



The accumulation of microplastics in fish from an important fish farm and mariculture area, Haizhou Bay, China



Zhihua Feng^{a,c}, Tao Zhang^{a,c}, You Li^{a,c}, Xinran He^d, Rui Wang^{a,c}, Juntian Xu^{a,c}, Guang Gao^{a,b,*}

^a Jiangsu Key Laboratory of Marine Bioresources and Environment, Jiangsu Ocean University, Lianyungang 222005, China

^b State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361005, China

^c Jiangsu Key Laboratory of Marine Biotechnology, Jiangsu Ocean University, Lianyungang 222005, China

^d Lianyungang Environmental Monitoring Center of Jiangsu Province, Lianyungang 222001, China

HIGHLIGHTS

- All fish in the Haizhou Bay had high microplastic (MP) abundance.
- The total number of MPs in skin or in gills was higher than in gut.
- The abundance of MPs exponentially increased with the decrease of MPs size.
- Scaleless fish had higher MPs abundance in skin than scaly fish.

GRAPHICAL ABSTRACT



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ABSTRACT

Marine fisheries and aquaculture can match growing demand for marine protein from an increasing population. However, the microplastics (MPs) in marine environments may pose a threat to human health through food chains by seafood consumption. The MPs have been found lodged in the digestive tracts and other tissues of various sea animals, nevertheless, little is known in regard to the accumulation of MPs in fish from major fish farms and mariculture areas, especially in non-digestive tissues of fishes. This study investigated the accumulation of MPs in six major wild fish species (including *Thryssa kammalensis*, *Amblychaeturichthys hexanema*, *Odontamblyopus rubicundus*, *Cynoglossus semilaevis*, *Chaeturichthys stigmatias* and *Collichthys lucidus*), both in digestive and non-digestive tissues, from an important fish farm and mariculture area, Haizhou Bay, China. All fishes had items that were identified as MPs. The highest abundance of MPs was 22.21 ± 1.70 items/individual or 11.19 ± 1.28 items/g in *T. kammalensis*, which is filter-feeding and usually inhabits in estuary. The lowest abundance of MPs was observed in *C. semilaevis* (13.54 ± 2.09 items/individual) and *C. stigmatias* (1.61 ± 0.56 items/g). The abundance of MPs exponentially increased with the decrease of MPs size. The MPs were dominated by fiber in shape, black or grey in colour and cellophane in composition. As to different tissues, the total number of MPs on skin (800) or in gills (746) was higher than that in gut (514). In terms of skin, the abundances of MPs in three species of scaleless fish with mucus (*A. hexanema*, *C. stigmatias* and *O. rubicundus*) were generally higher than other three fishes with scales (*C. lucidus*, *C. semilaevis* and *T. kammalensis*), implying the potential high risk of scaleless fish consumption for human health in Haizhou Bay. More in-depth studies need to focus on the scaleless fish through mucus adsorbing enormous MPs.

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* Corresponding author at: Jiangsu Key Laboratory of Marine Bioresources and Environment, Jiangsu Ocean University, Lianyungang 222005, China.
E-mail address: gaog@hhit.edu.cn (G. Gao).

1. Introduction

Since 1950s, plastics have been more and more widely used in industrial production and daily life (Barnes et al., 2009). It has been reported that the plastic materials account for 60–80% of all marine debris (Gregory and Ryan, 1997). Microplastics (MPs) were first described by Thompson et al. (2004) defined as any pieces of plastics in size < 5 mm and even smaller particles in the environment. The abundance of MPs have increased rapidly over the past decades in different environmental media including the water body, sediment, coast and table salts (Lusher et al., 2013; Yang et al., 2015; Zhao et al., 2015; Peng et al., 2017, 2018; Hu et al., 2018). Due to the divers densities of polymer (positively, neutrally or negatively buoyant), MPs are ubiquitous in seawaters and available for marine organisms in different depths (Lusher et al., 2013). Additionally, with the diversity of colours, sizes and shapes, some plastic particles may be similar to natural foods sources resulting in accidental ingestion finally (Moore, 2008). There is growing concern about the occurrence of MPs in fish in marine environments because of the importance of fish in food web and food supply for humans (Boerger et al., 2010; Lusher et al., 2013; Jabeen et al., 2017; Qu et al., 2018). Previous studies had shown that the uptake of MPs in fish is location-specific (Boerger et al., 2010; Bellas et al., 2016; Murphy et al., 2017; Baalkhuyur et al., 2018).

As the most populous country in the world, China is the largest plastic producer and consumer in the world (Plastics Europe, 2016). In recent studies, the Chinese coast is considered to be a hot spot for MPs pollution (Zhao et al., 2014; Yu et al., 2016; Jabeen et al., 2017; Li et al., 2016; Li et al., 2018; Zhu et al., 2018). The average abundance of MPs ingested by 21 species of sea fish from Yangtze estuary, East China Sea and South China Sea was from 1.1 to 7.2 items by individual and 0.2 to 17.2 items by gram (Jabeen et al., 2017). In North Yellow Sea, Zhu et al. (2018) found that the abundance of MPs was 545 ± 282 items/m³ in surface seawater and 37.1 ± 42.7 items/kg dry weight in sediments, and polyethylene (PE) and polypropylene (PP) were the dominant type of MPs. Su et al. (2019) found that MPs varied from 0.3 to 5.3 items/individual and from 0.3 to 2.6 items/individual in gut and gill, respectively, of 13 species of fishes from coastal/estuary areas of China. However, little is known about the current MP status for wild sea fish in major fish farms in China. Previous studies on MPs in fish mainly focus on digestive tissues (Foekema et al., 2013; Ory et al., 2017; Karthik et al., 2018), while some non-digestive tissues, such as skin and gills, may also accumulate MPs but are not completely removed when cooking.

The Haizhou bay, a typical semi-enclosed bay, situated at the western margin of the South Yellow Sea with an area of 820 km², is one of the most important fish farms and aquaculture areas in China. It is also the largest cultivation area for *Porphyra yezoensis* (nori), accounting for about 50% of production in China due to suitable environmental conditions (Lu et al., 2018; Gao et al., 2019). Intensive human activities and the extensive use of plastic farming equipment in this area may affect the abundance of MPs in the marine environment, leading to the accumulation of MPs in living organisms. Based on previous studies (Jabeen et al., 2017; Kolandhasamy et al., 2018; Su et al., 2019), we hypothesize that non-digestive tissues in wild fish in the Haizhou Bay can accumulate MPs, particularly for those scaleless species with visible massive mucus. In this study, a survey on MPs in wild fish was conducted in the Haizhou Bay, investigated the distribution and characteristics of MPs in the gut, skin and gills of different species of fish, focusing on the verification of the above hypothesis.

2. Materials and methods

The methodology in this study follows the latest recommendations for microplastics study (Hermsen et al., 2018; Dehaut et al., 2019).

2.1. Sample collection and preparation

The fish samples were caught at four locations of A (119°15.85' E, 34°57.72' N), B (119°23.06' E, 34°59.47' N), C (119°23.68' E, 34°52.68' N), D (119°18.73' E, 34°50.25' N) in the Haizhou Bay in April 2018 using a new polypropylene Agassiz trawl (2.2 m wide, 0.65 m high, and 4 m long; mesh size 20 mm) (see Fig. 1). The trawling of each site was performed at a speed of approximately 2 knots and lasted for 40 min. The sampling depth was 10.8–13.4 m at four locations. A total number of 124 dominant fishes were collected, which could be divided into six different species including *A. hexanema* (pinkgray goby), *C. stigmatias* (finespot goby), *O. rubicundus* (red eel goby), *C. lucidus* (light maigre), *C. semilaevis* (tonguefish) and *T. kammalensis* (rednose anchovy). Following sampling, the fishes were quickly sealed with a foil bag and transferred in a cooler (−5 °C) to the laboratory where they were stored at −20 °C pending processing and analysis. All fish samples were stored for no more than one week before dissection and digestion.

2.2. Quality assurance and control

The newly purchased drag net was used to minimize the contamination from the net. The caught fish was quickly loaded into the aluminum foil bag to reduce the atmospheric contamination. Because MPs are ubiquitous in indoor environments (Gasperi et al., 2018), suitable preventive measures must be taken to avoid plastic and fiber contamination in the laboratory. Firstly, the operator number in the laboratory and air circulation to the outdoors were minimized. All chemical reagents were filtered with 8 μm glass microfiber before use. Operators were equipped with white cotton lab coats, disposable latex gloves and face masks throughout the sample manipulation and processing and their hands and forearms were scrubbed three times before operation. And all instruments and equipment were first thoroughly washed by deionized water, and then cleaned three times with alcohol (75%). Solution preparation and biological dissection were always conducted in a laminar flow cabinet (SW-CJ-2F, SUJING, China). Five procedural blanks (without tissues and filtered water) were made during sample processing to determine the extent of microplastic contamination under laboratory conditions. Positive controls were not conducted because the method for digestion and identification has been proven successful with a very high recovery rate (Karami et al., 2017). All the same, it would be better to have both negative and positive controls in future MPs study.

2.3. Sampling dissection and digestion

After defrosting, the skin of the fish was washed with distilled water to remove large matters, such as seaweeds and sediments, while MPs in the skin were difficult to remove, and the body length of each individual was measured using a vernier caliper. The wet weight of fish was determined by a precision electronic balance (BS124S, Sartorius electronic balance, Beijing). Each individual was dissected in a metal tray using a scalpel, forceps and scissors and the gut, skin and gills were separated. During the anatomy, the skin was first peeled off for MPs identification to prevent MPs contamination from the gut and gills. The separated tissues were immediately placed into clean beakers, and covered with aluminum foil to minimize the risk of contamination. Tissue samples (~5–15 g) were digested by 100–200 ml of 10% KOH in oscillation incubators (DKZ-3, Shanghai Yiheng, China) at 40 °C with 60 rpm for 48 h according to the previous study (Foekema et al., 2013; Karami et al., 2017; Hermsen et al., 2018; Dehaut et al., 2019). Then, the digestion solution was transferred and filtered through glass microfiber (8 μm pore size, 47 mm, Haining Jinzheng, China) using a filtration unit with one Büchner funnel (AP-01P, Autoscience, Tianjin), with the residue kept in the beaker. Saturated NaCl solution was added to the residue for density separation. After 48 h of sedimentation, clean supernatant was

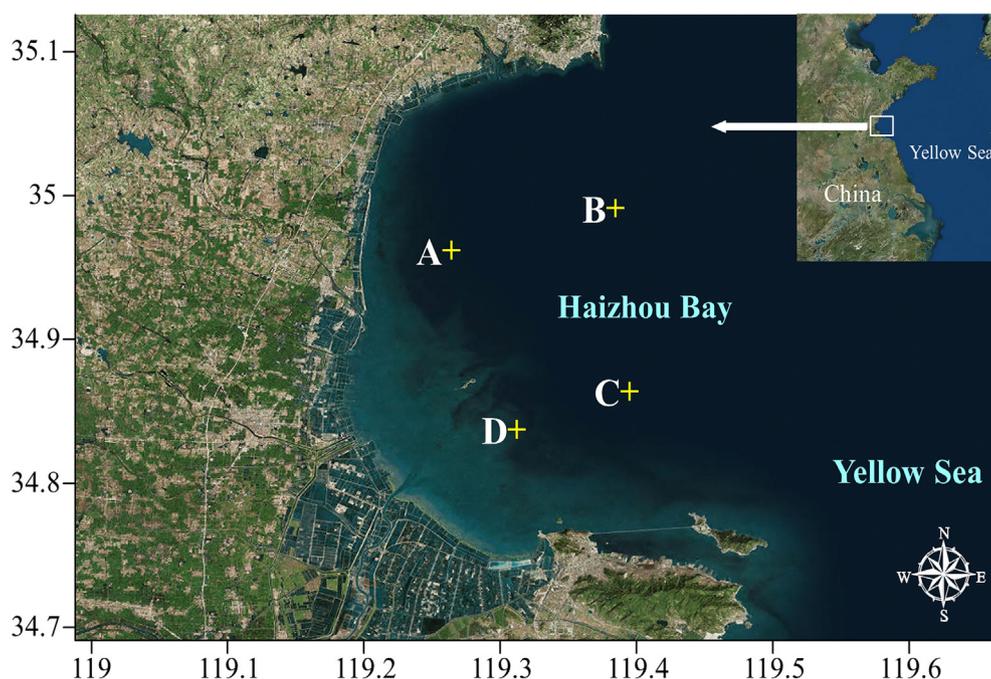


Fig. 1. Geographic position of four sampling sites (A, B, C and D) in the Haizhou Bay.

filtered as described above. Density separation was repeated three times to improve recovery of MPs. And then, the filter membranes were placed in clean Petri dishes with lids and dried at room temperature for further study.

2.4. Observation and identification of MPs

Nikon SMZ 1500N stereomicroscope (Nikon, Japan) with charge-coupled device (CCD) camera was used for isolating, photographing and measuring resembling MPs at largest cross section and categorizing according to their size, shape and colour. All potential mesoplastics and MPs were selected at middle area from the randomly selected filters and thus 200 potential MPs were identified by a FT-IR microscope (Thermo Nicolet iN10 MX, Thermofisher, America). The OMNIC software was used for the identification of polymer by compared with libraries of standard spectra. Polymers matching with reference spectra for >70% were validated (Thompson et al., 2004). As previous studies, this study followed the same procedure of Cai et al. (2019) or Jabeen et al. (2017) method, in which unknown particles was not conducted.

2.5. Data analysis

The data were analyzed using the software SPSS v.23. The data under every treatment conformed to a normal distribution (Shapiro-Wilk, $P > 0.05$) and the variances could be considered equal (Levene's test, $P > 0.05$). One-way analysis of variance (ANOVA) was conducted to assess difference of MPs abundance among different fish species. Two-way ANOVA was conducted to assess difference of MPs abundance among different fish species and organs. Least significant difference was conducted for ANOVA post hoc investigation. A confidence interval of 95% was set for all tests.

3. Results

3.1. Characteristic of fish

Due to overfishing, the abundance of fish is low in the Haizhou Bay. The number of fish in this study is what we were able to catch during sampling times. Among all the 124 fishes caught, 6 different species,

A. hexanema, *C. stigmatias*, *O. rubicundus*, *C. lucidus*, *C. semilaevis* and *T. kammalensis* were found (Table 1). Among them, *A. hexanema*, *C. stigmatias*, and *O. rubicundus* are scaleless and can secrete massive visible mucus on skin, while the other three are scaly. Only *T. kammalensis* is filter-feeding and the other five species of fish are all predatory. The body length of the total fish was from 6.2 to 29.1 cm, and the weight ranged from 4.7 to 261.4 g. The size of scaleless fish is generally bigger than scaly fish. The adult sizes for these six species of fish (*A. hexanema*, *C. stigmatias*, *O. rubicundus*, *C. lucidus*, *C. semilaevis* and *T. kammalensis*) are 6.7–15.5, 8.4–16.4, 11.1–23.4, 7.5–15.5, 13.2–50.0, and 5.5–9.0 cm, respectively (Ni and Wu, 2006). Therefore, all fish in this study are adult and could be considered to reach commercial size.

3.2. Abundance of microplastics in fish

The total number of suspected items was 2121; all mesoplastics were identified as plastic polymers, and 148 of the 200 potential MPs tested were identified as plastics. Excluding non-plastic items after recalculation, a total number of 2069 mesoplastics and MPs were left, and >99% of them are MPs (<5 mm). MPs were found in all six fish species while mesoplastics (9 items in total) were only found in the predatory fish (Table 1). Compared to the MPs in fish (~150–200 items/batch and 8–12 fish/batch), the MPs for the blank (0.57 ± 0.17 items/batch) is negligible. Therefore, the blank was not taken into account when calculating MPs in fish. MPs abundance in six species of fish was shown in Fig. 2. One-way ANOVA showed that MPs abundance in different fish species was significantly different for both items/individual ($F = 4.616$, $df = 5$, 118 , $P = 0.001$) and items/g ($F = 32.926$, $df = 5$, 118 , $P < 0.001$). The highest abundance of MPs was found in *T. kammalensis* which is 22.21 ± 1.70 items/individual and 11.19 ± 1.28 items/g. The lowest abundance of MPs was observed in *C. semilaevis* with 13.54 ± 2.09 items/individual and *C. stigmatias* with 1.61 ± 0.56 items/g.

The MPs distribution in different tissues of fish was also investigated (Table 1 and Fig. 3). The total numbers of 800 MPs on skin and 746 MPs in gills were found, higher than the number of 514 MPs in gut. Two-way ANOVA showed that both species ($F = 8.429$, $df = 5$, $P < 0.001$) and tissue ($F = 21.015$, $df = 2$, $P < 0.001$) affected the MPs abundance and these two factors had an interactive effect ($F = 9.632$, $df = 10$, $P < 0.001$). Specifically, the highest MPs abundance was detected on skin

Table 1
A summary of fish traits and plastics distribution in fish. The values in brackets are the ranges of length and weight for different fish species.

Fish species	<i>A. hexanema</i> (Bleeker, 1853)	<i>C. stigmatias</i> (Richardson, 1844)	<i>O. rubicundus</i> (Hamilton, 1822)	<i>C. lucidus</i> (Richardson, 1844)	<i>C. semilaevis</i> (Gunther, 1873)	<i>T. kammalensis</i> (Bleeker, 1849)	Total
Feeding behavior	Predatory	Predatory	Predatory	Predatory	Predatory	Filter-feeding	
Fish abundance	23	16	23	17	26	19	124
Length (cm)	20.3 (14.6–25.2)	19.7 (13.8–29.1)	19.2 (14.3–21.8)	9.1 (7.9–14.8)	15.6 (13.4–18.4)	7.4 (6.2–8.3)	6.2–29.1
Weight (g)	114.9 (20.2–261.4)	72.0 (35.3–99.9)	72.2 (23.3–149.7)	35.7 (13.8–55.4)	34.8 (15.6–77.6)	9.2 (4.7–13.2)	4.7–261.4
Skin type	Scaleless	Scaleless	Scaleless	Scaly	Scaly	Scaly	
Microplastics number	410	238	399	239	352	422	2060
Mesoplastics number	2	1	3	1	2	0	9
MPs number in gut	69	55	84	69	117	120	514
MPs number in gills	127	70	124	97	125	203	746
MPs number on skin	214	113	191	73	110	99	800

for the three scaleless fishes and significant difference among tissues was found for *A. hexanema* ($F = 85.574$, $df = 2$, $P < 0.001$), *C. stigmatias* ($F = 6.559$, $df = 2$, $P = 0.003$), and *O. rubicundus* ($F = 7.810$, $df = 2$, $P = 0.001$); for scaly fishes, the highest MPs abundance occurred all in gills, though significant difference among tissues was only found in *T. kammalensis* ($F = 39.911$, $df = 2$, $P < 0.001$), it was not statistically significant for *C. lucidus* ($F = 0.801$, $df = 2$, $P = 0.455$) and *C. semilaevis* ($F = 0.463$, $df = 2$, $P = 0.631$).

3.3. Morphotype, colour, size and material of microplastics in fish

Three morphotypes of microplastics were observed in the fish samples, including fiber, fragment and sheet (Fig. 4a). Fiber was the most abundant morphotype (97.9%), followed by fragment (1.3%) and sheet (0.8%) as shown in Fig. 4b. The colour of MPs can be classified into five categories (Fig. 4c). Black-grey (49.2%) and white-transparent (35.9%) were the dominating colours for MPs in fish, followed by blue-green (10.0%) and red-purple contributed (4.3%). Yellow-orange MPs accounted for the lowest abundance of about 0.6%.

In terms of MPs size, the distribution characteristics of the total were shown in Fig. 5. MPs with the size from 0 to 1000 µm contributed 68.4%

of the total MPs. The abundance of MPs exponentially decreased with the increase of size ($y = 1308e^{-0.526x}$, $R^2 = 0.9822$), with the proportion of MPs from 2501 to 5000 µm only 5.7%.

The size distribution of MPs in different tissues and species was also shown in Fig. 6. It ranged from 27 to 4932 µm, with the shortest one found in the gills of *C. stigmatias* and the longest one found in the skin of *A. hexanema*. For the total six species, the average MPs size on skin, in gut and in gills was 973 ± 803 µm, 906 ± 864 µm and 871 ± 784 µm respectively. Two-way ANOVA showed that the species ($F = 4.165$, $df = 5$, $P = 0.001$) affected the size of MPs significantly, while the tissues ($F = 2.887$, $df = 2$, $P = 0.056$) didn't. Post-hoc LSD test indicated that the average MPs sizes of *O. rubicundus* and *C. lucidus* were lower than other four species.

In addition to morphotype, colour and size, material of MPs in the fish samples was also identified (Fig. 7). Among the 200 potential MPs tested, cellophane (CP) was the major material, which accounted for 33.5%, followed by polypropylene (PP, 15.0%), polyethylene (PE, 13.0%), nylon (8.0%) and polyester (PET, 4.5%). Nonplastic particles (such as cotton, etc.) and unidentified particles accounted for 6.5% and 19.5% respectively.

4. Discussion

4.1. Microplastic pollution level in fishes of the Haizhou Bay

In this study, the abundance of plastics accumulated by 6 species of major fish in the Haizhou Bay was investigated. The result showed that all species, irrespectively of habitat and feeding behavior, were found to ingest MPs, indicating the ubiquitous distribution of MPs in the Haizhou Bay. The abundance of MPs ranged from 13.54 ± 2.09 items/individual in *C. semilaevis* to 22.21 ± 1.70 items/individual in *T. kammalensis*. Considering specific tissues, the abundance of MPs in

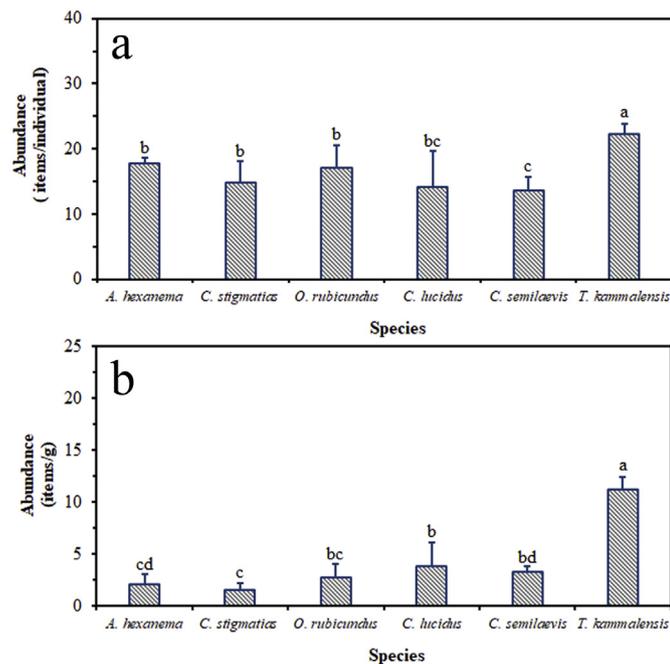


Fig. 2. Abundance of microplastics (mean ± 2SE) in the fish from the Haizhou Bay normalized to per individual (a) and per gram total fresh weight of the three tissues (gut, gills and skin) (b). Different letters above the bars indicate significant differences among tissues (One-way ANOVA, $P < 0.05$).

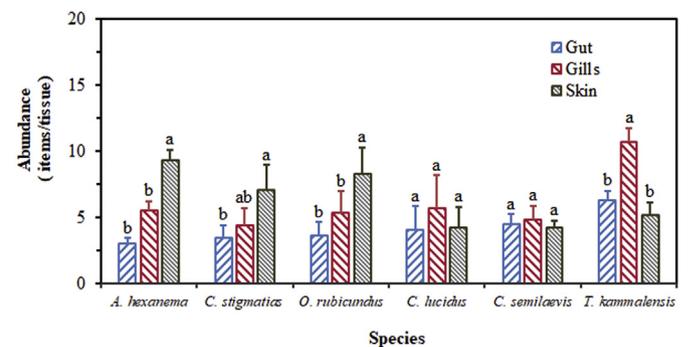


Fig. 3. Abundance of microplastics (mean ± 2SE) in different tissues (gut, gills and skin) of fish from the Haizhou Bay. Different letters above the bars indicate significant differences among tissues (One-way ANOVA, $P < 0.05$).

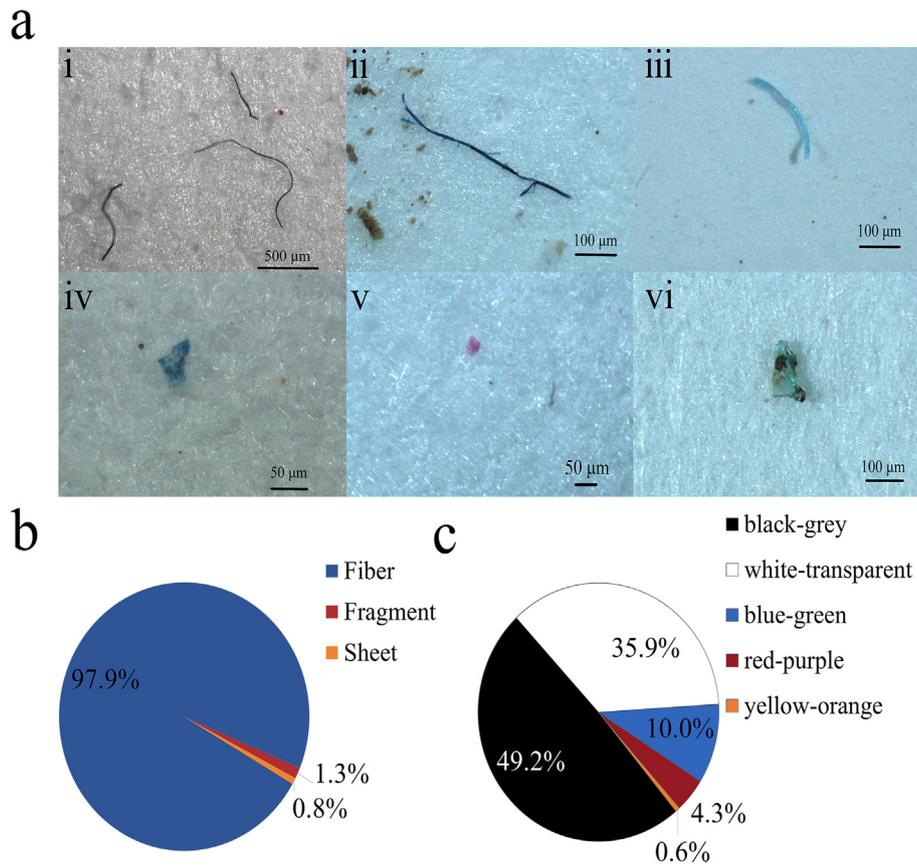


Fig. 4. Micrographs of several typical microplastics (a) and shapes (b), colours (c) distribution of the microplastics observed in the fish samples. (i) Black fiber, (ii) dark blue fiber, (iii) light blue fiber, (iv) blue fragment, (v) purple fragment, (vi) green sheet. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

gut, gills and skin ranged from 3.00 to 6.32 items/individual, 4.38 to 10.68 items/individual and 4.23 to 9.30 items/individual respectively.

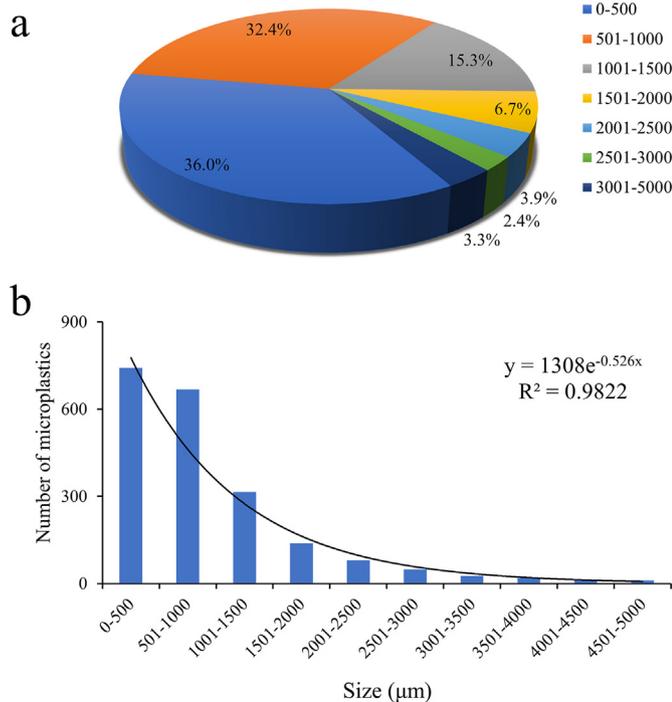


Fig. 5. Size distribution of the microplastics observed in the total fish samples.

The average MPs abundance in specific tissue gradually increased in the order: gut (4.16 ± 0.44 items/individual) < gills (6.08 ± 0.67 items/individual) < skin (6.40 ± 0.65 items/individual).

In comparison with the data normalized by biomass and counted as tissue-summing, the MPs abundance of one specific tissue standardized by individual is more reasonable to be selected for the evaluation of MPs pollution level in fish among different studies. The MPs abundances in main specific tissues of fish reported in previous and this study were summarized in Table 2. Jabeen et al. (2017) investigated MPs in 21 species of sea fish from Yangtze estuary, East China Sea and South China Sea and the tissues of stomach and intestine were studied. It was found that the average abundance of MPs varied from 0.72 to 3.30 items/individual in stomach and 0.93 to 2.70 items/individual in intestine. Abbasi et al. (2018) reported the MPs abundance in gut, gills and skin of fish from the Musa Estuary, Persian Gulf. The range is 1.53–4.00, 2.00–7.00, 1.50–6.83 items/individual and the average is 2.63 ± 1.04 , 3.81 ± 2.19 , 3.88 ± 2.06 items/individual respectively. MPs were even found in liver (Collard et al., 2017) and muscle (Abbasi et al., 2018). Furthermore, Zhang et al. (2019) and Su et al. (2019) also studied the MPs abundance in gut and gills of fish from the East China Sea. As is shown in Table 2, the values in this study at Haizhou Bay were higher than previous studies reported by specific tissue for fish. The results in this study showed that the pollution of MPs in major fish of the Haizhou Bay may be more serious than other areas in China and other countries. As the largest cultivation area for *P. yezoensis* and one of the most important fish farms aquaculture areas in China, the serious MPs pollution in the Haizhou Bay would threaten human health via the food chain.

It is well known that rivers are the main sources that can transport microplastics to adjacent oceans (Simon-Sánchez et al., 2019; Van Wijnen et al., 2019). The previous study reported that Linhong River,

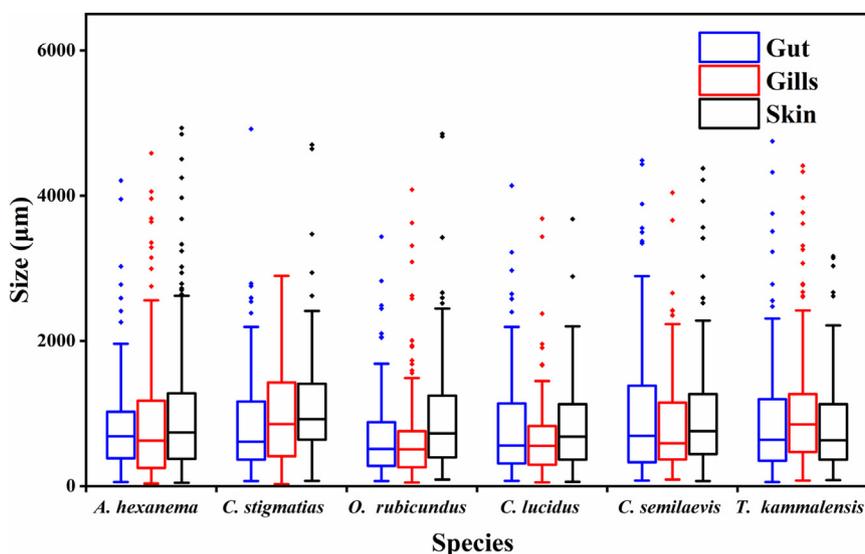


Fig. 6. Size distribution of the microplastics in different tissues (gut, gills and skin) of fish from the Haizhou Bay.

as one of the largest land rivers along the Haizhou Bay, carried 2.26×10^8 t of domestic sewage and industrial wastewater in 2010 and a considerable portion of plastics could be transferred to the Haizhou Bay (EPAL, 2011). The Haizhou Bay is a natural deep-water harbor with annual load and unload cargoes of about 210 million t (Zeng, 2017). The intense human activities should also contribute to the severe MPs pollution in the Haizhou Bay.

4.2. Characterization of plastics

Of the plastics detected, fibers were the most common morphotype in the fish from the Haizhou Bay, which was consistent with previous studies on MPs in fish and shellfish (Lusher et al., 2013; Jabeen et al., 2017; Murphy et al., 2017; Li et al., 2018; Qu et al., 2018). In this study, the materials composition was dominated by cellophane (CP) which was similar with MPs in fish, shellfish and sea cucumber in the seas of China (Li et al., 2016; Jabeen et al., 2017; Mohsen et al., 2019; Teng et al., 2019). Cellophane is a form of regenerated cellulose manufactured from wood pulp by the viscose process (Brasier, 1986). In recent years, cellophane had been extensively used as a packaging

material for food, pharmaceuticals and goods as a replacement for petrochemical plastics (Benyathiar et al., 2015). Unlike traditional petroleum-based plastics, semi-synthetic cellulose (such as cellophane and rayon) is also identified as plastic recently and has non-degradable properties (Li et al., 2016; Li et al., 2018). High proportions of PP, PE and Nylon were also detected in fish of this study. These MPs may originate from aquaculture industry, such as ropes, fishing nets as the Haizhou Bay is a major aquaculture area in China (Park et al., 2004; Andrady, 2011). The decomposed fishing gears in the marine environment can be easily ingested by marine organisms (Cheng et al., 2018). Meanwhile, polyesters are considered as from the main composition of clothes and thereby polyesters can come from domestic wastewater (Wang et al., 2019b). In terms of MPs size, $<1000 \mu\text{m}$ MPs accounted for more than two thirds of the total MPs in the fish samples. This should be related to the size distribution of MPs in seawater as Sun et al. (2018) has found that smaller MPs are the dominating type in coastal seawaters of the Yellow Sea. Compared to larger MPs, smaller MPs with same mass can carry more toxic matters and is more difficult to remove as well (Boris et al., 2015; Wang et al., 2018; Wang et al., 2019a; Zhu et al., 2019a, 2019b).

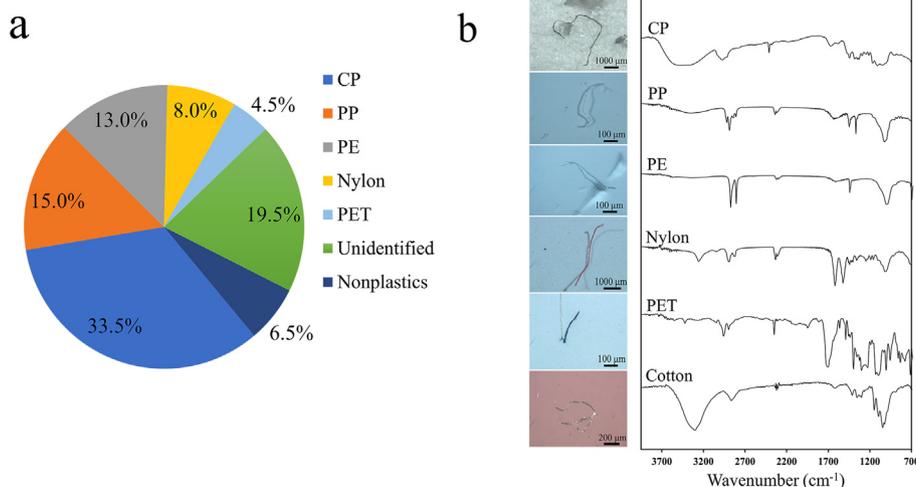


Fig. 7. Type distribution (a) and material spectra (b) identified by FT-IR microscope of the microplastics observed in the samples. CP, cellophane; PP, polypropylene; PE, polyethylene; PET, polyester.

Table 2

A summary of MPs abundance found in main specific tissues of fish in previous and present studies. Range and average of MPs abundance in different species of fish are given. GIT refers to gastrointestinal tract.

Area	Type of fish	Total species	Total individuals	Type of tissue	MPs abundance in gut (items/ind.)	MPs abundance in gills (items/ind.)	MPs abundance in skin (items/ind.)	References
Yangtze Estuary, East China Sea, South China Sea	Commercial	11	198	Stomach, intestine	Stomach: 0.72–3.30 Intestine: 0.93–2.70	/	/	Jabeen et al. (2017)
Musa Estuary, Persian Gulf	Wild caught	5	56	Skin, gut, gills, muscle, liver	1.53–4.00 2.63 ± 1.04	2.00–7.00 3.81 ± 2.19	1.50–6.83 3.88 ± 2.06	Abbasi et al. (2018)
Zhoushan fishery, East China Sea	Wild caught	11	193	GIT, gills	0–1.20 0.52 ± 0.90	0–1.93 0.77 ± 1.25	/	Zhang et al. (2019)
Hangzhou Bay, Yangtze Estuary	Wild caught	13	185	Gut, gills, muscle, liver	0.30–5.30 1.71 ± 1.50	0.30–2.60 1.12 ± 0.61	/	Su et al. (2019)
Haizhou Bay, Yellow Sea, China	Wild caught	6	124	Skin, gut, gills	3.00–6.32 4.16 ± 0.44	4.38–10.68 6.08 ± 0.67	4.23–9.30 6.40 ± 0.65	This study

4.3. Microplastics in different species and tissues

It is apparent that the abundance of MPs in fish is very variable both between species and within the same species. This reflects the changes in the quantity and type of food consumed by individuals of between different species or within the same species (Abbasi et al., 2018). The differences in MPs abundance in fish stem from the general assumption that the accumulation of plastics by fish and other organisms is mainly through ingestion and therefore depends on factors such as feeding strategy, intestinal structure and local plastics contamination (Romeo et al., 2015; Jabeen et al., 2017). High density plastic particles could be ingested by fish through predation in the previous reports (Brandao et al., 2011; Wright et al., 2013), and this phenomenon is very common in main commercial fish (Jabeen et al., 2017). In this study, the highest abundance of MPs (both items/individual and items/g) was found in *T. kammalensis*, which is a filter-feeding species. This could be attributed to the effect of habitat because *T. kammalensis* usually inhabits in the estuary where MPs is usually rich (Cheung et al., 2016; Lang et al., 2018; Nel et al., 2018).

Previous studies have suggested that ingestion is the essential way that fish accumulate MPs and thereby digestive tissues are used as an estimate of MPs in fish digestive tissues (Foekema et al., 2013; Neves et al., 2015; Bellas et al., 2016; Ory et al., 2017; Karthik et al., 2018). However, little is known regarding the adherence of MPs in fish. In the present study, the abundances of MPs on skin and gills are close to and even exceed the gut in the fish from the Haizhou Bay, suggesting that adherence is also an essential means for MPs contaminating fish. In particular, the abundance of MPs in gills was much higher than gut for *T. kammalensis*, which may be explained by its filter-feeding behavior. The high MPs abundance in gills also contributed most to the total MPs in *T. kammalensis*, making it the most MPs-contaminated species in the Haizhou Bay.

Furthermore, it was found that skins had highest abundance of MPs in three scaleless species of fish (*A. hexanema*, *C. stigmatias* and *O. rubicundus*) while gills generally had highest abundance of MPs in the other scaly fish (*C. lucidus*, *C. semilaevis* and *T. kammalensis*). Those scaleless species could adsorb MPs with visible massive mucus. These findings verified our hypothesis that non-digestive tissues in wild fish in the Haizhou Bay can accumulate MPs and it seems that scaleless species with visible massive mucus are likely to adsorb more MPs compared to scaly species. Mucus on fish skin is a viscous gel composed mainly of water and polysaccharides, and also contains other compounds in different proportions, such as proteins (Wotton, 2005). Mucus has a wide range of biological functions, such as lubricant, osmoregulative agent, and protective coating against desiccation, UV-radiation and predators (Wotton, 2011). In addition, fish mucus also

contains antibiotics that prevent microbial attack (Subramanian et al., 2008). However, fish mucus also binds potentially harmful metals such as Pb (Coello and Khan, 1996; Tao et al., 2000a), Cu (Tao et al., 2000b) and Al (Berntssen et al., 1997). The present study indicates that fish mucus can also adsorb MPs.

4.4. The risk of microplastics in fish

Ingesting MPs may block or damage the digestive tract, or decrease individual fitness, ultimately leading to death of marine animals (Luís et al., 2015; Lönnstedt and Eklöv, 2016). Apart from the direct effect, MPs are reported to exert their harmful effects by acting as a medium to facilitate the transport of other toxic compounds, such as persistent organic pollutants (POPs) and heavy metals and, to marine animals (Rochman et al., 2013; Brennecke et al., 2016). The higher abundance of MPs, finding in the major wild fish of the Haizhou Bay, may cause the decline of fisheries resources and pose negative effects on the long-term sustainability of the fishery in the major fish farm. The declined fish biomass may also affect the structure and stability of the food web (Sun et al., 2019).

Although there is no food chain among these six fish species, the MPs in these fish can be transferred to the higher trophic levels (such as *Miichthys miiuy*), leading to the accumulation in the food chain. Furthermore, these wild fish are directly consumed by humans. Although gut and gills are usually removed before cooking in some cases, the MPs on skins cannot be completely washed off, particularly for those scaleless fish with visible massive mucus, enhancing the possibility of MPs exposed to the human digestive tract through fish consumption. Although the evidence on the adverse effects on human health due to the consumption of fish containing MPs is very scarce, MPs could affect human health via their direct or indirect toxicity (Barboza et al., 2018). From this perspective, this study suggests that some scaleless fish with visible massive mucus on the skin should be worthy of additional attention both in MPs detection and cooking ways.

5. Conclusion

High abundance of fiber, fragment and sheet of microplastics in six major wild fishes were firstly reported in a major fish farm and mariculture area, Haizhou Bay, China. The study found microplastics adhered on non-digestive tissues (skin and gills) were as much important way as that ingested in digestive tissues of gut. The higher abundance of MPs on skin of fish should be paid more attention due to the potential risks of MPs to human health through fish consumption. This study supplies a new perspective of MPs pollution in fisheries environment, which will be helpful to the regional fisheries management and environmental

biomonitoring. Much more studies based on the MPs accumulation in the scaleless fish and non-digestive tissues in different fishes need to be investigated in the further work.

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.

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