Occurrence of Microplastics in the Gastrointestinal Tract and Gills of Fish from Guangdong, South China

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Abstract: Microplastic pollution has become a major global concern. Coastal areas are densely populated with human activity, commercial enterprises, and fishing, resulting in high incidences of fish microplastic pollution. It has been shown that microplastics exist in commercial fish in coastal areas of Guangdong, China. Most of the microplastics were less than 1 mm, white and blue fibers. The abundance, type, shape and color of microplastics in gills and in the gastrointestinal tract (GIT) of eight species of commercial fish in the coastal waters of central and western Guangdong Province were analyzed. The overall abundance of microplastic particles in fish was 6.6 items/individual, with an average of 2.2 particles in gills and 4.4 in the GIT. The GIT of carnivorous fish was less likely to ingest microplastics than that of herbivores or omnivores. Middle-water fish, on the other hand, are less likely to have their gills contaminated with microplastics. These results revealed the degree of microplastic pollution in fish tissues from the central and western coastal areas of Guangdong province. The quantity of microplastics in the GIT of fish in the Pearl River Delta was the highest compared with the surrounding areas.

Keywords: microplastics; fish; coast; gills; gastrointestinal tract

1. Introduction

Plastic pollution has recently become a major problem for global marine ecosystems by destroying the environment and harming organisms [1]. Microplastic pollution is widespread around the world and is widely found in soil [2], sediment [3], seawater, estuaries [4], freshwater lakes [5], and in the Arctic [6]. Previous field studies have reported severe plastic pollution in oceans and seas around the world, such as the Amazon River estuary [7], Pacific Ocean [8], Mediterranean Sea [3], and the Arctic Ocean [9]. Plastic breaks down into microplastics, which persist in various forms in our environment in sand, dust and water [10]. Microplastics are defined as plastic particles less than 5 mm. Microplastics can be classified as primary microplastics and secondary microplastics, based on different routes of entry into the environment [11]. Primary microplastics are microscopic plastics that invade the environment directly after use, such as cosmetics containing microplastic particles or plastic particles and resin particles used as industrial raw materials [12]. Secondary microplastics are plastic particles formed by splitting and reducing the volume of large plastic waste through physical, chemical and biological processes, such as plastic bags and packaging plastics [10]. It has been reported that
microplastics are more harmful than plastic due to their small size and persistent presence in the environment [13].

Microplastics can be ingested by a variety of marine organisms, including plankton, bivalve filter feeders, fish, and mammals [14–16]. The physical characteristics of microplastics allow for easy absorption of heavy metals, persistent organic pollutants, and other harmful substances [17]. These harmful substances will be released after being ingested by organisms, causing adverse effects in both the health of the organism, as well as the stability of the environment [18].

Accumulation of microplastics starts at the plankton level then moves up to higher nutrient levels. Some fish carry and transmit microplastics that have been transmitted up through the food web [19]. Humans may ingest microplastics from eating fish. For example, Jabeen et al. found that large amounts of microplastics were found in commercial fish from the Shanghai fish market [20]. However, market research on microplastics in contaminated seafood is still relatively rare [21]. Therefore, contamination of food with microplastics and related chemicals is an urgent problem due to potential harmful effects on human health [22].

Studies have shown that the intake of microplastics in fish is affected by many factors, such as local microplastic pollution levels, fish nutrition level and feeding strategy [23,24]. In addition, high microplastic abundance is also associated with strong human activities. For example, studies have shown that the quantity of microplastics in coastal fish may be much higher than in fish found in the open sea [25].

Coastal areas are important feeding and spawning grounds for fish, having high biodiversity. These specialized zones in the aquatic ecosystem are crucial to commercial aquaculture production [26]. China’s Gross Domestic Product reached 101 trillion RMB in 2020, with booming economic activity that has caused many environmental pollution problems. Guangdong is the southern-most part of the Chinese mainland with a coastline of 4114.3 km. The Pearl River Delta, on the central coast of Guangdong, is one of the most developed regions in China. In recent years, China’s coastal areas, such as the Pearl River Delta, have been targeted as “hot spots” of microplastic pollution [27]. However, in these coastal areas of Guangdong where the risk of microplastic pollution is high, data has been severely lacking.

Thus, the aim at the present study was to investigate the level of microplastic pollution in commercial fish located in the central and western coastal areas of the Guangdong Province. The shapes, colors, and sizes of microplastics found in wild commercial fish were counted. The data showed the accumulation of microplastics in commercial fish in this region, which may pose a food-safety risk. The data quantitating microplastic pollution in fish can provide a basis for future treatment of microplastic contamination in this area.

2. Materials and Methods

2.1. Sampling

Thirty sampling sites were assessed from 26 December 2019 to 8 January 2020 in seven cities along the coast of China’s Guangdong Province (Supplementary Table S1 and Figure 1). Fish were purchased from local fish markets. For this study, only fish (three per species) that were caught in the wild and not farmed were selected and used. Fish were placed in an incubator with ice packs, then transferred to a −20 °C freezer until use. Eight species of fish were selected for our study, including Clupanodon punctatus, Clupanodon thrissa, Siganus fuscescens, Leiognathus brevirostris, Alepes djedaba, Gerres lucidus, Sillago sihama and Terapon jarbua, since these eight species are common in the coastal areas of Guangdong (Supplementary Figure S1). The length and wet weight of each fish was measured and recorded (Supplementary Table S2).
2.2. Microplastic Extraction

One fish per species was selected at each sampling point, a total of eighty fishes being analyzed. Fish are divided into different feeding habits and different habitats. Both gill tissue and gastrointestinal tract (GIT) tissue were used to study the microplastic pollution of fish in different habitats, and fish with different diets. Fish were then thawed, and tissues were dissected. Gills and GIT tissues were placed in a digestion container containing an appropriate volume of 10% KOH solution. Digestion containers were 300 mL and 500 mL glass bottles. The digestion container was placed in a 60 °C water bath for two days [12]. The digestion solution was absorbed by vacuum filtration, and the residue was adsorbed on a 0.45 μm filter membrane. For a solution containing a large amount of sand, saturated NaCl was added to oscillate, mix, and suspend the upper layer of the solution, which was then adsorbed on a 0.45 μm filter membrane [20].

2.3. Microscopy

The filter membrane was analyzed with microscopy (CNOPTEC SZ680, Chongqing, China). Microplastics were classified and counted. Microplastics less than 1 mm were grouped as: <0.5 mm and 0.5–1 mm; larger than 1 mm were classified as a group at intervals of 1 mm. The microplastics in the sample were classified as six colors: black, yellow, red, green, blue and white. Both transparent and white were classified as white since it was difficult to distinguish the clearly in the microscope. We squeezed the suspect with iron tweezers, sifting out materials that were damaged or deformed, and substances that have a certain elasticity are considered microplastics. The microplastics were divided into fiber type, sphere type, fragment type, and film type. Fiber judgment criteria: fiber is required be in a long strip; uniform thickness is required; the overall color should be uniform; terminal non-bifurcation. Sphere microplastics are smooth, spherical or granular microplastics. Fragments: Grains must have clear boundaries, uniform overall color,
irregular shape, sharp edges and no metallic color. Film: thin sheet, no metallic luster, uniform color. Microplastics of typical shapes observed in the microscope in this study are shown in Figure 2.

Figure 2. Shapes of microplastics in fish samples from the coastal areas of central and western Guangdong province: (A–C) fragments; (D, E) sphere; (F) film; (G–I) fibers. Black arrows indicate microplastics.

2.4. Quality Assurance and Control

In order to reduce the influence of external microplastics on the experiment, no plastic tools were used on samples during the whole experiment. The same experimental operations were carried out by the same experimenter to achieve a unified operating standard. To further prevent the influence of microplastics in the experiment, anatomical tools and experimental utensils were rinsed with clean water before use.

2.5. Statistical Analysis

ArcMap 10.2 was used to draw maps, Microsoft Office Excel and SPSS 19 were used to analyze data. There were no correlations found between microplastics and fish length/weight by the computations of Pearson’s correlation coefficients two-tailed test.

3. Results

3.1. Microplastics in Fish

Of the 80 fish studied, only three had no microplastics, and the average quantity was 6.6 items/individual (Table 1). The majority of microplastics were fibers (78%), followed by fragments (20%), then spheres and films (only 1% each) (Figure 3a). The proportion of white to blue was identical (36%), with black particles as the next most abundant (22%) (Figure 3b). The maximum length of all microplastics counted was less than 5 mm. Small microplastics were in a large portion of the fish samples. The microplastics <0.5 mm in size were predominant (50%), with 0.5–1 mm, 1–2 mm and 2–3 mm as the next abundant
(constituting of 21%, 14% and 7%, respectively). Only a small amount of microplastics 3–4 mm (4%) and 4–5 mm (4%) in size were observed (Figure 3c).

Table 1. Microplastics (MPs) in fish from the coastal areas of central and western Guangdong province, China.

| Species                   | Living Habitats | Feeding Habitats | Number of Fish | Body Length (cm) | Wet Weight (g) | Number of MPs | Average (Items Individual⁻¹) |
|---------------------------|-----------------|------------------|----------------|------------------|----------------|---------------|-----------------------------|
| Clupanodon punctatus      | Pelagic         | Herbivorous      | 9              | 13.6 ± 1.74      | 47.3 ± 15.12   | 85            | 9.4                         |
| Clupanodon thrissa        | Pelagic         | Herbivorous      | 6              | 17.8 ± 3.03      | 92.3 ± 38.93   | 50            | 8.3                         |
| Siganus fuscesens         | Pelagic         | Herbivorous      | 14             | 12.7 ± 1.74      | 46.6 ± 23.44   | 158           | 11.3                        |
| Leiognathus brevirostris  | Midwater        | Omnivorous       | 10             | 7.8 ± 0.88       | 11.5 ± 4.08    | 41            | 4.1                         |
| Alepes djedaba            | Midwater        | Omnivorous       | 5              | 11.2 ± 1.61      | 21.8 ± 7.63    | 36            | 7.2                         |
| Gerres lucidus            | Midwater        | Carnivorous      | 7              | 8.1 ± 1.48       | 14.3 ± 7.43    | 13            | 1.9                         |
| Sillago sihama            | Demersal        | Carnivorous      | 15             | 12.4 ± 1.76      | 18.9 ± 8.57    | 72            | 4.8                         |
| Terapon jarbua            | Demersal        | Carnivorous      | 14             | 11.3 ± 1.42      | 34.5 ± 14.16   | 73            | 5.2                         |

Figure 3. Proportion of shape (a), color (b) and size (c) of microplastics in fish samples from the coastal areas of central and western Guangdong province.

Among all fish in this study, Siganus fuscesens (11.3 items/individual) had the highest average abundance of microplastics. Relatively high levels of microplastics were found in Clupanodon punctatus (9.4 items/individual), Clupanodon thrissa (8.3 items/individual), and Alepes djedaba (7.2 items/individual), while relatively less of Leiognathus brevirostris (4.1 items/individual), Sillago sihama (4.8 items/individual) and Terapon jarbua (5.2 items/individual). Gerres lucidus (1.9 items/individual) was the lowest (Table 1).

3.2. Characteristics of Microplastics in the Gills of Fish from Different Habitats

The microplastics in fish gills were mainly fibers, which accounted for more than 70% of fish samples different habitats (Figure 4A). Microplastic fragments were found in the gills of fish all habitats. Except for sphere particles found in pelagic gills, sphere and film were not found in the gills from other species. More abundant microplastics were found in the gills of pelagic and demersal fish than from midwater species. In pelagic fish, microplastics were detected in the gills of Siganus fuscesens (3.1 items/individual), Clupanodon thrissa (3 items/individual) and Clupanodon punctatus (2.7 items/individual). In demersal fish, microplastics in the gills of Terapon jarbua (2.9 items/individual) and Sillago sihama (2.4 items/individual) were nearly identical. In midwater fish, Alepes djedaba (1.2 items/individual) was slightly higher than Leiognathus brevirostris (0.9 items/individual) with Gerres lucidus having the lowest (0.4 items/individual) (Figure 5A).

White and blue colored microplastics accounted for the most abundant microplastics in the gill samples. Green and red microplastics were not observed in midwater and demersal fish gills. In pelagic fish, the proportion of blue microplastics was greater than white. In the midwater and demersal fish species, the proportion of white was greater than blue, and the proportion of black was greater than the other colors (Figure 4B).
Small microplastics accounted for the majority of the microplastics in fish gills, and microplastics smaller than 1 mm accounted for at least 50% in the gills from species in all habitats. The proportion of microplastics larger than 2 mm in gills in different habitats...
was not more than 28%, and the microplastics larger than 2 mm in pelagic, demersal, and midwater fish were 10%, 28%, and 22%, respectively (Figure 4C).

3.3. Characteristics of Microplastics in the GIT of Fish from Different Feeding Habits

Omnivorous fish had the least proportion of fiber microplastics. However, fish with other feeding habits had primarily fiber microplastics in the GIT. Neither sphere particles nor films were found in carnivorous fish, and no film microplastics were found in omnivorous fish. Microplastics of all shape types were found in herbivores, and the proportion of fiber microplastics was the highest in herbivores (82%) (Figure 4D).

The average content of microplastics was relatively high in the GIT of herbivores. The microplastic content of herbivorous fish was higher for Siganus fuscescens (8.2 items/individual) than Clupanodon thrissa (5.3 items/individual) and Clupanodon punctatus (6.8 items/individual). The microplastic content of omnivorous fish was about the same for Alepes djedaba (6 items/individual) and Leiognathus brevirostris (5.3 items/individual). Lower microplastics were found in the carnivorous fish Terapon jarbua (2.7 items/individual), Sillago sihama (2.4 items/individual), and Gerres lucidus (1.4 items/individual) (Figure 5B).

The proportion of fiber microplastics in fish with different feeding habits was more than 60% (Figure 4D). Sphere and film microplastics in the GIT of fish samples were not observed in carnivorous fish. The microplastics in the GIT were primarily white and blue, and the total proportion of these two colors were more than 67% in fish having different diets. Green microplastics were not found in the GIT of omnivorous fish (Figure 4E). Approximately 50% of the microplastics from the GIT of fish with different feeding habits were <0.5 mm, and only less than 22% of the microplastics were >2 mm (Figure 4F).

3.4. Characteristics of Microplastics in Fish Isolated from Three Regions

Thirty sampling points were used to analyze the spatial distribution characteristics of microplastics among three regions of the west and central coast of the Guangdong Province. Points W1-8 (the coastal area of the Pan-Pearl River Delta), W9-19 (Yangjiang, Maoming and Zhanjiang east coastal), and W20-30 (the eastern coast of the Beibu Gulf) were divided according to their geographical location and economic status. The average quantity of microplastics found in the GIT and gills of fish in the Pan-Pearl River Delta coastal area was significantly higher than the Yangjiang, Maoming and Zhanjiang eastern coast of the Beibu Gulf (Supplementary Figure S2). The proportion of fiber microplastics from W1-8 was higher than from the W9-19 and W20-30 regions (Supplementary Figure S3a). The proportion of white and blue total microplastics from W1-8 was lower than from W9-19 and W20-30 (Supplementary Figure S3b). Microplastics less than 1 mm were observed in 60–80% of the fish samples of different regions, with W9-W19 (78%) having the highest (Supplementary Figure S3c).

4. Discussion

4.1. Characteristics of the Microplastics in Fish

Microplastics were primarily found in the digestive tracts of fish. The average quantity of microplastics in the GIT was slightly lower in the fish from the east coast of Guangdong (5.4 items/individual) [28] and lower than wild freshwater fish in inland rivers of the Guangdong (7.0 ± 23.8 items/individual) [29] and Beijiang regions (average 5.6 items/individual) [30]. However, microplastics in these regions were higher than microplastics in the deep fish in the South China Sea (stomachs 1.96 ± 1.12 and intestines 1.77 ± 0.73 items/individual) [31] the Beibu Gulf, South China Sea (0.228 ± 0.080 items/individual) [32] and the Nanxun Reef in Nansha Islands (3.1 items/individual) [33]. The results also showed that the Pearl River Catchment had the highest levels of microplastic pollution among fish in all of the Guangdong and the South China Sea. The Pearl River Delta region of Guangdong has a large urban population, as well as highly developed production and consumption fisheries. Therefore, this region poses a potential safety hazard for microplastic pollution in commercially sold fish.
The microplastics found in this experiment were primarily white and blue fibrous particles of <1 mm. Other microplastics found in fish studies have reported similar results [27,28]. Fiber microplastics in fish gills may be related to the anatomical structure of the fish gill. The gills of fish can collect more fibers due to their comb-like structure. In the color category, transparent microplastics were classified as white because transparent microplastics may scatter blue light under the light microscope. This phenomenon might be the reason why blue and white microplastics accounted for a larger abundance in the data. The proportion of colors other than white, such as blue and black was very low. For example, the report by Lin Zhu et al. was consistent with the results of our study [31]. Because fiber microplastics may mimic target food of plankton for fish in the ocean, this may account for the high proportion of fiber microplastics found in the fish GIT. The majority of the microplastics were found to be <1 mm. In a related study, Isobe et al. reported that due to factors such as mechanical action, photochlorination, and biodegradation, large pieces of plastic debris become smaller and lighter, such that the number of small pieces of plastic debris increases exponentially [34]. In the present study, the amount of microplastics in fish increased in the decreasing size of the microplastic particles, which consistently conformed to this trend.

In studies of microplastic pollution in the wild fish, microplastics are found more in the gastrointestinal tract, gills, and skin, with less abundance in other organs such as muscle and liver [35]. In this study, a total of 351 microplastics were found in the GIT and 177 microplastics were found in the gills. In different coastal areas in western Guangdong, the content of microplastics in the GIT of fish was twice as high as the amount found in the gills (Supplementary Figure S2). In two reports on 2020, Park et al. and Koongolla et al., the situation was similar to the present study in that the amount of microplastics in the GIT was found to be higher than in the gills [32,36]. In a study of fish microplastics in Haizhou Bay, China, the microplastics in the gut were lower than in the gills and even lower than the skin [37]. This result is different from the results of our study. Due to the biological activities of fish, the gills of demersal fish may accumulate more microplastics in the bottom mud. The presence of many microplastics in the GIT of fish is justified by the intentionally or accidental intake of microplastics in water or sediment, or by the predation of prey that are polluted with microplastics with low nutrient levels [38]. Therefore, microplastic pollution in the gills is primarily affected by the habitat of the fish, while the microplastic pollution in the GIT may be more affected by feeding habit. In the present study, the finding that microplastics were in higher abundance in the GIT than in the gills requires further study. It is suggested that the GIT, gills, and skin of fish can be used as the research target organs for the accumulation of microplastic pollution in the study of wild fish.

Fish play a very important role in the biological food chain. If people directly use contaminated commercial fish, microplastics are likely to accumulate in the human body, thus affecting people’s health. Therefore, it is very important to select a typical fish as a surveillance organism for fish microplastic pollution. In this study, *Siganus fuscescens* had the highest abundance of microplastics in the gills and GITs, so we considered it as a candidate for microplastics contamination monitoring in commercial fish. At present, microplastic pollution in commercial fish food has not received enough attention. This study provides survey data of potential food safety hazards caused by microplastic pollution in commercial fish, which can provide data support for the government in formulating policies to deal with microplastic pollution in commercial fish in the future.

4.2. Microplastics in the Different Feeding Habits and Different Habitats of Fish

Pelagic fish gills are vulnerable to floating and microplastic contaminated plankton. Demersal fish gills are vulnerable to sediment. There is less microplastic in the midwater, which can suspend to the surface, settle to the bottom of the ocean or attach to reefs. This may be the reason why there were more microplastics in the gills of demersal fish and pelagic fish than midwater fish.
The dietary habits of fish were classified into carnivorous, omnivorous, and herbivorous, and their nutritional grades were successively reduced. In terms of nutrition, herbivore and omnivore fish need to ingest more food, so the risk of microplastic exposure is also increased. The minimum value of omnivorous fish species was also higher than the maximum value of carnivorous fish species. Herbivores typically have more than twice as much microplastic pollution as carnivores. These results are consistent with previous studies [39]. The accumulation of microplastics in carnivorous fish was less than that in herbivorous fish and omnivorous fish, which may be due to the fact that the accumulation of microplastics in herbivorous fish and omnivorous fish did not reach the average value in the environment, and the microplastics in the prey fish were less likely to accumulate in the gastrointestinal tract than the microplastics on the plant surface.

5. Conclusions

The levels of microplastic contamination were analyzed in gills and in the GIT of wild commercial marine fish from the central and western coast of the Guangdong province. A total of eighty wild marine fish was divided into eight species and microplastics were found in seventy-seven specimens (with an average 6.6 items/individual). The quantity of microplastics in gills and in the GIT was 0–23 (average 2.2) and 0–21 (average 4.4), respectively. In this study, *Siganus fuscescens* had the highest abundance of microplastics in the gills and in the GIT. The particles found were predominantly white and blue fiber microplastics that were <1 mm. The effects of different feeding habits and different zonal habitats on uptake of microplastics were also discussed. In the GIT of fish with different feeding habits, microplastics were predominantly found in herbivores and omnivores compared to carnivorous fish. Midwater dwelling fish showed fewer microplastics than fish in zones. The data in this study can provide a reference to the study of microplastic pollution in coastal fish in central and western Guangdong Province, and can also provide data for governmental agencies to formulate microplastic pollution control policies.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jmse9090981/s1. Figure S1. Fish samples: (A) Clupanodon punctatus; (B) Clupanodon thrissa; (C) Siganus fuscescens; (D) Leiognathus brevirostris; (E) Alepes djedaba; (F) Gerres lucidus; (G) Sillago sihama; (H) Terapon jarbua. Information about the sampling sites. Figure S2. Abundance of microplastic in W1–W8 (the coastal area of the Pan-Pearl River Delta), W9–W10 (Yangjiang, Maoming and Zhanjiang east coastal) and W20–W30 (the eastern coast of Beibu Gulf) in fish samples from the coastal areas of central and western Guangdong province. Figure S3. Proportion of microplastic shape (a), color (b) and size (c) in W1–W8 (the coastal area of the Pan-Pearl River Delta), W9–W19 (Yangjiang, Maoming and Zhanjiang east coastal) and W20–W30 (the eastern coast of Beibu Gulf) in fish samples from the coastal areas of central and western Guangdong province. Table S2. Information about the sampling fishes.

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