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# Food-web transfer of microplastics between wild caught fish and crustaceans in East China Sea



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ARTICLE INFO	A B S T R A C T
Keywords: Microplastic Wild fish Trophic transfer Marine food chain	Plastic pollution, including microplastics (MPs), poses a global threat to environmental and human health. Studies on the transference of MPs along marine food webs are limited. In the present study, we investigated MP pollution in 11 wild fish species (193 individuals) and 8 wild crustacean species (136 individuals) captured from the Zhoushan fishing ground, off the East China Sea. The average abundance of MPs found in two main tissues, the gill and gastrointestinal (GI) tract, were $0.77 \pm 1.25$ and $0.52 \pm 0.90$ items/individual, respectively. The MPs we found were predominantly fiber-shaped, blue, and composed of polyester polymers. Our results suggest that MP pollution is ubiquitous in the East China Sea. We suggest that MPs are likely aggregated in the higher trophic level fish species throughout the marine food web. Furthermore, we suggest that marine organisms

which occupy higher trophic levels might be suitable MP indicator species.

#### 1. Introduction

Marine plastic pollution poses a considerable threat to marine ecosystems across the globe (Dubaish and Liebezeit, 2012; Collignon et al., 2012; Desforges et al., 2014), from the tropical equator to the poles (Obbard et al., 2014; Lusher et al., 2015; Bergmann et al., 2017; Tekman et al., 2016). Microplastics (MPs) are plastics < 5 mm in diameter (Andrady, 2011). Primary microplastics are manufactured as microbeads, fragments, fibers or pellets, and secondary microplastics was derived from the breakdown of macroplastics, which are retained in a range of marine and freshwater ecosystems, and from inter-tidal to abyssal environments (Andrady, 2011; Cole et al., 2011; Ja and Costa, 2014). Previous field studies have reported severe plastic pollution in seas and oceans around the world, such as the Pacific Ocean (Hipfner et al., 2018), Amazon River estuary (Pegado et al., 2018), Mediterranean Sea (Bellas et al., 2016; Güven et al., 2017; Romeo et al., 2015; Romeo et al., 2016), and the Arctic Ocean (Morgana et al., 2018). Peng et al. (2017) reported a concentration of up to 121  $\pm$  9 MP items per kg of dry weight in the sedimentary environment of the Changjiang Estuary. In addition, Xu et al. (2018) recorded an average concentration of 23.1  $\pm$  18.2 MP items/100 L in surface waters of the East China Sea. MPs are commonly entangled and ingested by plankton, crustaceans, fish, turtles and seabirds, leading to physical injury (including internal and/or external abrasions and ulcers, starvation, and smothering) (Gassel et al., 2013) and physiological effects (including reduced

growth rates, blockage of enzyme production, diminished feeding stimulus, and reproductive failure) (Lusher et al., 2013). Boerger et al. (2010) reported an average abundance of 2.1  $\pm$  5.78 MP items per fish among six fish species collected in the North Pacific Central Gyre. Comparatively, Cannon et al. (2016) recorded low MP contamination in 21 fish species from the Southern Hemisphere, two acrylic resins were identified. Ingestion of MPs was also identified in a study of Norway lobsters; plastic pollution was recorded in up to 83% of the stomachs of studied animals (Murray and Cowie, 2011). However, investigations into the abundance of MPs in wild fish species along Chinese coastal areas remain scarce. Jabeen et al. (2017) examined fish samples purchased from a local fishery market in Shanghai and found an abundance of 1.1-7.2 MP items/individual. It is difficult to ascertain whether these samples were contaminated by MPs before being purchased, and the data may therefore not reflect actual pollution exposure in the ocean. Given the potential threat of MPs to the health of the marine environment, information regarding the abundance of MPs ingested by marine organisms is required urgently.

A few studies that are available show that marine MPs are ubiquitous in the East China Sea. Hence, we investigated MPs in wild caught fish and crustacean from the Zhoushan fishing ground to add our understanding of the abundance of MPs in wild marine organisms. Zhoushan fishing fround was the largest fishing ground in China, where occupied frequent fishing activity. Data recorded by continuous plankton recorders demonstrated fisheries played a major part in plastic

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Received 17 April 2019; Received in revised form 27 May 2019; Accepted 27 May 2019 Available online 14 June 2019 0025-326X/ © 2019 Published by Elsevier Ltd. pollution (Ostle et al., 2019). In addition, we hypothesized that MPs may be transferred up the marine food chain through different trophic levels (TLs) (Setälä et al., 2014; Farrell and Nelson, 2013). Commonly, producers (green plants) are the first TL, followed by herbivores, primary carnivores, secondary carnivores, and top carnivores. Several controlled-feeding studies have been conducted that demonstrate the trophic transfer of MPs along artificial food chains in laboratory settings (Santana et al., 2017; Farrell and Nelson, 2013). Many harmful chemical substances or phycotoxins can leak from MPs as they are transferred through the marine food web (Lithner et al., 2009), and studies that investigate TLs are highly valued (Pauly et al., 2001).

Our study aimed to increase knowledge regarding the distribution of MPs and to quantify variability among different species. We measured the potential of different marine organisms to ingest MPs and evaluated the potential for MPs to enter the marine food chain. Overall, we aimed to ascertain the relationship between MP abundance and TL in marine organisms from the East China Sea.

#### 2. Materials and methods

#### 2.1. Sample collection

All samples were captured by a bottom trawl on September 18–19, 2017. The survey method was conducted in accordance with the *Marine Survey Specification* (GB12736.6-1991). The bottom trawl employed a 32 m long net with mesh that became progressively smaller, ranging from 18.0 to 2.2 cm. During the sampling, the fishing boat maintained 3.1 knots/h. The sampling area is displayed in Fig. 1. If > 30 individuals of a given species were found, 30 were collected and the species was labelled Group 1. If < 30 individuals were found, all individuals of the species were collected and the species was labelled Group 2. A total of 193 fish and 136 crustaceans from 19 different species were captured from the bottom trawl samples. For each species habitat and trophic level was assigned according to the available data

from FishBase (Froese and Pauly, 2016) and regional articles (Jiahua, 2015; Ji, 2011; Cai et al., 2005; Zhang and Tang, 2004; Min et al., 2005; Li et al., 2017; Kai et al., 2010; Yan et al., 2016; Zhou-Ting et al., 2011). With the exception of Collichthys niveatus, the ecological behaviors of these species are well known (Table 1). All samples were preserved at - 20 °C prior to analysis. For each individual, an electronic balance (TB-2002, DENVER INSTRUMENT, USA) and electronic Vernier calipers were used to record weight (g) and total length (mm), respectively. Other basic biological data were collected, including a fullness index (0-5, where 0 = empty and 5 = full stomach), gender, and maturity stage. The gills and gastrointestinal (GI) tract from each individual were removed and stored in glass bottles at -20 °C until further analysis. Analysize the abundance of MP in both gill and GI tract was necessary. The names and taxonomic status of each fish and crustacean species were recorded based on the Checklist of Marine Biota of China Seas (Ruiyu, 2008) and the SealifeBase database (http://www.sealifebase. org).

#### 2.2. Digestion procedures

Potassium hydroxide (KOH), sodium iodide (NaI) and hydrogen peroxide ( $H_2O_2$ ) were purchased from SINOPHARM GROUP CO. LTD. (CHINA). Solutions of KOH (20% *w/v*), NaI (4.4 M) (Karami et al., 2017) and  $H_2O_2$  (30% v/v) were prepared by dissolving powder or pellets in Milli-Q water. Filter membranes were supplied by Whatman Inc. (GF/A No. 1820-047, 1.6 µm pore size, 47 mm diameter).

The digestion method used for fish samples was as per Karami [28]. The gills and the GI tracts of fish samples were separately transferred into 100 mL pre-combusted glass bottles filled with filtered 10% KOH solution. The glass bottles were placed at 40 °C for 48 h in a thermostatic water bath (HWS28, CHINA). The digestion method used for crustacean samples was as per Masura et al. (2015), which used 30%  $H_2O_2$  with added Iron (Fe)-II solution (0.05 M), incubated for 24 h at 60 °C. Each digestate was placed into a pre-combusted 600 mL glass

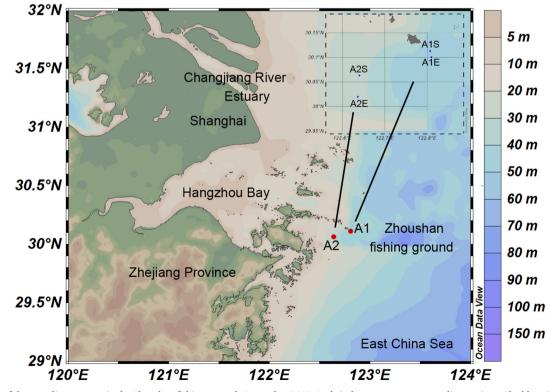


Fig. 1. Location of the sampling transect in the Zhoushan fishing ground, September 2017. Red circles represent two sampling stations. The blue circle represents the location of the start (A1S,A2S) and the end (A1E,A2E) of the transect. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

#### Table 1

Basic biological data collected for each sampled species from the Zhoushan fishing ground.

	Species	Feeding features	Ν	Body weight $\pm$ SD (g)	Fork length $\pm$ SD (range) (mm)	Fullness index (0-5)
Zhoushan fishing ground	Fish species Mesopelagic					
	Johnius spp.	Benthos; nekton	30	38.45 ± 13.30	$125.80 \pm 16.70$ (84.80–153.60)	0
	Larimichthys crocea	Plankton; benthos; nekton	30	37.00 ± 12.00	$133.80 \pm 14.80$ (104.10–170.00)	$1.4 \pm 0.2$
	Harpadon nehereus	Benthos; nekton	30	85.70 ± 27.70	$220.90 \pm 21.20$ (171.90-254.90)	$2.8 \pm 2.0$
	Pennahia argentata	Benthos; nekton	4	89.95 ± 29.98	$17.63 \pm 12.45$ (4.60–33.10)	0
	Collichthys lucidus	Benthos; nekton	5	$123.80 \pm 10.42$	$28.08 \pm 3.91$ (27.30-31.40)	0
	Demersal					
	Chrysochir aureus	Benthos	30	$40.20 \pm 10.30$	$127.00 \pm 15.70$ (102.70–193.90)	0
	Cynoglossus robustus	Benthos	30	$14.90 \pm 4.50$	$147.10 \pm 16.50$ (84.80–185.80)	0
	Muraenesox cinereus	Plankton; benthos; nekton	30	$145.40 \pm 170.30$	$447.50 \pm 127.40$ (204.00-853.60)	$3.5 \pm 1.6$
	Polydactylus sextarius	Benthos; nekton	2	62.39 ± 13.83	$6.15 \pm 3.15$ (3.00-9.30)	0
	Pennahia macroephalus Unknown	Benthos; nekton	1	132.44	46.70	0
	Collichthys niveatus Crustacean species	Benthos; nekton	1	154.26	54.00	0
	Oratosquilla oratoria	Benthos	64	13.96 ± 6.61	$103.96 \pm 24.09$ (11.94–172.04)	-
	Portunus trituberculatus	Benthos	30	109.00 ± 88.66	-	-
	Carcinoplax vestita	Benthos	18	$4.44 \pm 3.05$	_	-
	Charybdis bimaculata	Benthos	15	$2.34 \pm 2.11$	-	-
	Charybdis variegata	Benthos	4	$2.11 \pm 0.54$	-	-
	Portunus gracilimanus	Benthos	3	$4.94 \pm 1.59$	_	-
	Charybdis japonica	Benthos	1	31.62	-	-
	Oratosquilla kempi	Benthos	1	13.63	-	-

beaker, to which 400 mL NaI solution (4.4 M) was then added and stirred for 2 min with a glass rod. The mixture was kept undisturbed for 48 h. The supernatant was then filtered over a filter membrane to collect putative MP.

#### 2.3. Quality control

All the solvents (including Milli-Q water) used for sample processing and analysis were filtered over a glass-fiber filter (GF/A,  $1.6 \mu m$ , Whatman). All the glassware was washed with dishwashing liquid, followed by Milli-Q water, and then dried in an electric thermostatic drying oven (DGG-9070A, China) covered with aluminum foil for 8 h at 60 °C. Glassware was then combusted in a muffle furnace at 450 °C for 3 h. The procedure was carried out in a horizontal laminar flow cabinet (SW-CJ-1FB) to prevent potential contamination with airborne MPs. The working surface area was thoroughly cleaned with 70% ethanol prior to starting work. Dissecting tools were rinsed with pre-filtered Milli-Q water three times after dissecting each biological compartment to prevent cross-contamination. Procedural blanks were performed in parallel with the samples, and no microplastic was found.

#### 2.4. Observation, identification and validation of MPs

Membranes with suspected MPs were observed and photographed under a Leica stereoscope (MDG33, Singapore) after being dried in a desiccator for 24 h. Visual identification was used to quantify and sort the suspected MPs based on their physical properties. They were classified into fibers, fragments, pellets, foams, and films.

Fourier-transform infrared spectroscopy (FT-IR, Nicolet<sup>M</sup> iN<sup>M</sup>10, Thermo Fisher Scientific) was used to accurately identify the type of MP removed from the gills and GI tracts under the transmittance-mode (Yang et al., 2015). OMNIC software produced output spectra that

could be compared with databases from Thermo Fisher (Thermo Fisher Scientific, USA). Plastic items with a level of certainty (match degree higher than 70 with reference spectra) were accepted as a MP (Thompson et al., 2004). The length of each MP was measured using ImageJ (version 1.48) and MP color was identified visually. The shape factor (SF) of each particle was generated using the following formula (Zhao et al., 2018):

#### $SF = (4\pi \cdot A)/P^2$

where A represents the 2D surface area and P represents the perimeter.

#### 2.5. Data analysis

Differences in MP abundance between species, and between the gills and GI tract, were analyzed by analysis of variance (ANOVA). Pairwise comparisons were conducted using Tukey's HSD, where appropriate. Differences in the size of MPs between the gills and GI tract were examined using a general linear model, followed by Tukey's HSD post-hoc tests. If there was no significant difference found after an ANOVA test, *t*tests were conducted among Group 1 species. Based on size and shape factors, the *k*-medoid algorithm was employed to group MPs between the different species. A linear correlation analysis was conducted to examining the relationship between MPs and TL (Spearman correlation coefficient was used). All statistical analyses were performed in SPSS (version 22.0) and R 3.4.3. In all tests, an  $\alpha$  level of 0.05 was used. Unless otherwise indicated, data are reported as mean  $\pm$  SD.

#### 3. Results

#### 3.1. Abundance of MPs in fish

MP particles, based on spectrometric analysis, were found in 111

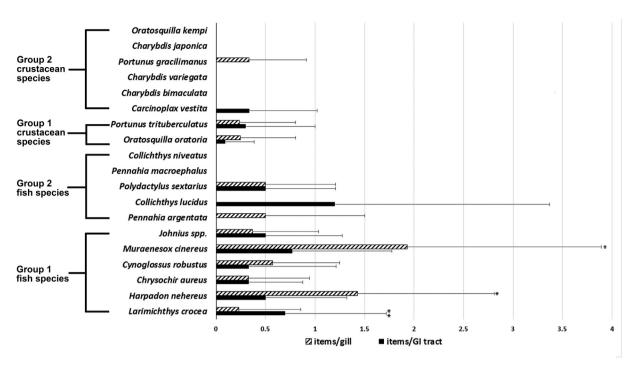


Fig. 2. The average abundance of MPs ( $\pm$  SD) in the gills and GI tracts of fish and crustacean species from Zhoushan fishing ground. There were no MP particles detected if there is no column on the figure.

\*indicates a significant difference in the abundance of MPs between gills or GI tracts for a given species, 0.01 .

\*\*indicates a highly significant difference in the abundance of MPs between gills or GI tracts for a given species, p < 0.01.

individual fish (57.5%). The highest MP abundance was 8 items per individual.

#### 3.1.1. Abundance of MPs in the gills of Group 1 species

The abundance of MPs found in the gills of Group 1 species ranged from 0.23  $\pm$  0.63 to 1.93  $\pm$  1.96 items per sample. The highest average MP abundance in gills was 1.93  $\pm$  1.96 items/gill, found in the dagger-tooth pike conger, with a range of 0–7 items/gill. The next highest values were recorded in *Harpadon nehereus* (1.43  $\pm$  1.38 items/gill) and *Cynoglossus robustus* (0.57  $\pm$  0.68 items/gill) (Fig. 2). There was a significant difference in the abundance of MPs found among Group 1 species (ANOVA, p < 0.01, the results of the pairwise comparisons are shown in Table 2).

#### 3.1.2. Abundance of MPs in the GI tract of Group 1 species

The abundance of MPs in the GI tracts of Group 1 species ranged from  $0.33 \pm 0.55$  to  $0.77 \pm 1.01$  items per sample (Fig. 2). The highest average abundance of MPs in GI tracts was  $0.77 \pm 1.01$  items/GI tract, found in the *Muraenesox cinereus*, with a range of 0–3 items/GI

Table 2

The results of	pairwise (	comparison	between	two	species	in	Group	1
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tract). The next highest values were recorded in *Larimichthys crocea* (0.70  $\pm$  1.02 items/GI tract) and *H. nehereus* (0.50  $\pm$  0.82 item/GI tract) (Fig. 2). There was no significant difference in the abundance of MP particles found among the six Group 1 species (ANOVA, p < 0.01, Table 2). According to the results of pairwise comparisons, there are a significant different in abundance of MP particles found between *Chrysochir aureus* (p < 0.05) and *Larimichthys crocea* (p < 0.01), *C. aureus* and *Muraenesox cinereus* and *C. aureus* and *Harpadon nehereus* (p < 0.05).

#### 3.1.3. Abundance of MPs in Group 2 species

Two MPs were found in one *Pennahia argentata* gill sample (n = 4). Two MPs were found in the GI tract and gill, respectively, from one individual *Polydactylus sextarius* (n = 2). Six MPs were found in the GI tracts of *Collichthys lucidus* (n = 5). No MPs were found in either *Collichthys niveatus* (n = 1) or *Pennahia macroephalus* (n = 1).

In gill	Larimichthys crocea	Muraenesox cinereus	Chrysochir aureus	Johnius spp.	Harpadon nehereus	Cynoglossus robustus
Larimichthys crocea	1	$0.000^{\rm b}$	0.295	0.193	$0.000^{b}$	0.041 <sup>a</sup>
Muraenesox cinereus	/	/	$0.000^{b}$	0.000 <sup>b</sup>	0.188	0.001 <sup>b</sup>
Chrysochir aureus	/	/	/	0.795	0.001 <sup>b</sup>	0.310
Johnius spp.	/	/	/	/	0.001 <sup>b</sup>	0.450
Harpadon nehereus	/	/	/	/	/	0.003 <sup>b</sup>
In GI Tract	Larimichthys crocea	Muraenesox cinereus	Chrysochir aureus	Johnius spp.	Harpadon nehereus	Cynoglossus robustus
Larimichthys crocea	/	0.875	0.011 <sup>b</sup>	0.255	0.377	0.165
Muraenesox cinereus	/	/	0.003 <sup>b</sup>	0.162	0.262	0.110
Chrysochir aureus	/	/	/	0.080	0.045 <sup>a</sup>	0.509
Johnius spp.	/	/	/	/	0.774	0.599
Harpadon nehereus	/	/	/	1	/	0.462

<sup>a</sup> Indicates a significant difference in the abundance of MPs between gills or GI tracts for a given species, 0.01 .

 $^{\rm b}$  Indicates a highly significant difference in the abundance of MPs between gills or GI tracts for a given species, p < 0.01.

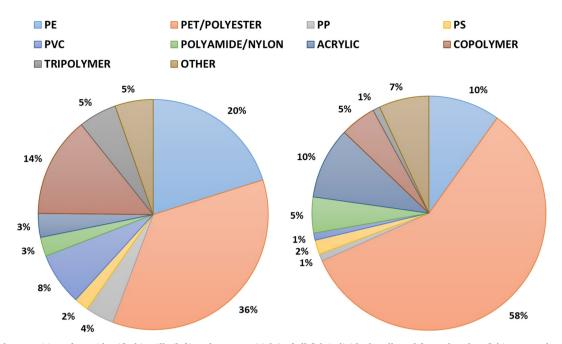


Fig. 3. Chemical composition of MPs identified in gills (left) and GI tracts (right) of all fish individuals collected from Zhoushan fishing ground. PE, polyethylene; PET, polyethylene terephthalate; PP, polypropylene; PS, polystyrene; PVC, polyvinyl chloride.

3.1.4. Comparison of MP abundances between the gill and GI tract within each species

The abundance of MPs was significantly higher in the gills than that in the GI tracts of *M. cinereus* (ANOVA, p < 0.05) and *H. nehereus* (ANOVA, p < 0.05); whereas, abundance was significantly higher in the GI tracts than the gills of L. *crocea* (ANOVA, p < 0.05) (Fig. 2). No significant differences in abundance of MPs were found between the gills and GI tracts of *C. aureus, C. robustus,* or *Johnius spp.* (ANOVA, p > 0.05).

#### 3.2. Chemical composition, size and color of MPs in fish

A total of 250 MP particles were found in the gills and GI tracts of fish samples, with 23 different polymer types identified (Fig. 3, Table S3). Polyethylene terephthalate (PET), including polyester, was the most frequent MP chemical composition found, at 44.8%, followed by polyethylene (PE) at 16.0% (Fig. 3). PET occurred in most of the samples, reaching an occurrence of 100% in the gill samples of P. argentata The total amount of other polymers combined was less than PET, including 2.8% polypropylene (PP), 2.0% polystyrene (PS), 4.8% polyvinyl chloride (PVC), 3.6% polyamide (PA), and 6.0% acrylic. Uncommon chemical compositions and MPs only found once in the study (e.g., polyacrylamide, polyvinyl ester, and poly tetra fluoroethylene) were classified 'other'. (Table S3). Copolymers (accounting for 10.4%) and tripolymers (accounting for 3.6%) were only detected in two species. Copolymers were detected in *M. cinereus* and the gills of *H.* nehereus, whereas tripolymers were only detected in the gills of M. cinereus and H. nehereus. Several types of copolymer and tripolymer were discovered: poly (propylene:ethylene), poly(acrylonitrile:butadiene), and poly(propylene: ethylene:diene) (Table S3).

Fiber and fragment particles were the most common MP shape found, both in GI tracts and gills, accounting for 59.6% (n = 149) and 38% (n = 95), respectively (Fig. S1). The percentage of fiber reached 100% in L. *crocea* gill samples, and 81.0% and 78.3% in *M. cinereus* gill and GI tract samples, respectively (Fig. S1). In contrast, film and foam were only found in *H. nehereus* gills, accounting for 9.3% and 2.3%, respectively, of the total number of items found (Fig. S1). Pellets were only found in the gills of *M. cinereus* s, accounting for 1.7% (Fig. S1). 655.39 ± 753.77 μm (ranging from 24.64 to 268.03 μm) and 727.03 ± 1148.22 μm (ranging from 32.90 to 4092.15 μm), respectively. The most common size of MPs was smaller than 1 mm, accounting for 74.7% and 78.7% of the total in gills and GI tracts, respectively (Fig. 4). Three fibers > 5 mm were detected in the GI tract of *H. nehereus* (5.178 mm) and L. *crocea* (7.388 mm), and in the gill of *M. cinereus* (5.307 mm). Marine debris that is 5 mm-2 cm in length or diameter is defined as mesoplastic [43]. No significant differences in MP size were observed between the gills (p > 0.05) and GI tracts of all fish species (p > 0.05). Twelve colors of MP were found in the investigated species; the dominant colors were blue, followed by black and red.

#### 3.3. Transfer of MPs along marine food webs

We found a high correlation between MP abundance and TL (Spearman correlation coefficient was 0.893, p < 0.01), with MP abundance increasing as trophic levels increased (Fig. 5, Fig. S4). The TLs of crustacean species were obviously less than those of fish species (Fig. S4), and MP abundances in crustacean species were significantly lower than in fish species (Fig. S5).

Of the individual crustaceans examined (n = 136), 34 (25%) contained 40 confirmed MP particles. The most prevalent polymer found was PET (65%), followed by PP (10%). The majority were fibers (n = 24; 60%) and the remaining 40% were comprised of fragments (n = 16).

According to Yu et al. (1986), *H. nehereus* is the dominant species in the Zhoushan fishing ground across all seasons. Mantis shrimp and gazami crab are economically important perennial crustaceans, and are the dominant species in offshore fisheries and one of the most important fisheries in coastal areas of China. Hence, two direct feeding relationships were listed: 1) *Muraenesox cinereus* (dagger-tooth pike conger) – *L. crocea* (large yellow croaker) – *Oratosquilla oratoria* (shako), and 2) *Harpadon nehereus* (bummalo) – *Collichthys lucidus* – *Portunus trituberculatus* (gazami crab). *M. cinereus* and *H. nehereus* were predators, and shako and gazami crab were preys. The TLs of each species were estimated according to studies on the food web structure in the East China Sea (Fig. 5) (Jiahua, 2015; Ji, 2011; Cai et al., 2005; Zhang and Tang, 2004; Min et al., 2005; Li et al., 2017; Kai et al., 2010;

The average size of MPs in the gills and GI tracts of fish samples was

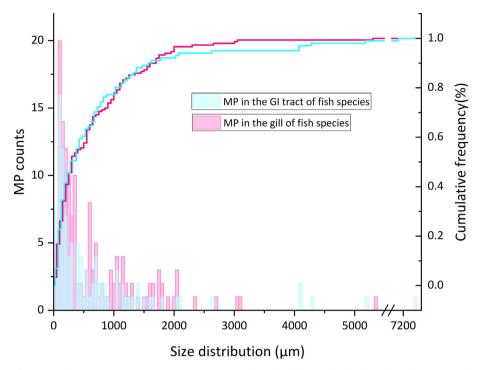


Fig. 4. Distribution of MP counts versus size (µm) in fish individuals sampled in Zhoushan fishing ground.

Yan et al., 2016; Zhou-Ting et al., 2011). The value of each species TL was taken the average on the historical data from different year.

Food relationships are among the most important interspecific relationships. The main economic fish species have different food composition preferences during different stages of growth. The size distribution of MPs was similar in *M. cinereus*, *L. crocea* and shako, with 73.9% (17 out of 23), 85.2% (23 out of 27) and 76.5% (13 out of 17) of the particles being smaller than 1000  $\mu$ m. In the same way, the size distribution of MPs was consistent in *H. nehereus, L. crocea* and gazami crab with 86.7% (13 out 15), 66.7% (4 out 6) and 75.0% (12 out of 16) of the particles being smaller than  $1000 \,\mu$ m. The *k*-medoids clustering plot showed that most MP particles in the predator and prey groups overlapped (Fig. 6).

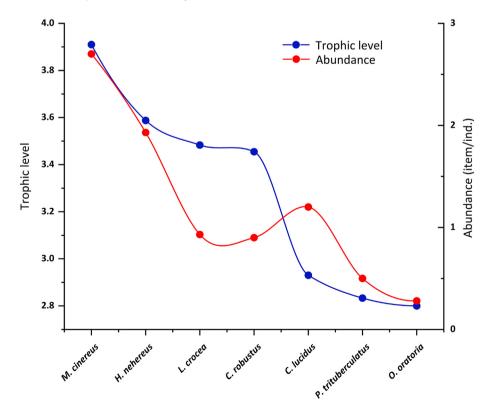


Fig. 5. The relationship between trophic level and MP abundance (items/individual) (correlation coefficient was 0.893, p < 0.01).

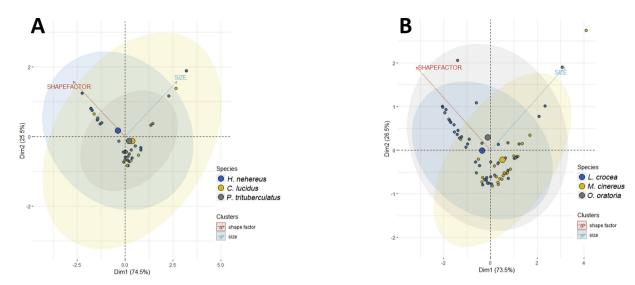


Fig. 6. Clustering using a k-medoids algorithm depicting the relationships of MPs, based on size and shape, in GI tract samples of: (A) Harpadon nehereus, Collichthys lucidus, Portunus trituberculatus; and (B) Muraenesox cinereus, Larimichthys crocea, Oratosquilla oratoria.

#### 4. Discussion

#### 4.1. MP transfer through TLs in the marine food chain

*M. cinereus* s and *H. nehereus* consumed marine debris more frequently than other species on our study (Fig. 2). This may be explained by the hypothesis that MPs are accumulated in higher TL fish species through marine food webs (Fig. 7), given that these species have TLs of 3.59 (Jiahua, 2015; Ji, 2011; Cai et al., 2005; Zhang and Tang, 2004;) and 3.91 (Jiahua, 2015; Ji, 2011; Min et al., 2005; Li et al., 2017), respectively (Fig. S4). Higher TL marine mammals have been found to interact with plastic particles on micro- and macro- scales. Lusher et al. (2015) reported mesoplastic pieces with a diameter of approximately 7 cm lodged in the accessory main stomach of beaked whales. *M. cinereus* and *H. nehereus* occupied the highest trophic niche in the present study, followed by L. *crocea* (TL = 3.48) (Min et al., 2005; Li et al., 2017; Kai et al., 2010), *C. robustus* (TL = 3.46) (Cai et al., 2005; Yan

et al., 2016), C. lucidus (TL = 2.9) (Jiahua, 2015; Cai et al., 2005; Li et al., 2017; Yan et al., 2016; Zhou-Ting et al., 2011), gazami crab (TL = 2.83) (Jiahua, 2015; Ji, 2011; Yan et al., 2016), and shako (TL = 2.8) (Zhou-Ting et al., 2011) (Fig. S4). During the correlation analysis between TL and MP abundance, P. argentata (TL = 3.57) (Jiahua, 2015; Ji, 2011; Cai et al., 2005; Zhang and Tang, 2004; Li et al., 2017) was excluded on account of the low quantities found (Table 1). Foekema et al. (2013) reported the highest abundance of MPs in cod (13%) among the examined individuals from the North Sea, followed by haddock. Cod occupied the highest TL among the sampled species; and haddock has a similar TL to cod in the North Sea food chain]. Jantz et al. (2013) showed marine debris was ingested by longnosed lancetfish, which often swallow mouthfuls of a variety of small and medium-sized bait. Our study agreed with the conclusion, which Nelms et al. (2018) presented, that TLs can act as a transfer pathway of micro-debris from lower TL fish species to higher TL marine top predators.

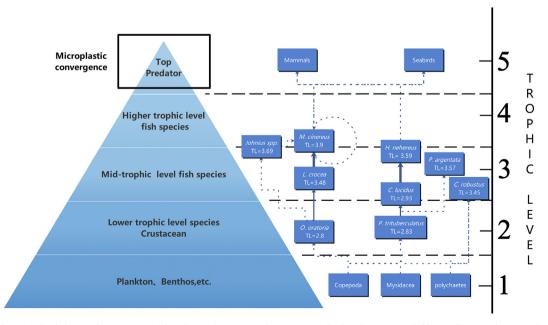


Fig. 7. A simplified marine food chain indicating potential pathways for MP transfer among trophic levels (TLs). A solid line indicates a direct predation relationship, and a dotted line indicates an indirect predation relationship. A circle with an arrow indicates cannibalism.

MP trophic transfer occurs between prey and higher predators, such as mussels and crabs (Farrell and Nelson, 2013), cod and fur seals (Eriksson and Burton, 2003), zooplankton and seabirds (*Alle alle*) (Amélineau et al., 2016). Through the analysis of gastric content, we found that MPs were delivered by feeding between adjacent TLs. Setälä et al. (2014) found plastic microspheres were ingested by different zooplankton taxa and particles may have several alternate transfer routes within the pelagic food web. Therefore, plastic debris could be introduced to various compartments of the marine food chain via several pathways (Wright et al., 2013).

It should be noted that the conclusion that MP concentration is amplified in the organism should be obtained by comparing the concentration of MPs within the whole organism. In view of the fact that MPs may be concentrated in certain organs and tissues, if MP contents are only analyzed in specific tissues, it is possible to draw erroneous conclusions. Whether there is a positive correlation between the abundance of MPs ingested or entangled and the trophic niche still requires further study. At present, there is limited information about the effects of MPs on TLs and no associated laboratory experiments have been conducted. In-depth understanding and prediction of the bioaccumulation of MPs in different aquatic organisms and related physiological processes is one of the challenges faced by researchers. In general, it is still unknown whether MPs are biomagnified or diluted through different TLs in benthic or plankton food chains. At present, research in this area is limited, and little is known about the difference in the transmission of different plastics in different marine food chains or in different marine ecosystems. We surmise that the chemical composition of MPs and the feeding types of different organisms will both affect MP transference in the food chain, thereby making predictions difficult regarding the fate of MPs in marine food webs.

#### 4.2. Abundance of MPs in different species

In the present study, 52.3% of fish GI tracts sampled contained MP particles, providing further evidence of the presence and distribution of MPs in marine food webs and implicating marine fish as an important intermediate carrier of MP particles in the marine environment. The abundance of MP particles in fish GI tracts were compared to results reported worldwide (Table 3). Only studies that examined particles in the GI tracts of fish using similar digestion procedures and density separations were considered. The average abundance of MPs detected in GI tracts in our study was 0.52  $\pm$  0.35 items/GI tract, which is comparable to that found in fish captured from the Saudi Arabian Red Sea coast (0.14  $\pm$  0.14 items/GI tract) (Baalkhuyur et al., 2018) and the North Sea (0.03  $\pm$  0.04 items/GI tract) (Foekema et al., 2013). Measurements of fish from an Arctic Sea near Northeast Greenland revealed an average MP abundance of  $1.1 \pm 0.3$  items/GI tract (Morgana et al., 2018). In contrast, the average MP abundance found in C. robustus in the present study is nearly five times lower than that found in GI tracts of S. C. robustus a from the Adriatic Sea (Pellini et al., 2018), although both belong to the Pleuronectoidei.

Gills are a well-known target organ in fish which have permanent contact with the aquatic environment, and are the first to react to disadvantageous environmental conditions (Poleksic, 1994).

Respiration, osmoregulation and excretion are performed by the gills, and gill rakers enable fish to filter MP particles from the water (Batel et al., 2018), but gills are not as protected as the skin and mouth. As expected, MPs were detected in the gills (77.2%) of fish collected from the Zhoushan fishing ground (Table 1). Significant differences in abundance of MPs was found among species, which could be explained by differences in gill raker structure. MP adherence in fish gills is probably a novel way for marine fish to uptake MPs beyond ingestion. Abbasi et al. (2018) demonstrated MPs were detected in different tissues of fish from the Musa Estuary, in the Persian Gulf. There were 180 MPs detected in fish gills among four fish species, and 828 MPs in total identified in four different tissues. Furthermore, MP abundances tended to be higher in gills than in the GI tracts among the four fish species. which is similar to results presented in the present study. Ding et al. (2018) conducted an exposure experiment on red tilapia (Oreochromis niloticus) with PS (0.1 µm) at different concentrations. Following 14 days exposure, residues of PS in the gills were much lower than in the GI tracts. MP contamination between GI tracts and gills varies dramatically among studies; however, in this case, differences can be explained by the size of the PS particles in the experiment. MP prevalence in fish gills seems to correspond with the pollution characteristics of contaminated habitats where fish live because of their direct exposure, although studies between fish gills and MPs remain scarce. MPs are ubiquitous and adherence of MPs to gills of mussels (Kolandhasamy et al., 2018), crabs (Watts et al., 2016), and larvae of xenopus (Hu et al., 2016) has been studied previously.

#### 4.3. Chemical composition, shape and size of MPs in gills and GI tracts

The chemical composition of MPs identified from fish samples was highly diverse (23 polymer types), with multiple sources of domestic and industrial uses. PET and PE made up over 60% of all MP particles identified in sampled GI tracts. Plastic bags, bottles, drinking straws, and milk jugs are commonly used products composed of PET and plastic beverage bottles are typically composed of PE. These two types of polymer are often detected in fish samples from the Baltic Sea (Rummel et al., 2016) and European seas (Collard et al., 2015). In contrast, some studies shown a low diversity of MPs; for example, nylon fragments (polyamide) were the only form of plastic detected in the stomachs of fish captured from the main channel of the Goiana Estuary (Ramos et al., 2012; Dantas et al., 2012). Kühn et al. (2018) identified epoxy resin and polymethylmethacrylate from the stomach samples of fish from the Arctic Ocean according to µFT-IR. The cause of discrepancies among samples from different regions is not clear, but it might be related to differences in MP contamination levels in the surrounding water. The considerable differences in the literature suggest that there are various origins of MPs, ranging from the terrene to the oceans. Therefore, the unique fate and effects of MPs in different ecosystems requires further study to be better understood (Rochman, 2018). A number of fibrous rayon particles were detected in the present study (Table S2); however, Rayon fibers composed of regenerated cellulose were not classified as a MP, to avoid skewing results (Christopher Blair Crawford, 2017).

Plastic waste is distributed globally across surface seawaters due to

#### Table 3

Summary of the prevalence of MPs found in fish in previous studies and the results reported in the present study for the East China Sea.

Area	Type of fish	Ν	% ingestion	Size of MP $\pm$ SD (mm)	Refs
1-Adriatic Sea	Commercial	125	28.0%	$1.78 \pm 0.97$	Avio et al. (2015)
2-English Channel	Pelagic and demersal	504	36.5%	$0.13 \pm 14.30$	Lusher et al. (2013)
3-Mediterranean Sea	Pelagic	121	18.2%	$1.51 \pm 16.50$	Romeo et al. (2015)
4-North Pacific Subtropical Gyre	Mesopelagic	141	9.2%	$2.20 \pm 1.90$	Davison and Asch (2011)
5-Portuguese coast	Commercial	263	32.7%	$2.11 \pm 1.67$	Neves et al. (2015)
6-Red Sea	Commercial and non-Commercial	178	14.6%	$2.39 \pm 0.28$	Baalkhuyur et al. (2018)
7-East China Sea	Wild caught	193	33.2%	$0.73 \pm 1.15$	This study. (Table S1)

its buoyancy. Previous studies have shown that oceanic MPs predominantly range from 1.01 to 5 mm (Cozar et al., 2014; Eriksen et al., 2014). The observed size distribution of MP particles within the GI tracts in the present study was mainly in the range < 1 mm, which was not consistent with these previous studies. Zhao et al. (2018) hypothesized that marine snow aggregates were a vector for the transfer of small MPs (< 1 mm) from the sea surface to the deeper water layers. Other plausible mechanisms that could explain the predominance of < 1 mm MPs include biofouling, ingestion by marine organisms, and MP aggregation leading to sinking (Andrady, 2011; Law et al., 2010). Results from the present study suggest that marine fish are also important in the vertical transfer of MP particles from the sea surface to the sediment. The fish samples examined in this study were mainly carnivorous, which leads to the hypothesis that carnivores may be second and/or third stage organisms in the marine environment. Ingestion by zooplankton and suspension feeders appears to be the first stage and the direct pathway for MPs to be introduced into marine food chain. Retention of MPs in ten natural zooplankton groups was systematically studied. As expected, > 90% of all MPs identified in different zooplankton taxa were < 1 mm (Sun et al., 2018a, 2018b). Sun et al. (2018a, 2018b) performed a comprehensive study of MPs in zooplankton and the surrounding seawater in the Yellow Sea. They found that over 82% of MP particles in the water column were longer than  $1200\,\mu m$ , which is in agreement with the global distribution of MPs. In contrast, MPs  $< 500\,\mu m$  accounted for 90% of the total measured across 11 zooplankton groups. Fibrous MPs were the predominant shape in the fish GI tracts in our study, accounting for 59.6% of all MPs. Similarly, fibrous MPs accounted for 65.8% of the total in commercial fish collected off the coast of Portugal (Neves et al., 2015). These results contrast with previously published data which reveal that fragments are the dominant shape of MPs found in GI tracts of fish (Boerger et al., 2010; Davison and Asch, 2011; Possatto et al., 2011).

#### 5. Conclusion

It is becoming overwhelmingly evident that severe pollution by plastic debris in the marine environment is impacting wild fish, and that the entry of MPs into marine food chains leads to accumulation in the top predators. Results of the present study verified that MPs can be found in the gills and GI tracts of both fish and crustacean species. The chemical composition, shape and size of MPs differed between these two tissues. This study provides further evidence of trophic transfer and more credibly comparable data regarding the abundance of MPs. However, the marine food web is intricate, and there are various MP chemical compositions, which makes research in this field challenging. Studies which utilize larger sample sizes should be conducted in the future to allow for more credible conclusions. Furthermore, effective global regulatory solutions for marine plastic pollution are urgently required.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2019.05.061.

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