Environmental Pollution 221 (2017) 141–149

Contents lists available at ScienceDirect

Environmental Pollution

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

Microplastics and mesoplastics in fish from coastal and fresh waters of China☆

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ARTICLE INFO

Article history:
Received 5 September 2016
Received in revised form
16 November 2016
Accepted 17 November 2016
Available online 7 December 2016

Keywords:
Microplastics
Mesoplastics
Fish
Marine debris
Ingestion

ABSTRACT

Plastic pollution is a growing global concern. In the present study, we investigated plastic pollution in 21 species of sea fish and 6 species of freshwater fish from China. All of the species were found to ingest micro- or mesoplastics. The average abundance of microplastics varied from 1.1 to 7.2 items by individual and 0.2–17.2 items by gram. The average abundance of mesoplastics varied from 0.2 to 3.0 items by individual and 0.1–3.9 items by gram. Microplastics were abundant in 26 species, accounting for 55.9–92.3% of the total number of plastics items in each species. *Thamnaconus septentrionalis* contained the highest abundance of microplastics (7.2 items/individual). The average abundance of plastics in sea benthopelagic fishes was significantly higher than in freshwater benthopelagic fishes by items/individual. The plastics were dominanted by fiber in shape, transparent in color and cellophane in composition. The proportion of plastics in the stomach to the intestines showed great variation in different species, ranging from 0.5 to 1.9 by items/individual. The stomach of *Harpodon nehereus* and intestines of *Pampus cinereus* contained the highest number of plastics, (3.3) and (2.7), respectively, by items/individual. Our results suggested that plastic pollution was widespread in the investigated fish species and showed higher abundance in comparison with worldwide studies. The ingestion of plastics in fish was closely related to the habitat and gastrointestinal tract structure. We highly recommend that the entire gastrointestinal tract and digestion process be used in future investigations of plastic pollution in fish.

1. Introduction

Plastics comprise the largest part of marine debris and have been reported as important pollutants in marine as well as freshwater environments (Thompson et al., 2004; Cole et al., 2011; Maximenko et al., 2012; Wagner et al., 2014; Dris et al., 2015). The larger plastics gradually degrade into mesoplastics (5–25 mm) and microplastics (<5 mm) (Andrady, 2011; OSPAR, 2014). Microplastics may also come from primary plastics, which are intentionally used as resin pellets or as ingredients of personal care products (Fendall and Sewell, 2009).

After small plastic particles enter the environments, the primary risks associated with them are their suspected bioavailability for marine organisms (Wright et al., 2013; Desforges et al., 2015). The ingestion of plastics has been reported in various groups of organisms such as invertebrates, fishes, seabirds, turtles and mammals (Di Beneditto and Awabdi, 2014; Lavers et al., 2014; Lusher et al., 2015; Nadal et al., 2016; Peters and Bratton, 2016; Welden and Cowie, 2016). Especially, small plastic particles are easily transported through water into ecosystems. Therefore, research regarding plastic pollution has focused on sources, fate and ecological effects of small particles in recent years (Cole et al., 2015; Hall et al., 2015; Rocha-Santos and Duarte, 2015).

Previous field studies have revealed that ingestion of plastic may lead to internal blockages and injury to the digestive tract of fish (Jackson et al., 2000; Cannon et al., 2016; Nadal et al., 2016). It has also been proven in the laboratory that exposure to plastic has negative impacts on fishes (Rochman et al., 2013; Pedà et al., 2016). For example, microplastics at an environmentally relevant concentration can significantly affect the survival of *Perca fluviatilis* during their early developmental stages (Lönnstedt and Eklöv, 2016). *Pomatochistus microps* juveniles show a decrease in predatory performance and efficiency after exposure to microplastics

☆ This paper has been recommended for acceptance by Maria Cristina Fossi.
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http://dx.doi.org/10.1016/j.envpol.2016.11.055
0269-7491/

(de Sa et al., 2015). In addition, the accumulation of chemicals on the surface of plastic material can cause adverse health effects on fish (Derraik, 2002; Boerger et al., 2010).

The ingestion of plastic has been reported in approximately 150 fish species (Supplementary Table 1). The local pollution level of plastics and the feeding strategy of fish are important factors affecting the ingestion of plastics in fish (Romeo et al., 2015; Battaglia et al., 2016). The severity of plastic pollution has been reported in many parts of the world, e.g., North Central Pacific Gyres, North Pacific Subtropical Gyres and the Mediterranean Sea. Several fishes of these areas are affected by plastic pollution (e.g., Boerger et al., 2010; Davison and Asch, 2011; Fossi et al., 2014; Cozar et al., 2014; Romeo et al., 2016). In global studies that target fishes, the highest percentage of plastic pollution has been reported in the sea fish *Boops boops* (68% of the selected samples) and freshwater fishes *Lepomis macrochirus* and *L. megalotis* (45% of the selected samples) (Nadal et al., 2016; Peters and Bratton, 2016). According to Nadal et al. (2016), the highest abundance of plastic reached 3.75 items/individual in Bogue (*Boops boops*). Fibers are the dominant composition pattern in most field studies (Lusher et al., 2013; Rochman et al., 2015; Neves et al., 2015; Nadal et al., 2016).

In previous studies, different methods have been used for the isolation, identification and validation of plastic pollution in fish (Foekema et al., 2013; Neves et al., 2015; Romeo et al., 2015, 2016; Bellas et al., 2016). For example, plastic items were usually extracted from the whole gastrointestinal tract (GIT) of fish (Lusher et al., 2013; Cannon et al., 2016; Nadal et al., 2016; Rummel et al., 2016). However, some researchers also studied the plastic levels in stomachs instead of the GIT. In addition, both microplastics and mesoplastics were considered in some studies, and digestion methods were used (Foekema et al., 2013; Avio et al., 2015; Bellas et al., 2016; Rochman et al., 2015). In other studies, however, no digestion was used, and only meso- or macroplastics were directly observed under a microscope (Sanchez et al., 2014; Romeo et al., 2015; Peters and Bratton, 2016). Obviously, these divergent methods make it difficult to obtain comparable data at international level.

The coast of China was suggested as a hotspot for microplastic pollution in recent studies (Zhao et al., 2014; Yu et al., 2016). High levels of ingested microplastics have been found in nine commercial bivalve species from fishery markets and in wild mussels caught along the coastal waters of China (Li et al., 2015, 2016). Intense anthropogenic activities were linked to higher abundances of microplastics. Additionally microplastic pollution has also been confirmed in estuarine waters and freshwater systems (Zhao et al., 2015; Su et al., 2016). However, very few data are available regarding plastic pollution in fish from coastal or fresh waters of China.

In the present study, micro- and mesoplastic pollution was investigated in sea and fresh water fishes from China. The abundance, morphotype, size and color of plastics were recorded in the whole GIT as well as in the intestines and stomachs of fish. Our aims were to determine the features of plastic pollution in fishes and the differences in the accumulation of plastic between intestines and stomachs.

2. Materials and methods

2.1. Sample collection

From May to December, 2015, fish samples of 21 sea species were purchased from the fishery markets of Shanghai. These fishes were collected from the Yangtze estuary, East China Sea and South China Sea (Supplementary Fig. 1). The fish of 6 freshwater species were purchased from local fishermen, who collected fish in a freshwater lake (Taihu Lake). Approximately 20–40 individual fish were purchased for each species and stored at −20 °C.

Eighteen individuals of approximately equal length were selected for each species. Weight and fork length of each fish were recorded to the nearest 0.1 g and 0.1 cm, respectively (Lusher et al., 2013; Romeo et al., 2015) (Table 1). The GIT was removed by dissecting fish ventrally, and weight (to the nearest 0.1 g) of the GIT was recorded using an electronic weighing balance (BSA224S, Sartorius, China).

For organ specific plastic analysis, 11 species with special GITs were selected. The shape, size, and internal structure of the stomach and intestine were observed and recorded. The stomach and intestine of each individual were removed, and three replicates (6 individuals in each replicate) of stomachs and intestines were used for analysis in each species to reduce the error within groups. Based on the structure of the stomach and intestine in different species, we divided the species into two classes: fish with complex GIT structure and fish with simple GIT structure. In detail, the complex class referred to internally folding or protruding stomachs as well as thin and coiled intestinal structures; the simple class referred to internally smooth wall stomachs as well as wide and uncoiled intestines (Supplementary Fig. 2). We concluded the critical procedure of our method to make it clear (Supplementary Table 2). The aim of this analysis was to determine the difference in the accumulation of plastics between the stomach and intestine.

2.2. Quality control of experiments

All apparatus (e.g., glass wares and dissection tools) were rinsed three times with filtered water to reduce the chances of contamination (Li et al., 2015; Yang et al., 2015; Lusher et al., 2015). Tap water, saline water and hydrogen peroxide were filtered with a 1-μm filter prior to use. Gloves and laboratory coats were worn during the experiments. The samples were immediately covered when not in use. The experimental procedures without any tissues were performed as blank experiments.

2.3. Hydrogen peroxide treatment

The whole GIT was digested to extract plastics. For organ specific accumulation of plastics, the stomach and intestine were put into 1 L clean glass bottles and digested separately. Extraction of plastics was carried out according to the method described by Li et al. (2015, 2016). To increase the efficacy of extraction and characterization of plastic from the tissue, a digestion method was used to treat the GIT. Based on the weight of samples, approximately 200–400 mL of 30% H_2O_2 was added to digest the organic matter. The volume of the liquid did not exceed 50% of the total volume of the bottle. Bottles were covered and placed in an oscillation incubator at 65 °C with 80 rpm for 24–72 h (depending upon the digestion level) for adequate digestion to obtain dissolved solution.

2.4. Saline (NaCl) solution floatation and filtration

A saturated saline solution (1.2 g/mL in density) was prepared and filtered. Approximately 800 mL of filtered NaCl solution was added to the bottle to separate plastics from dissolved solution of the GIT via floatation. The solution was mixed by stirring and kept overnight to observe the clearance level. The solution was filtered through a 5-μm pore size, 47-mm cellulose nitrate filter (Whatman AE98) using a vacuum with a pump. After the filtration process, filters were stored in cleaned petri dishes with lids for microscopic observation of plastic items. This procedure has been followed as described by Li et al. (2015, 2016).

Table 1
Abundance of microplastics and mesoplastics in fishes from China.

Fish Species	Feeding features	Body weight (g)	Fork length (Range) (cm)	Microplastics		Mesoplastics	
				items/g	items/individual	items/g	items/individual
Pelagic (sea water)							
Hyporhamphus intermedius (Cantor, 1842)	planktivore	98.2 ± 13.2	34.6 ± 0.8 (33.0–36.6)	3.4 ± 2.4	3.7 ± 2.2	1.5 ± 1.3	1.8 ± 1.2
Liza haematocheila (Temminck and Schlegel, 1845)	omnivore	88.9 ± 11.4	23.3 ± 0.5 (22.6–24.0)	0.6 ± 0.05	3.3 ± 0.3	0.2 ± 0.01	1.1 ± 0.1
Coilia ectenes (Jorden and Seale, 1905)	planktivore	28.8 ± 3.8	19.4 ± 0.4 (18.5–19.9)	11.5 ± 6.1	4.0 ± 1.8	1.8 ± 2.3	0.7 ± 0.8
Lateolabrax japonicus (Cuvier, 1828)	carnivore	94.0 ± 13.3	21.6 ± 0.9 (20.0–23.5)	0.5 ± 0.1	2.1 ± 0.3	0.1 ± 0.1	0.5 ± 0.2
Sillago sihama (Forsskål, 1775)	carnivore	25.1 ± 2.3	15.2 ± 0.3 (14.8–15.9)	5.7 ± 2.9	2.8 ± 1.5	2.6 ± 2.0	1.2 ± 0.1
Benthopelagic (sea water)							
Larimichthys crocea (Richardson, 1846)	carnivore	154.0 ± 13.5	23.7 ± 0.3 (23.2–24.3)	1.3 ± 1.5	4.6 ± 3.4	0.1 ± 0.2	0.7 ± 0.9
Psenopsis anomala (Temminck and Schlegel, 1844)	carnivore	68.1 ± 13.9	16.9 ± 0.8 (15.5–18.5)	0.5 ± 0.2	1.1 ± 0.3	0.4 ± 0.2	0.8 ± 0.4
Pampus cinereus (Euphrasen, 1788)	carnivore	105.8 ± 15.9	19.3 ± 1.4 (15.8–21.0)	0.5 ± 0.2	3.0 ± 0.8	0.4 ± 0.2	2.2 ± 1.1
Harpodon nehereus (Hamilton, 1822)	carnivore	60.3 ± 12.7	23.9 ± 1.0 (22.1–25.8)	1.9 ± 0.1	3.8 ± 2.0	0.8 ± 0.6	1.8 ± 1.3
Demersal (sea water)							
Mugil cephalus (Linnaeus, 1758)	omnivore	102.4 ± 13.4	22.8 ± 0.9 (21.0–24.5)	0.5 ± 0.2	3.7 ± 1.0	0.2 ± 0.1	1.6 ± 0.5
Muraenesox cinereus (Forsskål, 1775)	carnivore	145.9 ± 10.3	43.2 ± 1.2 (38.0–55.0)	0.4 ± 0.2	2.4 ± 0.6	0.2 ± 0.1	1.1 ± 0.6
Terapon jarbua (Forsskål, 1775)	omnivore	87.9 ± 10.8	18.9 ± 0.8 (17.5–20.5)	0.9 ± 0.3	3.0 ± 0.7	0.2 ± 0.2	0.8 ± 0.4
Sebastiscus marmoratus (Cuvier, 1829)	carnivore	36.9 ± 4.1	13.6 ± 0.3 (13.2–14.1)	3.3 ± 1.2	4.2 ± 1.3	0.9 ± 1.2	1.1 ± 1.4
Photopectoralis bindus (Valenciennes, 1835)	omnivore	20.9 ± 2.6	11.3 ± 0.2 (11.0–11.8)	10.1 ± 4.9	4.1 ± 2.1	3.8 ± 2.5	1.5 ± 0.9
Cynoglossus abbreviatus (Gray, 1834)	carnivore	62.4 ± 13.1	22.7 ± 1.1 (21.0–25.0)	9.4 ± 5.1	6.9 ± 2.4	3.9 ± 4.4	2.9 ± 3.1
Thamnaconus septentrionalis (Günther, 1874)	carnivore	107.3 ± 9.2	19.3 ± 0.7 (18.0–21.0)	4.0 ± 1.7	7.2 ± 2.8	1.1 ± 0.8	2.0 ± 1.4
Oxyeleotrix marmorata (Bleeker, 1852)	carnivore	46.2 ± 5.3	16.8 ± 0.6 (16.0–17.9)	1.3 ± 1.1	4.2 ± 2.4	0.3 ± 0.4	0.9 ± 1.1
Synechogobius ommaturus (Richardson, 1845)	carnivore	21.5 ± 5.7	14.0 ± 1.1 (12.8–17.3)	12.6 ± 9.5	5.3 ± 2.9	0.9 ± 1.4	0.4 ± 0.6
Collichthys lucidus (Richardson, 1844)	carnivore	29.4 ± 3.7	14.7 ± 0.6 (13.3–15.3)	17.2 ± 9.7	6.2 ± 2.4	1.8 ± 3.4	0.7 ± 1.1
Branchiostegus japonicus (Houttuyn, 1782)	carnivore	50.5 ± 4.9	15.4 ± 0.4 (14.5–16.0)	8.1 ± 5.2	4.6 ± 2.8	1.4 ± 1.8	0.7 ± 0.9
Benthic (sea water)							
Callionymus planus (Ochiai, 1955)	carnivore	36.4 ± 5.3	20.7 ± 1.0 (19.0–22.4)	3.6 ± 1.8	4.8 ± 2.3	1.3 ± 1.3	1.7 ± 1.9
Benthopelagic (freshwater)							
Cyprinus carpio (Linnaeus, 1758)	omnivore	271.0 ± 150.8	28.0 ± 5.7 (21.0–24.0)	0.5 ± 0.3	2.5 ± 1.3	0.1 ± 0.2	0.5 ± 1.0
Carassius auratus (Linnaeus, 1758)	omnivore	59.5 ± 20.5	16.0 ± 1.7 (14.0–20.0)	1.7 ± 1.0	1.9 ± 1.0	0.1 ± 0.4	0.2 ± 0.5
Hypophthalmichthys molitrix (Valenciennes, 1844)	planktivore	39.6 ± 19.8	16.1 ± 1.9 (14.0–22.0)	2.1 ± 1.1	3.8 ± 2.0	0.7 ± 0.6	1.3 ± 1.0
Pseudorasbora parva (Temminck and Schlegel, 1846)	omnivore	14.5 ± 1.8	11.4 ± 0.6 (10.0–12.0)	5.6 ± 3.9	2.5 ± 1.8	1.8 ± 2.5	0.7 ± 1.0
Megalobrama amblycephala (Yih, 1955)	herbivore	109.0 ± 59	23.5 ± 3.1 (21.0–28.0)	0.2 ± 0.1	1.8 ± 1.7	0.3 ± 0.2	3.0 ± 1.4
Hemiculter bleekeri (Warpachowski, 1888)	planktivore	43.0 ± 10.8	17.3 ± 1.5 (15.0–19.0)	1.1 ± 0.5	2.1 ± 1.1	0.2 ± 0.3	0.3 ± 0.5

2.5. Observation, identification and validation of microplastic

Filters were observed under a Stereo microscope (Carl Zeiss Discovery V8, MicroImaging GmbH, Göttingen, Germany), and images of plastic items were taken with an AxioCam digital camera at different (25–80) magnifications. Particles were assessed visually first (Hidalgo-Ruz et al., 2012). Plastics were classified according to Li et al. (2016) and categorized by type according to their physical characteristics into fibers (elongated), fragments (small angular pieces), pellets (spherical, ovoid), sheets (irregular flat, flexible) and films (thin, soft, transparent). The longest or widest dimensions of each particle was measured to the nearest millimeters (Choy and Drazen, 2013; Jantz et al., 2013; Phillips and Bonner, 2015). When maximum particle size was smaller than 5 mm, the plastic sample was considered as microplastic; on the contrary plastics larger than 5 mm were categorized as mesoplastics. All plastic items were grouped into small microplastics (<2 mm), large microplastics (2–5 mm) and mesoplastics

(5–25 mm) following the size categories described by Collignon et al. (2014) and Romeo et al. (2016).

Some particles from each morphotype were randomly selected and identified with a micro-Fourier Transformed Infrared Spectroscope (μ-FT-IR, Thermo Nicolet iN10 MX) under the transmittance mode according to Yang et al. (2015). The selected particles represented the most common types of visually identified particles from all filters. The spectrum range was 4000–675 cm^{-1} with a collection time of 3 s and 16 co-scans for each measurement. All spectra were post-processed under an automatic baseline correction mode via the OMNIC software. To verify the polymer type, all spectra were compared with Hummel Polymer and Additives and Polymer Laminate Films (Thermo Fisher Scientific, USA). The abundance of plastics was recalculated by excluding all of the verified non-plastic items.

2.6. Statistical analysis

Data were analyzed using SPSS 16.0 software. Independent-Samples T test was performed to determine mean differences of plastics abundance between two groups, sea benthopelagic and freshwater fishes, stomachs and intestines at 95% confidence level. Significant differences in the abundance of plastics among sea fishes was observed through one way analysis of variance (ANOVA) followed by Tukey test's HSD test (homogeneous variances) and Dunnett's T3 (heterogeneous variances). Significant differences were recorded at $^{*} = p < 0.05$ and $^{**} = p < 0.01$.

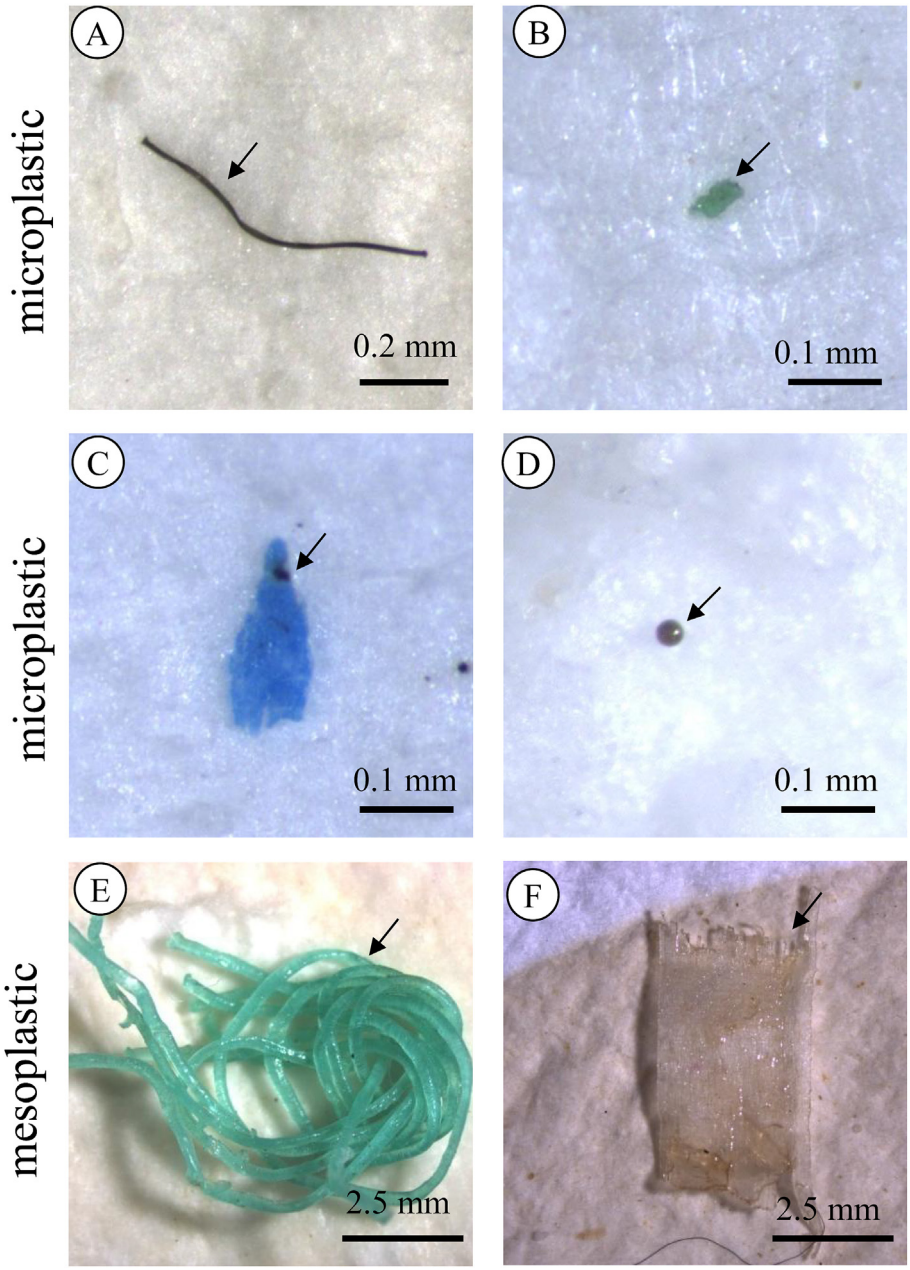

Fig. 1. Photographs of micro and mesoplastics in fish from China. The morphotypes included fiber (A), fragments (B, C), pellet (D), meso fibers (E) and meso sheet (F). Scale bar = 0.2 mm (A), 0.1 mm (B–D), 2.5 mm (E, F).

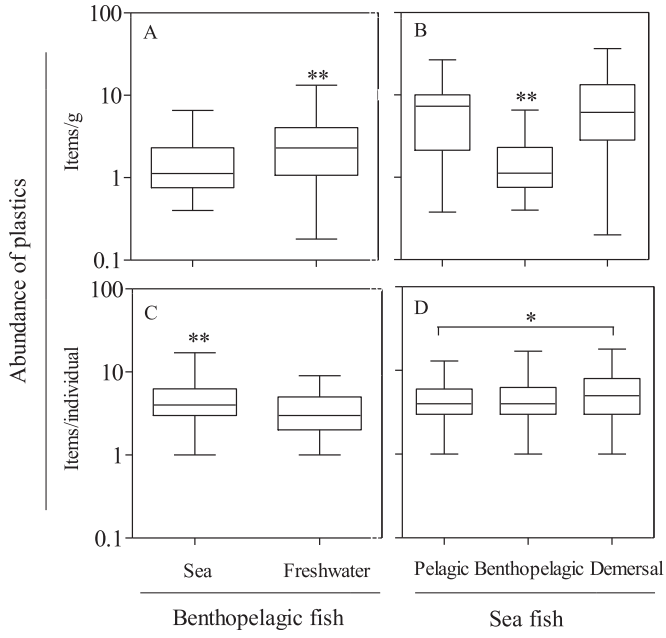

Fig. 2. Comparison of abundance of plastics between different groups. Benthopelagic fishes from sea water and freshwater (A, C); pelagic, benthopelagic and demersal fishes from sea water (B, D). * means $p < 0.05$ and ** means $p < 0.01$.

3. Results

3.1. Abundances of microplastics and mesoplastics in fish

Plastics were found in all fishes from 21 sea species and 6 freshwater species. Microplastics were found in 100% of sea fish and 95.7% of freshwater fish, while mesoplastics occurred in 70.9% of sea fish and 43.5% of freshwater fish. Different morphotypes of micro- and mesoplastics were observed in fish samples. Microplastics included fiber, fragment and pellet (Fig. 1A–D). Mesoplastics included fibers and sheet (Fig. 1E and F).

Contamination from the laboratory was effectively prevented, and the procedural blanks only contained 0.25 ± 0.05 items/filter of plastic contamination, representing less than 5% of the average abundance of plastics detected in fish. The abundance of microplastics varied from 1.1 to 7.2 items/individual (i.e., 0.2 to 17.2 items/g) and that of mesoplastics varied from 0.2 to 3.0 items/individual (i.e., 0.1 to 3.9 items/g) (Table 1). The average abundance of microplastics was the highest by individual in *Thamnaconus septentrionalis* (7.2 ± 2.8 items/individual) and by weight in *Collichthys lucidus* (17.2 ± 9.7 items/g) (Table 1). The abundance of mesoplastics was the highest in *Megalobrama amblycephala* (3.0 ± 1.4 items/individual) by individual and in *Cynoglossus abbreviatus* (3.9 ± 4.4 items/g) by weight (Table 1).

The abundance of plastics by items/individual was significantly higher in sea benthopelagic fishes than in freshwater benthopelagic fishes, while the abundance by items/g was higher in freshwater benthopelagic fishes ($p < 0.01$). The abundance of plastics by items/individual was significantly higher in sea demersal fishes than that in pelagic fishes ($p < 0.05$). Benthic fish (n = 1) were not included in the statistical analysis because only one species was available (Fig. 2).

3.2. Types, sizes and colors of plastics in fish

Of all plastics, the most common morphotype was fiber, followed by fragment (Table 2). The percentage of fiber reached 100% in *Cyprinus carpio* and *Hemiculter bleekeri* and 90.3% in *Muraenesox cinereus*. The average percentage of fragments in all species was 15.4%. Film was only found in *C. abbreviatus* and *Psenopsis anomala*, accounting for 1.1% and 5.9% of the total number of items in each species, respectively.

Microplastics accounted for 36.8–92.3% of the total number of plastics in each specific species (Table 2). The size of microplastics ranged from 0.04 mm to 5 mm and that of mesoplastics ranged

Table 2
Types and sizes of microplastics and mesoplastics in fishes from China.

Fish Species	Microplastics (%)					Mesoplastics (%)		Sizes (%)		
	Fibers	Fragments	Pellets	Sheets	Films	Fibers	Sheets	<2 mm	2–5 mm	5–25 mm
H. intermedius	38.4	29.3	0	0	0	32.3	0	41.4	26.3	32.3
L. haematocheila	65.4	10.2	0	0	0	24.4	0	35.9	39.7	24.4
C. ectenes	52.9	31.8	0	0	0	15.3	0	52.9	31.8	15.3
L. japonicus	60.8	19.6	0	0	0	19.6	0	45.7	34.8	19.6
Si. sihama	43.7	26.7	0	0	0	29.6	0	39.4	31.0	29.6
L. crocea	40.4	45.7	1.1	0	0	12.8	0	58.5	28.7	12.8
Psen. anomala	44.1	5.9	0	0	5.9	44.1	0	8.8	47.1	44.1
Pa. cinereus	40.9	17.2	0	0	0	41.9	0	28.0	30.1	41.9
H. nehereus	48.5	18.8	0	0	0	32.7	0	33.7	33.7	32.7
M. cephalus	50.0	18.8	1.0	0	0	30.2	0	36.5	33.3	30.2
M. cinereus	59.7	9.7	0	0	0	30.6	0	27.4	41.9	30.6
T. jarbua	60.9	17.4	0	0	0	21.7	0	42.0	36.2	21.7
Se. marmoratus	58.9	15.8	4.2	0	0	21.1	0	38.9	40.0	21.1
P. bindus	61.4	10.9	1.0	0	0	26.7	0	23.8	49.5	26.7
C. abbreviatus	50.9	16.9	0	1.1	1.1	30.0	0	30.5	39.5	30.0
T. septentrionalis	62.0	15.1	0	1.2	0	21.7	0	37.3	41.0	21.7
O. marmorata	59.3	20.9	1.1	1.1	0	17.6	0	45.1	37.5	17.6
S. ommaturus	62.5	22.1	7.7	0	0	7.7	0	54.8	37.5	7.7
C. lucidus	75.0	11.3	3.2	0	0	10.5	0	40.3	49.2	10.5
B. japonicus	75.0	11.5	0	0	0	13.5	0	50.0	36.5	13.5
C. planus	54.7	16.2	1.7	0.9	0	25.6	0.9	23.9	49.6	26.5
C. carpio	83.3	0	0	0	0	16.7	0	0	83.3	16.7
C. auratus	86.5	0	5.4	0	0	8.1	0	43.2	48.6	8.1
H. molitrix	57.6	6.5	9.8	0	0	26.1	0	32.6	41.3	26.1
Pseu. parva	70.7	6.9	0	0	0	22.4	0	22.4	55.2	22.4
M. amblycephala	26.3	10.5	0	0	0	63.2	0	26.3	10.5	63.2
H. bleekeri	88.2	0	0	0	0	11.8	0	35.3	52.9	11.8

from 5.1 mm to 24.8 mm. The plastics smaller than 5 mm were the most common size of plastics, accounting for 76.3% of the total number of plastics ($p < 0.01$). Nine colors of plastic were found in sea fishes, and six were found in fresh water fishes (Supplementary Table 3). The dominant plastics were transparent, followed by black and blue plastics ($p < 0.01$). The variety of colored plastics was higher in sea fishes, but no preference for a particular color was found in the specific fish.

3.3. Accumulation of plastics in stomach and intestine

By item/individual, the average number of plastics was highest in the stomachs of *Harpodon nehereus* (3.3 items/individual) and in the intestines of *Pampus cinereus* (2.7 items/individual) (Fig. 3A). By item/g, it was the highest in the stomachs of *Larimichthys crocea* (5.5 items/g) and the intestines of *H. nehereus* (6.6 items/g) (Fig. 3B). More than 50% of fish species showed significant differences in the abundance of plastics between the stomachs and intestines ($p < 0.05$) (Fig. 3). In particular, the abundance of plastics by items/individual was significantly higher in the intestines than that in the stomachs in *Liza haematocheila* and *Psen. anomala* ($p < 0.01$), but it was significantly higher in the stomachs than in the intestines in *L. crocea* ($p < 0.05$).

Stomachs and intestines showed a similar distribution of plastic types. Fiber (>5 mm) was the highest in the stomachs of *Pa. cinereus* (61.4%) and in the intestines of *Psen. anomala* (36.4%) (Fig. 4). Sheet was only found in the stomach of *L. crocea*, and film was only found in the intestines of *Psen. anomala* (Fig. 4).

3.4. Identification and validation of plastics

Out of 2557 visually identified plastics, 227 items were selected for identification using μ-FT-IR. In total, 26 polymer types were identified (Supplementary Table 4). Approximately 95.2% were cellophane (49.1%), polyethylene terephthalate (10.6%) and polyester (7.9%), etc. Non-plastic particles (4.8%) such as vermiculite and diethanolamine were also identified.

4. Discussions

4.1. Plastic pollution in fishes

In our study, demersal species showed significantly higher abundance of plastics than pelagic fishes ($p < 0.05$). This result contrasts with the results reported in fishes from the North Sea, Baltic Sea and English Channel (Lusher et al., 2013; Rummel et al., 2016). Wright et al. (2013) and Brandao et al. (2011) suggest that fouling and high density plastic items can be ingested by fish through prey. This ingestion of plastic probably happens during the normal feeding activity of fish. Feeding habits and habitat play important roles in the ingestion of debris, and an increase in the abundance of plastics also increases the bioavailability of plastics. Microplastic ingestion is closely related to different feeding strategies (Anastasopoulou et al., 2013; Romeo et al., 2015; Battaglia

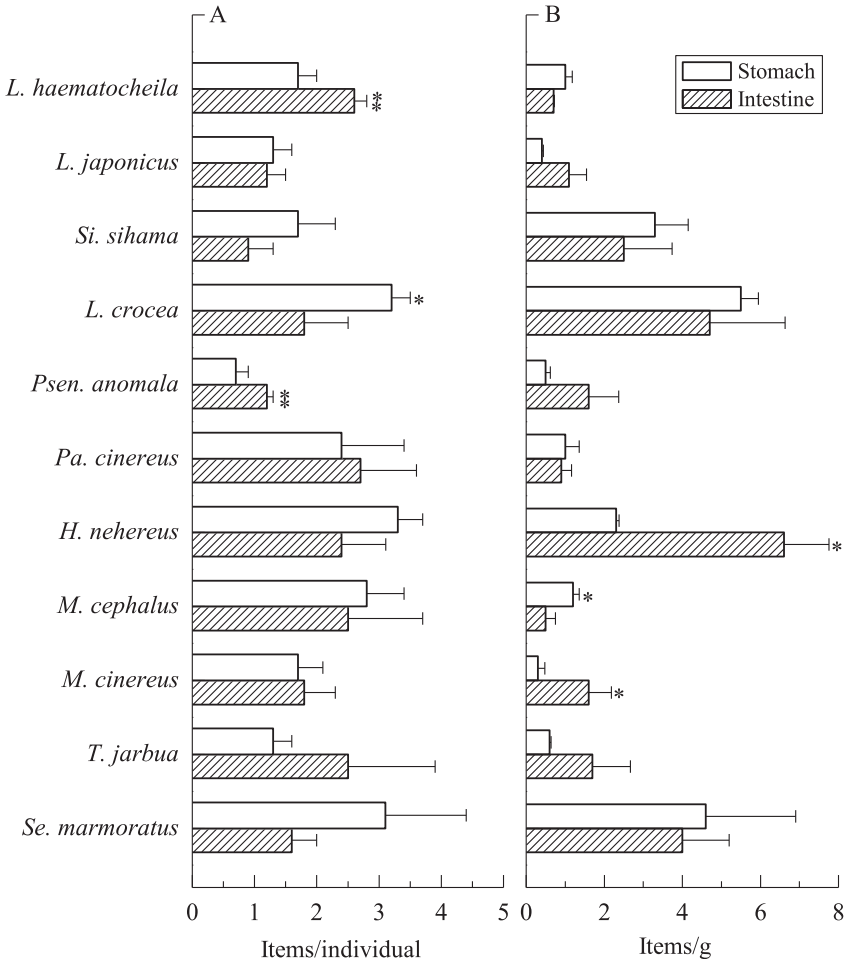

Fig. 3. Abundance of plastics in the stomach and intestine of fishes from China.

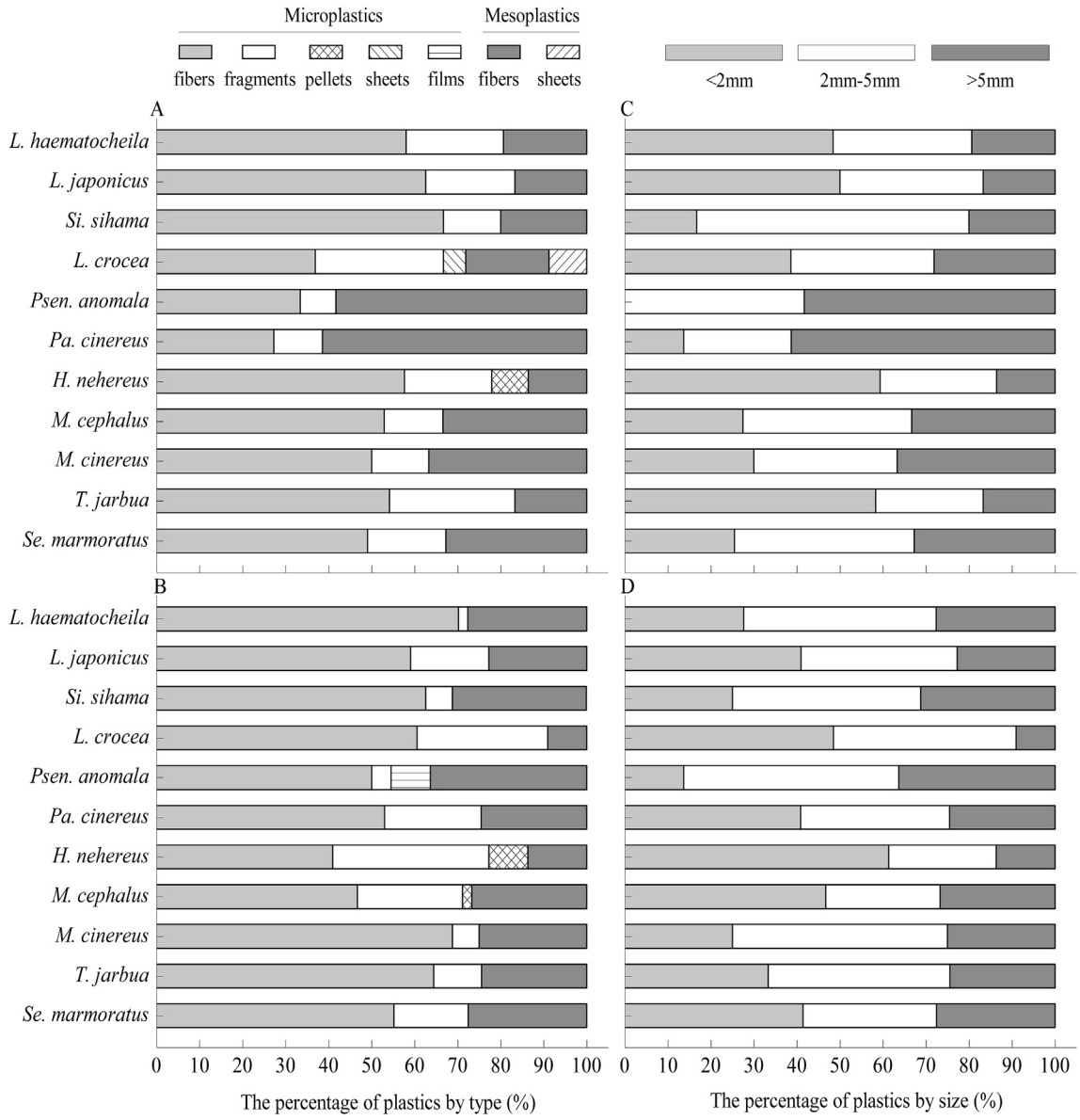

Fig. 4. Composition of plastics by type and size in the stomach (A, C) and intestine (B, D) of fishes from China.

et al., 2016). The higher abundance may be related to different habitats of fishes and the presence of plastic debris near the seabed (Woodall et al., 2014). This evidence also supports the relationship between plastics ingestion and feeding behaviour. We will focus on the plastic ingestion and ecology of species in our future studies.

Fish are known to ingest marine plastics with different shapes, sizes and colors, which have been widely spread throughout the water column (Possatto et al., 2011; Reisser et al., 2014; Romeo et al., 2016). In the present study, we found the abundance of microplastics was higher than mesoplastics in all investigated fish species with the exception of *Megalobrama amblycephala* ($p < 0.01$). Fibers were the most common morphotypes of plastics in the present study, which is similar to the results reported in previous studies (Lusher et al., 2013; Neves et al., 2015; Nadal et al., 2016). More types of plastic particles ingested by sea fishes than freshwater fishes might indicate that a greater variety of plastic particles are present in the marine environment compared with the freshwater environment, thus increasing the availability of plastics to sea fishes. In the present study, the composition of plastic polymers found in fish is highly similar to that found in mussels along the coastline of China reported by Li et al. (2016).

4.2. Organ-specific location of plastics in fish

To our best knowledge, this is the first report on the organ-specific location of plastics with a special emphasis on the structure of the digestive tract in fish in China. Significant variations of plastic abundance were found between the stomachs and intestines of fishes. Moreover, the use of different units (i.e., item/individual and item/g) also led to variations in the calculation of plastic abundance, even in the fish of the same species. The average abundance of plastics in the stomach in the present study is similar to that found by Boerger et al. (2010) and lower than that discovered by Choy and Drazen, 2013 (Supplementary Table 1). The abundance of plastics in the intestines has never been reported in previous studies. Our study suggested that the abundance of plastics in the intestines was even higher than in the stomachs in some fish species. The analysis of the entire digestive tract can then

provide additional information about the real amount of plastic ingestion by fish species.

Morphological variations exist in the GIT of different fishes due to feeding habits (Banan Khojasteh, 2012; Khalaf Allah, 2013; Chakrabarti and Ghosh, 2014). In the present study, the plastics were likely to accumulate in the coiled structures of the intestines (e.g., *Pa. cinereus*, *L. haematocheila* and *Mugil cephalus*). Especially, higher percentages of mesoplastics were found in the complex stomachs (e.g., *Pa. cinereus* and *Psen. anomala*). In addition, stomachs with a narrow opening to the intestines seemed to retain more plastics, e.g., sheets in the stomachs of *L. crocea* and absent from the intestines. The irregular and sharp edges of sheets could damage the stomach wall and create stress in the case of accumulation of plastics. Therefore, our results indicate that the complex stomachs and intestines increased the chances of plastic accumulation in the GIT.

4.3. Methods to investigate plastic pollution in fish

Currently, it is urgent to develop a uniform and effective method for isolation and identification of microplastics from biotic samples, including fish (Song et al., 2015). Previous studies have proposed different protocols to investigate plastic in fish (Neves et al., 2015; Peters and Bratton, 2016; Bellas et al., 2016). For example, in the protocol of Marine Strategy Framework Directive Technical Subgroup on Marine Litter, the stomach was chosen as the investigated organ for plastics, and the digestion method was recommended (MSFD-TSGML, 2013). In previous investigations, the digestion method has been used to extract microplastics from the stomach (e.g., Bellas et al., 2016) or from the GIT of fish (e.g., Foekema et al., 2013; Avio et al., 2015; Rochman et al., 2015). In contrast, extraction has been carried out without digestion in the stomach or GIT of fish in some other studies (Romeo et al., 2015, 2016; Battaglia et al., 2016; Cannon et al., 2016; Peters and Bratton, 2016; Rummel et al., 2016). Therefore, the methods should be further optimized; two aspects are discussed as follows based on our results.

On one hand, it is important to determine which organ should be investigated in fish. In most previous studies, the stomach was considered for plastic estimation (Choy and Drazen, 2013; Neves et al., 2015; Bellas et al., 2016). Our results strongly suggested that the whole GIT, rather than only stomach, should be used to avoid the under-or overestimation of plastic pollution. On the other hand, it is necessary to use the digestion process so that all size classes of plastics can be discovered. In general, smaller microplastics are too minute to be distinguished from the GIT using microscopic observation directly. Digestion and filtration make it possible to extract microplastics from the tissue and easily identify them.

5. Conclusion

In the present study, we reported plastic pollution in 21 sea fishes and 6 freshwater fishes from China for the first time. We found that micro and mesoplastic pollution was ubiquitous and relatively high levels were present in the investigated fish species, both in the stomachs and intestines. The abundance of microplastics was higher than that of mesoplastics in most species. The abundance of plastics in the intestines was even higher than in the stomachs in some species. We highly recommend that the whole GIT and digestion process be used in the future investigation of plastic pollution in fish.

Acknowledgements

This work was supported by grants from the National Key Research and Development (2016YFC1402204) and the Natural Science Foundation of China (41571467).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.11.055.

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