Environmental Pollution 357 (2024) 124426

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Exploring the trophic transfer and effects of microplastics in freshwater ecosystems: A focus on *Bellamya aeruginosa* to *Mylopharyngodon piceus*☆

Ming Zhang [a], Yijie Jin [a], Cenyi Fan [a], Yiwen Xu [a], Jiateng Li [a], Wenjing Pan [a], Ziyang Lou [b], Huili Chen [a], Binsong Jin [a,*]

[a] School of Life and Environmental Science, Hangzhou Normal University, 2318 Yuhangtang Road, Hangzhou, Zhejiang 311121, China
[b] School of Environmental Science and Engineering, Shanghai Jiao Tong University, Shanghai Engineering Research Center of Solid Waste Treatment and Resource Recovery, Shanghai 200240, China

ARTICLE INFO

Keywords:
Fluorescent microplastics
Trophic transfer
Bioaccumulation
Gut microbiota
Food safety

ABSTRACT

Microplastics (MPs) can enter aquatic food webs through direct ingestion from the environment or indirectly via trophic transfer, but their fate and biological effects within local freshwater food chains remain largely unexplored. In this study, we conducted the first investigation on the trophic transfer and impacts of fluorescently labeled polystyrene microplastics (PS-MPs) (100-nm and 10-μm) in a model freshwater food chain consisting of the snail *Bellamya aeruginosa* and the commercially important fish *Mylopharyngodon piceus*, both prevalent in Chinese freshwater ecosystems. Quantitative analysis revealed substantial accumulation of MPs in *B. aeruginosa*, reaching an equilibrium state within 12 h of exposure. While steady-state was not observed, a pronounced time-dependent bioaccumulation of MPs was evident in *M. piceus* over a five-week period following dietary exposure through the consumption of contaminated *B. aeruginosa*. Notably, MPs of both sizes underwent translocation from the gastrointestinal tract to the muscle tissue in *M. piceus*. High-throughput sequencing of the gut microbiota revealed that exposure to 100-nm MPs significantly altered the microbial community composition in *M. piceus*, and both particle sizes led to increased relative abundance of potentially pathogenic bacterial genera. Our findings provide novel insights into the trophic transfer, tissue accumulation, and biological impacts of MPs in a model freshwater food chain, highlighting the need for further research to assess the ecological and food safety risks associated with microplastic pollution in freshwater environments.

1. Introduction

Microplastic (MP) pollution has emerged as a pervasive environmental issue in freshwater ecosystems worldwide, with growing evidence of their widespread ingestion by aquatic organisms and potential risks to food safety and ecosystem health (Ali et al., 2024; Saemi-Komsari et al., 2023; Yi et al., 2024). Microplastics (MPs), defined as plastic particles smaller than 5 mm in size, can enter aquatic food webs through direct consumption by organisms at various trophic levels, from zooplankton to fish (Abdolahpur Monikh et al., 2022; Ashrafy et al., 2023; ECHA, 2019; Sulaiman et al., 2023; Wu et al., 2024; Zheng et al., 2023). For example, a global survey revealed that 49% of fish samples showed signs of MP ingestion (Wootton et al., 2021), with even higher prevalence rates reported in certain regions and species, such as 72.68% in commercial fish from the Persian Gulf (Gholizadeh et al., 2023) and 85% in the Bering Sea (Ding et al., 2023). Similarly, the ingestion of MPs has been observed in various aquatic invertebrates, such as the bivalve species *Asthenometis asthenodon*, with an occurrence of 84% (Rojas-Jimenez et al., 2022), and the holothurian species *Actinopyga crassa*, with an occurrence of 100% (Abd-Elkader et al., 2023). The considerable evidence of MP ingestion by aquatic organisms makes it reasonable to assume the likelihood of their transfer and potential bioaccumulation to higher-level consumers via the food chain.

In contrast to the studies on the trophic transfer of MPs in marine food webs (mostly focused on algae to zooplankton or zooplankton to fish (Junaid et al., 2024)), limited attention has been paid to the trophic transfer of MPs within freshwater food chains, especially for the transfer from invertebrates to fish. Currently, there are few publications addressing the trophic transfer of MPs from freshwater invertebrates to fish and the subsequent bioaccumulation in these fish. Yan et al. (2024)

☆ This paper has been recommended for acceptance by Maria Cristina Fossi.

* Corresponding author.
E-mail address: jin.binsong@gmail.com (B. Jin).

https://doi.org/10.1016/j.envpol.2024.124426
Received 16 March 2024; Received in revised form 1 June 2024; Accepted 21 June 2024
Available online 23 June 2024

reported the trophic transfer and tissue accumulation of polystyrene MPs from the brine shrimp (*Artemia nauplii*) to zebrafish (*Danio rerio*). Polystyrene MPs have also been shown to transfer along a food chain consisting of alga *Chlamydomonas reinhardtii*, water flea *Daphnia magna*, secondary-consumer fish *Oryzias sinensis*, and end-consumer fish *Zacco temminckii* (Chae et al., 2018).

Moreover, several studies have investigated the trophic transfer of MPs in simplified freshwater food chains under laboratory conditions, such as from water flea (*D. magna*) *to* zebrafish (*D. rerio*) (Yu et al., 2022), from *Neocaridina denticulate* to crucian carp (*Carassius auratus*) (Zhang et al., 2024), and from *Artemia franciscana* to Australian Bass (*Macquaria novemaculeata*) (Afrose et al., 2024). Even though these studies provided evidence of the trophic transfer of MPs via freshwater food chains, most of them focused on simplified food chains involving model organisms or small-sized fish species, such as zebrafish (*D. rerio*) (weight: 0.4 ± 0.03 g) (Yu et al., 2022), crucian carp (*C. auratus*) (weight: 23.7 ± 5.2 g) (Zhang et al., 2024) and snowy sculpin (*Myoxocephalus brandti*) (weight: 6.5 ± 0.8 g) (Hasegawa & Nakaoka, 2021). These findings may not be directly applicable to larger, commercially relevant fish species, which occupy higher trophic levels in freshwater food webs and may exhibit different patterns of MPs accumulation and biological responses. Moreover, none of the mentioned publications investigated the impact of the trophic transfer of MPs on the gut microbiota of the fish, which is valuable information for assessing their possible risks to the health and performance of freshwater fish (Bhattacharjee et al., 2022; Li et al., 2023).

Additionally, another area that lacks knowledge is related to the trophic transfer and effects of MPs in food chains involving commercially important freshwater mollusk and fish species. Importantly, in China, freshwater aquaculture plays a vital role in food security and rural livelihoods, with the country being the world's largest producer of freshwater aquaculture products (Xu et al., 2023). Among the various species cultured in Chinese freshwater systems, the freshwater snail *Bellamya aeruginosa* and the black carp *Mylopharyngodon piceus* are of significant economic importance. *B. aeruginosa* is widely consumed as a delicacy and is highly valued for its nutritional and medicinal properties (Wang et al., 2023). The black carp (*M. piceus*) is a large, commercially important fish species in China, with an annual production of approximately 700,000 tons that contributes significantly to the country's total freshwater aquaculture output (Yue et al., 2024; Tang et al., 2024). Given their significance in Chinese cuisine and the potential risks associated with MP pollution, understanding the trophic transfer and biological effects of MPs in a food chain involving these species is of utmost importance and urgency. To our knowledge, up until now, no study has focused on freshwater ecosystems concerning the trophic transfer and impacts of MPs in a food chain consisting of *B. aeruginosa* and *M. piceus*. The lack of published studies on this topic and the inconsistent trophic transfer results highlights the need for further studies on the trophic transfer of MPs in freshwater food webs, especially for food chains involving commercially important species.

In this study, *B. aeruginosa* and *M. piceus* were used to investigate the trophic transfer of fluorescently labeled polystyrene MPs (PS-MPs) and the associated effects on fish. The snails were first exposed to PS-MPs of two sizes (100 nm and 10 μm) via water, and then the fish were fed with the exposed snails for five weeks. The choice of these MP sizes was made to compare the influence of nanoplastics (NPs) and MPs, an approach employed in existing research on the size-dependent effects of MPs on aquatic organisms (Afrose et al., 2024). Afterward, the accumulation and distribution of PS-MPs in fish tissues were quantified using fluorescence microscopy, a promising tool for detecting and quantifying MPs in environmental and biological samples (Lee & Chae, 2021; Singh & Kumar, 2024; Zhou et al., 2022). Compared to other analytical methods, such as Fourier-transform infrared (FTIR) or Raman spectroscopy, fluorescence microscopy offers greater sensitivity and speed, with the ability to detect MPs down to the submicron range (0.1–1 μm) (Adhikari et al., 2022; Morgana et al., 2024; Singh & Kumar, 2024). The effects on fish gut microbiota were assessed using 16S rRNA gene sequencing. The objectives of this study are to investigate (1) the trophic transfer and tissue-specific accumulation of PS-MPs from snails to fish, (2) the effects on fish gut microbiota associated with the trophic transfer of PS-MPs, and (3) the potential implications for food safety. The findings of this study will help to improve the understanding of the trophic transfer of MPs in a freshwater food chain involving commercially important species and the subsequent effects on fish health and food safety. This will provide important information about the ecological and food safety risks of MP pollution in freshwater ecosystems.

2. Materials and methods

2.1. Polystyrene microplastics (PS-MPs)

Green fluorescent PS-MPs with diameters of 100 nm and 10 μm were purchased from Jiangsu Zhichuan Technology Co., Ltd. (Jiangsu, China) (Fig. S1). The chemical composition of the MPs was confirmed using a Nicolet iS50 Fourier-transform infrared (FTIR) spectrometer, which revealed characteristic peaks consistent with the reference spectrum of PS (Fig. S2). Prior to the exposure experiments, the MPs were further characterized by scanning electron microscopy (SEM) using a ZEISS Sigma 500 microscope to verify their size, morphology, and purity, and the particle size distribution was analyzed using a Malvern Mastersizer 3000 laser particle size analyzer (Fig. S3). SEM images confirmed that both the 100-nm and 10-μm MPs were spherical in shape and had uniform size distributions, with no visible impurities or agglomeration (Fig. S4). Dynamic light scattering (DLS) analysis further validated the size distribution of the MPs in aqueous suspensions, revealing polydispersity indices (PDI) below 0.1, indicating high monodispersity and stability in aqueous media (Table S1).

Sun et al. (2021) conducted a meta-analysis to quantitatively evaluate the impact of MPs on the locomotor activity of aquatic organisms at environmentally relevant concentrations, which they defined as those less than or equal to 1 mg/L. Based on their findings and the need to balance environmental realism with experimental feasibility, we selected a MP exposure concentration of 1 mg/L for our study. This aligns with the upper bound of environmentally relevant concentrations proposed by Sun et al. (2021) and has been frequently employed in previous toxicological investigations on aquatic biota (Cui et al., 2024; Li et al., 2020; Nam et al., 2023; Wu et al., 2023).

The MPs were dispersed in ultrapure water purchased from Hangzhou Wahaha Group Co., Ltd., which had been filtered through a 50-nm pore size polycarbonate membrane filters (Whatman® Nuclepore™ Track-Etched Membranes, GE Healthcare Life Sciences, Marlborough, MA, USA) to remove any background particles. The ultrapure water had a resistivity of 18.2 MΩ cm and a total organic carbon (TOC) content of less than 10 ppb. Prior to the exposure experiments, the ultrapure water was analyzed for the presence of MPs using a Nikon SMZ1270 stereomicroscope, and no visible particles were detected. The MP suspensions were prepared by adding the required amount of MPs to the filtered ultrapure water and sonicating for 20 min to ensure homogeneous dispersion. The suspensions were then diluted to the desired concentration of 1 mg/L and used immediately for the exposure experiments. The stability and homogeneity of the MP suspensions were visually checked using the Nikon SMZ1270 stereomicroscope before and during the exposure experiments, and no obvious settling or aggregation was observed.

2.2. Experimental organisms and treatment

2.2.1. Test organisms

The organisms used in this experiment were collected from a fish farm located in Deqing County, Huzhou City, Zhejiang Province, China. Individuals of *M. piceus* (body length of 26 ± 1.2 cm) and *B. aeruginosa* (shell height of 2.3 ± 0.2 cm) were captured from ponds on the fish farm

using a cast net and a D-shaped dip net, respectively. These animals were acclimatized in 500-L aquaria for 7 days, with 1/3 of the water exchanged every 3 days by static renewal. The acclimatization conditions included a temperature of 25 ± 1 °C and a photoperiod consisting of 12 h of light followed by 12 h of darkness. During the acclimatization, *B. aeruginosa* was fed with *Chlorella vulgaris*, and *M. piceus* was fed with fresh *B. aeruginosa*. Each aquarium housed approximately 300 individuals of *B. aeruginosa* or 5 individuals of *M. piceus*.

2.2.2. Ingestion of MPs by B. aeruginosa

Individuals of *B. aeruginosa* were exposed to suspensions of MPs in the two particle sizes (100 nm and 10 μm), and fecal samples were collected at different time points (0, 4, 8 and 12 h) following exposure. The selection of fecal collection time points for *B. aeruginosa* post-microplastic exposure was informed by initial experiments indicating that *B. aeruginosa* can ingest and subsequently excrete MPs within the established timeframe. This schedule facilitated the observation of how *B. aeruginosa* ingests and eliminates MPs. Subsequent analysis revealed the consistent presence of MPs (Fig. S5). *B. aeruginosa* was treated for different exposure durations. The experiment included six replicates for each particle size and 12 individuals of *B. aeruginosa* in each replicate, along with a blank control group that was not exposed to MPs. Individuals of *B. aeruginosa* were not fed during the exposure period and were then collected 0.5, 4, 8, 12, 16, and 20 h after exposure (8 individuals were collected at each time point). The collected *B. aeruginosa* individuals were immediately washed with pure water to remove MPs adsorbed on their surfaces, followed by dissection for the analysis of accumulated MPs.

2.2.3. Transfer of MPs from B. aeruginosa to M. piceus along the food chain

Based on the analysis of MPs accumulated in *B. aeruginosa*, individuals of *B. aeruginosa* that reached an equilibrium between MP ingestion and excretion were used to feed *M. piceus* (Fig. 1). To focus solely on the trophic transfer of MPs, a control group consisting of *M. piceus*, which were not fed *B. aeruginosa* containing MPs, was established parallel to the group undergoing trophic transfer. Each treatment included four replicates with five individuals in each replicate, and the fish were fed once daily for 5 weeks. Before the MPs exposure experiment, the *M. piceus* individuals underwent a 48-h fasting period. During each feeding session, around 20 g (wet weight) of *B. aeruginosa* laden with MPs were offered to each *M. piceus,* resulting in a feeding success rate of over 90%. The *B. aeruginosa* consumed by *M. piceus* were completely ingested within 1.5 h, leaving behind only their empty, fragmented shells (refer to Movie S1). To avoid the re-ingestion of expelled MPs, the water in aquarium was replaced with freshwater 8–10 h following each feeding session, aligning with the fish's digestion and excretion process completion. Daily tank cleaning was conducted to eliminate feces and any leftover food. Samples were taken weekly following the MPs exposure period. Prior to dissection, *M. piceus* specimens were carefully rinsed three times with distilled water to detach any adhering surface MPs, and their wet weights were recorded. The fish were dissected to extract samples of the gastrointestinal tract and dorsal muscle tissue for the quantitative analysis of MPs. Muscle tissue samples were specifically collected from areas away from the gastrointestinal tract to prevent cross-contamination.

To reduce the risk of MPs exposure through non-dietary pathways, we adopted various procedural measures. These measures included enforcing a period of fasting for the fish (Hasegawa and Nakaoka, 2021), conducting frequent changes of the aquarium water (Rochman et al., 2017), daily suction of feces and any leftover food (Zhang et al., 2019), and thoroughly rinsing the fish's exterior (Lu et al., 2016). Moreover, to avoid cross-contamination, muscle and gastrointestinal tract samples were collected and processed separately (Lu et al., 2016). These steps were crucial in validating the quantitative analysis of MPs, confirming that the accumulation detected in fish tissues primarily resulted from dietary intake via the food chain, rather than from respiratory uptake or environmental contamination.

2.2.4. Quantification and validation of MP accumulation in biological tissues

To evaluate MP accumulation, muscle and gastrointestinal tract samples were processed separately. Each tissue sample was cleaned with ultrapure water and digested at 60 °C for 48 h using a 10% potassium hydroxide (KOH) solution. The efficiency of the digestion method was validated by spiking control tissue samples with known amounts of 100-nm and 10-μm MPs, resulting in recovery rates of 96.0 ± 2.5% and 98.0 ± 1.6%, respectively (Table S3). Validation experiments were conducted to assess the impact of digestion on the integrity of MPs. SEM images and DLS results showed no significant changes in morphology, size, or shape of the MPs after digestion (Fig. S6, Table S2), indicating that the 10% KOH digestion process did not cause significant degradation or alterations in the physical properties of PS-MPs.

A dye release test was performed to verify whether the observed fluorescence signals originated from PS-MPs or free fluorescent dyes. MPs were incubated in simulated gastrointestinal fluids and fish serum at 60 °C for 48 h. No significant fluorescence signals were detected in the filtrates, confirming that the fluorescent dyes remained stably

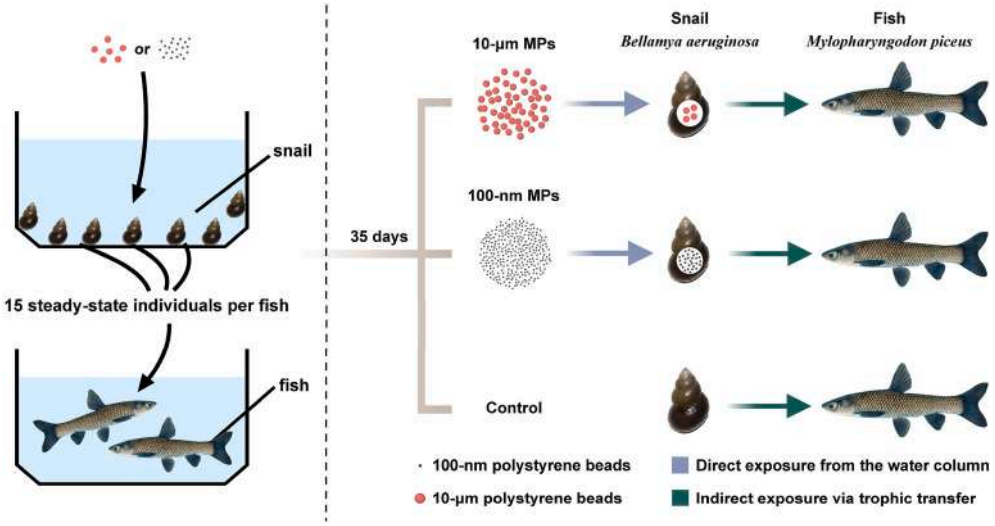

Fig. 1. Experimental design evaluating the trophic transfer of MPs along the food chain from *Bellamya aeruginosa* to *Mylopharyngodon piceus*.

incorporated within the PS matrix (Table S4).

Following digestion, samples were diluted with ultrapure water (1:1.5 v/v) and vacuum filtered through 50-nm pore size polycarbonate membrane filters (Whatman® Nuclepore™ Track-Etched Membranes). Filtered samples were analyzed using a fluorescence microscope (Nikon Eclipse Ti2) equipped with a GFP filter cube. For each sample, a minimum of five replicate filters were analyzed, capturing photographs of ten randomly chosen fields of view. Fluorescent MPs were manually counted using ImageJ software (Fig. S7). Negative controls were processed to mitigate background fluorescence. The method's accuracy was confirmed by recovery rates of 98.2% ± 2.3% and 96.1% ± 3.7% for 10-μm and 100-nm MPs in control samples, respectively (Table S5).

2.3. Analysis of the gut microbiota

Intestinal contents were collected from *M. piceus* following the 5-week exposure to MPs. Genomic DNA was extracted from the contents using the chaotropic method (Gerasimidis et al., 2016). The quality and concentration of DNA were determined by agarose gel electrophoresis and ultraviolet spectrophotometry. High-quality DNA samples were subjected to PCR amplification using primers targeting the V3–V4 region of the bacterial 16S rRNA gene (F: 5′-ACTCCTACGGGGAGGCAGCA-3'; R: 5′-GGACTACHVGGGGTWTCTAAT-3′). The PCR products were purified, and their concentrations were determined. A library was then constructed, and paired-end sequencing was carried out on the Illumina platform. The raw sequencing data were preprocessed using quality control, denoising, splicing, and chimerism removal algorithms. The α diversity indices (Chao and Shannon–Wiener) were calculated using QIIME2 software. Differences in α diversity indices and intestinal microbial composition at various taxonomic levels were evaluated using the Kruskal–Wallis test.

2.4. Statistical analysis

The distribution of data was assessed for normality using the Shapiro-Wilk test. Data conforming to a normal distribution were subjected to one-way ANOVA, with subsequent analysis via Tukey's post hoc test for pairwise comparisons. For data not adhering to normal distribution, the Kruskal-Wallis H test was employed, followed by Dunn's post hoc test for detailed analysis. A p-value of less than 0.05 was considered indicative of statistical significance. All results are expressed as mean ± standard deviation. Statistical evaluations were carried out using SPSS Statistics version 26.0.

2.5. Quality assurance and quality control (QA/QC)

A specialized QA/QC protocol was developed and implemented for the use of 100 nm and 10 μm fluorescent PS-MPs in this controlled laboratory study. The protocol focused on preventing cross-contamination between the different sizes of fluorescent MPs and ensuring result accuracy. Experimental procedures were conducted in a dedicated laminar flow hood (Class 100, Labconco, Kansas City, MO, USA), cleaned with 70% ethanol and UV-sterilized for at least 25 min before each use. Glassware, tools, and reagents (including KOH and ethanol) were thoroughly cleaned, filtered, or sterilized to eliminate any potential MP contamination. Ultrapure water used in the experiments was filtered through 50 nm pore size polycarbonate membrane filters (Whatman® Nuclepore™ Track-Etched Membranes) before use. Fluorescent MPs were stored in separate, clearly labeled containers, with their suspensions prepared in a dedicated area within the laminar flow hood. Personnel handling MPs wore disposable, powder-free nitrile gloves to prevent contamination.

Procedural blanks, consisting of ultrapure water, were included in each sample batch to monitor potential contamination during processing and analysis. These blanks were subjected to the same digestion, filtration, and microscopic analysis procedures as the actual samples, confirming that sample processing and analysis were free of contamination. Recovery efficiency was assessed by spiking known amounts of 100 nm and 10 μm fluorescent PS-MPs into clean fish tissue samples, which were then subjected to the same processing and analysis procedures as the actual samples. High recovery rates, as reported in Section 2.2.4, demonstrated the accuracy of the method for the specific types and sizes of MPs used in this study.

Despite the rigorous QA/QC measures employed in this study, there are some limitations to consider. First, the use of fluorescence microscopy, while highly sensitive and specific for detecting the fluorescent PS-MPs used in this study, may not be suitable for identifying non-fluorescent MPs or determining the chemical composition of the particles. Future studies could benefit from the use of additional analytical techniques, such as Raman spectroscopy or Fourier-transform infrared spectroscopy (FTIR), to provide more comprehensive information on the polymer types of the detected MPs. Second, the controlled laboratory conditions, while allowing for the minimization of potential contamination and the isolation of the effects of MPs on the studied organisms, may not fully represent the complex environmental factors that influence the behavior and fate of MPs in natural freshwater ecosystems. Future research should consider the use of more environmentally realistic exposure conditions to better understand the impacts of MPs on aquatic organisms in the field. Finally, this study focused on two specific sizes of fluorescent PS-MPs (100 nm and 10 μm), and the findings may not be directly applicable to MPs of different sizes, shapes, or polymer compositions. Additional studies investigating a wider range of MP types and characteristics would provide a more comprehensive understanding of their trophic transfer and biological impacts in freshwater ecosystems.

2.6. Calculation of MP transfer ratios

In this study, we investigated the trophic transfer of MPs from the snail *B. aeruginosa* to the commercially important fish *M. piceus* in a freshwater food chain. Due to the large body size of *M. piceus* (average body length of 26 ± 1.2 cm and body weight of 0.9 ± 0.2 kg), homogenizing and analyzing the entire fish body for MP concentration was not feasible, as is commonly done in studies using smaller fish species with body weights less than 250 g (Chen et al., 2022; Clere et al., 2022; Foo et al., 2022). Moreover, the use of bioconcentration factor (BCF) or bioaccumulation factor (BAF), typically calculated as the ratio of MP concentration in the whole organism to that in the surrounding media or food (Elizalde-Velázquez et al., 2020), may not be the most appropriate approach for assessing the trophic transfer and accumulation of MPs in large commercial fish species like *M. piceus*. Therefore, We incorporated our own insights by adapting the approach used by Elizalde-Velázquez et al. (2020) and Mackay et al. (2018) to calculate the transfer ratios of MPs from the prey (*B. aeruginosa*) to specific tissues (muscle and gastrointestinal tract) of the predator (*M. piceus*). The muscle tissue is the main edible part of the fish, directly relevant to human consumption and potential health risks (Ochiai & Ozawa, 2020). The gastrointestinal tract (GIT) is the primary site of MP ingestion and accumulation, with its MP content providing insights into dietary exposure and uptake of these contaminants (Turroni et al., 2021). By focusing on these two key tissues, this approach allows for a more targeted and ecologically relevant assessment of MP trophic transfer and accumulation in commercial fish species.

The transfer ratios of MPs in the muscle and GIT of *M. piceus* were calculated using the following equations:

$$\text{Muscle Transfer Ratio} = \frac{C_{muscle}}{C_{snail}} \quad (1)$$

$$\text{GIT Transfer Ratio} = \frac{C_{GIT}}{C_{snail}} \quad (2)$$

Where C_{muscle} and C_{GIT} represent the MP concentrations in the muscle and gastrointestinal tract (GIT) of *M. piceus*, respectively, and C_{snail} represents the MP concentration in the whole body of *B. aeruginosa*. The MP concentrations are expressed as either the number of particles per gram of tissue (particles/g) or the mass of MPs per gram of tissue (μg/g).

3. Results and discussion

3.1. Bioaccumulation of MPs in B. aeruginosa

The ingestion of PS-MPs by *B. aeruginosa* was observed starting from 0.5 h after exposure, and maximal concentrations were noted at 4 h (Fig. 2). The mass concentrations of 10-μm and 100-nm MPs in *B. aeruginosa* reached 776.80 ± 12.67 μg/g and 0.14 ± 0.01 μg/g, respectively. The ingestion rate was higher than the excretion rate during this period; however, after 4 h of exposure, the excretion rate exceeded the ingestion rate, and the concentrations of MPs in *B. aeruginosa* gradually decreased. Notably, the ingestion rates exhibited different time courses between the two groups exposed to different MP particle sizes. After 12 h of exposure, the accumulation of MPs within *B. aeruginosa* individuals reached an equilibrium, with measured mass concentrations of 222.95 ± 12.56 μg/g for 10-μm particles and 0.053 ± 0.01 μg/g for 100-nm particles. These values were 28.70% and 37.86% of the maximum concentrations, respectively.

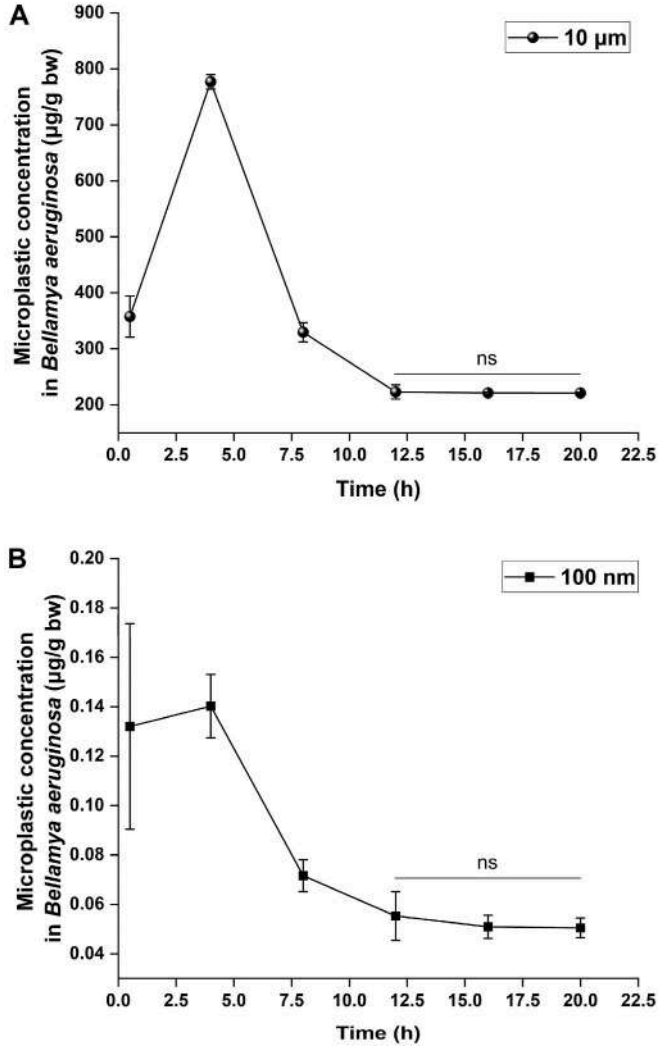

Fig. 2. Time courses of 10-μm (A) and 100-nm (B) concentrations of MPs in *Bellamya aeruginosa* (ns, non-significant difference). Error bars = means ± standard deviations.

As a pivotal component of the aquatic food web, serving as a primary consumer, *B. aeruginosa* is instrumental in energy and nutrient transfer to higher trophic levels (Bao et al., 2018; Liu et al., 2019). Its propensity for MP accumulation underscores the risk of these pollutants permeating and magnifying through freshwater ecosystems (Bao et al., 2018; Liu et al., 2019). The species' broad habitat range, ecological significance, and sensitivity to pollutants render *B. aeruginosa* an exemplary model for ecotoxicological research (Bao et al., 2018; Liu et al., 2019). Typically, *B. aeruginosa* ingests MPs within the 10–100 μm range in natural settings (An et al., 2022), and laboratory studies corroborate its ability to accumulate even NPs as small as 82.48 ± 1.26 nm (Luo et al., 2022). Echoing previous research (Weber et al., 2021), our study reveals *B. aeruginosa*'s rapid MP ingestion, with excretion rates varying according to particle size, suggesting slower excretion for larger particles.

The differential accumulation of 10-μm over 100-nm MPs in *B. aeruginosa* may result from the interplay of particle size with ingestion, egestion dynamics, and biological surface interactions. The 10-μm particles are likely more effectively captured and ingested due to *B. aeruginosa*'s filter-feeding behavior, fitting within the optimal retention range of its gill filtration system (Weber et al., 2021). Conversely, the nimble 100-nm particles are ingested but possibly expelled more swiftly, facilitated by their ability to navigate through biological barriers due to higher diffusivity (Kogel et al., 2020; Sharma et al., 2022; Stock et al., 2022). Moreover, the surface characteristics of particles, such as charge and hydrophobicity, may alter their gut interaction, where particles exhibiting increased charge or hydrophobicity might bind more strongly to mucus layers or epithelial cells, prolonging gut residency (Bucci et al., 2020; Foley et al., 2018).

3.2. Trophic transfer and bioaccumulation of MPs in M. piceus

M. piceus was exposed to *B. aeruginosa* at the point in the experiment when the ingestion and excretion of MPs had reached equilibrium (12 h). No fluorescent particles were detected in tissues from the blank control group throughout the experiment (Fig. S8). In contrast, fluorescent MPs were detected in the muscle and gastrointestinal tract tissues of exposed *M. piceus* (Fig. 3). The mass concentration of MPs was then measured, which revealed that MPs of both particle sizes had accumulated in tissues in a time-dependent manner within 35 days after exposure (Fig. 4). At 35 days of exposure, the maximum concentrations of 100-nm MPs in the muscle and gastrointestinal tract tissues were (8.50 ± 0.69) × 10^{-6} and (9.24 ± 0.39) × 10^{-5} μg/g, respectively. In comparison, the maximum concentrations of 10-μm MPs in these two tissues were 0.0036 ± 0.0001 and 1.72 ± 0.25 μg/g, respectively. Notably, the maximum concentrations of 10-μm particles were significantly higher than those of 100-nm particles in both tissues ($p < 0.001$). These experiments revealed the time-dependent bioaccumulation of MPs in the study organisms, with the concentration of MPs in *M. piceus* increasing continuously until the end of exposure. Similar trends have been observed for *M. piceus* in the bioaccumulation of other pollutants (Jing et al., 2020). Our results further demonstrated the potential capacity of *M. piceus* to accumulate ingested pollutants. Time-dependent bioaccumulation of PS-MPs was also observed in Nile tilapia (*Oreochromis niloticus*) (Ding et al., 2018). However, Lu et al. (2016) reported that MPs with a particle size of 5-μm reached a steady state in zebrafish within 48 h. The observed differences may be attributed to variations in fish body size and feeding behaviors (McNeish et al., 2018; Scherer et al., 2017). The maximum mass concentrations of 10-μm MPs were 423.5- and 1861.5-fold higher than those of 100-nm MPs in the muscle and gastrointestinal tract tissues of *M. piceus*, respectively. However, the numbers of 100-nm MPs were 2362.5- and 53.7-fold higher than those of 10-μm MPs in the muscle and gastrointestinal tract tissues, respectively (Table S6). These patterns align with the trends of the masses and numbers reported for 100-nm and 2-μm MPs ingested by *D. magna* (Rist et al., 2017). The discrepancy between mass concentrations and

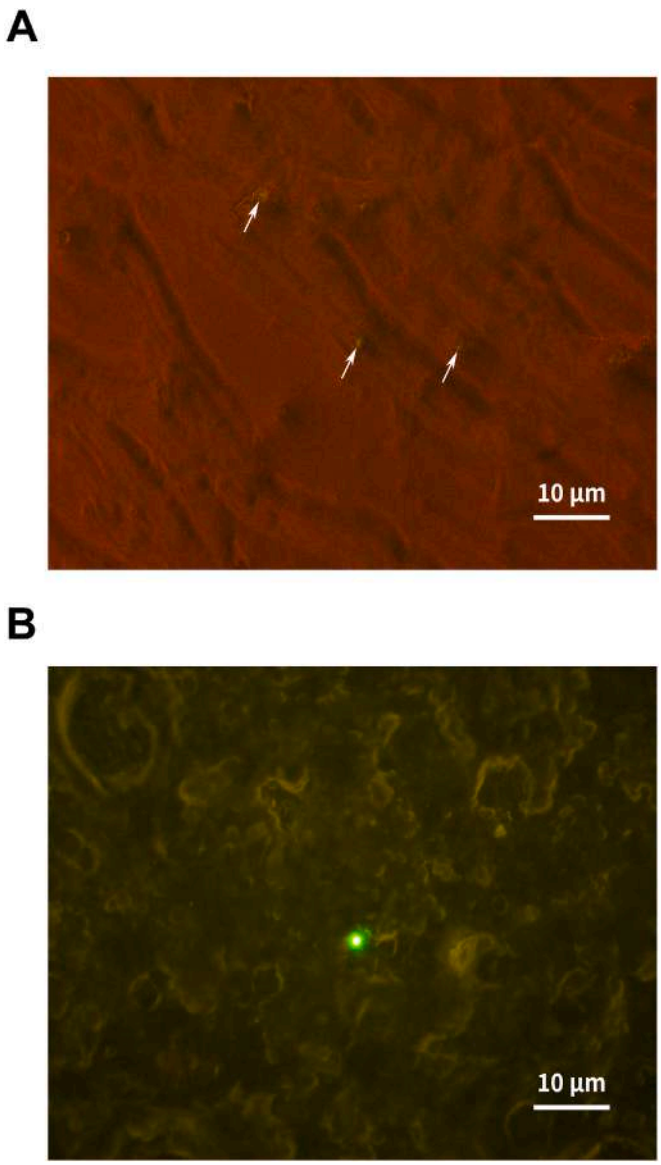

Fig. 3. Fluorescent MPs in *Mylopharyngodon piceus* muscle (A) and the gastrointestinal tract (B). Bar = 10 μm. White arrows indicate fragmented fluorescent MPs.

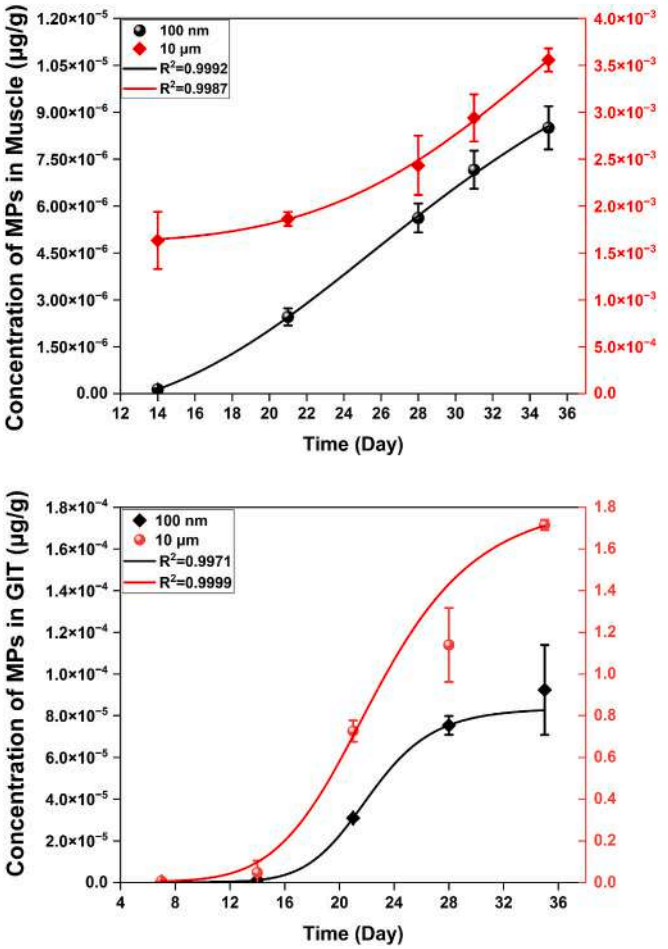

Fig. 4. Fitted time courses of concentrations of MPs accumulated in the muscle and the gastrointestinal tract of *Mylopharyngodon piceus*. GIT, gastrointestinal tract. Error bars = means ± standard deviations.

numbers can be attributed to the varying effects of MP sizes on absorption and excretion rates (Mattsson et al., 2015; O'Connor et al., 2022). Specifically, the smaller size of 100-nm particles provides larger surface area-to-volume ratios, allowing them to permeate more readily through the intestinal epithelial gaps into tissues (Koelmans et al., 2013; Walczak et al., 2015). However, their smaller size also facilitates easier excretion from the tissues (Batel et al., 2018). In contrast, despite lower intake numbers, the larger 10-μm MPs have slower excretion rates, leading to greater mass accumulation in muscle and gastrointestinal tissues over time. Additionally, the complex anatomy of the intestinal lining, with varying epithelial cells and tight junctions across segments, may impact the absorption and excretion of the two differently-sized MPs (Jabeen et al., 2017; Welden & Cowie, 2016). Further quantitative research on the dynamic profiles of MPs across digestive system tissues is needed to elucidate the effects of microenvironment and anatomical locations on the biokinetics of MP bioaccumulation (Jovanovic et al., 2018).

The identification of 10-μm PS-MPs within the muscle tissue of *M. piceus* prompts an exploration into how larger MPs migrate within organisms. Although the specific mechanisms are yet to be fully understood, our findings offer persuasive evidence of the journey of 10-μm MPs from the gastrointestinal tract to the fish muscle. We propose several mechanisms that might underlie this process. First, MP exposure could deteriorate the intestinal epithelium's integrity, enhancing permeability and enabling the larger MPs to cross the gut barrier (Hirt & Body-Malapel, 2020; Huang et al., 2021). This theory gains support from recent research showing MPs' capability to impair gut barrier functions and provoke inflammation in fish (Lei et al., 2018; Qiao et al., 2019). Second, MPs might be engulfed by macrophages or other immune cells within the gut's lamina propria and transported to the muscle through the lymphatic or circulatory systems (Ramsperger et al., 2020; Zhu et al., 2023), a plausible scenario given the documented phagocytosis of MPs by immune cells in various aquatic species (Abihssira-García et al., 2020; Limonta et al., 2019). Lastly, the possibility exists that MPs could disperse through organs and tissues via coelomic fluid (Farrell & Nelson, 2013; Mohsen et al., 2020), although direct evidence for this pathway is scarce. Nevertheless, MPs have been found in invertebrates' hemolymph and coelomic fluid, indicating systemic distribution could occur (Browne et al., 2008).

By quantitatively analyzing the time-dependent transfer ratios of MPs in the muscle tissue and gastrointestinal tract of *M. piceus*, the absorption rate constants (k_a) were estimated, allowing for comparison of the uptake kinetics of different-sized MPs. As shown in Fig. 5, the transfer ratios of both 100 nm and 10 μm MP in the muscle and gastrointestinal tract of *M. piceus* increased over the five-week dietary exposure period, indicating a gradual accumulation of MPs in fish

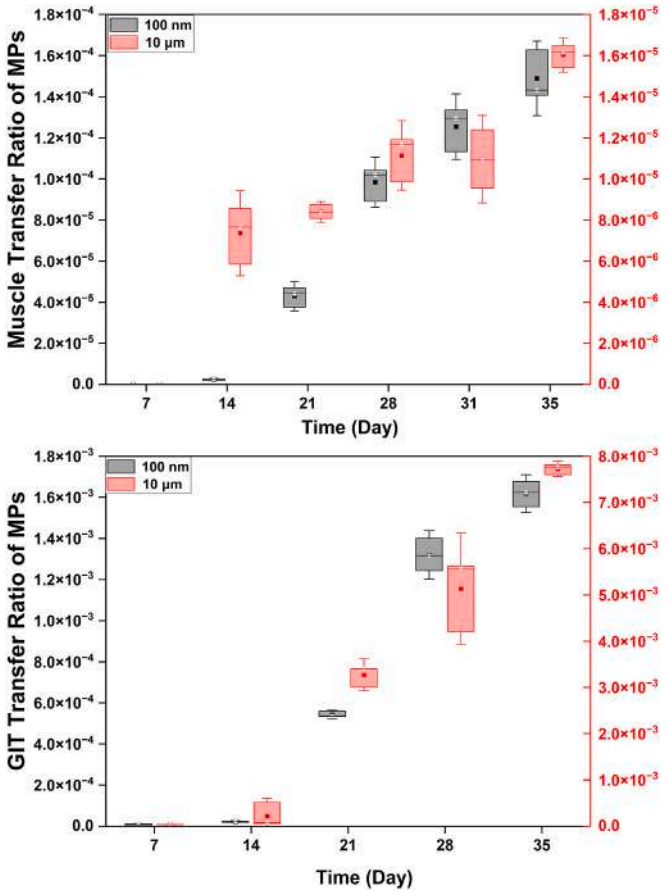

Fig. 5. Transfer ratios of MPs in the muscle and gastrointestinal tract of *Mylopharyngodon piceus* during a five-week dietary exposure period. GIT, gastrointestinal tract.

tissues. The absorption rate constants (k_a) of 100 nm MP in the muscle and gastrointestinal tract were 0.1416 ± 0.0110 d^{-1} and 0.2475 ± 0.0167 d^{-1}, respectively, higher than those of 10 μm MP (0.0881 ± 0.0068 d^{-1} and 0.1746 ± 0.0131 d^{-1}, respectively) (Table S7). These findings suggest that NPs (100 nm) were absorbed more rapidly by *M. piceus* than MPs (10 μm), possibly due to their larger specific surface area and higher bioreactivity (Kogel et al., 2020; Sharma et al., 2022; Stock et al., 2022). Moreover, the absorption rate constants were higher in the gastrointestinal tract than in muscle tissue for both particle sizes, indicating that the gastrointestinal tract is the primary site of MP uptake and accumulation in *M. piceus* (Pan et al., 2021). The steady-state transfer ratios (Css) of 100 nm MP in the muscle and gastrointestinal tract were estimated to be $4.0874 \times 10^{-3} \pm 1.6821 \times 10^{-4}$ and $1.7287 \times 10^{-4} \pm 4.8821 \times 10^{-6}$, respectively, higher than those of 10 μm MP ($2.6016 \times 10^{-3} \pm 1.3527 \times 10^{-4}$ and $1.2440 \times 10^{-4} \pm 4.5354 \times 10^{-6}$, respectively) (Table S7). These results suggest that NPs may accumulate to higher levels in fish tissues than MPs over extended exposure periods.

It is important to note that this study focused on the absorption kinetics of MPs and did not directly measure the elimination rate constants (k_e). The continuous increase in transfer ratios throughout the five-week exposure period (Fig. 5) suggests that MP concentrations in fish tissues had not reached a steady state within the timeframe of this experiment. To accurately predict long-term accumulation and potential biomagnification of MPs in the freshwater food chain, future studies should measure both absorption and elimination rate constants over extended exposure durations (Yu et al., 2022). This approach would allow for the development of more comprehensive kinetic models that account for the dynamic balance between MP uptake and excretion in aquatic organisms (Ma & You, 2021).

Another limitation of this study is that only the trophic transfer of PS-MPs with two specific particle sizes (100 nm and 10 μm) was investigated. Accumulation kinetics and tissue distribution patterns may vary for MPs with different physicochemical properties, such as shape, density, and polymer composition (Wang & Wang, 2023). Future research should explore a wider range of microplastic types and characteristics to better understand their fate and impacts in freshwater food webs (Bank et al., 2022). Furthermore, this study focused on a simplified two-level food chain consisting of *B. aeruginosa* and *M. piceus* under controlled laboratory conditions. While this approach allowed for quantitative assessment of the trophic transfer and accumulation of MPs, it may not fully capture the complexity of natural freshwater ecosystems, where multiple trophic levels and environmental factors can influence the behavior and fate of MPs (Krause et al., 2021). Future studies should investigate the trophic transfer and accumulation of MPs in more diverse and realistic food webs, considering factors such as species interactions, environmental variability, and the presence of other contaminants (Bucci et al., 2020; Piarulli et al., 2020).

In conclusion, this study provides novel insights into the trophic transfer and accumulation dynamics of MPs in a freshwater food chain by quantitatively analyzing the absorption kinetics and tissue-specific distribution patterns in *M. piceus*. It was found that NPs exhibited higher absorption rates compared to MPs, and the gastrointestinal tract was the primary site of microplastic accumulation. However, it is crucial to recognize that research into the movement and distribution of MPs within organisms is still in its infancy, with varied findings across studies (Thacharodi et al., 2024). These variances may stem from differences in experimental approaches, MP properties, and species-specific responses. For example, the form, surface properties, and weathering of MPs significantly influence their biological interactions, thereby affecting their assimilation and dispersal (Ali et al., 2024). Additionally, the method of exposure, duration, and concentration of MPs impact their accumulation and potential toxicity in organisms (Adhikari et al., 2022). The lack of elimination kinetics data and the simplified experimental design in this study limit the ability to fully predict the long-term fate and impacts of MPs in complex freshwater ecosystems. Future research should address these limitations by measuring both absorption and elimination rate constants, investigating a wider range of MP types and characteristics, and exploring more diverse and realistic food web scenarios (Ali et al., 2024).

3.3. *Effects of MPs transferred along the food chain on the gut microbiota of M. piceus*

The Kruskal–Wallis test revealed no statistically significant differences in the α diversity values for the gut microbiota between groups (Chao index: N = 12, $p = 0.338$, Fig. 6A; Shannon–Wiener index: N = 12, $p = 0.179$, Fig. 6B). In terms of β diversity, principal coordinate analysis revealed that though the 95% confidence intervals of the 10-μm and control groups partially overlapped, the 100-nm group was notably separated from the other two groups (Fig. 6C).

Further taxonomic analysis revealed that at the phylum level, Proteobacteria (40.08 ± 1.44%), Firmicutes (30.30 ± 1.90%), and Bacteroidetes (10.77 ± 0.62%) dominated the gut microbiota in *M. piceus* (Fig. 6D). Compared to the control group, exposure to 10-μm MPs did not significantly alter the dominant phyla of gut microbiota. However, exposure to 100-nm MPs resulted in a notable impact on the composition of the gut microbiota in *M. piceus*. Specifically, the relative abundance of Proteobacteria increased by 14.18% ($p < 0.05$) (Fig. 6F), whereas the relative abundances of Firmicutes and Bacteroidetes decreased by 9.57% ($p < 0.05$, Figs. 6G) and 3.53% ($p < 0.05$, Fig. 6H), respectively. At the genus level (Fig. 6E), notable increases in the relative abundances of specific pathogenic bacterial genera were observed during exposure to both particle sizes, as exemplified by *Plesiomonas* ($p < 0.05$, control vs. 10 μm vs. 100 nm: 0.011 ± 0.00004% vs. 0.16 ± 0.0007% vs. 0.14 ± 0.028%, Fig. 6I).

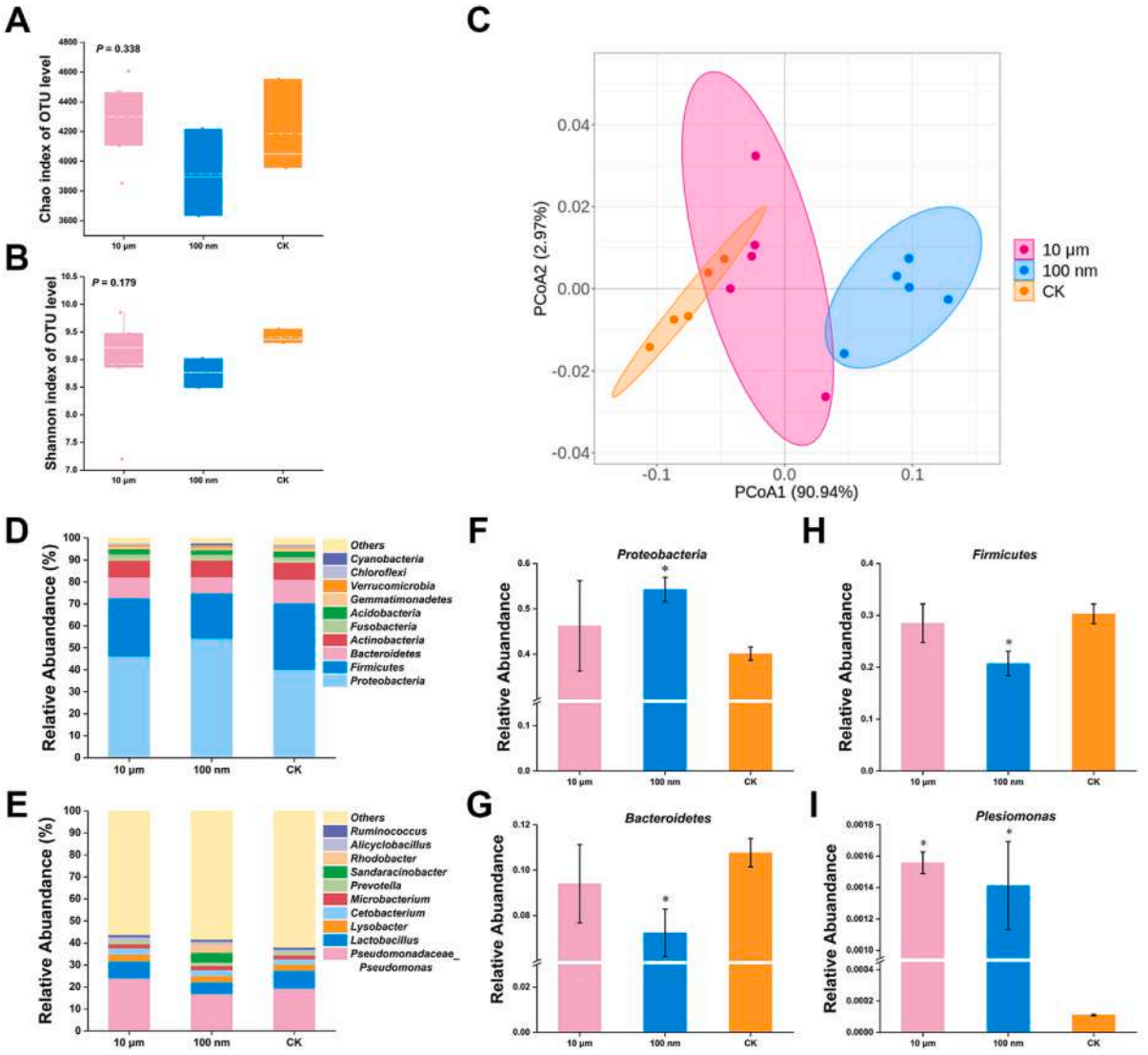

Fig. 6. Effects of MPs on the gut microbiota of *Mylopharyngodon piceus*. (A) Chao index, a measure of species richness, and (B) Shannon–Wiener index, a measure of species diversity, showed no significant differences among the treatment groups (Kruskal–Wallis test, $p > 0.05$). (C) Principal coordinate analysis (PCoA) based on the Bray-Curtis distance matrix revealed a clear separation of the gut microbial communities exposed to 100 nm NPs from those exposed to 10 μm MPs and the control group. (D) Composition of gut microbiota at the phylum level, showing the dominance of Proteobacteria, Firmicutes, and Bacteroidetes across all treatment groups. (E) Composition of gut microbiota at the genus level, highlighting the differences in the relative abundances of various genera among the treatment groups. Relative abundances of (F) Proteobacteria, (G) Bacteroidetes, (H) Firmicutes, and (I) *Plesiomonas* in different treatments. Exposure to 100 nm NPs significantly increased the relative abundance of Proteobacteria and decreased the relative abundances of Bacteroidetes and Firmicutes compared to the control group (Kruskal–Wallis test followed by Dunn's post hoc test, *$p < 0.05$). The relative abundance of the potentially pathogenic genus *Plesiomonas* was significantly higher in both 100 nm and 10 μm MP treatment groups compared to the control (Kruskal–Wallis test followed by Dunn's post hoc test, *$p < 0.05$). OTU, operational taxonomic unit.

Its intricate and dynamic composition, along with its high variability and environmental adaptability, distinguishes the gut microbiota as a valuable subject in ecotoxicological investigations (Evariste et al., 2019). Prior studies have demonstrated a reduction in the diversity of gut microbiota in zebrafish following exposure to PS-MPs (Jin et al., 2017). However, in the present study, exposure to PS-MPs did not significantly impact the α diversity of the gut microbiota in *M. piceus*. This discrepancy may be attributed to differences in fish species, body size, MP types, concentrations, and exposure durations. For example, exposure to MPs resulted in a significant increase in the α diversity of gut microbiota in medaka (Kang et al., 2021), though concentrations of MPs mimicking environmental conditions showed no significant effects on the α diversity values of crucian carp and grass carp (Li et al., 2023; Ouyang et al., 2021). These factors can significantly impact the interactions between MPs and the gut microbiome, underscoring the necessity for an in-depth evaluation of the ecological and health consequences of MP pollution among various species and under diverse exposure conditions.

Members of the Bacteroidetes phylum in the gut microbiota play pivotal roles in maintaining host health and complex homeostasis (Gibiino et al., 2018). Firmicutes bacteria are closely associated with nutrient absorption in the host (Gibiino et al., 2018). In the present study, exposure to NPs significantly reduced the abundances of Firmicutes and Bacteroidetes in the gastrointestinal tract of *M. piceus*. This observation implies that NPs may exert stronger adverse effects on *M. piceus* gut microbiota compared to MPs, which is consistent with the results from a study performed in zebrafish (Xie et al., 2021) and is further supported by the significant separation of gut microbiota

induced by NPs than by MPs. The heightened impact of NPs on the gut microbiome could stem from their larger surface area-to-volume ratio, enhanced reactivity, and a greater ability to breach the intestinal barrier compared to their larger MP counterparts (Chae & An, 2017; Gigault et al., 2021).

The elevated presence of *Plesiomonas*, a facultative or opportunistic pathogen, in the gastrointestinal tract of *M. piceus* after exposure to MPs of both sizes, underscores potential health concerns linked to the trophic movement of these pollutants. *Plesiomonas* has been associated with various gastrointestinal ailments in fish, such as hemorrhagic septicemia, ascites, and enteritis, leading to substantial disease and death in aquaculture environments (Cortes-Sanchez et al., 2021). Additionally, *Plesiomonas* is recognized as a zoonotic pathogen, capable of causing foodborne diseases in humans who consume contaminated fish products (Levin, 2008; Stock, 2004). The surge in *Plesiomonas* within *M. piceus*' gut could result from multiple factors. Firstly, MPs may act as carriers for the attachment and growth of pathogenic bacteria like *Plesiomonas*, enhancing their accumulation and survival in the gastrointestinal system (Feng et al., 2020). Secondly, consuming MPs might inflict physical harm on the intestinal lining, weakening the gut barrier and fostering conditions favorable for opportunistic pathogen proliferation (Jin et al., 2019). Lastly, changes induced by MPs in the gut's microbial balance, such as the decrease in beneficial bacteria like *Firmicutes* and *Bacteroidetes*, might weaken the host's defenses against pathogenic invasion and sickness (Zafar & Saier, 2021). The prevalence of *Plesiomonas* in *M. piceus*' gut could affect not just the fish's health and survival but also pose risks to humans. Given *M. piceus*' significance in the commercial and dietary habits in China and elsewhere in Asia, pathogen presence in its digestive system might contaminate fish products during their processing and handling (Novoslavskij et al., 2015; Tang et al., 2024b).

In summary, the increased abundance of *Plesiomonas* in *M. piceus* following MP exposure underscores the ecological and health risks associated with the trophic transfer of these pollutants. Future studies should explore the interaction mechanisms between MPs, pathogens, and the gut microbiome and evaluate the food safety implications.

4. Conclusions

Our study demonstrates that MPs can be efficiently transferred along a freshwater food chain from *B. aeruginosa* to *M. piceus*, resulting in size-dependent accumulation in various tissues and significant alterations in the gut microbiota composition of *M. piceus*. The higher accumulation of 10-μm MPs compared to 100-nm NPs in both the gastrointestinal tract and muscle tissue of *M. piceus* underscores the importance of considering particle size when assessing the trophic transfer and fate of MPs in aquatic organisms. Additionally, the significant increase in the relative abundance of potentially pathogenic bacterial taxa and the marked alteration of the gut microbiota structure in *M. piceus* exposed to 100-nm NPs highlight the potential ecological and health risks associated with nanoscale plastic pollution. To further elucidate the factors influencing the toxicity and accumulation of MPs in freshwater food webs, future research should investigate the role of polymer type, shape, surface properties, and the physiological characteristics of exposed organisms. Additionally, future studies should explore the interactive effects between MPs and other environmental stressors, assess the long-term consequences of chronic microplastic exposure on the fitness, reproduction, and population dynamics of freshwater species, and develop standardized methods for detecting, quantifying, and characterizing MPs in complex environmental matrices and biological samples.

CRediT authorship contribution statement

Ming Zhang: Writing – original draft, Project administration, Methodology, Conceptualization. **Yijie Jin:** Software, Investigation, Data curation. **Cenyi Fan:** Validation, Supervision. **Yiwen Xu:** Visualization, Validation. **Jiateng Li:** Software, Investigation. **Wenjing Pan:** Investigation, Formal analysis. **Ziyang Lou:** Writing – review & editing, Resources. **Huili Chen:** Writing – review & editing, Resources. **Binsong Jin:** Writing – review & editing, Supervision, Resources, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We thank the editor and the three anonymous reviewers for their help in improving the manuscript. We acknowledge financial support from the National Natural Science Foundation of China (31960254), the National Key R&D Program of China (2018YFD0900906, 2018YFD0900806), the Basic and Commonweal Programme of Zhejiang Province (LGN21C140009), the Hangzhou Normal University Undergraduate Innovation Capability Enhancement Project (CX 2023142), and the Hangzhou Normal University Starlight Plan 2023. We thank LetPub (www.letpub.com) for linguistic assistance and pre-submission expert review.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.124426.

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