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The Study of the Accumulation Characteristics of Environmental Characteristic Microplastics in Organisms at Different Trophic Levels

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Abstract: Microplastics are widely present in aquatic environments and, due to their high hydrophobicity, can carry organic pollutants while affecting their accumulation and toxicity in organisms at different trophic levels. The ingestion of microplastics by organisms can be divided into direct and indirect ingestion. Direct ingestion refers to organisms directly consuming microplastics present in the environmental medium, while indirect ingestion can be understood as microplastics being ingested by organisms along the transfer of trophic levels. This study aimed to determine the distribution of microplastics in the surface water of the Yangtze River Estuary and the accumulation characteristics of microplastics in organisms at different trophic levels. In 2021, the study selected typical sampling stations in the Yangtze River Estuary and its adjacent waters for observation, analyzing the concentration and characteristics of microplastics in surface water and samples from nine different trophic level organisms (two types of gastropods and seven types of fish). The abundance of microplastics in the surface water samples collected from the Yangtze River Estuary was 661.2 ± 220.5 items/m^3. The average abundance of microplastics in the gills of organisms was 1.1 ± 0.4 items/g w.w., and in the gastrointestinal tract, it was 0.3 ± 0.1 items/g w.w. Based on the calculation of the bioconcentration factor of microplastics, we found that the bioconcentration factor of higher trophic level organisms (fish) (2.6 ± 0.5 m^3/kg w.w.) was significantly greater than that of gastropod organisms (0.87 ± 0.4 m^3/kg w.w.). In terms of feeding types, the bioconcentration factor of carnivorous fish organisms was significantly greater than that of omnivorous fish. This paper determined the trophic level of organisms through stable nitrogen isotopes (δ^{15}N), and the biomagnification factor of microplastics was calculated to be 4.2 based on the linear regression equation of microplastic concentration and organism trophic level. Therefore, microplastic concentrations can be transferred along different trophic levels in the food chain, and the accumulation level of microplastics in organisms significantly increases with the rise of trophic levels, indicating the potential for biomagnification of microplastics in gastropods and fish organisms.

Keywords: Microplastics; Viral mechanisms; Trophic levels of organisms; Bioaccumulation; Biomagnification

1 Introduction

At the beginning of the 21st century, microplastics have gradually come into the sight of scientists and the public [1]. As a representative environmental pollutant in the development of modern human society [2, 3], plastics do not "degrade" and disappear from the marine environment. Instead, they break down into microplastics, which are preserved in the marine environment [4]. The ultimate destination of large pieces of trash is to break down into microplastics [5]. The pollution of marine microplastics has gradually attracted the attention of more and more researchers, government departments, and public organizations. According to previous studies, microplastics are distributed in the atmosphere [6], various water bodies (rivers, lakes, and oceans) [7, 8], soil, and within organisms [9]. They are ubiquitous in different ecosystems due to their transfer and accumulation through the food chain and natural cycling [10, 11]. Microplastics have also been proven to serve as carriers for various pathogens and pollutants. Their small size and large surface area facilitate the attachment of various microorganisms, which are then carried into the process of food chain transmission [12, 13].

According to current research, more than 200 species have been affected by microplastic pollution [14, 15], especially economically important edible marine organisms such as mussels (0.9–5.4 items/g), oysters (2.4–8.6

items/individual), shrimp, and clams. In addition, edible fish in the ocean are also severely polluted by microplastics, including species like *Thryssa kammalensis* [16], *Larimichthys polyactis* [17], *Thamnaconus septentrionalis* [18], and *Boleophthalmus pectinirostris* [19]. So far, 230 marine species and 26 freshwater species have been surveyed in the estuarine and coastal areas of China, with over 90% of organisms tested positive for microplastics. Fish, being the most frequently consumed by humans, are also considered one of the new problems threatening human health due to microplastic pollution in edible marine life [20, 21]. In addition to direct ingestion, microplastics are also ingested indirectly, which can also be referred to as the transfer of microplastics along the trophic levels [22, 23]. Studies have shown that microplastics are transferred from lower to higher trophic levels through the behavior of the food chain [24].

Although there are many field studies on the accumulation of microplastics in organisms at different trophic levels, research on the transfer process of microplastics in the food chain, based on an understanding of the structure of the food web, is currently neglected. In recent years,more and more studies have applied stable carbon isotopes ($\delta^{13}C$) and stable nitrogen isotopes ($\delta^{15}N$) to determine the structure of the food chain and trophic levels in ecosystems [25, 26]. Research on the transfer process of various pollutants in the food chain through stable isotopes is also becoming increasingly mature. At present, foreign countries have applied the stable nitrogen isotope measurement technique to the study of the transfer and accumulation of microplastics along the food chain and in organisms at various trophic levels. Based on the determination of the trophic levels of large invertebrates and fish in the Galon River using stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$), researchers have analyzed the accumulation and transfer characteristics of microplastics in organisms at different trophic levels. The study proved that there are differences in the abundance of microplastics ingested by large invertebrates and fish, and there is no significant relationship with the concentration of microplastics in the water environment. However, there are few domestic reports on the application of carbon and nitrogen stable isotopes to construct the food chain structure in the Yangtze River Estuary and its adjacent waters, and to study the transfer process and accumulation characteristics of microplastics [27, 28].

The Yangtze River, as the largest river in China, its coastal estuary and coast are the main sources of land-based pollutants entering the sea. There are many human activities around the Yangtze River Estuary, with a high level of urbanization, making the Yangtze River Estuary an important area for assessing the ecological risks of microplastics [29, 30]. At the same time, the Zhoushan Fishing Ground is located at the confluence of rivers such as the Yangtze and Qiantang, surrounded by thousands of islands of various sizes. The unique geographical location and water environmental conditions make the Zhoushan Fishing Ground rich in nutrients and suitable temperatures, attracting most economically important aquatic organisms. However, the large amount of land-based pollutants carried by rivers such as the Yangtze into the ocean has made marine pollution much more severe than it was decades ago, greatly exceeding the ocean's "self-purification" capacity. This has led to a sharp deterioration in the water quality around the Zhoushan Fishing Ground, destroying the ecological conditions of various aquatic breeding and fattening grounds, and directly affecting the local fishery resource production capacity. Compared to previous studies, our research uniquely integrates stable nitrogen isotope-based trophic level assessment with microplastic accumulation data in the Yangtze River Estuary, one of the most urbanized and industrially impacted river deltas in East Asia. Moreover, we adopt a finer-resolution species-specific analysis, capturing regional dynamics not covered in earlier works.

2 Research Methods

2.1 Research Area

In October 2021, water and biological samples were collected at three typical stations near the Zhoushan Fishing Ground in the Yangtze River Estuary (Figure 1, Table 1) to observe the correlation between the distribution of microplastics and the trophic levels of organisms. The distribution of microplastics in estuarine water and biological samples was analyzed in conjunction with the characteristics of microplastic concentrations in the waters of the Yangtze River Estuary and its adjacent areas. During actual sampling, a single water layer can often collect no more than several hundred liters of water samples. At the same time, smaller volume water samples are often directly filtered onto a membrane, which helps to study smaller-sized microplastics. Combining previous research on the flux of microplastics in nearshore waters, small volume sampling methods often focus on a range of 5–20 liters. However, due to the large variability in microplastic abundance between small volume samples, parallel samples are needed to more accurately quantify concentrations in the environment. Considering factors such as the hydrological environment and sampling conditions of the Yangtze River Estuary, this study selected the small volume sampling method, collecting 25 L of surface seawater at a depth of 30 cm in buckets at each station, with three parallel samples taken at each station, stored at low temperature, and processed upon return to the laboratory. A total of 9 types of biological samples were collected, including 7 species of fish and 2 species of gastropods. The collection and processing methods of the biological samples comply with the relevant provisions of the 'GB/T 12763.6-2007 Marine Investigation Specifications Part Six: Marine Biological Investigation'. The collected biological samples were identified, their body lengths were measured (Table 1), and after being sorted by species, they were stored at -20°C in the laboratory until further analysis.

Figure 1. Microplastic collection at three stations near the Zhoushan Fishing Grounds near the Yangtze River Estuary

Table 1. Species, number of organisms samples and the mean length

Category	Type of Organism	Number of Samples	Body Length/Centimeters
Fish	*Pampus argenteus*	6	15.2 ± 0.8
	Lagacephalu spadiceus	6	19.5 ± 1.2
	Conger muxiaster	6	54.0 ± 4.3
	Miichthys miiiux	5	30.5 ± 4.2
	Johnius carouna	6	17.1 ± 0.6
	Sillago japonica	6	18.2 ± 1.4
	Platucephalus indicus	6	37.2 ± 0.7
Gastropod	*Rapana venosa*	6	12.3 ± 1.2
	Turbo petholatus L.	6	2.2 ± 0.9

2.2 Research Methods

2.2.1 Sample treatment

In the laboratory, to determine the content of microplastics in water samples, each parallel sample (15 L) was filtered (glass filter, Tianjin Jinteng, Tianjin) onto a nylon membrane (pore size 20 μm, diameter 47 mm, NY2004700, Millipore, Burlington, MA). The filtration process was repeated several times depending on the residue in the visible water samples. Each membrane was placed in a clean glass petri dish and stored in a desiccator for further microscopic observation. At the same time, to determine the stable nitrogen isotope values ($\delta^{15}N$) in the water samples, the well-mixed surface water samples were immediately filtered using pre-combusted (450°C, 4 hours) glass fiber membranes (pore size 0.7 μm, diameter 47 mm, Whatman, GF/F). All utensils used during the filtration process were thoroughly cleaned with Milli-Q water at least three times and then rinsed with sample water. Three background blank control groups (same volume of Milli-Q water) were set up throughout the sample filtration process to ensure data quality.

For each type of organism, six individuals with similar body lengths were selected for dissection experiments on a super-clean laboratory bench. For gastropod organisms, the shell was removed to obtain muscle and digestive gland tissues. For each fish, the gills, gastrointestinal tract, and muscle below the first dorsal fin were also taken. After weighing all sample tissues (electronic balance, BSA224S, Sartorius, Germany), they were placed in glass bottles, sealed, labeled, and stored in a 4°C refrigerator for further processing.

2.2.2 Stable isotope analysis

Surface Water Samples: The glass fiber membranes used for stable isotope analysis were dried in an oven (digital electric heat and air circulation drying oven, DHG-9140A, Shanghai Yiheng Scientific Instrument Co., Ltd., Shanghai) at 50°C for 48 hours until a constant weight was reached to ensure the removal of moisture from the surface of the membranes. The dried membranes were then wrapped in tin boats and tightly packed for analysis.

Biological Samples: Muscle tissues from both gastropod and fish organisms were used for stable isotope analysis. All samples were washed with Milli-Q water, placed in centrifuge tubes, and dried for 72 hours using a freeze dryer (Trx-FD-1A-50, Shanghai YunGuan Electromechanical Equipment Co., Ltd., Shanghai). After drying, the samples were ground into powder, wrapped in tin boats, and tightly packed for analysis.

A stable isotope mass spectrometer (DELTA PLUS XP, Thermo, Germany) was used to determine the nitrogen stable isotope values of particulate nitrogen (PN) in both surface water samples and biological samples. The nitrogen stable isotope samples were measured repeatedly, with a method precision better than 0.2% and an isotopic abundance relative deviation of less than 1.5%. Additionally, atmospheric nitrogen (Air-N2) was used as a reference standard for sample quality control during the measurement process. To ensure the accuracy of the δ^{15}N data measurement results, the N integral area corresponding to all reported δ^{15}N values should be above 25 Vs.

The results are expressed in δ^{15}N, and the calculation formula is:

$$\delta^{15}\mathrm{N} = \frac{\mathrm{R_{Sample}} - \mathrm{R_{Standard\ Product}}}{\mathrm{R_{Standard\ Product}}} \times 100\%$$

In this context, R represents the isotope ratio, specifically the ratio of ^{15}N/^{14}N. The trophic level (TL) of different organisms can be calculated using the δ^{15}N values, with the calculation formula as follows:

$$\mathrm{TL} = \frac{\delta^{15}\mathrm{N_{Consumer}} - \delta^{15}\mathrm{N_{baseline\ biology}}}{3.4\%} + 2$$

In general, filter-feeding gastropods are used as the reference organisms, with their TL set at 2 (primary consumers), and the difference in stable nitrogen isotope values between adjacent TL is taken as 3.4%.

A linear regression analysis is conducted between the logarithmic values of microplastic concentrations within various organisms from the Yangtze River Estuary and their TL, with the calculation formula as follows:

$$\log(\text{Microplastic Concentration}) = \mathrm{a} + \mathrm{b} \times \mathrm{TL}$$

In this formula, a represents the intercept of the linear regression equation, while b denotes the slope. The trophic magnification factor (BMF) can be calculated based on the slope:

$$\mathrm{BMF} = 10^{\mathrm{b}}$$

Wherein, a BMF greater than 1 indicates the presence of a biological magnification effect of microplastics among organisms at different trophic levels; whereas a BMF less than 1 suggests a biological dilution effect. The use of stable nitrogen isotopes (δ^{15}N) provides a robust framework for TL assignment, as δ^{15}5N typically increases by 3–4‰ per TL, enabling precise differentiation between primary consumers and higher predators. By correlating δ^{15}N with microplastic burden, this study captures the trophic transfer potential with higher confidence.

2.2.3 Analysis of microplastic content and characteristics

Surface Water Samples: Nylon membranes used for the identification and analysis of microplastics were observed and photographed under a Zeiss stereo microscope (Zeiss Discovery V8, Micro Imaging GmbH, Göttingen, Germany) at a magnification of 40 times after the moisture on the filter surface had evaporated. The color of the microplastics was determined based on the microscopic photography, and Image J software was used to measure the size of the microplastics. Subsequently, the composition was identified using a Fourier-transform infrared microscope (μ-FT-IR, Thermo Fisher, MA, USA) in transmission mode (spectral range of 4000–675 cm^{-1}), selecting polymer types with a spectrum match greater than 70% (Figure 2). To minimize exogenous contamination, it is necessary to avoid airborne pollution during sampling and laboratory processing, and strict requirements should be imposed on the laboratory environment, containers used during the experiment, and personnel. For example, utensils should be rinsed with Milli-Q water and then sealed for storage. Glassware should be wrapped in aluminum foil and placed in a muffle furnace (MF-4-12N, DuTe Company, Shanghai) and calcined at 450°C for 2–3 hours. Clean filters should be placed in the laboratory environment to filter the same volume of Milli-Q water for blank quality control analysis. According to the blank results, only one white fiber was found on all the blank filters, which was identified as a cotton fiber by Fourier micro-infrared spectroscopy, likely fallen from a white pure cotton lab coat, as cotton products are not within the scope of microplastics reported in this study. Therefore, no microplastic contamination occurred during the experiment.

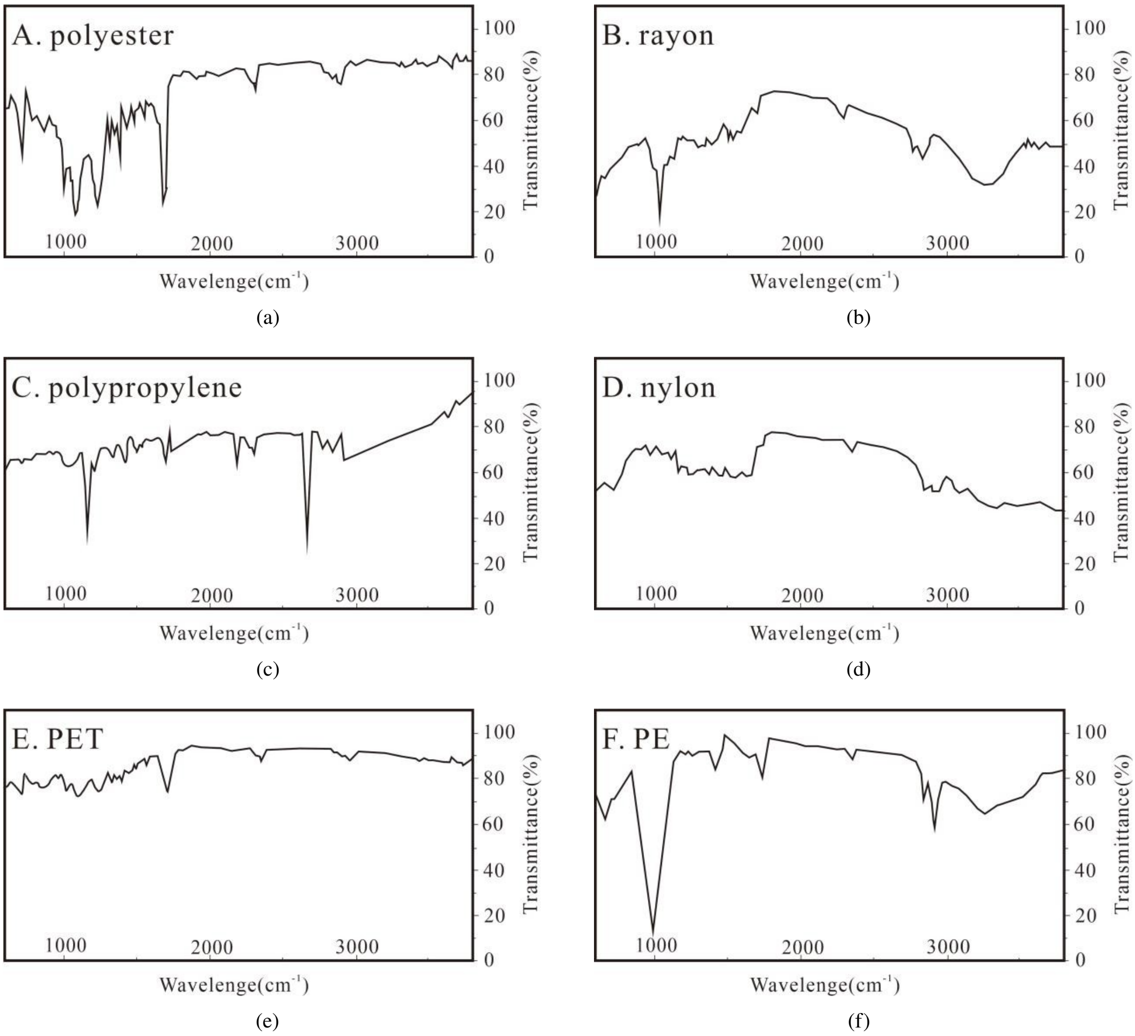


Figure 2. Identification results of microplastic polymer types

Note: PET: polyethylene terephthalate; PP: polypropylene.

Biological Samples: Glass bottles containing the digestive gland tissues of gastropod organisms and the gills and gastrointestinal tracts of fish organisms were filled with a 30% H_2O_2 solution (Sinopharm Chemical Reagent Co., Ltd., Shanghai) and the caps were tightly sealed. They were then placed in a constant temperature shaker (HZ-9612K, DaCang Laboratory Instrument Company, Taicang) and oscillated at 65°C for 48 hours to completely digest the biological tissues. The solution from the sample bottles was filtered, and the filters, like the surface water samples, used nylon membranes with a pore size of 20 μm and a diameter of 47 mm. The filtered membranes were placed in a desiccator to thoroughly dry the surface moisture. Further microplastic content determination and characteristic analysis were conducted using the same microscopic examination and identification methods as for the surface water samples. Although the total number of biological samples is relatively limited (56 individuals), the inclusion of diverse trophic groups (7 fish species and 2 gastropods) ensures coverage of the local food web structure. Furthermore, all sampling occurred under consistent environmental conditions in October, minimizing temporal bias. Future studies should include multi-seasonal campaigns to capture seasonal variability in microplastic transport linked to river discharge and monsoonal flow.

3 Result Analysis

3.1 Distribution Characteristics of Microplastics in the Surface Waters of the Yangtze River Estuary

In the study of plastic samples from three typical stations in the Zhoushan Fishing Ground of the Yangtze River Estuary, natural materials (such as wool, cotton, etc.) were not included in the reported results. The study found that the average concentration of microplastics in the surface waters of the Yangtze River Estuary was 661.2 ± 220.5 items/mm^3, and there were significant differences in the distribution of microplastics among the three sampling stations (ANOVA variance analysis, $p < 0.01$). Among them, the station closest to the outer sea, station 3, had the highest concentration of microplastics (1083.8 ± 87.5 items/m^3), which was significantly higher than that of the coastal stations (station 1: 333.8 ± 16.7 items/m^3 and station 2: 583.8 ± 21.7 items/mm^3) (Figure 3a). Although the freshwater discharge from the Yangtze River carries waste plastics from the land to the East China Sea, causing severe pollution in the areas near stations 1, 2, and 3, it is worth noting that there is a significant increasing trend in the concentration of microplastics from the estuary to the outer sea. This may be due to the fact that during the transportation process, environmental factors (such as waves, currents, and wind) intensify the degree of plastic fragmentation, increasing the likelihood of the presence of small-sized plastics (such as microplastics). The level of microplastic concentration reported in this study is lower compared to the study by Fan et al. [8] (4137.8 ± 2461.5 items/mm^3), and slightly higher than their 2019 report on the same area (157.7 ± 75.8 items/mm^3) [8]. The reason for such a significant difference lies in the fact that this study only collected water samples from three typical stations in one quarter of the Yangtze River Estuary, while the latter study collected surface water samples from 95 stations over three quarters in 2019. Since both time and space can affect the level of microplastic concentration to a certain extent, the data range reported in the latter is more varied. Compared to the lower monthly runoff in October, the increased runoff in July can carry more terrestrial plastics to the Yangtze River Estuary and further into the East China Sea shelf area [26], which may have led to an overestimation of the microplastic concentration in the surface waters of the Yangtze River Estuary based on the July data. Therefore, the limited sampling and single-quarter sampling may lead to inaccurate reporting of microplastic concentrations. More research is needed for long-term, large-scale systematic observations of the spatiotemporal distribution characteristics of microplastics in the Yangtze River Estuary. The size range of microplastics in the Yangtze River Estuary is 60–4460 μm, mainly dominated by smaller particle sizes (Figure 3c). Microplastics in the range of 0–500 μm and 500–1000 μm account for 35% and 30%, respectively. Very few microplastic particles >2000 μm were found. At the same time, there are also differences in the size distribution of microplastics among the stations. As speculated in the previous paragraph, the closer to the outer sea, the smaller the size of the microplastics. The particle size of microplastics at station 3 (716 ± 122 μm) is significantly smaller than that at station 1 near the estuary mouth (1837 ± 922 μm) ($p < 0.05$) (Figure 3b). This is largely due to the fact that during the transportation of plastics from the estuary mouth to the outer sea, they have undergone a fragmentation process caused by physical factors in the environment, with most plastics breaking down into smaller microplastic particles.

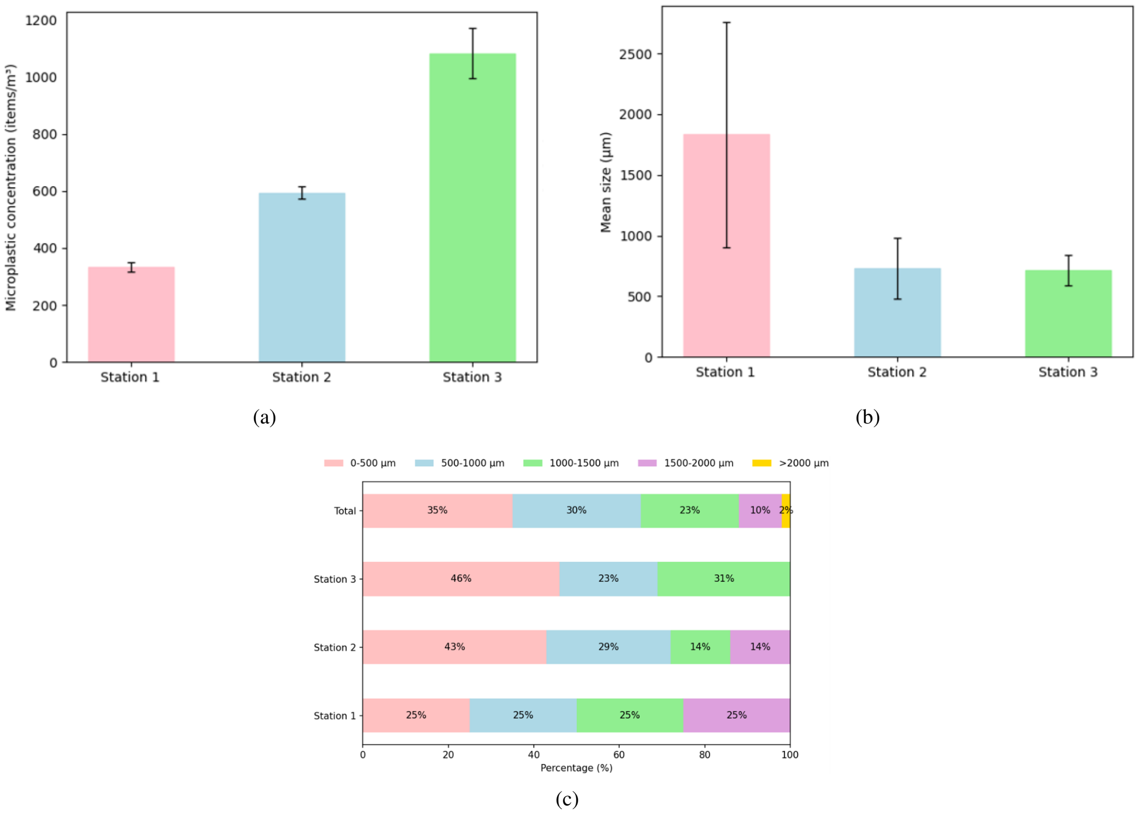
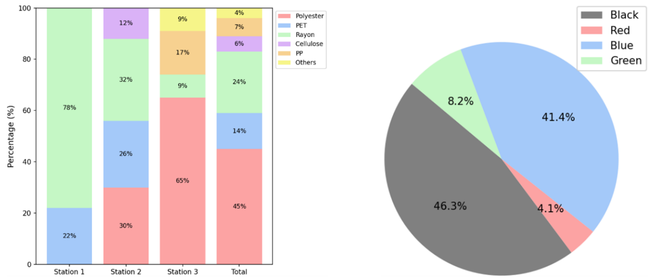


Figure 3. Characteristics of microplastic occurrence in surface waters at typical stations

Identified by Fourier infrared spectroscopy, a total of five types of polymers were found in the surface waters at three typical stations in the Yangtze River Estuary. The most abundant was polyester fiber (45%), followed by rayon (24%), polyethylene terephthalate (14%), polypropylene (7%), and cellulose compounds (6%) (Figure 4a). Polyester fiber is a common polymer in microplastics research, primarily used in the textile industry. With the development of the textile industry and frequent human activities around the Yangtze River Estuary and Hangzhou Bay, the strong hydrological conditions transport waste plastics from the land to the East China Sea, leading to severe microplastic pollution in the areas near the three typical stations, especially with polyester fiber and rayon as the main components. It is worth noting that although polyethylene particles are often considered the main type of microplastic polymer in many reports, we did not find any polyethylene particles in this study. Some polymer components identified at station 3 were not detected in the samples from the nearshore stations, leading us to speculate that the microplastics at the offshore stations of the Yangtze River Estuary may be mostly secondary microplastics. The weathering and fragmentation of plastics during transportation could be one of the sources of microplastics at this station. Based on the above considerations, we speculate that the estuary can act as a 'sink' for terrestrial input microplastics carried by rivers, and at the same time, due to the unique hydrological and sedimentary environmental conditions of the area, the estuary can also become a 'source' of secondary plastics generated by weathering during the transportation process. In this study, among the collected microplastic samples, colored particles (including easily recognizable colors such as blue, red, and green) accounted for more than half (50.7%), and the rest were black (46.3%) (Figure 4b), with no transparent microplastics found in this study. There were no significant differences in the colors of microplastics among the stations. Studies have shown that brightly colored microplastics are more likely to be ingested by aquatic organisms and enter the body, causing toxic effects, compared to black and transparent plastic particles. Black and transparent microplastics are likely the result of colored plastics weathering and fading. Therefore, based on the identification of polymer types and colors of microplastics at different stations in the Yangtze River Estuary, we found that the characteristics of microplastics at the estuary mouth station 1 are mainly influenced by human activities, while the characteristics of microplastics at the offshore station 2 and station 3 are mainly affected by the degree of weathering. Thus, identifying the characteristics of microplastics in the Yangtze River Estuary is of great significance for understanding the 'source and sink' characteristics of microplastics in the aquatic environment of the estuary.



(a) (b)

Figure 4. Distribution of different polymer types: (a) and color distribution of microplastics; (b) in the surface waters at typical stations of the Yangtze River Estuary

Note: PET: polyethylene terephthalate; PP: polypropylene.

3.2 Characteristics of Microplastic Contamination in Various Organisms of the Yangtze River Estuary

Among all the biological samples from the Zhoushan Fishing Ground in the Yangtze River Estuary, 35 samples (61.9%) were found to be contaminated with microplastic particles by spectral analysis (Figure 5). Among the seven species of fish, the average abundance of microplastics in the gills was 1.2 ± 0.3 items/g w.w., with the *Conger myriaster* (3.7 ± 1.3 items/g w.w.) and the *Pampus argenteus* (1.9 ± 0.4 items/g w.w.) having significantly higher average abundances of microplastics in their gills compared to other organisms ($p < 0.01$). The abundance of microplastics ingested into the gastrointestinal tract varied from 0.08 to 0.83 items/g w.w. The highest average abundance of microplastics in the gastrointestinal tract was 0.80 ± 0.29 items/g w.w. (*Sillago japonica*). It is noteworthy that, aside from the *Sillago japonica*, which had a significantly higher average abundance of gastrointestinal microplastics compared to the *Lagocephalu spadiceus* (0.20 ± 0.04 items/g w.w.), the *Conger myriaster* (0.21 ± 0.05 items/g w.w.), the *Miichthys miiuy* (0.13 ± 0.06 items/g w.w.), and the *Platycephalus indicus* (0.10 ± 0.03 items/g w.w.), no significant differences in the abundance of microplastic particles were found among these seven species of fish (ANOVA one-way analysis, $p > 0.05$). When comparing the abundance of microplastics between two different parts of the same species, the *Pampus argenteus* ($p < 0.01$), the *Conger myriaster* ($p < 0.01$), and the *Platycephalus indicus* ($p < 0.05$) had significantly higher abundances of microplastics in their gills than in their intestines. In contrast, the *Sillago japonica* and the *Johnius carouna* had higher abundances of microplastics in their gastrointestinal tract, but the difference was not significant.

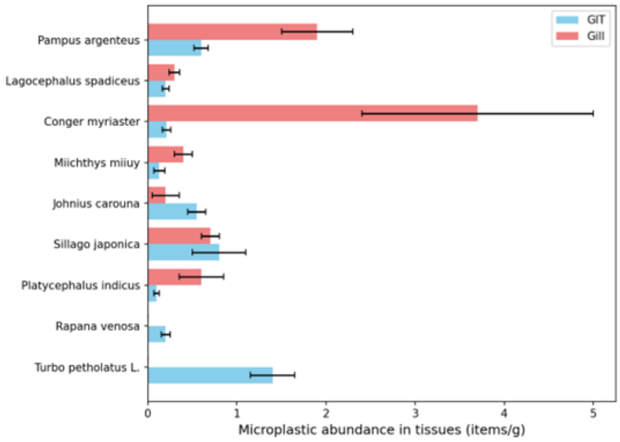
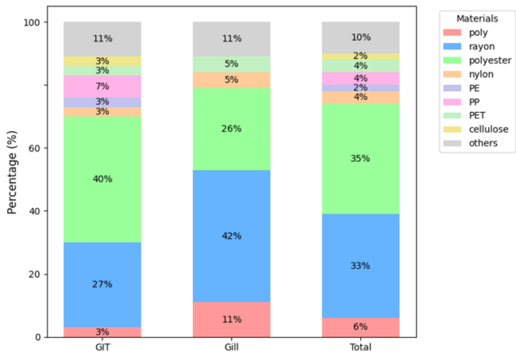
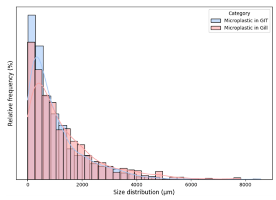


Figure 5. Abundance of microplastics (items/g w.w.) in the gills and gastrointestinal tract of biological samples from typical stations in the Yangtze River Estuary

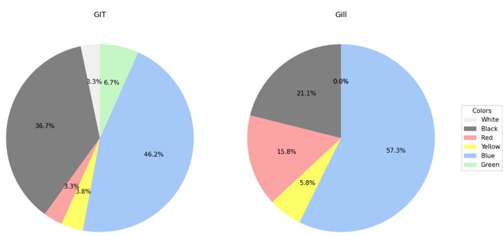
Nine different polymer types were identified in the biological samples (Figure 6a), with polyester being the most common type of microplastic, accounting for 35%, followed by rayon at 33%. These two types of microplastics were present in the majority of samples, making up 68% even in the gills of fish. The combined amount of other polymer types was less than these two, including polypropylene (4.0%), polyethylene (2.0%), polyethylene terephthalate (4.0%), nylon (4.0%), and cellulose compounds (2.0%). Copolymers were detected in only two species (6.0%, in the gills of the *Conger myriaster* and the gastrointestinal tract of the *Pampus argenteus*). In addition, some uncommon chemical compositions of microplastic particles were categorized as 'others'. Overall, the size distribution of microplastics in the gills and gastrointestinal tract of the samples was mainly between 300–500 μm (Figure 6b), with the average distribution in the gills and gastrointestinal tract being 1320 ± 295 μm (range 304–4515 μm) and 1120 ± 114 μm (range 190–2285 μm), respectively, indicating that the plastics found in the gills were slightly longer than those in the intestines. The microplastics detected in the gastrointestinal tract of the *Sillago japonica* and the *Johnius carouna* were significantly smaller than those in the gills. No significant differences in microplastic sizes were observed between the gills (Kruskal-Wallis test, $p = 0.64$) and the gastrointestinal tract of all samples (Kruskal-Wallis test, $p = 0.92$).



(a)



(b)



(c)

Figure 6. Different polymer types: (a) and color distribution; (b) of microplastics in the gill and gastrointestinal tissues of biological samples from the Yangtze River Estuary

Note: PE: polyethylene; PET: polyethylene terephthalate; PP: polypropylene.

Six colors of microplastics were found in the gills and gastrointestinal tract of the studied biological species (Figure 6c), with the most common color being blue (gastrointestinal tract: 46.2%; gills: 57.3%), followed by black and red. The higher microplastic concentration observed in *Conger myriaster* may be attributed to its benthic predatory habits and prolonged habitat contact with sediment microplastics. Its larger body size and higher metabolic intake may also contribute to increased exposure and ingestion rates.

3.3 Trophic Structure and Nutritional Relationships in the Yangtze River Estuary Food Web

This study collected a total of two species of gastropods and seven species of fish from the Yangtze River Estuary. The results of the stable nitrogen isotope (δ^{15}N) and trophic levels of different organisms in the Yangtze River Estuary are shown in Table 2. The δ^{15}N value for gastropod organisms in the Yangtze River Estuary was 8.7 ± 0.5‰, and for fish species, it was 11.9 ± 1.1‰. Using the purely filter-feeding gastropod species (*Rapana venosa*) as the reference organism (defining its trophic level as 2), this study calculated the average trophic levels of various organisms in the Yangtze River Estuary (Table 2): gastropods at 8.9, fish at 12.3, with the highest trophic level being 14.5 ± 0.9 (*Conger myriaster*).

Table 2. Values of δ^{15}N, trophic levels and microplastic concentrations in organisms

Category	**Type of Organism**	**δ^{15}N (‰)**	**Trophic Level**	**Microplastic Content (items/g)**
Gastropod	*Rapana venosa*	9.0 ± 0.5	2.0 ± 0.2	0.05 ± 0.03
	Turbo petholatus L.	8.4 ± 0.5	1.8 ± 0.2	0.68 ± 0.31
Fish	*Pampus argenteus*	10.7 ± 0.4	2.7 ± 0.3	0.73 ± 0.53
	Lagocephalu spadiceus	11.3 ± 0.5	2.8 ± 0.4	0.40 ± 0.30
	Conger myxiaster	14.5 ± 0.9	3.4 ± 0.2	4.05 ± 1.14
	Miichthys miiux	12.7 ± 0.2	3.1 ± 0.2	0.54 ± 0.22
	Johnius carouna	11.6 ± 0.5	2.7 ± 0.3	0.34 ± 0.14
	Sillago japonica	12.7 ± 0.5	3.2 ± 0.1	0.65 ± 0.26
	Platycephalus indicus	11.6 ± 0.7	2.7 ± 0.2	0.45 ± 0.22

A linear regression analysis was conducted between the logarithmic values of microplastic concentrations (Table 2) within various organisms of the Yangtze River Estuary and their trophic levels (Figure 7). A linear model was chosen for its interpretability and established use in trophic magnification studies. While nonlinear models may better fit certain complex dynamics, our dataset exhibited a reasonably linear relationship ($R^2 = 0.45, p < 0.05$), justifying the use of a linear regression to estimate the BMF. Future work may explore nonlinear alternatives with expanded datasets. The results indicated that the accumulation levels of microplastics within organisms gradually increased with the rise in trophic levels, demonstrating a significant trophic magnification effect of microplastics ($R^2 = 0.45$, $p < 0.05$). Through the linear regression equation ($y = 0.68\text{TL} - 1.88$), the biomagnification factor (BMF) for microplastics during their transfer through the food chain was calculated (BMF = 4.2). Comparing the BMF value, which is greater than 1, it was found that microplastics can be transferred along different trophic levels and exhibit biological magnification within the aquatic food web of the Yangtze River Estuary. Carnivorous fish tend to have higher BCF values due to consumption of prey species already containing microplastics, leading to a cumulative transfer effect. Additionally, carnivorous species often occupy habitats closer to microplastic accumulation zones (e.g., sediment-rich bottoms), increasing their exposure likelihood.

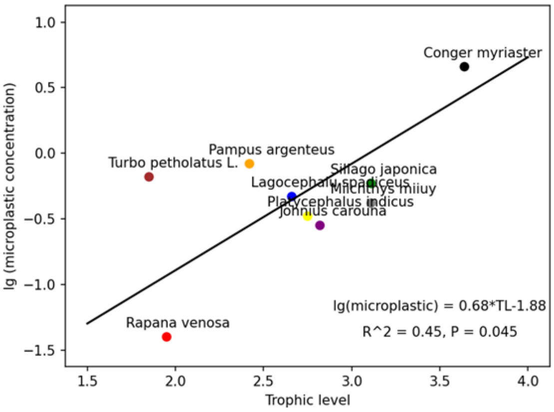


Figure 7. Correlation between microplastic concentration and trophic levels in biological samples from the Yangtze River Estuary

4 Discussion and Conclusions

4.1 Discussion

The accumulation of microplastics within organisms and their impact on these organisms depend not only on the physical and chemical properties of the microplastics themselves but also on the exposure conditions and the characteristics and feeding preferences of the organisms. Since microplastics are mostly in the form of particles and exist primarily as individuals, the feeding preferences and predatory behavior of exposed organisms have a more pronounced effect. Generally, organisms with regular feeding selection are more likely to ingest microplastics that resemble their natural food. Considering the impact of exposure concentration on the abundance of microplastics ingested by organisms, the study further established a unified evaluation index, the bioconcentration factor, to reflect the accumulation of microplastics within organisms relative to the increase in environmental concentrations in the Yangtze River Estuary. This study used the calculation method of the bioconcentration factor to compute the relevant data on microplastic concentrations in biological samples collected from the Yangtze River Estuary and compared different types of organisms to attempt to compare the impact of biological characteristics on the accumulation of microplastics within their bodies.

Overall, the bioconcentration factors of microplastic accumulation in most organisms from the Yangtze River Estuary are greater than 1, meaning that the concentrations within different organisms exceed the concentrations in the surrounding environment. Therefore, most organisms in the Yangtze River Estuary exhibit phenomena of internal accumulation, but no significant bioconcentration effect (bioconcentration factor less than 1000) is observed. This is consistent with the results summarized from different field conditions regarding the bioconcentration effect of microplastics. Combining actual observations from the Yangtze River Estuary and summarizing different field research results, it can be concluded that the accumulation of microplastics within organisms may only be a temporary presence in a single tissue space and may not necessarily lead to continuous accumulation within organisms. However, whether this temporary residence will have an impact on the organisms themselves or whether this impact will be transmitted along the food chain still lacks dual research from field and laboratory experiments. At the same time, as humans are the top consumers of the aquatic food chain, it is also crucial to explore the trophic magnification effect of microplastics along the aquatic food chain.

4.2 Conclusions

(1) In October 2021, the abundance of microplastics in the surface water samples of the Yangtze River Estuary showed significant spatial variation, with the highest concentration at the offshore station 3 (1083.8 ± 87.5 items/m^3), significantly higher than the coastal stations (1 and 2). Considering the characteristics of microplastics at each station, it is speculated that the weathering and fragmentation of plastics during transportation in the Yangtze River, reducing their size and increasing their concentration, is one of the sources of microplastics at station 3, which is different from the other two stations.

(2) Among the biological samples collected from the Yangtze River Estuary, 35 samples (61.4%) were found to be contaminated with microplastic particles by spectral analysis. The *Conger myriaster* (3.7 ± 1.3 items/g w.w.) and the *Pampus argenteus* (1.9 ± 0.4 items/g w.w.) had significantly higher average abundances of microplastics in their gills compared to other organisms. The highest average abundance of microplastics in the gastrointestinal tract was 0.80 ± 0.29 items/g w.w. (*Sillago japonica*). Comparing the abundance of microplastics between two different parts of the same species, the *Pampus argenteus,Conger myriaster*, and *Platycephalus indicus* had significantly higher abundances of microplastics in their gills than in their intestines.

(3) Using the calculation method of the bioconcentration factor, the relevant data on microplastic concentrations within biological samples collected from the Yangtze River Estuary were calculated. It was found that the concentrations within organisms exceeded the concentrations in the surrounding environment, indicating a phenomenon of internal accumulation, but no significant bioconcentration effect was observed. The bioconcentration factor for higher trophic level organisms (fish) was significantly greater than that for gastropod organisms. Additionally, in terms of feeding behavior, the bioconcentration factor for carnivorous fish species was significantly greater than that for omnivorous fish.

(4) The trophic levels of organisms in the Yangtze River Estuary were determined using stable nitrogen isotope technology. Comparing the accumulation concentrations of microplastics within organisms at different trophic levels, the results showed that microplastics can be transferred within the food chain of the Yangtze River Estuary, and there is a significant positive correlation between the concentration of microplastics within organisms and their trophic levels. At the same time, looking at the accumulation patterns within the Yangtze River Estuary food web, microplastics have the potential for biological magnification in gastropod and fish species (BMF value of 4.2). However, due to the limited number of samples from lower trophic level organisms in this study, there is a certain impact on the accurate reflection of the accumulation patterns of microplastics within the Yangtze River Estuary food web.

4.3 Practical Implications

The observed biomagnification potential of microplastics in the Yangtze River Estuary highlights the need for targeted mitigation strategies. Policy recommendations include: (1) implementing stricter waste management controls along urbanized estuary zones; (2) regular monitoring of high-risk fish species for human consumption; (3) establishing seasonal fishing restrictions during periods of high microplastic flux. These measures could reduce the trophic transfer of microplastics and associated contaminants to both aquatic organisms and humans.

Author Contributions

Conceptualization, T.W. and M.S.E.; methodology, T.W. and Y.G.; software, T.W.; validation, T.W., Y.G., and M.S.E.; formal analysis, T.W.; resources, M.S.E.; data curation, T.W. and Y.G.; writing—original draft preparation, T.W.; writing—review and editing, T.W., Y.G., and M.S.E.; visualization, T.W.; supervision, M.S.E.; project administration, M.S.E.; funding acquisition, M.S.E. All authors have read and agreed to the published version of the manuscript.

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Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflict of interest.

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