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Characterization of ingested MPs and their relation with growth parameters of endemic and invasive fish from a coastal wetland

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Abstract

Microplastic (MP) contamination is a persistent and ubiquitous threat to aquatic ecosystems. This study quantifies MP ingestion by fish inhabiting the Anzali Wetland (Iran), a hotspot of biodiversity. Growth parameters have been monitored in endemic demersal fish (Caspian spined loach, *Sabanejewia caspia*), and invasive benthopelagic species (Prussian carp, *Carassius gibelio)* in the wetland and compared with their internal content of MPs. MPs were extracted from the gastrointestinal (GI) tracts following digestion of the samples in alkaline medium and observation of the extracts with microscopy (Scanning Electron Microscopy equipped with an Energy-Dispersive X-ray microanalyzer (SEM-EDS) and confocal Raman microscopy). A total of 84.6% of the study fish (n=26) were contaminated with MPs. Fibres were the only type of MPs found in the GI tracts, and these were mainly dark blue and made of polycarbonate and nylon in both investigated species. The mean number of MPs in the GI tracts of the carp and the loach was 3.6 and 3.7 respectively. MPs had smooth surfaces in most cases although some presented brittle, fragmented, and uneven surfaces and signs of degradation. The growth rate of *Carassius gibelio* and *Sabanejewia caspia*, measured with the *b* value (growth factor), was 2.91 and 2.15. *Carassius gibelio* can play a significant role in the transport of MPs to other aquatic organisms inhabiting the Anzali wetland, and hence can cause potential harm to them. *Carassius Gibelio's* MP contamination was more pronounced with increasing gut mass in older specimens. Due to the presence of MPs and in fish that can be consumed, there could be a trophic transfer to humans. Regarding *Sabanejewia caspia*, although not statistically significant, their uptake of MPs tends to increase in older specimens with smaller size and body weight. This can imply that MP pollution causes inappropriate conditions and results in negative growth . The findings of this work provide new insights into MP contamination in the Anzali wetland, specifically in endemic fish. These results will be important in conservation and management programs.

Keywords demersal, benthopelagic, trophic level, pollutant, endemic, invasive species

1. Introduction

Plastic is widely used: the global plastic production was ~ 367 million metric tonnes in 2020 (Li et al., 2018; Tiseo, 2021). In a single year, 2010, ~4.8–12.7 million tonnes of plastic waste were accumulated in the ocean, while both plastic production and accumulation are continuing (Jambeck et al., 2015). About 20 million tonnes of plastics litter were transferred to marine environments every year (Vannela, 2012) and this has raised worldwide concerns about plastics pollution (Zhu et al., 2019). Pieces of any type of plastic with a greatest dimension < 5 mm are known as microplastics (MPs) (Frias and Nash, 2019). MPs are entering natural ecosystems from various sources. They are emerging pollutants that have been found in different environmental matrices of marine and coastal ecosystems, where they can generate harmful ecological impacts (Garcés-Ordóñez et al., 2022). There is widespread concern regarding the effect of MPs on wildlife. MPs are remaining at high levels in the environment, especially in aquatic ecosystems (both freshwater and marine), and their prolonged exposure to the environment causes their further degradation that can result in the release of contaminants such as smaller plastic debris, plasticizers, metals, and potentially, substances that MPs had adsorbed (Smith et al., 2018).

The accumulation of plastic waste in the environment has increased (Jambeck et al., 2015; Tiseo, 2021; Lamichhane et al., 2022; Chen et al., 2022). Therefore, there is concern about the direct effect of plastic and its degradation products on aquatic organisms, as well as the indirect effect of them entering the food cycle of organisms related to the aquatic environment (e.g., seabirds). MPs with sizes between a few millimeters to a few micrometers can be mistakenly eaten, filtered or confused for prey by different aquatic organisms (Guzzetti et al., 2018; Hall et al., 2015; Li et al., 2016; Lusher et al., 2017; Saeed et al., 2020; von Moos et al., 2012; Vroom et al., 2017). Their ingestion may cause negative effects on fish (Prinz and Korez, 2020). The accumulation of MPs in the gut can cause gastrointestinal obstruction leading to the reduction of optimal conditions and mass loss of fish, which can eventually result in death (Jovanović, 2017). The toxicity of MPs is under study. A recent review has assessed the toxicity of MPs and nanoplastics in organisms including fish (Rahman et al., 2021). The toxicity of plastic particles appears related to their size (Rahman et al., 2021). Macroplastics can obstruct internal parts of organisms or be excreted (Ma et al., 2020), while smaller plastics can cause biochemical damage. However, the relation size/effect of MPs on organisms is not yet established. Among the toxic effects caused by MPs observed there is tissue damage, oxidative stress and changes in immune-related gene expression neurotoxicity, growth retardation and behavioral abnormalities (Bhuyan, 2022). MPs can affect predatory behavior in fish (de Sá et al., 2015), leading to malnutrition and MP storage in key organs such the gills, gut, and stomach (Güven et al., 2017; Greven et al., 2016). MPs can also act as a vector of pollutants such as heavy metals, endocrine-disrupting chemicals, persistent organic pollutants (POPs) (Ashton et al., 2010; Neves et al., 2015; Rios et al., 2007), and the toxicity of these combinations with MPs can influence biological processes, including motility, reproduction and development of cancer (Thiele and Hudson, 2021; Fossi et al., 2018; Lithner et al., 2011). Recent studies reported that the association of polyethylene MPs (PE-MPs) with a mix of emerging pollutants induces adverse genotoxic, mutagenic and unbalances redox effects in adult zebrafish and these were as severe as when the exposure was to the MPs and mix of pollutants separately (Araújo et al., 2022). It was an organ-dependent biochemical response caused by the exposure to pollutants (Araújo et al., 2022). Induced stress was also observed in tadpoles exposed to MPs alone and in combination with a mix of pollutants. Both cases lead to significant changes in physiological and biochemical responses (Araújo et al., 2023).

Ingestion of MPs can cause acute and chronic inflammation and irritation. This may potentially lead to DNA damage and promote cancer (Rahman, et al. 2021). Some genes related to carcinogenesis became up-regulated when exposed to MPs and nanoplastics. Such is the case of *tcim* gene (promoter of cell proliferation, apoptosis inhibitor), involved in thyroid and lung cancer in humans. In contrast MPs down-regulated *cers2b* (encoding in humans *tumor metastasis-suppressor gene 1* protein, liver regeneration promoter); *tp53inp1* (p53 inhibitor implicated in cancer progression); *agr2* (involved in cell migration, transformation and metastasis) and *wwox* (tumor suppressor that plays a role in apoptosis) genes in humans when exposed to plastic particles (Limonta et al., 2019).

The number of studies focused on MP pollution in freshwater ecosystems has been small compared to studies in marine ecosystems in most parts of the world including Iran as there is increasing evidence about the occurrence of MPs in freshwater systems (Lin et al., 2018b; Sighicelli et al., 2018). In particular, wetlands, due to their inherently high productivity, processes affecting MPs' fate and decomposition might differ from the marine environment. The Anzali Wetland (Talab) complex, ~15,000 ha, is located in the Western part of the Southern Caspian Sea basin (Northern Iran). This wetland is fed by several rivers (Chafroud, Bahambar, Morghak, Masal, Palangvar, Masolehrodkhan, Pasikhan, Siahdarvishan, Lakanroud, and Siahroud) and separated from the sea by a dune system, supporting extensive reedbeds, abundant submerged and floating vegetation (Ramsar Site 2020). The wetland is close to densely populated urban and rural areas, large-scale industries and agricultural activities (Sadeghi Pasvisheh et al., 2021; Birami et al., 2022). It is exposed to various water and soil pollutants such as industrial sewages, agricultural runoff and hospital effluents (Darabi-Golestan et al., 2019; ALabdeh et al., 2020). In addition, high sedimentation takes place in various of the rivers connected to the wetland, barriers have been constructed (36°45′31.27″N 49°23′16.03″E, 36°59′24.05″N 49°34′04.25″E, 37°07'05.6"N 49°43'53.9"E), channelization are among the treats that have affected the population size of several species there (Esmaeili and Abbasi, 2021). Nevertheless, it has very diverse flora and fauna including 75 species of fish (Esmaeili and Abbasi, 2021; Jamshidi-Zanjani and Saeedi, 2013). The wetland is known as a shelter for anadromous fishes and migratory birds (Pourang 1996; Ashoori et al., 2021). Therefore, the food chain cycle is considerable, especially when it provides spawning ground, nursery and feeding habitats for residual and anadromous fish. The Anzali wetland is habitat of some endemic aquatic species such as *Sabanejewia caspia* (Eichwald, 1838), which exclusively live there and in the surrounding water bodies (Esmaeili and Abbasi, 2021). The *Carassius gibelio* (Bloch, 1782), another cypriniform fish (Cyprinidae), is an invasive fish that lives in the Anzali Wetland and shows high resistance to environmental changes. The present study aims to characterize the MPs in the gastrointestinal tract (GI) of *S. caspia*, and *C. gibelio,* and investigate the effect of MP abundance on some biological parameters of the fish to assess the impact of MPs in the wetland. Our study is one of the firsts to report MPs pollution in the wetland via the analysis of GI tract of its inhabiting fish. We hypothesized that MPs could cause adverse effects in the habitat of some of the study fish, and that could cause imbalance in biological factors related with their survival. To the best of our knowledge, this is the first study reporting on MP ingestion by *S. caspia*. The assessment in this work will help to establish ecological impact of MPs in wetlands.

2. Material and methods

2.1. Taxon sampling

Fish specimens were sampled from the Anzali wetland (37.45′to 37.48′N and 49.37 to 49.32′E) in the southwest Caspian Sea (Fig. 1). Specimens were collected through sampling over consecutive three days in August 2021. A total of 26 fish specimens including the endemic *Sabanejewia caspia* (10 individuals) with ecological and ornamental value, and an invasive consumed species *Carassius gibelio* (16 samples) were examined for their exposure to MPs. *Sabanejewia caspia* is an endemic rare species very difficult to find in groups. For this reason, this study included them in low number. The collected fishes were wrapped in aluminum foil at the spot, and then kept in ice packs, for subsequent analyses. Characteristics of the examined fish are presented in Table 1. Age estimation was determined by counting annual rings in scales of the specimens of *Carassius gibelio*, and opercula of *Sabanejewia caspia* (Campana, 2001).

2.2. Growth parameters

Two common growth parameters including the *b* value of length-weight relationship and condition factor were estimated. The length-weight relationship is generally expressed by equation 1

$$W = aL^b \qquad \text{Equation 1}$$

Here *a* describes body shape, and the *b* value gives information about the balance of the dimensions. Specifically, *b* can be < 3 (negative allometry: fish growing faster in length than in weight), or > 3 (positive allometry: fish growing faster in weight than in length), or equal to 3 (isometry) (Koutrakis and Tsikliras, 2003; Froese, 2006).

Fulton’s condition factor (K) was calculated according to Htun-Han equation (2), where, W=mass of fish (g), L=Length of fish (cm).

$$K = \frac{W\ x\ 100}{L} \qquad \text{Equation 2}$$

2.3. Necropsy and detecting MPs

Fish samples were left to reach room temperature. Each specimen was dissected using scalpel, scissors and forceps, all cleaned with deionized water. The whole gastrointestinal tract of each specimen was removed and transferred to a pre-cleaned and pre-weighted beaker. The samples were digested with 10% KOH (Merck, Germany) (Rochman et al., 2015; Karami et al., 2017). The volume of KOH solution used to soak the specimens was at least three times of the volume of each sample. The beakers were covered with aluminum foil and then transferred to oven at 60 ˚C for 72 h (Nematollahi et al., 2021). After chemical digestion, the contents of jars were filtered using filter papers (S&S blue band, grade 589/3 with 2 µm pore size) using vacuum. The filters were transferred to the Petri dish and dried in an oven at 40 ˚C (Nematollahi et al., 2021).

2.4. Identification of MPs

The search of MPs in the digestate samples was carried out with a binocular microscope (Carl-Zeiss, Weet Germany) (Hidalgo-Ruz et al., 2012). All shapes of microplastics were investigated. Criteria which help to distinguish MP fibres visually were the thickness of fibres, homogeneity of colors throughout the particles and absence of organic or cellular structures on them (Hidalgo-Ruz et al., 2012). MPs were classified as 1000 µm ≤L <5000 µm, 500 µm ≤L <1000 µm, 250 µm ≤L <500 µm, 100 µm ≤L <250 µm, and 60 µm ≤L<100 µm. The color of the MPs was classified as blue, green, red-pink, black-gray, and yellow-orange. Images of the particles identified as MP were taken by a digital camera (Canon EOS 7D) followed by calibration of the EOS utility software (connect camera to computer). Digimizer Image Analysis Software (Erceg et al., 2020) was used to measure the size of the particles.

High vacuum scanning electron microscope (SEM, TESCAN Vega 3, Czech Republic, resolution of 2 nm at 20 kV) was used to determine the morphology of MPs. MPs were mounted onto double-sided copper adhesive tapes, coated with gold (using a gold coater unit, SC7640 SPUTTER COATER, Model: FISONS) before analysis. SEM equipped with an energy-dispersive X-ray microanalyzer (EDS) was used to identify the elemental composition of MPs (10% of the recovered MPs). A confocal Raman microscope (XploRA PLUS, HORIBA France) equipped with a 785 nm laser was used to support the identification of the polymer type of selected MPs (10% of all observed MPs).

2.5. Quality control

To avoid contamination from MP in the analytical facilities, an isolated laboratory was chosen (only the researcher working with the fish samples was allowed to access the lab), and all laboratory equipment and glassware were washed three times with pre-filtered deionized water and then covered with aluminum foil. Chemical solutions, reagents were pre-filtered by filters (S&S, 2 μm pore size, and grade 589/3). Benches were rinsed with pre-filtered ethanol before using. One analyst performed all the determinations. Control and blank tests, consisting of empty Petri dishes (blank control dishes) (n=3) and reference materials (n=3), were carried out during the full length of the analysis.

2.6. Statistical analysis

SPSS software (version 20.0) was used to perform two-way ANOVA tests at the significance level of 0.05 for assessing differences in MP intake by the two different fish species. The Tukey post hoc test was used to identify significant differences among the means. Prior to the ANOVA analysis, data was examined for normality and homogeneity of variances using the Shapiro-Wilk and Levene tests, respectively (p 0.05). To determine differences between biological factors of the two species, an independent t-test with parametric data and a Mann–Whitney U test wtih nonparametric data were carried out. Student t correlation test (p 0.05) followed by Person correlation coefficient were used to assess correlation between number of MPs detected in the GI tract and biological factors

3. Results and discussion

There are limited studies on the impact of MP pollution on fish in wetlands. In this study carried out in southern Caspian Sea wetlands, the GI tracts of 22 out of 26 fish specimens (84.6%) were found contaminated with MPs.

Mass was a main difference in the study fish as it can be observed in Fig. 2a. Their whole mass was 2.49-13.41g (mean 6.52 g) for *Carassius gibelio*, and 0.23-1.41g (mean 0.859 g) for *Sabanejewia caspia*. Their corresponding GI tracts were 0.2 -1.42 g, and 0.189 - 0.57 g. The numbers of MPs extracted were 0-11 and 0-8 in the GI tract of the *Carassius gibelio*, and *Sabanejewia caspia*, respectively, with very similar mean values 3.56 and 3.70 respectively (Fig. 2e). Most *C. gibelio* individuals were younger (1-3 years old) than the *S. caspia* specimens (3-4 years old) (Fig. 2f), with respective average age of 2.56 and 3.8 years. The relationship between the numbers of MPs extracted from their GI tracts and the specimens' biometric factors are examined in Table 1. Between the two groups, the data showed normality in biological factors except in MPs, total length, body mass and gut mass ($p>0.05$) respectively. The only parameters that did not show normality were age and condition factors between the two groups ($p<0.05$). However, data within every specie showed normality for age and condition factor. Independent t-test tests were carried out to find out if there was difference between MPs, total length, body mass, and gut mass between the two species. The GI tract, between the two species showed differences ($F=5.168$; $df=24$; $p=0.032$). The total length, did not identify differences between the two groups ($F=1.61$; $df=24$; $p=0.003$). The body weight between the two species showed differences in the significance level ($F=12.518$; $df=24$; $p=0.002$). Besides, there were no differences in the level of significance between the two groups for the number of MPs in the GI tract ($F=0.0044$; $df=24$; $p=0.836$). A Mann–Whitney U test showed that age and condition factors between the species were significantly different ($p<0.05$). A positive correlation was found between the number of MPs and the GI tract (Pearson correlation coefficient= 0.581, $p=0.018$) and age (Pearson correlation coefficient= 0.598, $p=0.014$) in *Carassius gibellio*. This means that in the larger GI tracts found in older *C. gibelio,* specimens presented more accumulation of MPs in the GI tract. Although biological factors did not show a strong correlation with the significant level found, an interesting trend identified the total length and weight, was correlated with the increment of MPs in GI tract. Overall, all present data showed that *C. gibellio* in older age (which corresponded to larger size) showed increasing accumulation of MPs in GI tract. In the case of *S. caspia,* there was not a strong correlation ($p>0.05$). However, a negative correlation has been observed frequently. The study found negative growth allometry for *S. caspia* and a negative correlation between the number of MPs and body weight and total length. Weak positive correlation was found with age, whih led to the effect of MPs on growth factor causing smaller body size in older specimens. A two way ANOVA was used to identify significant effects on the frequency of MPs in the gut and biological parameters (as control factors) including total length, weigh and age, CF (condition factor=K) and the two species (as factors of interest) ($p>0.05$). Also, a linear regression model was carried out between biological factors and the frequency of MPs in the gut and no significant difference between the number of MPs and biological parameters was found ($r=0.694$, $p=0.530$). The MPs in the GI tracts of both species presented normal distribution ($p>0.05$) and homogeneous variance (p>0.0.5). No significant difference was found between the level of MPs in the GI tracts of both species (ANOVA, $p>0.05$) despite their different living habitat and eating habits. Moreover, multivariant analysis between weight, total length and the number of MPs showed positive significant relationships in the case of *C. gibelio* but not for *S. caspia* ($p<0.05$ and $p>0.05$ respectively). This means that bioaccumulation of MPs in *C. gibelio* could be proven. Regarding the fish health, the Power function of the length-weight relationships estimated the *b* value of *Carassius gibelio* and *Sabanejewia caspia* 2.91 and 2.15 respectively (Fig. 3a, b). Our results led to a *b* value lower than 3. Exponent '*b*' provides information on fish growth. Our data estimated $b=2.91$ for *C. gibelio* that showed approximately an isometric growth. While *b* value of *S. caspia* showed <3 indicating negative allometric growth ($p<0.0001$, $t= 5.221$). In terms of length (Fig. 2d), most *S. caspia* specimens were longer than *C. gibelio*.

The study of MPs in endemic fish with narrow distribution range (e.g., *S. caspia*) can be particularly valuable to capture the impact of pollution. Indeed, the average number of MPs in omnivorous demersal (*S. caspia*) tended to be higher, although without significant differences (assessed at $p>0.05$), than in the omnivorous benthopelagic species (*C. gibelio*). However, it seems that greater number of specimens were needed to establish statistical difference.

Sabanejewia caspia is located in the trophic level 3 hence this omnivorous fish is closer to primary carnivores. In contrast, *C. gibelio* is in the trophic level 2, therefore it is an omnivorous fish closer to primary consumers or herbivores. The fish mass and growth rate of *S. caspia* was lower than *C. gibelio* ($p<0.05$). Recent studies proved that feeding strategy and abundance of MPs in their living environment are factors that could influence the ingestion of MPs by different fish species (Romeo et al., 2015; Battaglia et al., 2016; Wieczorek et al., 2018), in addition to methodology employed in the study (e.g., using protocols such as visual extraction of MPs from gut contents; differences in staining or extraction) as proposed by Wieczorek et al. (2018).

Our study found a total of 94 MPs in GI tracts of 26 specimens. MP pollution was abundant in the sediments of the study habitat (113–3690 items/kg Anzali wetland dry weight sediment) (Rasta et al., 2020). Hence, *S. caspia*, which lives near the sediment, may be more exposed to MPs compared to *C. gibelio* which feeds in the benthic zone. However, the density of the MPs and water currents will play a role in their distribution in the water column and species affected by such type of pollution. According to recent studies on abundance of gut contents in mesopelagic species (Wieczorek et al., 2018), MP abundance and characteristics can have implications in the cycle of carbon and nutrients (Wieczorek et al., 2018). Generally, there are significant differences in amounts of carbon and nutrient cycling in fish inhabiting surface, middle and bottom of a water body (Radchenko, 2007; Wieczorek et al., 2018). Organic material released as feces or from dead and decaying fish (e.g., mesopelagic fish), sinks very slowly from the upper surface to the deep region of a water body. A large proportion of this organic material is recycled by other organisms and re-released before it can reach the floor. Fish such as *C. gibelio*, with diurnal migration, travels long distances quickly, from the epipelagic layer where they feed to the deeper region where they deposit their feces. Therefore, these play a key role in speeding up the downward flux of carbon and nutrients to deeper depth and circumvent recycling by other organisms (Radchenko, 2007; Irigoien et al., 2014; Wieczorek et al., 2018). Therefore, benthopelagic fish (e.g., *C. gibelio*) expected to have greater effect on the changes in carbon and nutrient cycling than a benthic fish (e.g., *S. caspia*).

The growth rate of *S. caspia* presented a shrinking trend although no significant differences in the level of condition factor were found ($p>0.05$). *Carassius gibelio* has larger biomass (in terms of number and size of individuals), reproductive strategy and wider distribution range than *Sabanejewia caspia*. It also had unaltered growth. It could be that the endemic fish shrunk to adapt to MP pollution. If that was the case, this strategy could have negative effects on its future generation as they tend to become smaller in size to keep suitable body condition to their environment. It could also be that their digestive system got obstructed by plastic pollution. The accumulation of MPs could also lead to their transfer in the food chain. More MPs were reported in carnivores than omnivores in the Zhanjiang mangrove wetland and feeding habits were important factors in ingesting MPs (Huang et al., 2020). In contrast, in a previous study in the in the Anzali wetland, greater concentration of MPs were omnivores in comparison with carnivores (Rasta et al., 2021). Such apparently incoherent results indicate that it is likely that the ingestion of MPs is affected by the species.

Examining the MPs of these two contrasting fish species inhabiting the same wetland evidences the effect of MP contamination on ecologically different species. Biotic and abiotic factors such as habitat, geographical location, feeding behavior and trophic level could influence concentration of fibre MPs in the fish. However, our data in Table 1 did not show significant relationships between studied biological parameters such as length, weight, and age of the fish and MPs. *Carassius gibelio* is considered as an available economic food source for local people due to its low price and high availability, especially in the recent economic crisis in Iran. Therefore, there could be transference of MPs to humans directly, or indirectly through the food chain e.g., consuming carnivorous fish (e.g., northern pike, *Esox lucius*) or birds preying on *C. gibelio.* Also there is overlap in some food items between *S. caspia* and *C. gibelio* including macro-invertebrates (Özdilek and Jones, 2014).

Carassius gibelio feeds mainly from small size MPs (<50 mm) including zooplankton, detritus, filamentous algae, phytoplankton, and in less number benthos and fish larvae. The main food items of *Sabanejewia caspia* include detritus, chironomid larvae and oligochaetes. Therefore, there might be overlap of food resources in these two species during *C. gibelio* vertical movements to the benthic zone. Due to presence of MPs in the water and sediment of the Anzali wetland, MPs uptake of planktons and benthos, accumulation of planktons and benthos in the gut of *C. gibelio* and *S. caspia*, fish larvivorous behavior of *C. gibelio*, and direct/indirect human consumption, the possible trophic transfer of MPs during the feeding process and along the aquatic food chains can be considered (Fig. 8). The trophic transfer of MPs from aquatic plants to freshwater invertebrates as part of the feeding process has already been demonstrated in a very different setting (Mateos-Cardenas et al., 2022).

Also, the varied diet of *C. gibelio* specimens and their lower trophic position make them less susceptible to anthropogenic threats (Duffy, 2003) when compared with *S. caspia*. Overall, even if the accumulation of MPs did not show significant impact between the two species, the ecological behavior of the benthopelagic and benthic species in *C. gibelio* and *S. caspia*, as well as the isometric and negative allometric growth in both species, respectively, indicate that *S. caspia* can be more susceptible to pollution including MPs.

The characteristics of MPs found in the GI tracts, including their size, shape and color were examined. In the GI tract of *C. gibelio* and *S. caspia*, fibres were the only type of MPs found (Fig. 4a). The size of MP fibres in *C. gibelio* (60 and 3800 µm) and in *S. caspia* (90-3650 µm) was similar. There was normality in size of the MP fibres ($p>0.05$) and similar size classes were observed in the two studied species. The predominant size ranged between 1000-5000 µm (mm), followed by 500-1000, 250-500, and 100-250 (µm) (Fig. 5a). MP particle size can important in MPs bioavailability and bioaccumulation (Marin et al., 2022).

Fibre MPs were classified into blue/green, red/pink, black/grey, and yellow/orange colors (Fig. 5b). The most abundant MPs were dark blue, which were considered in the blue-green category (84.1, and 83.3% in *C. gibelio,* and *S. caspia*). A range of light to dark blue MPs was observed in the gut of the studied fish. The red/pink (12.2 and 13.9%, respectively), black/gray (2.439, and 2.778%, respectively) and yellow/orange (1.220%, 0), categories comprised a smaller percentage of the rest of the observed MPs respectively (Fig. 5b). Previous studies also reported high MP contamination rates in fish GI tracts (e.g. 30 out of 32 fish samples (93.7%) in the Zhanjiang mangrove wetland (Huang et al. 2020). In contrast, somewhat lower contamination rates of MPs have been reported in sea fish: 73% of marine mesopelagic fish samples from the Northwest Atlantic (Wieczorek et al., 2018); 11% of the fish samples in a study sampling in the North Atlantic; 9 and 35% in the North Pacific Gyre regions (Boerger et al., 2010; Davison and Ash, 2011); 35% of the pelagic and demersal fish examined in the English channel (Lusher et al. 2013); 83% of the Norway lobster *Nephrops norvegicus* (Murray and Cowie, 2011). Also the accumulation of MPs in the GI tract of different species complied in Table 2 indicate that *S. caspia* should be considered in conservative programs. They have small size and small GI tract in comparison with other species. Hence they presented high accumulation of MPs in the GI tract.

Fig 6 show six selected MPs representative of different texture and morphology. MPs had smooth surfaces in most cases, although uneven surfaces with cracks were also observed (Fig. 6). The composition of MPs included oxygen carbon (as most abundant components) and they had traces of aluminum, silicon and calcium according to EDS analysis (Fig. 6). The main detected polymers with Raman spectroscopy were nylon (Fig. 7a, b) and polycarbonate (PC) (Fig. 7 c, d), however this assessment was based on the characterization of 10% the MPs and gives limited view of the overall contamination.

While dark blue MPs fibres accounted for the majority of MPs, the red MP fibres showed the same polymer composition as dark blue ones. On the other hand, the different color fibres were not distinguishable according to polymer compounds. More than 90 % of the observed particles from GI fish were confirmed as MPs by Raman spectrometry. There were no significant differences between the type of polymers in the MP fibres found in the GI tract in the two investigated species ($p>0.05$).

Importantly, fibres were the only type of MPs detected in the GI tracts of the study fish. Recent studies confirmed that there are many species in the Anzali wetland where the investigation of GI tracts showed higher percent of fibres including *Esox lucius, Abramis brama, Tinca tinca, C. gibelio, Rutilus kutum, Chelon saliens, Rutilus kutum* (Rasta et al., 2021; Nematollahi et al., 2021).

This points that a main source of pollution may be water from washing cloths (hence inefficient wastewater treatment). Atmospheric deposition has to be considered, although the distance of the wetland from urban centers (about 3 Km) indicates that it is a less important pathway. Equivalent distribution MP sizes was found in both studied species and it could be because of potentially homogeneous distribution of MPs in the water column of the Anzali wetland. Although different polymers were detected in the MP fibres and they can signify different sources, polycarbonate and nylon were dominant in the GI tract of the investigated species (60% nylon; 40% polycarbonate). Polycarbonate is used in the construction industry, in electrical components, automotive parts, compact discs, anti-break lenses and windows whereas nylon is widely used in textiles (PlasticsEurope, 2015). However, the differences in type of MPs could be related to the time of sampling. Other recent studies analysing sediment of the Anzali Wetland reported additional type of MPs than the present study (Rasta et al., 2020; Birami et al., 2022). Moreover, lower concentration of MPs in the sediment was reported from the region where we have sampled. (Birami et al., 2022). Therefore, it is assumed that spatial and temporal variability of sampling might have an effect on the results.

4. Conclusions

Plastic microfibres were the only MP found in the GI tract of fish in the Anzali wetland. These were mainly dark blue, covering approximately 60-3800 μm range in both fish populations studied. The type of microfibres found was not dependent on the fish species, however the analysis of more specimens would support finding more representative information and less overlap between the study fish species. Nylon and polycarbonate were the main constituents of the fibres. There was frequent contamination of fish (84.6%) (including endemic and invasive fish species) with MP fibres in their GI tracts. The average number of fibres found in the GI tract per fish was 3.63, and no significant differences were found between the levels of MPs in the GI tracts of both species (ANOVA, p>0.05) despite different habitats and eating habits (omnivorous demersal versus omnivorous benthopelagic species). The size range of the fibres found in GI tract was not dependent on the specie. The *b* value of *C. gibelio* indicated isometric growth, approximately. In contrast, *S. caspia* presented negative allometric growth. *Sabanejewia caspia*, as primary demersal, became more contaminated and potentially carries MPs to higher levels of the food chain. The two fish species studied presented different changes in biological parameters that are related to their survival in that habitat. *C. gibelio* showed lower number of MPs in older specimens while their body condition factors decreased. However, the b values showed they are in the good condition to continue in the environment. In contrast, *S. caspia* may be suffering more from the impact of pollution (such as MPs) because they showed negative growth allometry. It is likely that they are shrinking in size and weight, and becoming thinner and shorter in length to provide essential energy for continuing in their inhabitants. Future studies quantifying MP ingestion by predatory fish species should also consider sampling their putative prey to investigate trophic transfer of MPs.

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Credit authorship contribution statement

The manuscript was written by MSK and HRE, and it reviewed/edited through contributions of all authors. All authors have given approval to the final version of the manuscript.

Ethics statement

The project was approved by Research and Technology, Iran, basic sciences research fund (No. BSRF-bio-399-09), and Ethic Committee of Biology Department, Shiraz University (2594473081).

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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Figures

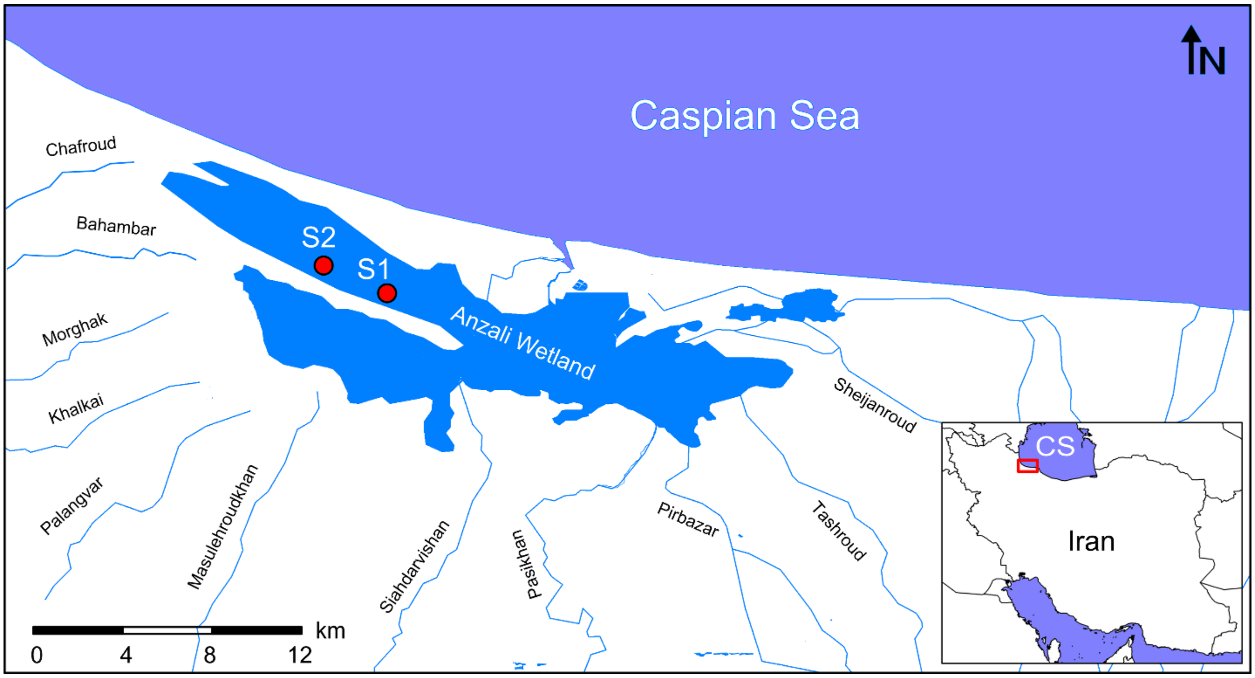


Fig. 1. Map of the Anzali wetland showing the sampling sites situated in the southern Caspian Sea (S1 and S2).

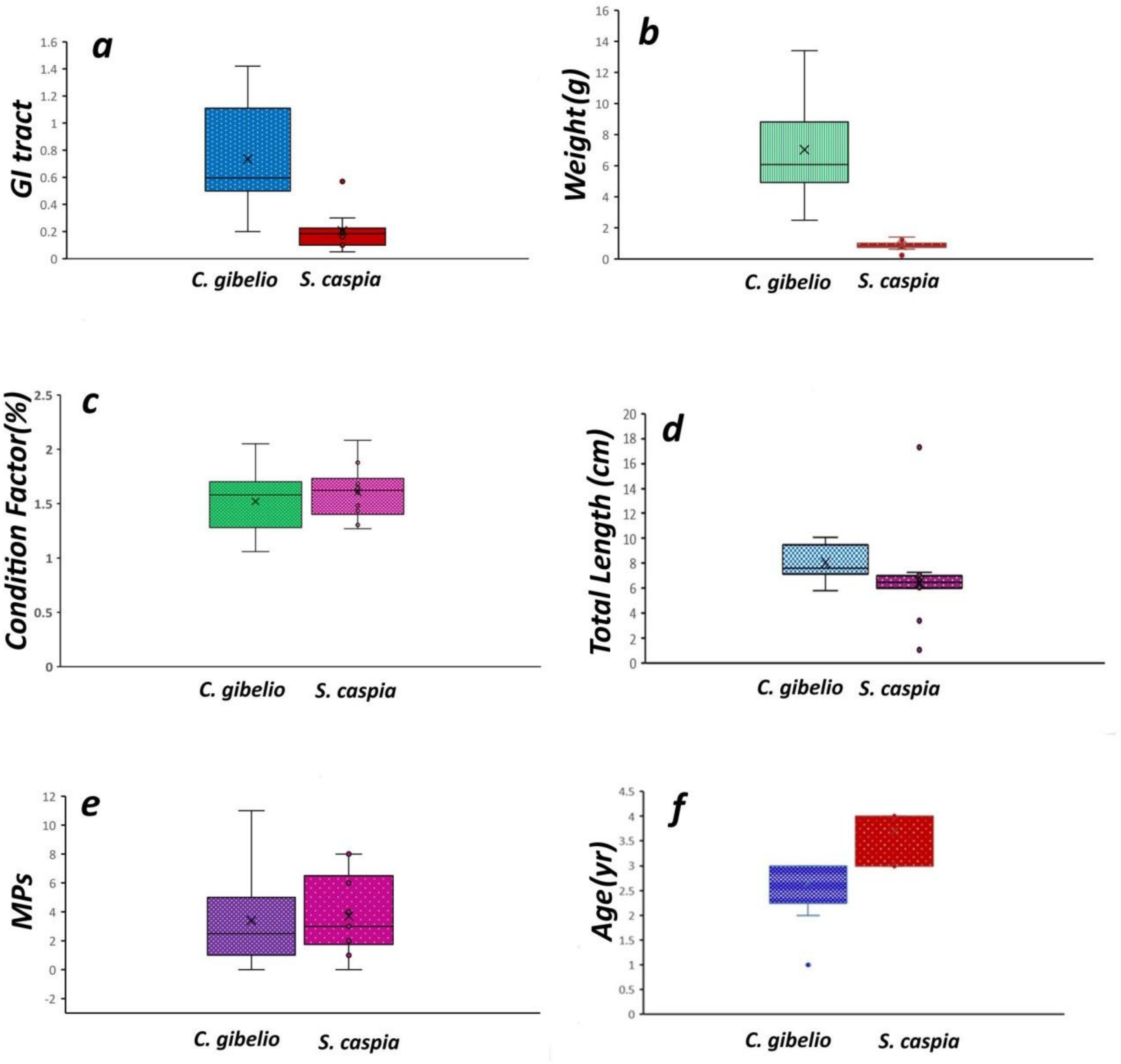


Fig. 2. Box plots of a) GI tract mass (g); b) body weight (g); c) condition factor; d) total length (TL) (cm); e) distribution of the number of MPs; and f) age of the fish expressed in years for the two species *Carassius gibelio* and *Sabanejewia caspia*. Error bars: standard deviation (SD). In the box pot: median value; ×: the mean value; o: maximum and minimum values.

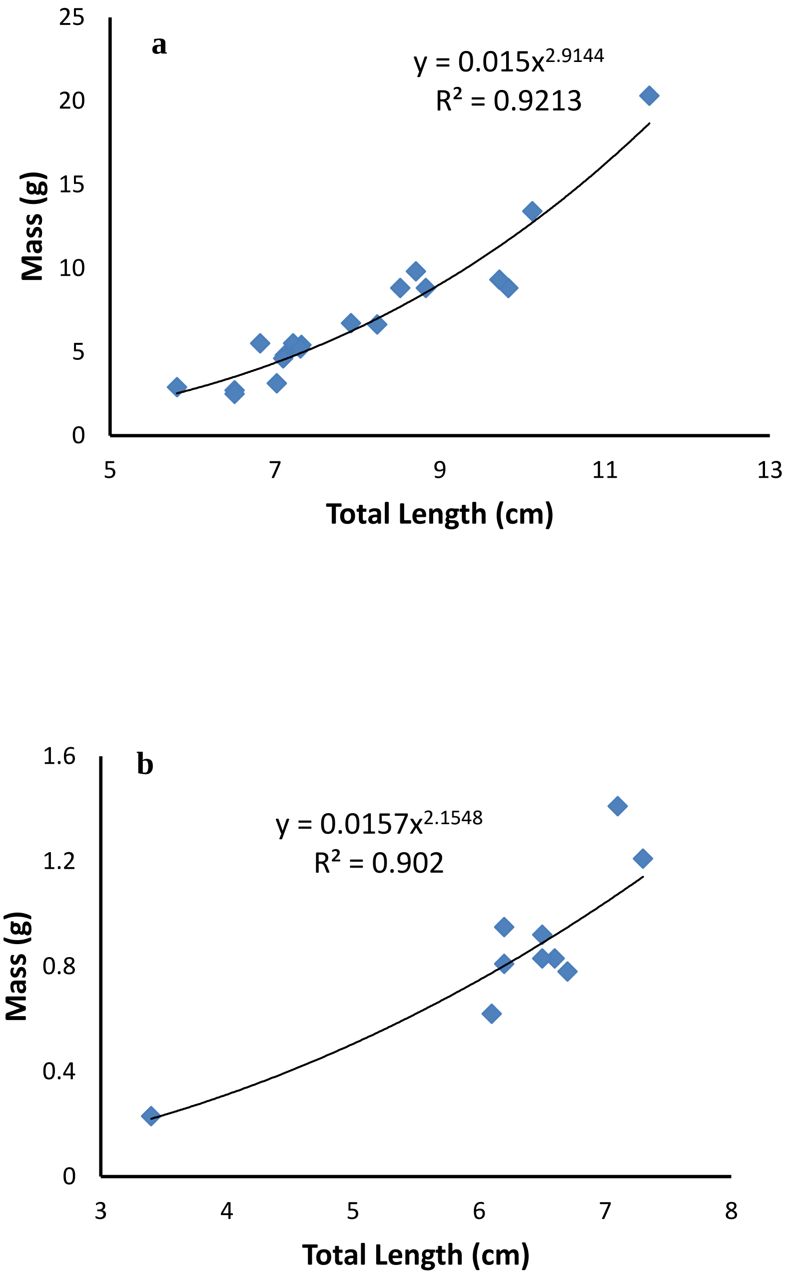


Fig. 3. Power function of length-mass relationships in the two studied fish species (a) *Carassius gibelio* (b) *Sabanejewia caspia*. b values were 2.9144 and 2.1548; and r^2 were 0.8821 and 0.902, for both species, respectively.

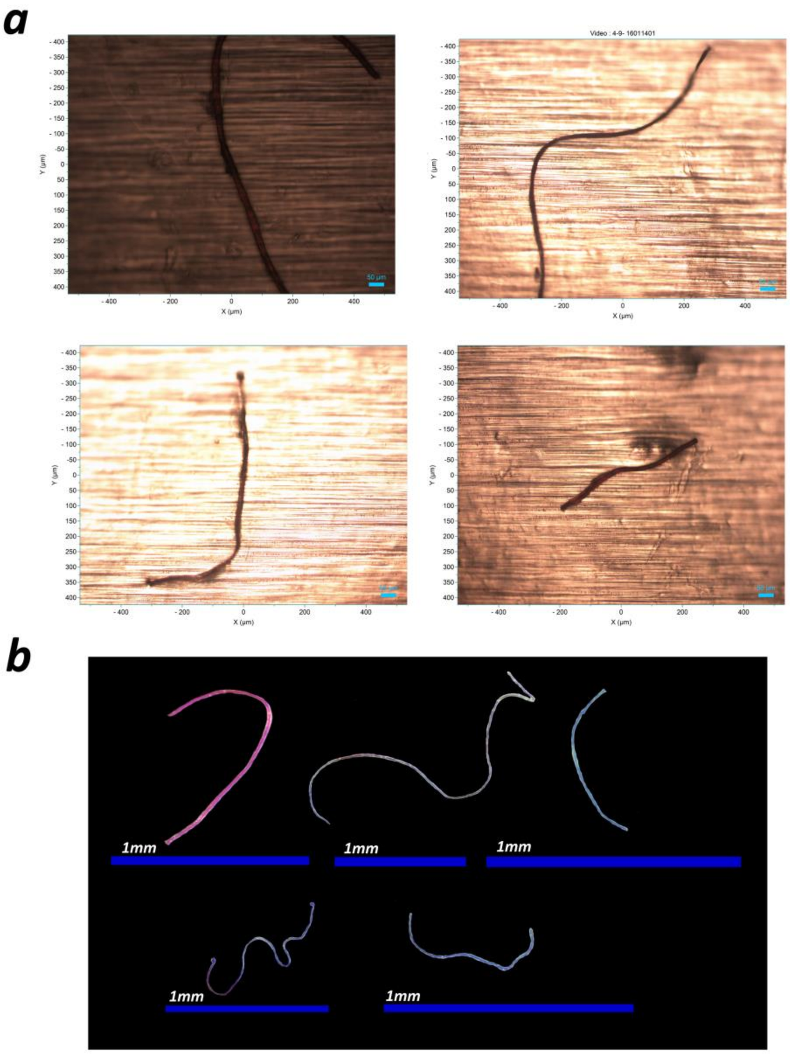


Fig. 4. Representative micrographs of microfibers detected in the GI tract of *Carassius gibelio* and *Sabanejewia caspia* with (a) binocular microscopy and (b) stereo microscopy.

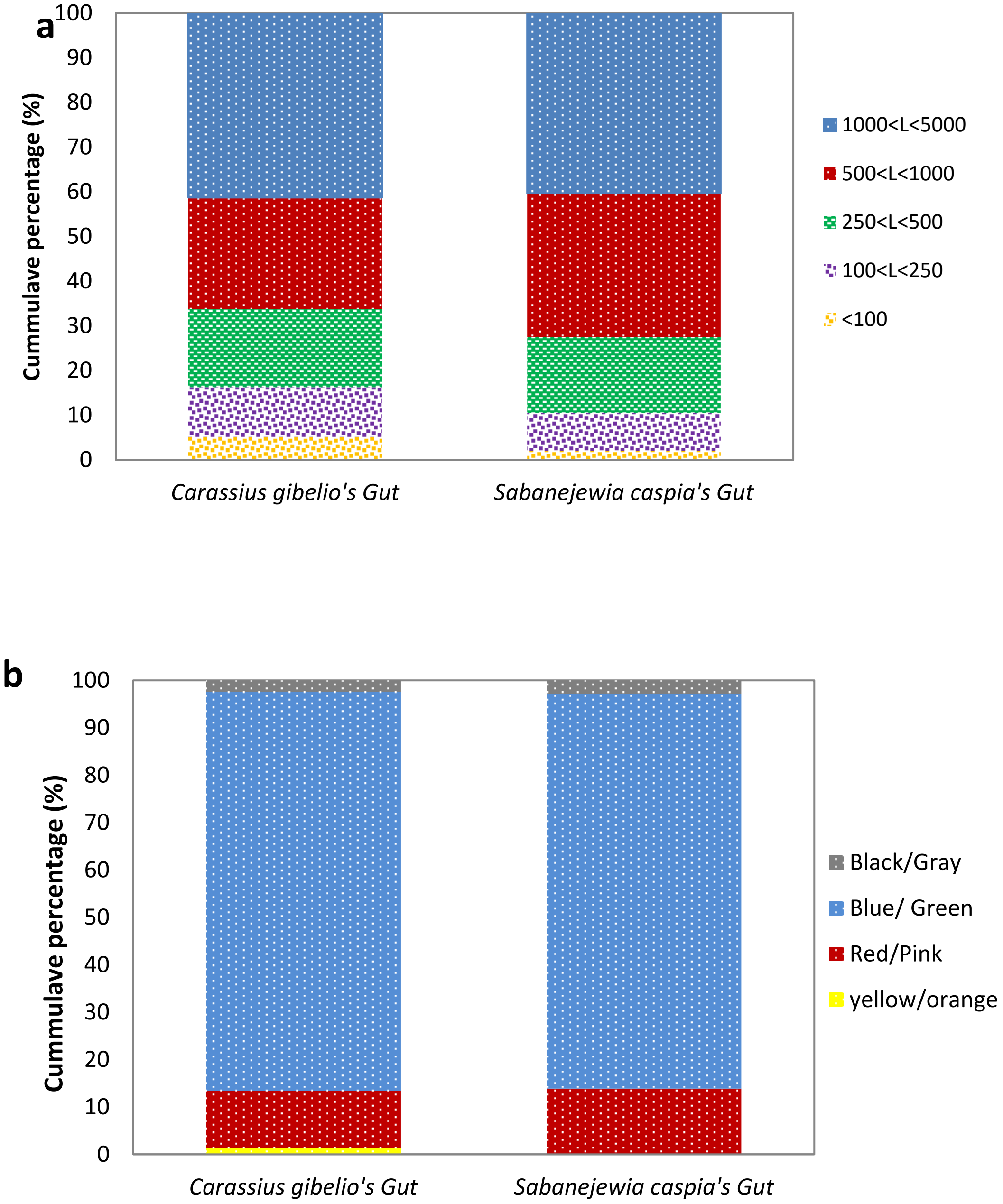
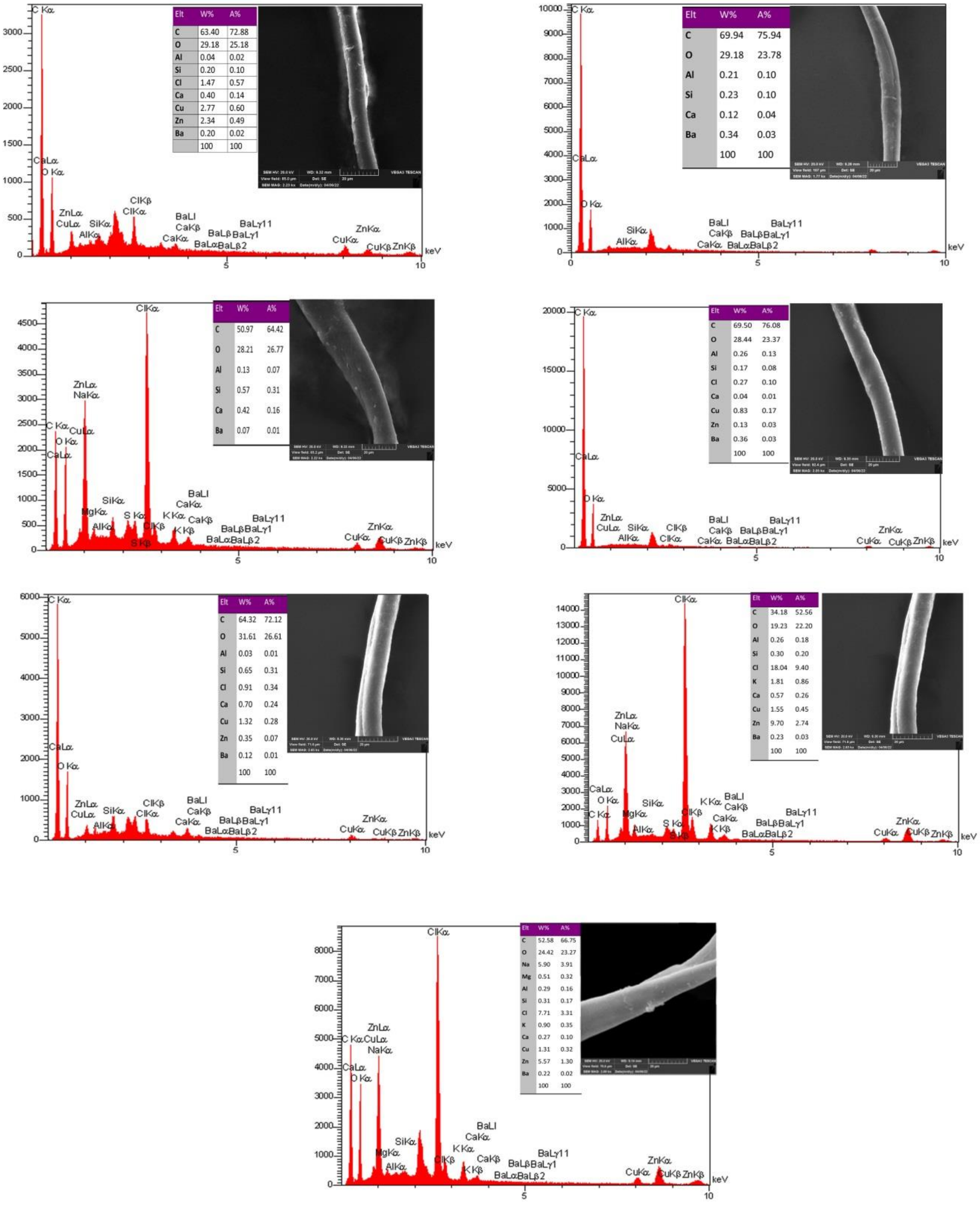


Fig. 5. Cumulative percentage of a) size classes (in µm) and b) colors of microfibres in *Carassius gibelio* and *Sabanejewia caspia*.



Elt	W%	A%
C	63.40	72.88
O	29.18	25.18
Al	0.04	0.02
Si	0.20	0.10
Cl	1.47	0.57
Ca	0.40	0.14
Cu	2.77	0.60
Zn	2.34	0.49
Ba	0.20	0.02
	100	100

Elt	W%	A%
C	69.94	75.94
O	29.18	23.78
Al	0.21	0.10
Si	0.23	0.10
Ca	0.12	0.04
Ba	0.34	0.03
	100	100

Elt	W%	A%
C	50.97	64.42
O	28.21	26.77
Al	0.13	0.07
Si	0.57	0.31
Ca	0.42	0.16
Ba	0.07	0.01

Elt	W%	A%
C	69.50	76.08
O	28.44	23.37
Al	0.26	0.13
Si	0.17	0.08
Cl	0.27	0.10
Ca	0.04	0.01
Cu	0.83	0.17
Zn	0.13	0.03
Ba	0.36	0.03
	100	100

Elt	W%	A%
C	64.32	72.12
O	31.61	26.61
Al	0.03	0.01
Si	0.65	0.31
Cl	0.91	0.34
Ca	0.70	0.24
Cu	1.32	0.28
Zn	0.35	0.07
Ba	0.12	0.01
	100	100

Elt	W%	A%
C	34.18	52.56
O	19.23	22.20
Al	0.26	0.18
Si	0.30	0.20
Cl	18.04	9.40
K	1.81	0.86
Ca	0.57	0.26
Cu	1.55	0.45
Zn	9.70	2.74
Ba	0.23	0.03
	100	100

Elt	W%	A%
C	52.58	66.75
O	24.42	23.27
Na	5.90	3.91
Mg	0.51	0.32
Al	0.29	0.16
Si	0.31	0.17
Cl	7.71	3.31
K	0.90	0.35
Ca	0.27	0.10
Cu	1.31	0.32
Zn	5.57	1.30
Ba	0.22	0.02
	100	100

Fig. 6. SEM/EDS analyses of MPs in the GI tract tissues of *Carassius gibelio* and *Sabanejewia caspia*. All the MPs found were microfibers.

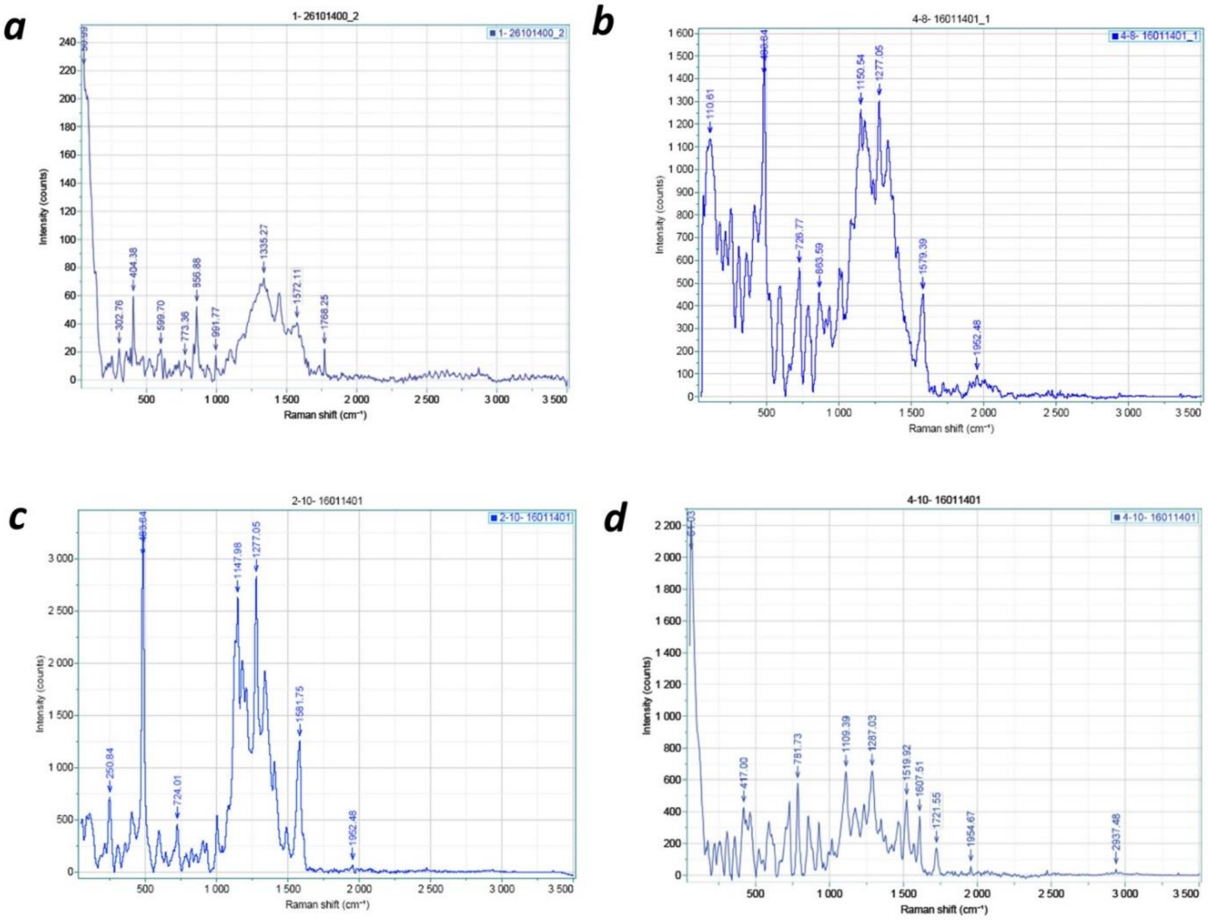


Fig. 7. Prevalence (%) of identified polymers in the GI tracts; Raman spectra of representative samples identified (a, b: nylon; c, d: polycarbonate) in *Carassius gibelio* and *Sabanejewia caspia*.

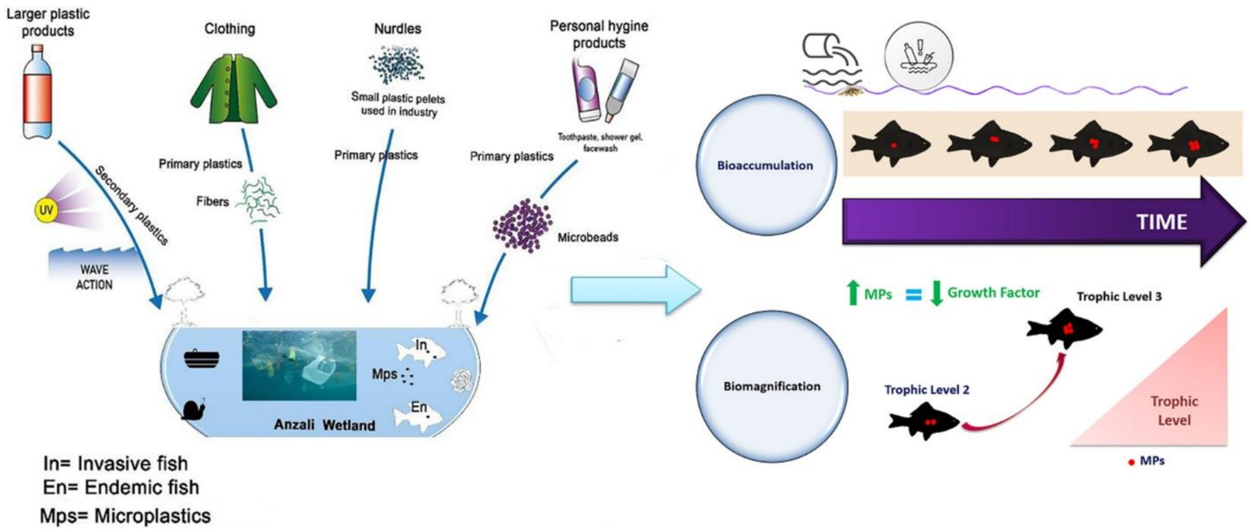


Fig. 8. Schematic presentation of MP contamination and the possible trophic transfer of MPs during the feeding process.

Table 1. Biological parameters and MPs frequency in the GI tract of *Carassius gibelio* and *Sabanejewia caspia* from the Anzali wetland, southern Caspian Sea. GI= Gastrointestinal tract, TL = total length, A = age (y), W = mass (g), K= Condition Factor, MP = number in the GI tract, Med = median, SD = standard deviation, Skew = skewness, Kurt = kurtosis, S-W = Shapiro-Wilk.

Species		GI (g)	TL (cm)	A (years)	W (g)	K	MPs/GI (number)
Carassius gibelio	Average	0.65	7.97	2.56	7.016	1.46	3.56
n=16	SD	0.33	1.25	0.73	2.75	0.87	3.05
	Min	0.20	5.81	1.00	2.49	0.26	0.00
	Max	1.42	11.54	3.00	20.31	4.50	11.00
	Med	0.55	7.32	3.00	5.52	1.33	3.00
	CV	0.58	0.16	0.28	0.59	0.39	0.86
	Skew						0.957
	Kurtosis						0.916
	Sum						57
	S-W						p>0.05
Sabanejewia caspia	Average	0.24	6.25	3.80	0.86	1.61	3.70
n=10	SD	0.12	1.08	0.42	0.32	0.25	2.79
	Min	0.19	3.40	3.00	0.23	1.27	0.00
	Max	0.57	7.30	4.00	1.41	2.08	8.00
	Median	0.20	6.50	4.00	0.83	1.65	3.00
	CV	0.51	0.16	0.13	0.36	0.26	0.75
	Skewness						0.577
	Kurtosis						-0.851
	Sum						37
	S-W						p>0.05

Table 2. Comparison of microplastic accumulation in the GI tract of different fish species and the present study; mean MP/gut =mean number of microplastics in the gut, mean number of MPs/ g GI tract; N: number of specimens

Species	**Mean MP/GI**	**Mean MP GI (g)**	**Sampling Site**	**References**
Sillago sihama (N =17)	1.5	0.25	Musa Estuary, Persian Gulf	Abbasi et al., 2018
Platycephalus indicus (N =12)	2.3	0.59	Musa Estuary, Persian Gulf	Abbasi et al., 2018
Cynoglossus abbreviatus (N =11)	2.9	0.16	Musa Estuary, Persian Gulf	Abbasi et al., 2018
Saurida tumbil (N =4)	2.8	0.37	Musa Estuary, Persian Gulf	Abbasi et al., 2018
Carassius gibelio (N=54)	~1.5±3.03		Anzali Wetland, Caspian Sea	Rasta et a., 2021
Cyprinus carpio (N =31)	~2±2.01		Anzali Wetland, Caspian Sea	Rasta et a., 2021
Esox lucius (N =23)	~0.7±0.65		Anzali Wetland, Caspian Sea	Rasta et a., 2021
Tinca tinca (N =5)	~1.8±1.7		Anzali Wetland, Caspian Sea	Rasta et a., 2021
Perca fluviatilis(N =44)	~0.5±0.7		Anzali Wetland, Caspian Sea	Rasta et a., 2021
Vimba vimba (N =7)	~1±1.4		Anzali Wetland, Caspian Sea	Rasta et a., 2021
Chelon saliens (N =14)	4.2		Anzali Wetland, Caspian Sea	Nematollahi et a., 2021
Cyprinus carpio (N =10)	3.4		Anzali Wetland, Caspian Sea	Nematollahi et a., 2021
Rutilus kutum (N =11)	1.6		Anzali Wetland, Caspian Sea	Nematollahi et a., 2021
Carassius gibelio(N =16)	3.4±3.05	0.57±0.44	Anzali Wetland, Caspian Sea	Present study
Sabanejewia. caspia(N =10)	3.7±2.79	6.23±7.6	Anzali Wetland, Caspian Sea	Present study