. Environ. Sci. Technol. 48, 13451–13458. https://doi.org/10.1021/es501385y

Han, M., Niu, X., Tang, M., Zhang, B.T., Wang, G., Yue, W., Kong, X., Zhu, J., 2020. Distribution of microplastics in surface water of the lower Yellow River near estuary. Sci. Total Environ. 707, 135601. https://doi.org/10.1016/j.scitotenv.2019.135601

Horppila, J., Ruuhijarvi, J., Rask, M., Karppinen, C., Nyberg, K., Olin, M., 2000. Seasonal changes in the diets and relative abundances of perch and roach in the littoral and pelagic zones of a large lake. J. Fish Biol. 56, 51–72. https://doi.org/10.1111/j.1095-8649.2000.tb02086.x

Horton, A.A., Jürgens, M.D., Lahive, E., van Bodegom, P.M., Vijver, M.G., 2018. The influence of exposure and physiology on microplastic ingestion by the freshwater fish Rutilus rutilus (roach) in the River Thames, UK. Environ. Pollut. 236, 188–194. https://doi.org/10.1016/j.envpol.2018.01.044

Hurt, R., O'Reilly, C.M., Perry, W.L., 2020. Microplastic prevalence in two fish species in two U.S. reservoirs. Limnol. Oceanogr. Lett. 5, 147–153. https://doi.org/10.1002/lol2.10140

Ivar Do Sul, J.A., Costa, M.F., 2014. The present and future of microplastic pollution in the marine environment. Environ. Pollut. https://doi.org/10.1016/j.envpol.2013.10.036

Jabeen, K., Su, L., Li, J., Yang, D., Tong, C., Mu, J., Shi, H., 2017. Microplastics and mesoplastics in fish from coastal and fresh waters of China. Environ. Pollut. 221, 141–149. https://doi.org/10.1016/j.envpol.2016.11.055

Jacob, H., Besson, M., Swarzenski, P.W., Lecchini, D., Metian, M., 2020. Effects of Virgin Micro- and Nanoplastics on Fish: Trends, Meta-Analysis, and Perspectives. Environ. Sci. Technol. 54, 4733–4745. https://doi.org/10.1021/acs.est.9b05995

Kane, I.A., Clare, M.A., Miramontes, E., Wogelius, R., Rothwell, J.J., Garreau, P., Pohl, F., 2020. Seafloor microplastic hotspots controlled by deep-sea circulation. Science (80-.). 5899, eaba5899. https://doi.org/10.1126/science.aba5899

Kasamesiri, P., Thaimuangphol, W., Thaimuangpho, W., 2020. MICROPLASTICS INGESTION BY FRESHWATER FISH IN THE CHI RIVER, THAILAND. Int. J. GEOMATE 18, 114–119. https://doi.org/10.21660/2020.67.9110

Kim, S.W., Chae, Y., Kim, D., An, Y.J., 2019. Zebrafish can recognize microplastics as inedible materials: Quantitative evidence of ingestion behavior. Sci. Total Environ. 649, 156–162. https://doi.org/10.1016/j.scitotenv.2018.08.310

Kim, S.W., Kim, D., Chae, Y., An, Y.J., 2018. Dietary uptake, biodistribution, and depuration of microplastics in the freshwater diving beetle Cybister japonicus: Effects on predacious behavior. Environ. Pollut. 242, 839–844. https://doi.org/10.1016/j.envpol.2018.07.071

Koelmans, A.A., Mohamed Nor, N.H., Hermsen, E., Kooi, M., Mintenig, S.M., De France, J., 2019. Microplastics in freshwaters and drinking water: Critical review and assessment of data quality. Water Res. https://doi.org/10.1016/j.watres.2019.02.054

Kuśmierek, N., Popiołek, M., 2020. Microplastics in freshwater fish from Central European lowland river (Widawa R., SW Poland). Environ. Sci. Pollut. Res. 27, 11438–11442. https://doi.org/10.1007/s11356-020-08031-9

Lambert, S., Wagner, M., 2018. Microplastics are contaminants of emerging concern in freshwater environments: An overview, in: Handbook of Environmental Chemistry. Springer Verlag, pp. 1–23. https://doi.org/10.1007/978-3-319-61615-5_1

Lazzaro, X., 1987. A review of planktivorous fishes: Their evolution, feeding behaviours, selectivities, and impacts Katari-Titicaca lake (Bolivia) View project TITICACASENSORS View project. hidrobiologia 146, 97–167. https://doi.org/10.1007/BF00008764

Li, B., Su, L., Zhang, H., Deng, H., Chen, Q., Shi, H., 2020. Microplastics in fishes and their living environments surrounding a plastic production area. Sci. Total Environ. 727, 138662. https://doi.org/10.1016/j.scitotenv.2020.138662

Li, J., Liu, H., Paul Chen, J., 2018. Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection. Water Res. https://doi.org/10.1016/j.watres.2017.12.056

Liu, K., Wang, X., Fang, T., Xu, P., Zhu, L., Li, D., 2019. Source and potential risk assessment of suspended atmospheric microplastics in Shanghai. Sci. Total Environ. 675, 462–471. https://doi.org/10.1016/j.scitotenv.2019.04.110

Lusher, A.L., McHugh, M., Thompson, R.C., 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Mar. Pollut. Bull. 67, 94–99. https://doi.org/10.1016/j.marpolbul.2012.11.028

Lusher, A.L., Welden, N.A., Sobral, P., Cole, M., 2017. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Anal. Methods. https://doi.org/10.1039/c6ay02415g

Lv, W.W.W.W., Zhou, W., Lu, S., Huang, W., Yuan, Q., Tian, M., Lv, W.W.W.W., He, D., 2019. Microplastic pollution in rice-fish co-culture system: A report of three farmland stations in Shanghai, China. Sci. Total Environ. 652, 1209–1218. https://doi.org/10.1016/j.scitotenv.2018.10.321

Mateos-Cárdenas, A., O'Halloran, J., van Pelt, F.N.A.M., Jansen, M.A.K., 2020. Rapid fragmentation of microplastics by the freshwater amphipod Gammarus duebeni (Lillj.). Sci. Rep. 10, 1–12. https://doi.org/10.1038/s41598-020-69635-2

Mbachu, O., Jenkins, G., Pratt, C., Kaparaju, P., 2020. A New Contaminant Superhighway? A Review of Sources, Measurement Techniques and Fate of Atmospheric Microplastics. Water. Air. Soil Pollut. https://doi.org/10.1007/s11270-020-4459-4

Mcgoran, A.R., Clark, P.F., Morritt, D., 2017. Presence of microplastic in the digestive tracts of European flounder, Platichthys flesus, and European smelt, Osmerus eperlanus, from the River Thames. Environ.

Pollut. 220, 744–751. https://doi.org/10.1016/j.envpol.2016.09.078

McNeish, R.E., Kim, L.H., Barrett, H.A., Mason, S.A., Kelly, J.J., Hoellein, T.J., 2018. Microplastic in riverine fish is connected to species traits. Sci. Rep. 8, 11639. https://doi.org/10.1038/s41598-018-29980-9

Miranda, T., Vieira, L.R., Guilhermino, L., 2019. Neurotoxicity, behavior, and lethal effects of cadmium, microplastics, and their mixtures on pomatoschistus microps juveniles from two wild populations exposed under laboratory conditions—implications to environmental and human risk assessment. Int. J. Environ. Res. Public Health 16. https://doi.org/10.3390/ijerph16162857

Mizraji, R., Ahrendt, C., Perez-Venegas, D., Vargas, J., Pulgar, J., Aldana, M., Patricio Ojeda, F., Duarte, C., Galbán-Malagón, C., 2017. Is the feeding type related with the content of microplastics in intertidal fish gut? Mar. Pollut. Bull. 116, 498–500. https://doi.org/10.1016/j.marpolbul.2017.01.008

Moncheva, S., Racheva, E., Kamburska, L., D'hernoncourt, J., 2012. Environmental and Management Constraints on Tourism in Varna Bay, Bulgarian Black Sea Coast 17. https://doi.org/10.5751/ES-05107-170335

Mosello, R., Ambrosetti, W., Arisci, S., Bettinetti, R., Buzzi, F., Calderoni, A., Carrara, E., De Bernardi, R., Galassi, S., Garibaldi, L., Leoni, B., Manca, M., Marchetto, A., Morabito, G., Oggioni, A., Pagnotta, R., Ricci, D., Rogora, M., Salmaso, N., Simona, M., Tartari, G., Veronesi, M., Volta, P., 2010. Evoluzione recente della qualità delle acque dei laghi profondi sudalpini (Maggiore, Lugano, Como, Iseo e Garda) in risposta alle pressioni antropiche e alle variazioni climatiche 24, 167–177.

National Institute of Statistics, 2017. MOVIMENTO TURISTICO IN ITALIA.

Nor, N.H.M., Obbard, J.P., 2014. Microplastics in Singapore's coastal mangrove ecosystems. Mar. Pollut. Bull. 79, 278–283. https://doi.org/10.1016/j.marpolbul.2013.11.025

Ory, N.C., Gallardo, C., Lenz, M., Thiel, M., 2018. Capture, swallowing, and egestion of microplastics by a planktivorous juvenile fish. Environ. Pollut. 240, 566–573. https://doi.org/10.1016/j.envpol.2018.04.093

Ory, N.C., Sobral, P., Ferreira, J.L., Thiel, M., 2017. Amberstripe scad Decapterus muroadsi (Carangidae) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific subtropical gyre. Sci. Total Environ. 586, 430–437. https://doi.org/10.1016/j.scitotenv.2017.01.175

Pazos, R.S., Maiztegui, T., Colautti, D.C., Paracampo, A.H., Gómez, N., 2017. Microplastics in gut contents of coastal freshwater fish from Río de la Plata estuary. Mar. Pollut. Bull. 122, 85–90.

https://doi.org/10.1016/j.marpolbul.2017.06.007

Persson, L., Greenberg, L.A., 1990. Juvenile competitive bottlenecks: the perch (Perca fluviatilis)- roach (Rutilus rutilus) interaction. Ecology 71, 44–56. https://doi.org/10.2307/1940246

Peters, C.A., Bratton, S.P., 2016. Urbanization is a major influence on microplastic ingestion by sunfish in the Brazos River Basin, Central Texas, USA. Environ. Pollut. 210, 380–387. https://doi.org/10.1016/j.envpol.2016.01.018

Phillips, M.B., Bonner, T.H., 2015. Occurrence and amount of microplastic ingested by fishes in watersheds of the Gulf of Mexico. Mar. Pollut. Bull. 100, 264–269. https://doi.org/10.1016/j.marpolbul.2015.08.041

PlasticEurope, 2020. Plastics – the Facts 2020. PlasticEurope 1–64.

Poikane, S., Ritterbusch, D., Argillier, C., Białokoz, W., Blabolil, P., Breine, J., Jaarsma, N.G., Krause, T., Kubečka, J., Lauridsen, T.L., Nõges, P., Peirson, G., Virbickas, T., 2017. Response of fish communities to multiple pressures: Development of a total anthropogenic pressure intensity index. Sci. Total Environ. 586, 502–511. https://doi.org/10.1016/j.scitotenv.2017.01.211

Ricker, W.E., 1975. Computation and interpretation of biological statistics of fish populations. Bulletin of the Fisheries Research Board of Canada, Bulletin 191, Ottawa.

Roch, S., Friedrich, C., Brinker, A., 2020. Uptake routes of microplastics in fishes: practical and theoretical approaches to test existing theories. Sci. Rep. 10. https://doi.org/10.1038/s41598-020-60630-1

Roch, S., Walter, T., Ittner, L.D., Friedrich, C., Brinker, A., 2019. A systematic study of the microplastic burden in freshwater fishes of south-western Germany-Are we searching at the right scale? Sci. Total Environ. 689, 1001–1011. https://doi.org/10.1016/j.scitotenv.2019.06.404

Rogora, M., Buzzi, F., Dresti, C., Leoni, B., Lepori, F., Mosello, R., Patelli, M., Salmaso, N., 2018. Climatic effects on vertical mixing and deep-water oxygen content in the subalpine lakes in Italy. Hydrobiologia 824, 33–50. https://doi.org/10.1007/s10750-018-3623-y

Rogora, M., Kamburska, L., Mosello, R., Tartari, G., 2016. Lake Orta chemical status 25 years after liming: Problems solved and emerging critical issues. J. Limnol. 75, 93–106. https://doi.org/10.4081/jlimnol.2016.1320

Rogora, M., Mosello, R., Kamburska, L., Salmaso, N., Cerasino, L., Leoni, B., Garibaldi, L., Soler, V., Lepori, F., Colombo, L., Buzzi, F., 2015. Recent trends in chloride and sodium concentrations in the deep subalpine lakes (Northern Italy). Environ. Sci. Pollut. Res. 22, 19013–19026.

https://doi.org/10.1007/s11356-015-5090-6

Rösch R, 2014. Lake Constance fish and fisheries, in: Welcomme, R.L., Valbo-Jorgensen, J., H.A.S. (Ed.), Inland Fisheries Evolution and Management – Case Studies from Four Continents. FAO Fisheries and Aquaculture Technical Paper No. 579., Rome.

Rummel, C.D., Löder, M.G.J., Fricke, N.F., Lang, T., Griebeler, E.M., Janke, M., Gerdts, G., 2016. Plastic ingestion by pelagic and demersal fish from the North Sea and Baltic Sea. Mar. Pollut. Bull. 102, 134–141. https://doi.org/10.1016/j.marpolbul.2015.11.043

Sbrana, A., Valente, T., Scacco, U., Bianchi, J., Silvestri, C., Palazzo, L., de Lucia, G.A., Valerani, C., Ardizzone, G., Matiddi, M., 2020. Spatial variability and influence of biological parameters on microplastic ingestion by Boops boops (L.) along the Italian coasts (Western Mediterranean Sea). Environ. Pollut. 263, 114429. https://doi.org/10.1016/j.envpol.2020.114429

Sighicelli, M., Pietrelli, L., Lecce, F., Iannilli, V., Falconieri, M., Coscia, L., Di Vito, S., Nuglio, S., Zampetti, G., 2018. Microplastic pollution in the surface waters of Italian Subalpine Lakes. Environ. Pollut. 236, 645–651. https://doi.org/10.1016/J.ENVPOL.2018.02.008

Silva-Cavalcanti, J.S., Silva, J.D.B., França, E.J. de, Araújo, M.C.B. de, Gusmão, F., 2017. Microplastics ingestion by a common tropical freshwater fishing resource. Environ. Pollut. 221, 218–226. https://doi.org/10.1016/j.envpol.2016.11.068

Solovyev, M.M., Kashinskaya, E.N., Izvekova, G.I., Glupov, V. V., 2015. pH values and activity of digestive enzymes in the gastrointestinal tract of fish in Lake Chany (West Siberia). J. Ichthyol. 55, 251–258. https://doi.org/10.1134/S0032945215010208

Su, L., Nan, B., Hassell, K.L., Craig, N.J., Pettigrove, V., 2019. Microplastics biomonitoring in Australian urban wetlands using a common noxious fish (Gambusia holbrooki). Chemosphere 228, 65–74. https://doi.org/10.1016/j.chemosphere.2019.04.114

Tan, F., Yang, H., Xu, X., Fang, Z., Xu, H., Shi, Q., Zhang, X., Wang, G., Lin, L., Zhou, S., Huang, L., Li, H., 2020. Microplastic pollution around remote uninhabited coral reefs of Nansha Islands, South China Sea. Sci. Total Environ. 725, 138383. https://doi.org/10.1016/J.SCITOTENV.2020.138383

Thorpe, J.E., 1977. Morphology, Physiology, Behavior, and Ecology of Perca fluviatilis L. and P . flavescens Mitchill . J. Fish. Res. Board Canada 34, 1504–1514. https://doi.org/10.1139/f77-215

Tosetto, L., Williamson, J.E., Brown, C., 2017. Trophic transfer of microplastics does not affect fish personality. Anim. Behav. 123, 159–167. https://doi.org/10.1016/j.anbehav.2016.10.035

Volta, P., Jeppesen, E., Sala, P., Galafassi, S., Foglini, C., Puzzi, C., Winfield, I.J., 2018. Fish assemblages in deep Italian subalpine lakes: history and present status with an emphasis on non-native species. Hydrobiologia 824, 255–270. https://doi.org/10.1007/s10750-018-3621-0

Wagner, J., Wang, Z.M., Ghosal, S., Murphy, M., Wall, S., Cook, A.M., Robberson, W., Allen, H., 2019. Nonestructive Extraction an Ientification of Microplastics from Freshwater Sport Fish Stomachs. Environ. Sci. Technol. 53, 14496–14506. https://doi.org/10.1021/acs.est.9b05072

Walkinshaw, C., Lindeque, P.K., Thompson, R., Tolhurst, T., Cole, M., 2020. Microplastics and seafood: lower trophic organisms at highest risk of contamination. Ecotoxicol. Environ. Saf. 190, 110066. https://doi.org/10.1016/j.ecoenv.2019.110066

Wang, J., Liu, X., Li, Y., Powell, T., Wang, X., Wang, G., Zhang, P., 2019. Microplastics as contaminants in the soil environment: A mini-review. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2019.07.209

Wang, J., Qin, X., Guo, J., Jia, W., Wang, Q., Zhang, M., Huang, Y., 2020. Evidence of selective enrichment of bacterial assemblages and antibiotic resistant genes by microplastics in urban rivers. Water Res. 183, 116113. https://doi.org/10.1016/j.watres.2020.116113

Wang, W., Ge, J., Yu, X., 2020. Bioavailability and toxicity of microplastics to fish species: A review. Ecotoxicol. Environ. Saf. 189, 109913. https://doi.org/10.1016/j.ecoenv.2019.109913

Watts, A.J.R., Urbina, M.A., Corr, S., Lewis, C., Galloway, T.S., 2015. Ingestion of Plastic Microfibers by the Crab Carcinus maenas and Its Effect on Food Consumption and Energy Balance. Environ. Sci. Technol. 49, 14597–14604. https://doi.org/10.1021/acs.est.5b04026

Xue, N., Wang, L., Li, W., Wang, S., Pan, X., Zhang, D., 2020. Increased inheritance of structure and function of bacterial communities and pathogen propagation in plastisphere along a river with increasing antibiotics pollution gradient *. https://doi.org/10.1016/j.envpol.2020.114641

Yin, L., Chen, B., Xia, B., Shi, X., Qu, K., 2018. Polystyrene microplastics alter the behavior, energy reserve and nutritional composition of marine jacopever (Sebastes schlegelii). J. Hazard. Mater. 360, 97–105. https://doi.org/10.1016/j.jhazmat.2018.07.110

Yuan, W., Liu, X., Wang, W., Di, M., Wang, J., 2019. Microplastic abundance, distribution and composition in water, sediments, and wild fish from Poyang Lake, China. Ecotoxicol. Environ. Saf. 170, 180–187. https://doi.org/10.1016/j.ecoenv.2018.11.126

Zbyszewski, M., Corcoran, P.L., 2011. Distribution and degradation of fresh water plastic particles along the beaches of Lake Huron, Canada. Water. Air. Soil Pollut. 220, 365–372. https://doi.org/10.1007/s11270-

011-0760-6

Zhang, K., Xiong, X., Hu, H., Wu, C., Bi, Y., Wu, Y., Zhou, B., Lam, P.K.S., Liu, J., 2017. Occurrence and Characteristics of Microplastic Pollution in Xiangxi Bay of Three Gorges Reservoir, China. Environ. Sci. Technol. 51, 3794–3801. https://doi.org/10.1021/acs.est.7b00369