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RESEARCH ARTICLE

Microplastic size matters for absorption and excretion by *Artemia salina* and *Acipenser ruthenus* larvae in models of water pollution and food chain transfer

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Abstract

Microplastics pervade the hydrosphere and inevitably come into contact with aquatic organisms. The study reports quantitative data on absorption and excretion of polystyrene microspheres 2 and 10 μm in diameter by zooplankton and fish larvae on the example of *Artemia salina* L. and *Acipenser rithenus* L. At the initial concentration of 500 μg/L, *A. salina* accumulated 2 and 10 μm particles in amounts up to 0.103 and 0.151 ng/individual, respectively, at a similar rate. The mass content of large-sized particles in *A. salina* was significantly higher ($p < 0.01$) compared to small-sized particles throughout the experiment. Artemia salina and *A. rithenus* larvae did not accumulate microplastics in the gastrointestinal tract over a period of 96 and 72 h, respectively. Consumption of microplastics by *A. ruthenus* larvae with *A. salina* through the food chain was slower and less pronounced in mass than their direct absorption from water. The rates of absorption of 2 and 10 μm particles by fish attained 0.9 and 8.22 ng/individual/h from water, and 0.06 and 0.23 ng/individual/h with food, respectively. In the models of water pollution and food chain transfer, *A. ruthenus* larvae consumed more 10 μm particles in mass compared to 2 μm particles ($p < 0.05$) and at a higher rate. For 2 μm particles, the excretion time for 50% of particles from the gastrointestinal tract of fish (T_{50}) was 32–33 h, whereas for 10 μm particles, the excretion of particles consumed with food was slower (T_{50}=45 h) compared to that of particles

absorbed directly from water (T_{50}=25 h). The data obtained can be used to simulate transport and circulation of microplastics of different sizes in the environment.

Keywords
Microplastics, polystyrene, *Acipenser ruthenus*, *Artemia salina*, absorption, excretion, particle transfer, food chain

Introduction

Microplastics (MPs), solid synthetic polymer particles ranging from 1 to 5000 μm, are a ubiquitous pollutant of the hydrosphere, including terrestrial freshwaters (Frank et al. 2022; Triebskorn et al. 2019; Wong et al. 2020). MPs originate from both plastic debris fragmented in the aquatic environment and from micro-sized plastic particles entering water bodies with domestic and industrial wastewater, and through their transport from land with surface runoff (Schell et al. 2020; van Emmerick et al. 2022; Wang et al. 2022). MP pollution of continental freshwaters is a global phenomenon with varying degrees of severity in different regions of the world (Li et al. 2020; Talbot and Chang 2022). MPs have also been detected in Siberia, in particular, in Lake Baikal, Altai lakes, and Ob and Yenisei river basins (Frank et al. 2021a, 2021b; Il'ina et al. 2021; Malygina et al. 2021).

It is known that hydrobionts in freshwater ecosystems consume MPs at different trophic levels. MPs consumption is reported for phytoplankton (Hitchcock 2022), zooplankton (Setälä et al. 2014; Bulannga and Schmidt 2022; da Silva 2022), macrozoobenthos (Atici 2022; Hoellein et al. 2021; Pan et al. 2021), and fish with different food habits (McGoran et al. 2017; Roch et al. 2019; Vorobiev et al. 2024; Wang et al. 2020). Food transfer of MPs through food chains has also been evidenced. It was experimentally revealed that dragonfly and mysid shrimp larvae ingest MPs together with zooplankton (Setälä et al. 2014; Yildiz et al. 2022), while macrozooplankton absorb MPs together with copepods (Cole et al. 2013). Polyethylene particles 10–20 μm in size are consumed by fish larvae through trophic transfer from contaminated infusoria (Athey et al. 2020), and nano-sized polystyrene particles are transferred through a four-component freshwater food chain from microalgae to cladocerans and secondary consumer fish to the final consumer fish (Chae et al. 2018).

A number of review papers have documented adverse effects of MPs on freshwater living organisms from different systematic and ecological groups, including trophic transfer of MPs (Foley et al. 2018; Huang et al. 2021; Moyo 2022; Provencher et al. 2019; Wang W. et al. 2019; Zhang et al. 2022). Disruption of normal life processes in freshwater and marine invertebrates and fish ingested MPs includes behavioral and mechanical disorders, as well as physiological, biochemical and cellular disorders (Cole et al. 2015; Sole et al. 2019; Dyomin et al. 2023; Foley et al. 2018; Jeyavani et al. 2022; Jovanović 2017; Kim et al. 2021; Morgalev et al. 2022; Rochman et al. 2013). Hydrobionts absorb and excrete MPs in different manner and at differ-

ent rates (Sole et al. 2013; Roch et al. 2020; Yıldız et al. 2022), which causes a different physiological response in natural conditions and has a varying degree of impact on their well-being. The size of plastic particles can affect the rate and severity of these processes (Au et al. 2017; Roch et al. 2021; Cui et al. 2024). For example, different zooplankton organisms absorb 1 and 90 μm polystyrene (PS) microspheres with different intensity (Scherer et al. 2017). It was shown that a reduced size of polyethylene (PE) microspheres increases their toxicity to microalgae (Chae et al. 2019), while a reduced size of polyvinyl chloride (PVC) particles decreases their toxicity (Zhang et al. 2017). In rainbow trout experiments, 42.7 μm fragments of polymethyl methacrylate (PMMA) were retained in the gastrointestinal tract of fish much longer than 1,000 μm particles (Roch et al. 2021). Quantitative data on MPs consumption and excretion by living organisms are in demand to simulate particle transport between environmental components and are important for in-depth understanding of the MP global circulation.

Studies on consumption, excretion and bioaccumulation of MPs by hydrobionts are of high relevance; however, these studies have not yet been conducted for many groups of invertebrates and fish. It is also of interest to investigate different combinations of aquatic organisms in food chains to understand the versatility of the MPs trophic transfer. The aim of this study was to assess the amounts and rates of absorption and excretion of MPs of two sizes (2 and 10 μm) by zooplankton on the example of *Artemia salina* L. and by fish larvae using *Acipenser ruthenus* L. in the zooplankton-fish food chain and in the model of water pollution.

Materials and methods

Study objects

Artemia salina L., 1758 is a small planktonic crustacean of the Branchiopoda order that inhabits saline water bodies (Browne and MacDonald 1982). *Artemia nauplii* are often used as starter food for juveniles of different fish species in rearing. In this study, they were used as food for fish larvae and as a model object to assess MPs consumption by zooplankton organisms. *Artemia* cysts (Biotrade, Russia) were cultured to obtain nauplii using Weiss apparatus. Culturing was performed at 30 °C, water salinity of 30 g/L, and a 12D:12L photoperiod at 2,900 K with no feeding.

Acipenser ruthenus L., 1758, the smallest sturgeon species from the basins of large rivers flowing into the Black, Azov, Caspian and Kara seas, does not make long migrations (Orlov et al. 2022). Adult fish feed on benthos, and early juveniles feed on zooplankton (Popov 2007). Sterlet is a valuable commercial fish in Siberia, but its harvesting is strictly regulated (Interesova et al. 2018; Krokhalevsky et al. 2022). Three-week-old larvae of *A. ruthenus* of similar body weights (23.2 ± 4.02 mg) were used for the experiments. Sterlet larvae were bred under laboratory con-

ditions from the egg mass of wild fish in the Research and Production Company Tomsk-Ecologija SJC (Tomsk, Russia), which specializes in the Siberian river ichthyofauna restoration.

Experimental design

Fluorescent yellow-green monodisperse polysterene (PS) microspheres of 2 μm (XFNANO, China) and blue PS microspheres of 10 μm were used in the experiments. MPs were stored in the dark as an aqueous suspension of 1% (v/v) and gently vortexed before application.

Vessels with suspension containing *Artemia* nauplii 48 h after their emergence from cysts were divided into three groups (two parallel replicates in each group): (1) without PS introduction; (2) with 2 μm PS microspheres added at a concentration of 500 μg/L; (3) with 10 μm PS microspheres added at a similar concentration. That was the starting point of the experiment, and then the culture fluid from nauplii was sampled at 24 h intervals as shown in Figure 1 (time points '24 h', '48 h', '72 h' and '96 h') to assess MPs accumulation by *A. salina*. The small crustaceans were frozen and stored at –20 °C until subsequent processing and counting of MP particles. At each time point, 25 *Artemia* nauplii were randomly selected from two parallel experimental replicates and analyzed as described in Section 3.3. In total, 400 individuals were examined.

Experiments were conducted in two parallel replicates in aerated 25 L glass aquaria filled with 20 L of tap water as described in (Frank et al. 2023), with 50 sterlet larvae per experiment. Water circulation was performed by a filtered water pump, and the bottom was vacuum cleaned twice a day to remove faeces, residual organic matter, and MPs. Experiments were conducted under diffuse light, at a temperature of 15.9 to 16.3 °C, pH of 7.5 ± 0.1, and dissolved oxygen concentration of 7.43 to 8.81 mg L^{-1}. A series of experiments were carried out using 2 and 10 μm MP particles. An experimental design is shown in Figure 1. Two models were tested in each series.

In the food chain transfer model, sterlet larvae were fed twice on day 1 (at the start of exposure and after 12 h) with *Artemia* nauplii grown in the medium supplemented with MPs of appropriate size at an initial concentration of 500 mg/L (corresponds to 1.1×10^8 particles of 2 μm and 9.0×10^5 particles of 10 μm per liter). The larvae were sampled at '48 h' time point and contained PS in their intestines. The average content of MPs in *Artemia* nauplii at this time point is shown in Suppl. material 1: Table S1. In the experimental aquaria with sterlet larvae in the model of food transfer of 2 μm MPs, PS microspheres at a concentration of 7.52 ng/L or 1.7×10^3 items/L (a total of 15.0 ng or 3.4×10^3 items/L) entered the aquarium as part of Artemia nauplii with each of the two additions. Similarly, 70.3 ng or 126.7 PS microspheres of 10 μm per liter of water entered the aquarium with *Artemia* nauplii.

In the microplastic water pollution model, sterlet larvae were fed twice with *Artemia* nauplii, grown in the MP-free medium and not containing MPs in their

intestines, on day 1 in equivalent amounts. However, MPs of appropriate size were immediately added to the aquarium water at a concentration of 500 μg/L, which corresponds to 1.1×10^8 particles of 2 μm and 9.0×10^5 particles of 10 μm per liter.

After 24 h, the water in the aquaria was changed and the MP supply was terminated. To estimate the amount of MPs in sterlet larvae, samples were taken (7 randomly sampled larvae from each experimental aquarium at each time point) after 12 and 24 h at the particle ingestion stage and after 48 and 72 h at the particle excretion stage (Figure 1). A total of 112 fish species were analyzed at four time points, with a sample size of n=7 from each aquarium and two experimental replicates. Prior to MPs visualization, the fish were stored at –20 °C.

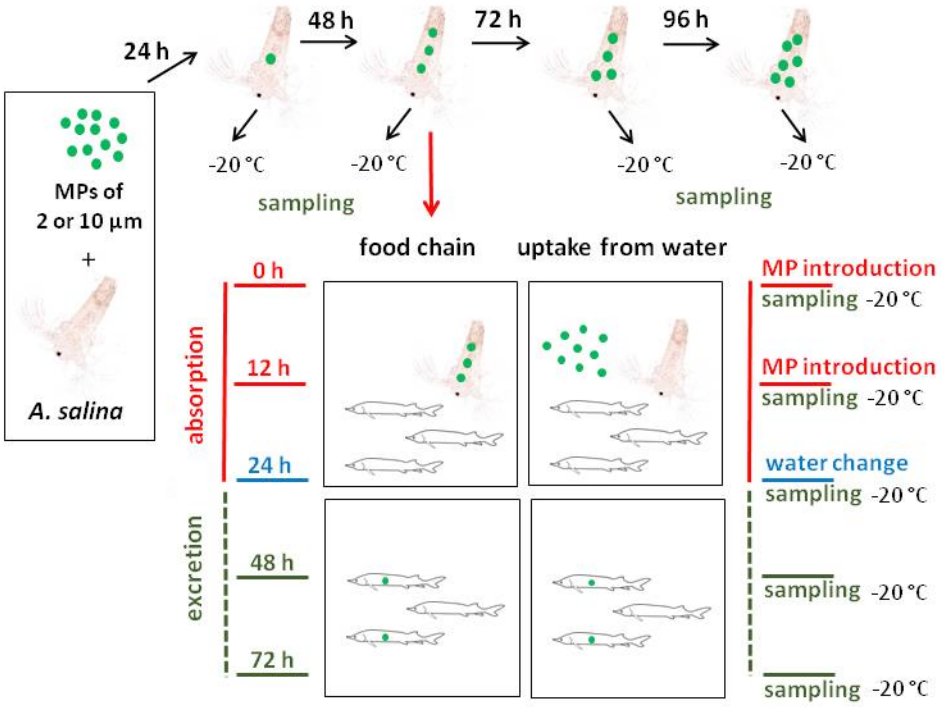

Figure 1. Experimental design.

Sample preparation and microplastic quantification

The quantity of MPs in *Artemia* nauplii and sterlet larvae were assessed by epifluorescence microscopy using an Axio Zoom.V16 fluorescence microscope (Carl Zeiss, Oberkochen, Germany). A 38 HP eGFP filter set with excitation/emission wavelengths of 450–490/500–550 nm and a 49 HP filter set with excitation/emission wavelengths of 365/445–450 nm were used to detect the presence of 2 and 10 μm PS

particles, respectively. The ZEISS Axiocam 712 mono digital camera and ZEN (Blue edition) software were used to take microphotographs.

Visualization and counting of fluorescent PS microspheres in the intestines of *Artemia* nauplii were carried out on intact individuals after double washing with distilled water and precipitation of nauplii by centrifugation at 8000 rpm. To count MPs ingested by sterlet larvae, each individual was washed with distilled water and dissected under a transmission microscope. The intestine was carefully extracted from each larva, fragmented with a needle in a drop of distilled water on a slide, and particles were counted using field partitioning into sectors (for 2 μm MPs), and ×64 magnification for larger and ×112 magnification for smaller PS microspheres using an appropriate filter.

Data analysis and statistical tests

For each sample of *Artemia* nauplii and sterlet larvae, the average content of MPs of each size was calculated and expressed as the ariphmetical mean and standard error (SE). Particle absorption and excretion rates were determined from the tangent of the slope of the trend line using Microsoft Excel software. The period, when sterlet larvae excreted 50% of consumed particles (T_{50}), was determined based on the decrease in the MP content in individuals over a certain period of time (Hou et al. 2023). For this purpose, the percentage of particles excreted within 48 h (from the removal of MPs from water at '24 h' time point to the end of the experiment at '72 h' time point) was calculated in each experimental version, and then T_{50} was calculated.

The nonparametric Mann-Whitney U-criterion was used to assess the reliability of differences in the content of PS particles of different sizes in samples of *Artemia* nauplii and sterlet larvae taken at the same time points, and the differences in the content of particles of similar size in the food transfer model and in case of their direct absorption from water. Differences were assessed at the confidence level of $\alpha = 0.01$ and $\alpha = 0.05$.

Result

Absorbtion of different-sized PS microspheres by *Artemia salina*

After introduction of both 2 and 10 μm PS microspheres to the water with the emerged *Artemia* nauplii, the latter began to ingest MPs. The ingested MPs were detected to localize in the gastrointestinal tract of the nauplii (Figure 2).

The average rate of MPs accumulation on day 1 after the start of exposure in the PS-supplemented medium was 0.788 items or 0.004 ng/h for 2 μm particles and 0.006 items or also 0.004 ng/h for 10 μm particles. After 24 h, 2 μm particles were

detected in all nauplii, whereas only 15% of the 50 *Artemia* nauplii from two replicates absorbed 10 μm particles after 24 h. Even after 96 h, not all nauplii contained 10 μm particles – three quarters of the larvae were free of MPs (Suppl. material 1: Table S1).

The absolute number of the absorbed particles of different sizes varied. PS particles of 2 μm were detected in amounts ranging from 18.9 ± 2.50 to 23.2 ± 3.03 items per individual (ind.), whereas 10 μm particles were detected at a maximum of one item per individual up to '72 h' time point (Figure 3, Suppl. material 1: Table S1). After 96 h, one *Artemia* individual out of the 25 examined individuals contained two large-sized PS particles. The average number of 10 μm particles per individual gradually increased from 0.15 ± 0.07 after 24 h to 0.27 ± 0.06 after 96 h (Figure 3, Suppl. material 1: Table S1).

Despite significantly higher consumption of 2 μm particles compared to 10 μm particles in terms of the number of MP particles per nauplii, absorption of larger particles in mass was significantly higher ($p < 0.01$) throughout the experiment (Figure 3). Over time, the amount of 10 μm particles in ng per *Artemia* individual was markedly higher compared to the amount of 2 μm particles (Figure 3, Suppl. material 1: Table S1). No statistically significant differences in the MP content were found after 24 h and 96 h ($p > 0.05$). The results obtained showed insignificant accumulation of 2 and 10 μm PS particles by *Artemia* nauplii both in absolute units and in mass during the observation period (96 h). The absorption and excretion of MPs of both sizes during this time interval were approximately equal.

Absorbtion and excretion of different-sized PS microspheres by sterlet larvae in models of food transfer and direct ingestion

It was shown that sterlet juveniles absorb 2 and 10 μm PS particles both directly from water and ingest it with food in the Artemia–fish food chain model. Epifluorescence microscopy detected PS particles of both sizes in sterlet larvae (Figure 4). The average amount of PS particles in sterlet larvae (items/ind. and ng/ind.) when absorbed directly from water 12 h after the start of exposure was higher than that of particles obtained through the Artemia–fish food chain. Accumulation of 2 μm particles from water was 13.2-fold higher than that through the food chain. Accumulation of 10 μm particles from water was 38.6-fold higher than that through the food chain (Figure 4, Suppl. material 1: Tables S2 and S3). The mean absorption rate (minus the excretion rate) for PS particles of both sizes was highest within the first 12 h (Table 1). During this period, sterlet larvae consumed 2 μm particles at a rate of 13.4 items/h with food and 203.2 items/h by absorbing them directly from water. For 10 μm particles, these values were only 0.41 and 14.9 items/h, respectively (Table 1).

The amount of 10 μm PS particles in fish (ng/ind.) was higher compared to 2 μm particles, which was particularly evident when MPs were absorbed directly from water (Figure 4, B and D, Suppl. material 1: Table S3). Significant differences

in the amount of PS microspheres of two sizes ($p < 0.05$) in sterlet larvae were recorded after 12, 48 and 72 h in the food chain transfer model and after 12, 24 and 48 h in the water pollution model (Figure 4). The absorption rate calculated for MPs with regard to the excretion rate was higher for 10 μm particles compared to smaller ones. During the first 12 h after feeding with MP-supplemented food, sterlet larvae absorbed large-sized particles at a rate of 0.23 ng/h, and the absorption rate for small-sized particles was lower and attained 0.06 ng/h (Table 1). When MPs were absorbed directly from water, the absorption rate for 10 and 2 μm particles was 8.22 and 0.90 ng/h, respectively, with regard to the excretion rate (Table 1). The amount of MPs of both sizes (ng/ind.) was significantly lower ($p < 0.01$) in the case of MPs ingestion from the MP-supplemented medium (12 and 24 h) through the food chain compared to MPs absorption directly from water (Figure 5). At '48 h' time point, the differences in the amount of 2 μm particles were significant ($p < 0.01$), while differences in the amount of 10 μm PS particles were negligible. At '72 h' time point, the amount (ng/ind.) of MPs of both sizes in the food chain transfer model was significantly lower ($p < 0.05$) compared to that in the water pollution model (Figure 5).

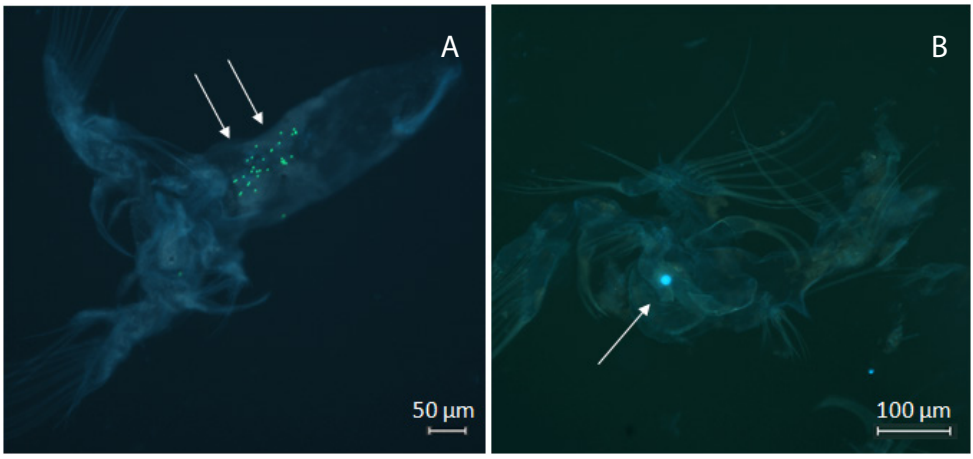

Figure 2. Micrographs of *Artemia* nauplii with (A) 2 μm and (B) 10 μm PS particles. Arrows show MPs in *Artemia* nauplii.

No statistically significant differences in the amount of MPs were found in sterlet larvae fed with MP-supplemented food (12 and 24 h), either in the case of MPs absorption from water or in the case of its trophic transfer ($p > 0.05$). This indicates insignificant accumulation of MPs in sterlet larvae in the models of water pollution and food chain transfer, both by the absolute number of particles and by the mass content.

The excretion rates varied depending on particle sizes and initial concentration of MPs in sterlet larvae. Of the total amount of 2 μm particles in the food chain, 75.6% were excreted after 72 h (48 h after MPs ingestion) at an average rate of 4.85

items/h. Of 2 μm particles absorbed directly from water, 72.8% were excreted after 72 h at an average rate of 37.3 items/h (Table 1, Suppl. material 1: Tables S2 and S3). Of the total amount of 10 μm particles in the food chain, 52.9% were excreted 48 h after MPs ingestion at an average rate of 0.05 item/h. Of 10 μm particles absorbed directly from water, 95.6% were excreted at an average rate of 3.01 items/h (Table 1, Suppl. material 1: Tables S2 and S3).

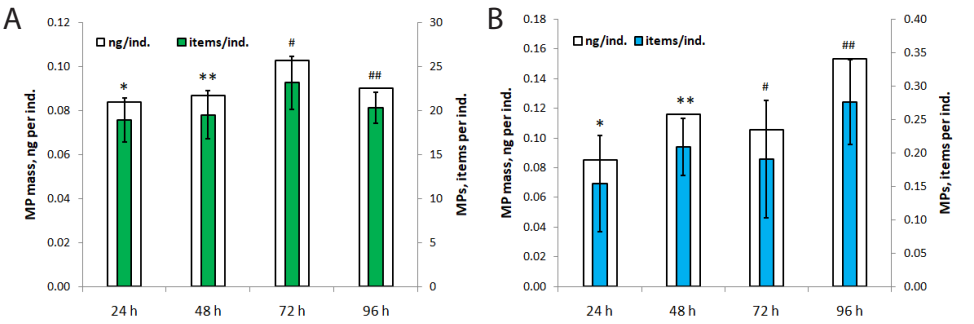

Figure 3. MPs of (A) 2 μm and (B) 10 μm in *Artemia* nauplii (arithmetic mean ± SE). The symbols '*' and '#' indicate significant differences in the content of different-sized particles (ng/ind.) at each time point ($p < 0.01$).

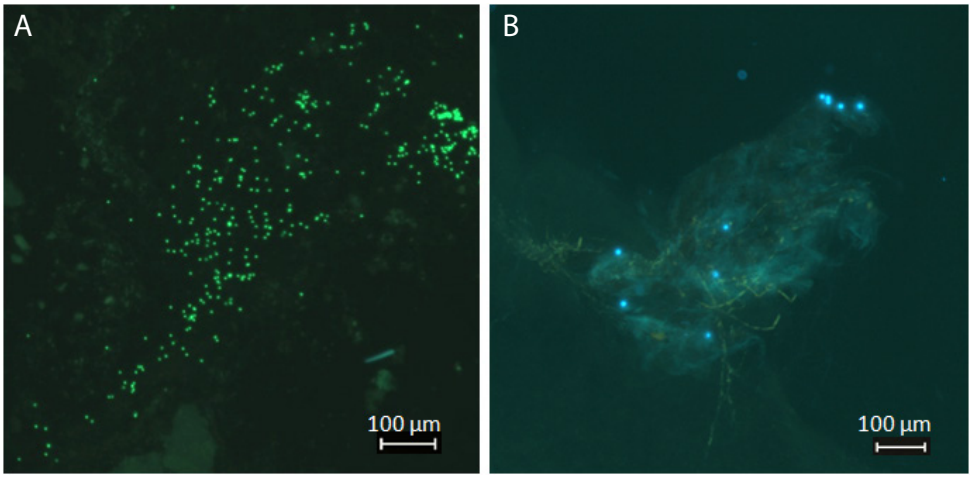

Figure 4. MPs of (A) 2 μm and (B) 10 μm in sterlet larvae.

In terms of the mass content (ng/ind./h), 10 and 2 μm MPs obtained through the food chain were excreted at the rates of 0.03 and 0.02 ng/h, respectively. Larger PS particles absorbed by fish directly from water were excreted much faster than smaller ones at the average rates of 1.72 and 0.12 ng/h, respectively (Table 1). In sterlet larvae, 2 μm particles were excreted from the gastrointestinal tract within

approximately the same time period in the models of food chain transfer and water pollution: T_{50} = 31.75 h and T_{50} = 32.98 h, respectively (Suppl. material 1: Tables S2 and S3). MPs of 10 μm consumed through the food chain were excreted much more slowly compared to direct absorption from water: T_{50} = 45.34 h and T_{50} = 25.11 h, respectively (Suppl. material 1: Tables S2 and S3).

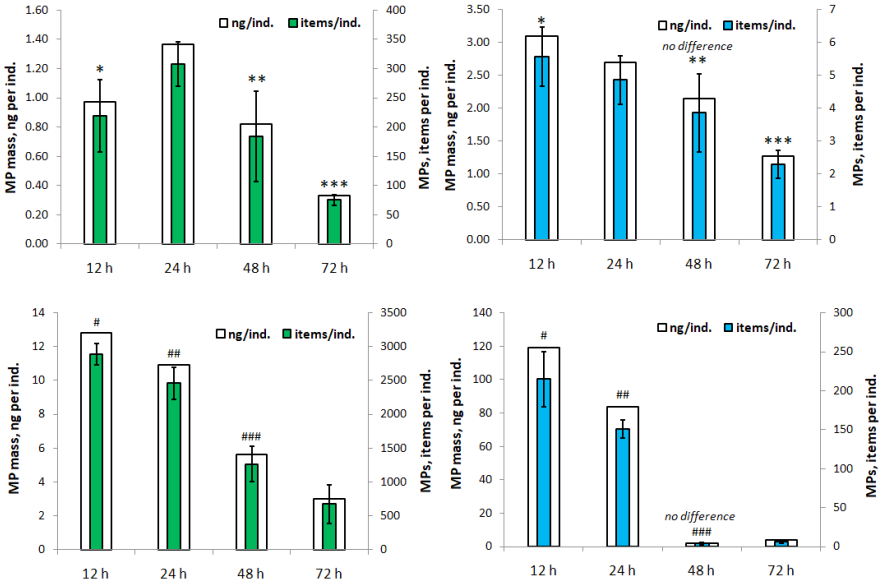

Figure 5. Content of MPs of different sizes in sterlet larvae in the models of food chain transfer and water pollution: A – 2 μm, food chain transfer, B – 10 μm, food chain transfer, C – 2 μm, water pollution, D – 10 μm, water pollution (arithmetic mean values ±SE). The symbols '*' and '#' indicate significant differences in the mass content of MPs of different sizes ($p < 0.05$),'no difference' indicates insignificant differences, in all other cases the amount of MPs absorbed from water is significantly higher ($p < 0.05$) than that obtained through the food chain.

Table 1. Average rates of absorption and excretion of MPs by sterlet larvae in the models of food chain transfer and water pollution

Units	Food chain transfer			Water pollution		
	Absorption*		Excretion 24–72 h	Absorption*		Excretion 24–72 h
	0–12 h	12–24 h		0–12 h	0–24 h	
			2 μm SP-MPs			
items/ind./h	13.372	2.5937	4.8507	203.19	97.619	37.281
ng/ind./h	0.0594	0.0116	0.0215	0.9000	0.2895	0.1700

Units	Food chain transfer			Water pollution		
	Absorption*		Excretion 24–72 h	Absorption*		Excretion 24–72 h
	0–12 h	12–24 h		0–12 h	0–24 h	
			10 μm SP-MPs			
items/ind./h	0.4107	-0.1131	0.0536	14.905	6.2500	3.0119
ng/ind./h	0.2280	-0.0628	0.0297	8.2210	1.1756	1.7230

Note: * the absorption rate is calculated with regard to the excretion rate.

Discussion

Quantitative data obtained on absorption and excretion of MPs by aquatic organisms are subsequently used for assessment of the extent and reconstruction of transport and circulation of MPs in the environment, including trophic transfer (Hou et al. 2023; Roch et al. 2021). In this regard, the quantitative characteristics of MPs absorption and excretion should be expressed in mass units. We employed similar mass contents of PS particles of different sizes (500 μg/L) in all the experiments to assess MPs absorption by brine shrimp and fish larvae directly from water.

The entry of MPs into the food chain and their trophic transfer can involve zooplankton organisms, which are primary consumers that connect producers and higher trophic levels (Richon et al. 2022; Serrão, Marques-Santos 2023). Zooplankton organisms are particularly susceptible to adverse impacts of MP pollution (Foley et al. 2018). Numerous studies focus on absorption of MPs by zooplankton, and the majority of studies have been performed on one model object *Daphnia magna* Straus; yet some data are available for *A. salina* (Serrão, Marques-Santos 2023). It was shown that *A. salina* ingests PP fragments of 11.9–44.6 μm at different stages of ontogenesis (Jeyavani et al. 2022). *Artemia* nauplii consume 10 μm PS particles and accumulate them in the amount up to 85.6 items/ind. at the MP content in water of 103 items/mL, and up to 306.2 items/ind. at the maximum level of water contamination of 104 items/mL (Albano et al. 2021). In this study, we estimated the absorption of 2 and 10 μm MPs at the initial level of water contamination of ~10^5 and ~10^3 items/mL, respectively; the mass content was similar and attained 500 μg/L. *A. salina* accumulated 2 μm particles in the amount of 23.2 ± 3.03 items/ind., and the maximum concentration was recorded after 72 h of exposure, while the average concentration of 10 μm particles during the entire experiment did not exceed 1 item/ind.; the differences are significant at $p < 0.01$ (Figure 1).

A more intensive absorption of small particles compared to larger ones can be due to the differences in the particle size. It was previously shown that zooplankton organisms *Chironomus riparius* Meigen and *D. magna* consume particles of different sizes (1, 30 and 90 μm) depending on the stage of ontogenesis: older and larger individuals ingested much more particles and gravitated toward larger ones

(Scherer et al. 2017). Zooplankton actively absorb particles of < 1 to 70 μm, which was shown on the example of Cladocera; however, the maximum size of spherical particles that can be ingested by zooplankton (y, μm) depends on the body length (x, mm) and is calculated by the equation y = 22x + 4.87 (Burns 1968). The body length of 3-day-old Artemia nauplii reaches 1.2 mm (Piper 2018). The body length of nauplii reared without food ranges from 0.70 to 0.92 mm 48 h after hatching (Claus et al. 1979), and from 0.92 to 0.98 mm 96 h after hatching (Albano et al. 2021). Therefore, individuals of this size are able to ingest particles larger than 20 μm. Apparently, the higher content of 2 μm MPs in *Artemia* nauplii is associated with a higher absolute number of particles in water (items per volume) compared to 10 μm MPs; the mass content was 500 μg/L in both cases. Over time, the mass content of 10 μm MPs was observed to increase in *Artemia* nauplii compared to that of 2 μm MPs. The maximum concentration was 0.103 ± 0.013 ng/ind. for 2 μm MPs after 72 h, while the maximum concentration of 10 μm particles was observed after 96 h and averaged 0.151 ± 0.035 items/ind.

It is apparent that the absorption of MPs by zooplankton depends on the particle concentration in the environment. However, the experiments with *Artemia partenogenetica* Bowen and Sterling revealed fluorescent PS microspheres with a diameter of 10 μm in invertebrates even at a very low content of these particles in the medium (1.1 ± 0.16 items/mL (0.61 ± 0.09 μg/L)) (Wang et al. 2019). MPs ingestion by zooplankton organisms may significantly depend on the population density, but the dependence has not yet been discussed. The density of *Artemia* nauplii in solution attained 607 ind./mL at '48 h' time point. In (Albano et al. 2021), a total of 6 individuals per mL of water were involved in the experiment with *Artemia* nauplii.

The average rate of MPs accumulation by *Artemia* nauplii (not the absorption rate, since the excretion rate was not considered) on the first day of exposure to the PS-supplemented medium was approximately 1 item or 0.004 ng/h for 2 μm particles and 0.006 item or 0.004 ng/h for 10 μm particles, which is relatively low. In (Albano et al. 2021), the average accumulation rate was 6.4 ± 4.39 items/ind. at the same initial concentration of 10 μm particles in the first 24 h, which corresponds to about 0.25 items/ind./h. The ciliates *Paramecium* and *Tetrahymena* from the South African stream showed even higher accumulation rates of 2, 5 and 10 μm PS microspheres, which ranged from 1,650 to 3,870 items/ind./h (Bulannga and Schmidt 2022). We calculated the accumulation rate of MPs by *A. salina* nauplii for two particle sizes in units of mass per individual per hour. Despite a more intensive absorption of 2 μm PS particles compared to 10 μm PS particles in absolute units of particles per individual, the absorption of larger particles in mass was significantly higher ($p < 0.01$) throughout the experiment (Figure 3).

Sterlet larvae ingested MPs both directly from water and indirectly by consuming *A. salina* nauplii that had absorbed plastic particles. In both models, fish absorbed MPs at a higher rate in the first 12 h compared to the 12–24 h period. The absorption rates calculated with regard to the excretion rates and the actual number of particles in the gastrointestinal tract were higher for 2 μm MPs compared to 10

μm MPs (items/ind./h). However, the absolute number of 2 μm MPs in the food chain transfer model and in the water pollution model was much higher than that of 10 μm particles, while the mass content per liter was similar. This was predictable due to a strong positive correlation ($r_s = 0.956$, $p < 0.01$) between the accumulation of 2 μm PS particles by *Coregonus peled* Gmelin larvae and the absolute number of particles in water shown previously in (Frank et al. 2023). The mass content of 10 μm MPs in sterlet larvae in both models was higher ($p < 0.05$) compared to that of smaller particles (Figure 3). A similar pattern was observed for the absorption rates, which were 9.1-fold higher for 10 μm MPs compared to smaller ones (ng/ind./h) in the water pollution model and 3.8-fold higher in the food chain transfer model with *A. salina*.

Artemia salina nauplii consumed by sterlet larvae in the food chain transfer model contained a relatively small number of 10 μm MPs, yet the larvae consumed food supplemented with these particles. As was previously noted, juvenile fish and larger aquatic invertebrate filter-feeders ingest a significant amount of MPs consuming zooplankton, although the amount of MPs ingested by zooplankton may be low (<1 items/ind.) (Desforges et al. 2015). PMMA microspheres with a diameter of 48 μm were detected in the food chain when fish *Danio rerio* Hamilton ingested planktonic organisms *D. magna* and *Ch. riparius* in the model experiment (Hanslik 2020). MPs were found in the invertebrate–fish food chain in natural populations from River Slaney, Ireland, including Amphipoda, Coleoptera, Diptera, Ephemeroptera, Gastropoda, Plecoptera, Trichoptera, and *Salmo trutta* L. as the final consumer (O'Connor et al. 2022). At the same time, no biomagnification of MPs through food chains was observed. However, there is no sufficient evidence on the versatility of the MPs trophic transfer, and the content of MPs in food chains may increase for other combinations of living organisms or, for example, at a more pronounced pollution of the aquatic environment.

The residence time of MPs in the gastrointestinal tract of fish can be directly related to particle sizes in case of relatively large particles. Irregularly shaped plastic fragments larger than the intestinal lumen can be retained in the gastrointestinal tract of fish for quite a long time (Capone et al. 2020). We did not obtain evidence that 2 and 10 μm PS microspheres accumulate in the gastrointestinal tract of *A. salina* and fish in any of the models nor did other published works. Previously, it was shown that gobies (*Neogobius melanostomus* Pallas) completely excrete 1-mm long polyacrylate (PA) microfibers 18–25 h after ingestion (Hou et al. 2023). In this case, no significant differences in the specific rate of MPs excretion were found after either a single ingestion of PA microfibers with food or their daily consumption for 7 days. MPs were also completely excreted from the gastrointestinal tract of goldfish; T_{50} = 10 h for a mixture of 50–500 μm PA microfibers and 200 μm PE microspheres (Grigorakis et al. 2017). Experiments on fish with different structure of the gastrointestinal tract showed faster excretion rates for larger PMMA particles. For rainbow trout *Oncorhynchus mykiss* Walbaum, T_{50} = 4.0 h and T_{50} =12.1 h for 1,086 and 42.7 μm PMMA particles, and for carp *Cyprinus carpio* L., T_{50} = 4.6 h and T_{50} =

7.3 h, respectively (Roch et al. 2021). In this study, the half excretion time of PS microspheres for sterlet larvae was longer and reached 45 h in the excretion of 10 μm particles transferred through the food chain via *A. salina*. In the two models of MPs absorption, the pattern of excretion of different-sized particles by fish was different. The average rate of MPs excretion by sterlet larvae in each exposure varied from 0.05 to 37.3 items/ind./h (from 0.02 to 1.72 ng/ind./h) and depended on both the initial concentration and the amount of particles consumed, and on particle sizes. The excretion rate and T_{50} were similar for 2 μm particles absorbed directly from water and indirectly through the food chain and amounted to 32–33 h; the excretion rate of 10 μm particles in the food chain transfer model was significantly higher compared to the water pollution model and amounted to 45 and 25 h, respectively.

It is known that MPs smaller than 5 μm can be translocated from the gastrointestinal tract to fish organs, such as the liver (Lu et al. 2016; Zeytin et al. 2020). Yet, we did not find 2 μm fluorescent PS particles outside the intestines of sterlet larvae.

Conclusion

MP pollution is a relatively new challenge faced by the humanity since the beginning of the industrial revolution and the creation of artificial materials. We are still not aware of the risks, the nature, and the impact of MPs on living organisms inhabiting the Earth. Therefore, it is of relevance to clarify individual aspects of MP circulation in nature in order to determine patterns and gain a general understanding of the paths and mechanisms of MP transport. In this study, we did not obtain evidence on accumulation of PS microspheres with a diameter of 2 and 10 μm in the gastrointestinal tract of *Artemia salina* and *Acipenser ruthenus* in any of the models. At the same time, we showed that particle size matters for the absorption rate of MPs by *A. salina* and *A. ruthenus* larvae; in particular, the content of larger MPs in *A. salina* nauplii (ng/ind.) throughout the experiment was significantly higher compared to small particles. The mass content of 10 μm MPs absorbed by *A. ruthenus* larvae is higher compared to 2 μm MPs, both when absorbed directly from water and when transferred through the food chain. In addition, particle size affected the excretion rate: a lower excretion rate in *A. ruthenus* was found for larger particles absorbed through the food chain.

An important novel finding is the suggestion that the absorption of MPs by organisms may depend on their population density, which has not been previously discussed. This study may resolve the contradictions in published data on different MP absorption rates.

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Supplementary material 1

Table 1S. Content of microplastics (MPs) in the nauplii of *Artemia* in the experiment on the absorption of PS microspheres from water
Table 2S. MP content in sterlet larvae in a food chain PS microsphere consumption experiment
Table 3S. MP content in sterlet larvae in the PS microsphere uptake experiment from water
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