

Microplastics in seawater and zooplankton from the Yellow Sea[☆]Xiaoxia Sun^{a, b, c, d, *}, Junhua Liang^{a, b}, Mingliang Zhu^{a, b}, Yongfang Zhao^{a, b}, Bo Zhang^b^a Jiaozhou Bay National Marine Ecosystem Research Station, Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao, 266071, China^b Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266071, China^c University of Chinese Academy of Sciences, Beijing, 100049, China^d Center for Ocean Mega Science, Chinese Academy of Sciences, Qingdao, 266071, China

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ABSTRACT

Marine plastic pollution is a worldwide problem. Microplastics (MPs) are the predominant form of marine plastic debris, a form small enough to be ingested by and potentially harm marine organisms. It is urgent to develop ecologically relevant metrics for the risk assessment of MPs based on *in situ* data, especially for coastal areas. For the first time, we performed a comprehensive study of the characteristics of MPs in seawater and zooplankton in the Yellow Sea. For MPs in seawater, the average concentration is 0.13 ± 0.20 pieces/m³, dominated by fragments (42%). The average size is 3.72 ± 4.70 mm, with the most frequent size appearing at 1200 μ m. The major polymer types are polypropylene and polyethylene, accounting for 88.13% in total. The distribution of MPs in seawater is patchy, with high MP concentrations close to the coastal cities. The average concentration of MPs in 11 total zooplankton groups is 12.24 ± 25.70 pieces/m³. The average size is 154.62 ± 152.90 μ m, with 90% being <500 μ m. Fiber is the dominant shape of MPs found in zooplankton, accounting for 46%, but the composition of the polymer type is diverse. The retention of MPs in zooplankton depends on the taxa and their abundance in the Yellow Sea. Siphonophorea, Copepoda, Euphausiacea and Amphipoda are the main repositories compared to other groups, achieving 3.57, 2.44, 1.41 and 1.36 pieces/m³, respectively. The high concentration area of MPs in zooplankton appeared near the adjacent waters of the Yangtze estuary. These results prove that zooplankton act as a repository for MPs in coastal waters. The retention of MPs in zooplankton is recommended as a key index for further ecological risk assessment of MPs.

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1. Introduction

Plastic production has increased dramatically worldwide over the past 60 years (Avio et al., 2017). Accordingly, marine plastic pollution has become a significant global problem after only half a century due to the widespread use of plastic materials (Cózar et al., 2015). It was estimated that 4.8–12.7 million metric tons (MT) of plastic waste entered the ocean in 2010, and the cumulative amount of plastic waste that could enter the ocean is predicted to increase one order of magnitude by 2025 unless suitable waste management and infrastructure improvements take effect (Jambeck et al., 2015).

Microplastics (MPs, plastic particles <5 mm in diameter) are the predominant form of marine plastic debris, occurring from coastal waters to mid-ocean gyres (Amaral-Zettler et al., 2016; Arthur et al., 2009). The presence of MPs in the marine environment poses a great threat to the entire ecosystem, greatly impacting oceans, and therefore, has received much attention (Auta et al., 2017). Moreover, MPs comprise a complex mixture of polymers, additive chemicals, absorbed organic compounds, and living substances to which organic material and contaminants can successfully bind (Galloway et al., 2017). Although the contribution of absorbed chemicals in MPs to the organisms by partitioning is controversial (Koelmans, 2015), there are still potential threats to the health of marine life, including commercial species sold for human consumption (Rochman et al., 2015). Thus, the ecological and societal risks of MPs are a matter of significant concern.

To evaluate the risk and the potential impacts of MPs on marine organisms, a number of laboratory experiments have been performed over recent years to mimic environmental exposure (Nobre et al., 2015; Phuong et al., 2016; Van Seville et al., 2015). However,

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more scientists began to question if the ecological risk from MPs was overstated because of laboratory tests using unrealistically high concentrations or unrealistic exposure scenarios (Burton, 2017; Koelmans et al., 2017; Phuong et al., 2016). With this knowledge, there is a need to define the ecologically relevant metrics (ERMS) for risk assessment of plastic debris and test environmentally realistic concentrations over long-term exposures (Koelmans et al., 2017).

Currently, there is some understanding on the distribution, concentration, and composition of MPs in seawater and sediments; however, this does not meet the demands of rational ecological risk assessments. For example, coastal biomes are under-sampled with respect to MPs (Van Seville et al., 2015). The MPs interact closely with marine organisms, but the quantified data of MPs in marine biota *in situ* remain largely unknown (Clark et al., 2016), especially for those groups that may directly interact with MPs. Biological interactions are key to understanding the movement, impact, and fate of MPs in the oceans (Clark et al., 2016). Data on the retention of MPs in the marine biota *in situ* is crucial to conduct the rational ecological relevant risk assessment and study the trophic transfer through the food web.

Zooplankton is ubiquitous in the marine environment. The most important role of zooplankton, as the main grazers in the ocean food web, is to provide the principal pathway for energy from primary producers to consumers at higher trophic levels (Richardson, 2008). Because zooplankton feed near the surface (Turner, 2015), they are more susceptible to ingesting MPs (Moore, 2008). Ingestion of MPs by zooplankton can be considered the fundamental link for MPs entering the food web since the millimeter-sized MPs are similar in size to zooplankton prey. Both laboratory studies and field observations detected the ingestion of MPs by zooplankton (Cole et al., 2013; Desforjes et al., 2015; Setälä et al., 2014; Sun et al., 2017), but the quantified data *in situ* are very limited due to the high biodiversity of zooplankton taxa. It is important to study the retention of MPs in different zooplankton groups and the accumulation through the entire zooplankton community in the field, as well as their relationship with environmental MPs.

China's coast was thought to be a typical area for MPs pollution (Jambeck et al., 2015; Zhao et al., 2014). China's coast belongs to the high biological productivity area and shelf seas that are adjacent to densely populated coastal towns and cities. The interactions between MPs and zooplankton were thought to be more frequent in such regions (Clark et al., 2016). By choosing the Yellow Sea as the target area, we conducted comprehensive observations of MPs in seawater and 11 zooplankton groups. We aim to explore the following questions: 1) the concentration and characteristics of MPs in seawater of the Yellow Sea; 2) the concentration and characteristics of MPs in different zooplankton groups in the Yellow Sea; 3) the accumulation of MPs in zooplankton in the Yellow Sea. We hope to provide references for associated controlled experiments and to support future studies on MP transfer and ecological risk assessment.

2. Materials and methods

2.1. Research area and sampling stations

The Yellow Sea is a semi-enclosed marginal sea with depths ranging from 20 to 90 m, bounded by China and the Korean Peninsula. It is separated from the West Pacific Ocean by the East China Sea in the south, and is linked with the Bohai Sea. The Yellow Sea is influenced by the East Asian Monsoon, the Kuroshio Current and riverine input (Liu et al., 2015). The Yellow Sea shows typical characteristics of a large marine ecosystem, shallow but rich in

nutrients and resources (Tang et al., 2016). As shown in Fig. 1, the Yellow Sea has a warm northward current (the Yellow Sea Warm Current, YSWC). Southward currents (the Yellow Sea Coastal Current, YSCC) prevail near the sea coast. Sampling was conducted in the Yellow Sea from the research vessel *Beidou*. The sampling stations are shown in Fig. 1 and the supplemental material 1. A total of 50 stations were designed in order to cover the coastal and the offshore area. The MPs in seawater and zooplankton were sampled at the 50 stations in the period Aug. 20–Sep. 10, 2015.

2.2. Sample collection for MPs detection from seawater and zooplankton

Horizontal tows from behind the research vessel were performed using bongo nets (Hydro-Bios, Kiel, Germany) in order to sample MPs and zooplankton from the surface (0–60 cm) of the ocean. The net aperture was 60 cm in diameter, with a mesh size of 500 μm . With this mesh size, most MPs collected from seawater are larger than 500 μm , which is underestimated in terms of abundance and not comparable with the MPs ingested by zooplankton. The nets were towed at 2–3 knots for a period of 15 min at each station. We towed the net once for each station. The towed volumes were calculated from the readings of a flowmeter installed in the mouth of the net, ranging from 213 to 293 m^3 for different stations. The samples were then preserved immediately after collection in a 5% formaldehyde solution.

2.3. Isolation of MPs from seawater

For the MPs in seawater, the plastic-like fragments were carefully picked out from the collected samples by hand under a stereomicroscope (Stemi SV11, ZEISS, Shanghai, China). They were washed with deionized water, dried at room temperature, and archived for further analysis.

2.4. Analysis of zooplankton abundance

After isolation of MPs from seawater, the zooplankton abundance was analyzed with a ZooScan digital imaging system (Gorsky et al., 2010). Image standardization, separation, and data matrix acquisition were performed using the Zooprocess software. Automatic recognition by supervised-learning was performed with the Plankton Identifier software. The automatic classification of the zooplankton groups was manually validated to ensure it was correct (Sun et al., 2017). All zooplankton were classified into 12 groups, including 11 taxonomic groups and one other group for rare species. The abundance of each group was calculated by dividing the zooplankton abundance per net by the volume of filtered seawater. The 11 taxonomic groups make up the primary zooplankton composition, accounting for over 90% of the total zooplankton abundance in the study area.

2.5. Isolation of MPs from zooplankton

After the analysis of zooplankton abundance, the samples were used for isolating MPs from zooplankton. The classification of the zooplankton groups used for MP retention analysis was consistent with the 11 taxonomic groups used for abundance analysis (as shown in Fig. 3). Depending on the abundance of each zooplankton taxonomic group in each station's sample, 50–100 individuals from each group of each sample were selected by hand (all individuals were chosen if there were less than 50) under a stereomicroscope (Stemi SV11, ZEISS, Shanghai, China). The selected individuals were rinsed several times with deionized water, checked for the exterior part to make sure that no MPs were attached, and then placed in a

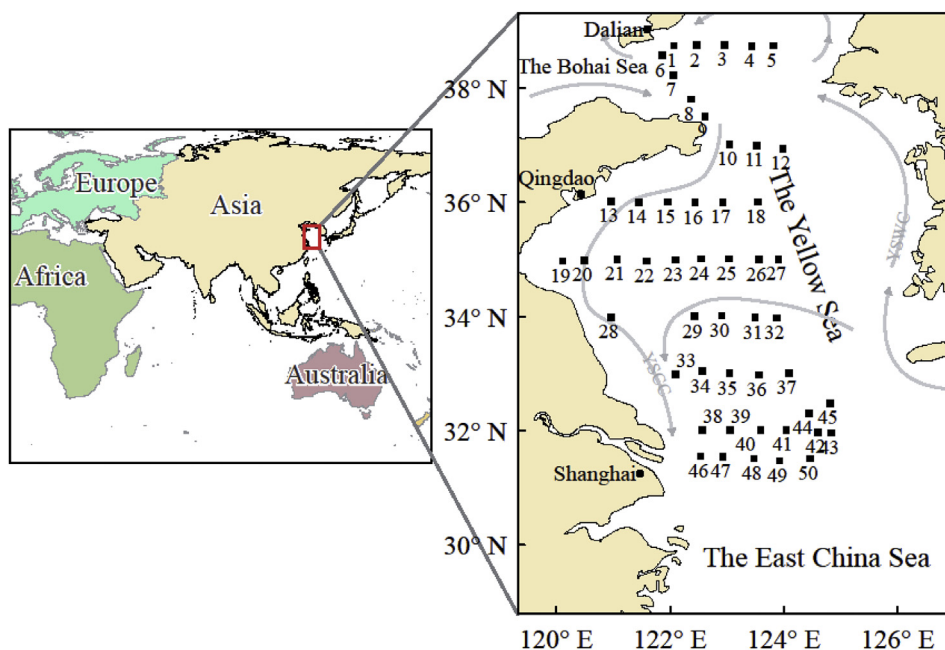


Fig. 1. Sampling stations for MPs in seawater and zooplankton in the Yellow Sea in the summer of 2015. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

20 mL scintillation vial. According to Desforges et al. (2015) 100% HNO_3 was added to each vial, and the vials were covered and heated in a water bath at approximately 80°C for 3 h until all tissue was digested. The digested samples were filtered through $0.45\text{-}\mu\text{m}$ mixed-cellulose ester filter membranes, and were then checked for MPs under a stereomicroscope. The major component of the mixed-cellulose ester filter membranes is refined nitrocellulose and cellulose acetate, which can avoid the contamination of the MPs from zooplankton due to their different component with plastic. Three blanks were run for each batch of samples to correct for potential airborne MPs deposition in the laboratory. Nylon, polyethylene terephthalate, and biopolymers (e.g., acetal, polyetheretherketone) may be affected by concentrated nitric acid (Desforges et al., 2015). The results we present here are thus conservative estimates of MP retention in zooplankton.

2.6. Measurements of the MPs detected from seawater and zooplankton

All MPs detected from the seawater and zooplankton samples were counted and imaged with an AxioCam HRc (Zeiss) connected to a stereomicroscope. The shape, size and chemical composition were measured. For shape composition, all MPs from seawater were divided into six groups, including fragment, fiber, foam, film, pellet and other. All MPs from zooplankton were divided into three groups: pellet, fiber and fragment (Supplemental material 2). For size composition, the length and width of each MP were measured manually using ImageJ software. After the image analysis, MPs were chosen randomly from each station and analyzed using $\mu\text{FT-IR}$ (Thermo Scientific Nicolet iN10 Infrared Microscope, Thermo Fisher, USA) to confirm their chemical composition ($n = 255$) (Supplemental material 3). The detection limit of the $\mu\text{FT-IR}$ is $10\text{ }\mu\text{m}$.

The bioaccumulated concentration of MPs for each taxonomic group at each station was determined by dividing the number of MPs found in zooplankton by the number of digested zooplankton (MP/zooplankton). At each station, the retention of MPs by the

zooplankton community as a whole was calculated as:

$$\sum_{i=1}^{11} (\text{MP/zooplankton of group } i \times \text{abundance of zooplankton group } i).$$

2.7. Data analysis

Seawater temperature and salinity were used to classify the survey area into different regions according to oceanographic characteristics of the water masses. The concentration of MPs in seawater and retention in zooplankton were compared among the classified areas. Multivariate analysis was conducted to test the potential influence of oceanographic variables on the distribution of MPs. The Getis-OrdGi was used to confirm the high and low value areas for the spatial distribution of MPs. Plots were created using Surfer 12.0 and Microsoft Excel 2010. All data are given as means \pm SD.

3. Results

3.1. Concentration and distribution of MPs in seawater and zooplankton

MPs were detected in seawater in 80% (40 of 50) of the Yellow Sea sampling stations. As shown in Fig. 2, the distribution of MPs across the study site is patchy, with high MPs concentrations close to the city of Dalian, Qingdao, and the Bohai Channel. The MPs concentration in seawater ranged from 0.00 to 0.81 pieces/m^3 , and the average concentration in the Yellow Sea was $0.13 \pm 0.20\text{ pieces/m}^3$.

MPs were detected in all of the 11 zooplankton taxonomic groups examined. The number of MPs per individual of the different groups varied from 0.07 MP/zooplankton for the Thaliacea, to 1.17 MP/zooplankton for the larger stomatopods (mantis

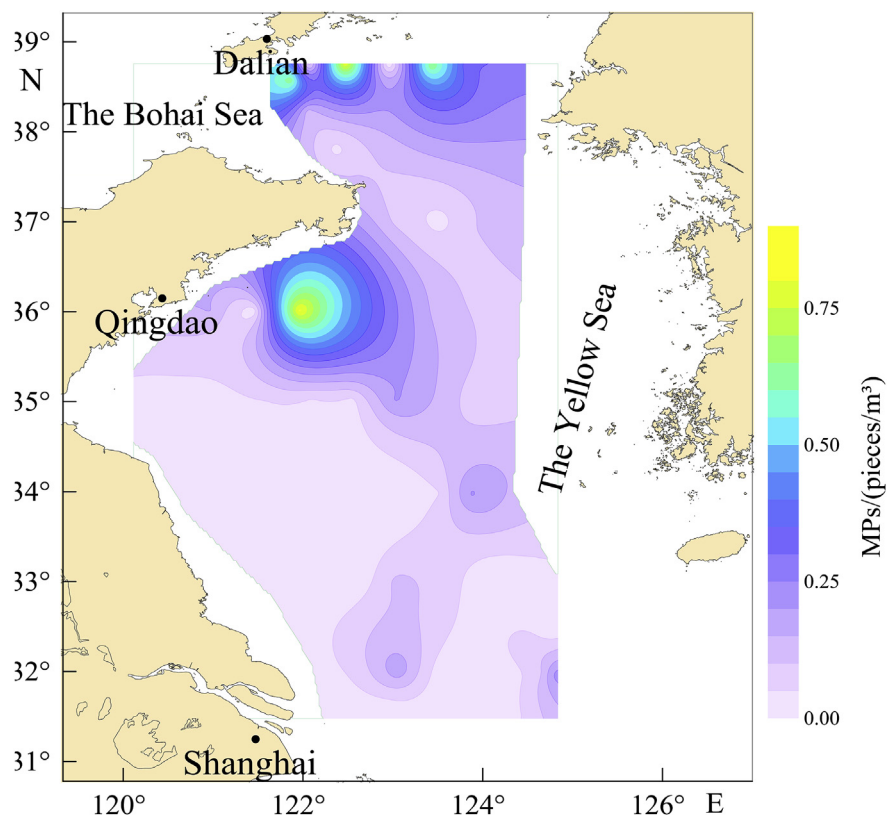


Fig. 2. Distribution of MPs in the surface water of the Yellow Sea. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

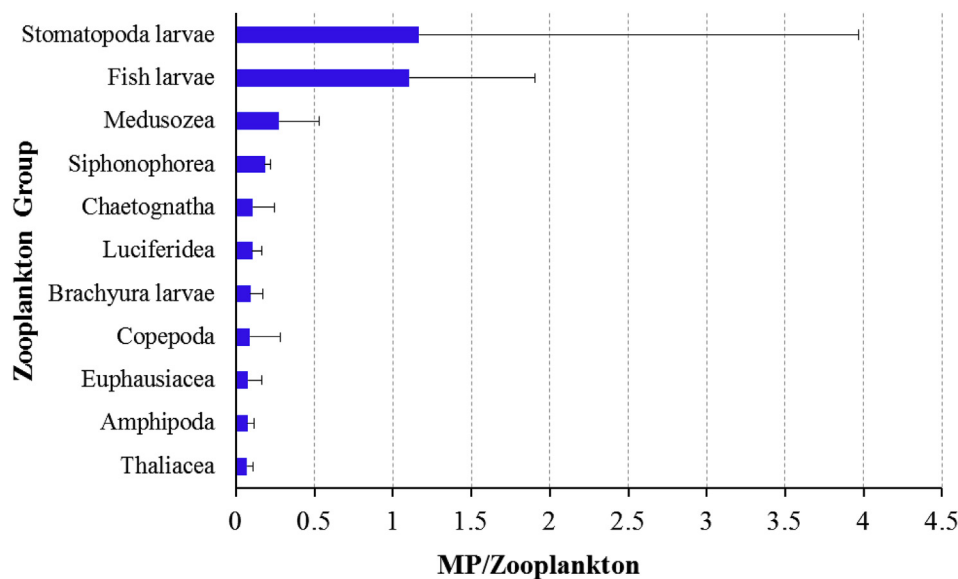


Fig. 3. Number of MPs per individual zooplankton, based on taxonomic group.

shrimps larvae; Fig. 3). The distribution and composition of zooplankton in the Yellow Sea is shown in Supplemental Material 4. The 11 groups tested for MPs accounted for more than 90% of the total zooplankton biomass based on abundance and biovolume. Copepoda, Euphausiacea, Chaetognatha and Siphonophorea were relatively higher in biomass than other zooplankton groups. By combining the MP/zooplankton and zooplankton abundances, the

number of MPs retained in different zooplankton groups and in the zooplankton community as whole are shown in Figs. 4–5. All distributions showed patchy characteristics. The number of MPs retained in the zooplankton community ranged from 0 to 151.34 pieces/m³, with an average of 12.24 ± 25.70 pieces/m³. The high concentration area appeared near the adjacent waters of the Yangtze estuary.

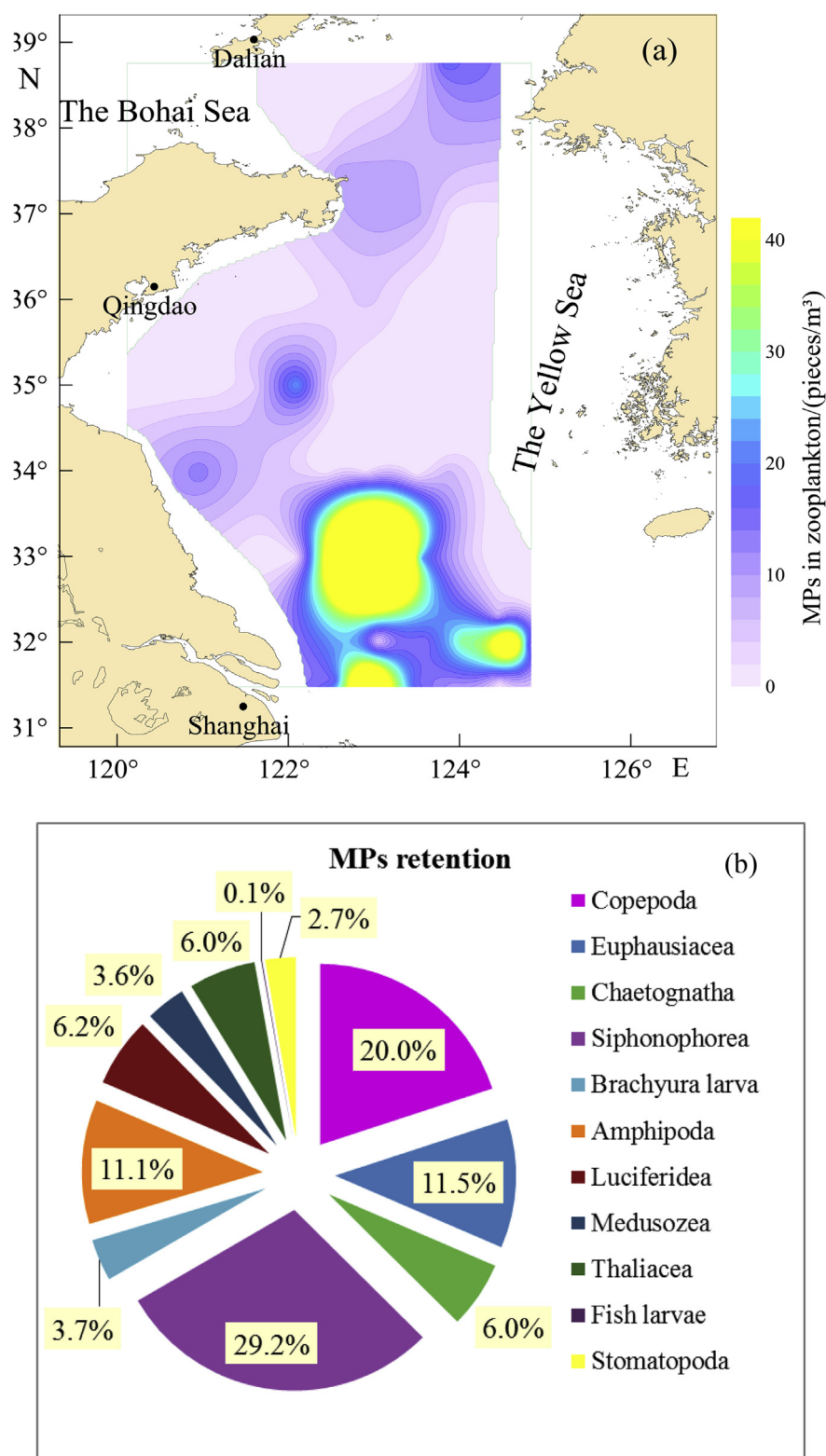


Fig. 4. Number of MPs retained in the zooplankton community as a whole in the Yellow Sea. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

a. Spatial distribution of MPs retention in total zooplankton community, b. Zooplankton group distribution of MPs retention.

By comparing the various zooplankton groups, it was found that most MPs were retained in Siphonophorea, Copepoda, Euphausiacea, and Amphipoda, accounting for 29%, 20%, 11%, and 11%,

respectively. The average retentions of MPs in these four dominant groups were 3.57 ± 9.57 , 2.44 ± 4.90 , 1.41 ± 3.87 , and 1.36 ± 7.39 pieces/m³. The spatial distribution of MPs depended on their

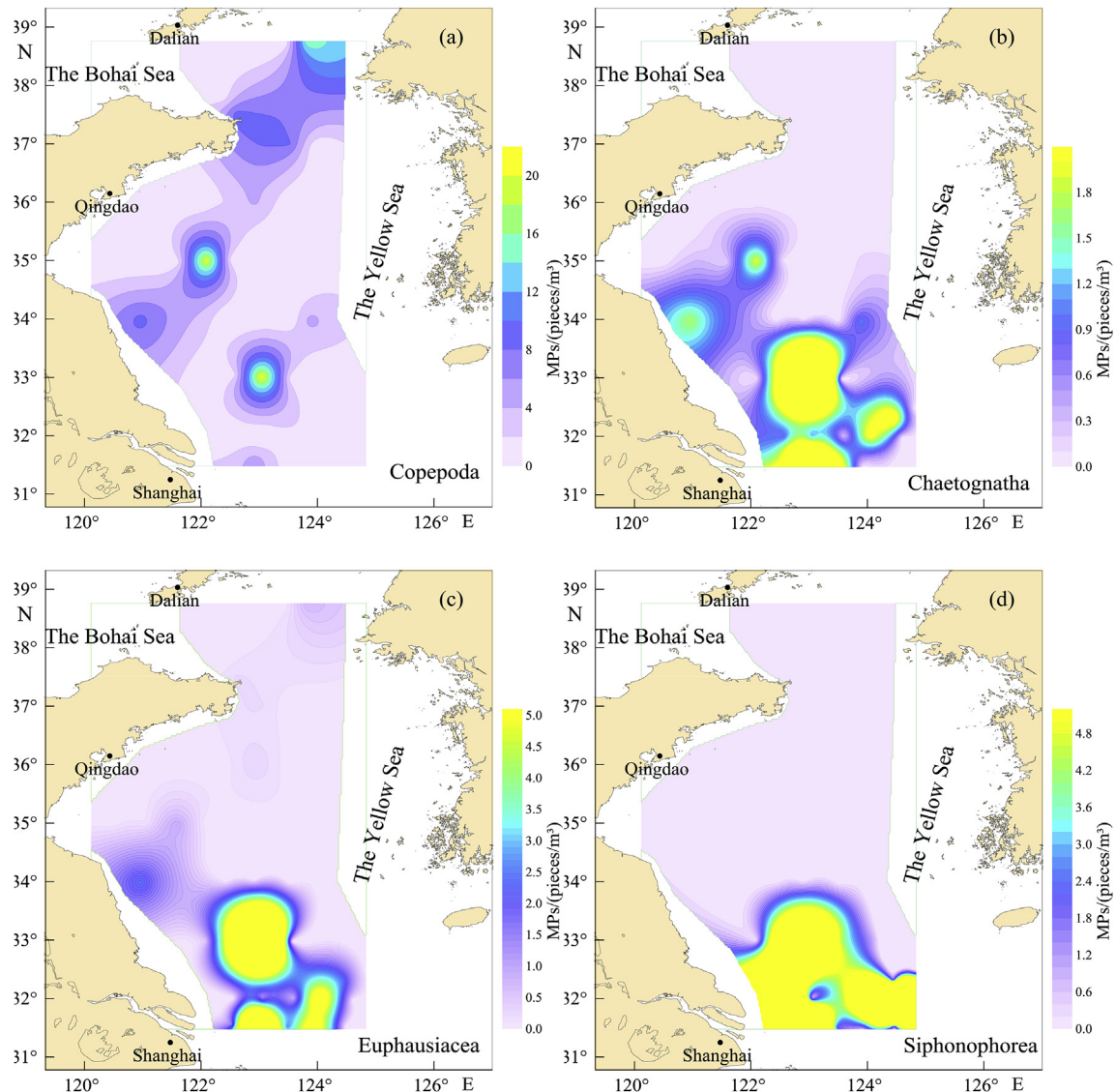


Fig. 5. Spatial distribution of MPs in different zooplankton taxa. a. Copepoda, b. Chaetognatha, c. Euphausiacea, d. Siphonophore.

abundance and the number of MPs per zooplankton in different areas; therefore, the MP retention varied for each group (Fig. 5).

3.2. Shape composition of MPs in seawater and zooplankton

The MPs in seawater are mainly fragments, accounting for 42% (Fig. 6). The percentage of film, foam and fiber was 22%, 19%, and 16% respectively. The major shape of MPs detected in zooplankton was fiber, accounting for 46%. The percentage of both fragment and pellet was 27%. For different zooplankton groups (Fig. 7), the percentage of fiber, fragment, and pellet varied. As the dominant shape, fiber ranged from 28.6% (Brachyura larva) to 55.9% (Siphonophorea).

3.3. Size composition of MPs in seawater and zooplankton

The size composition of MPs in seawater and zooplankton is shown in Fig. 8. Owing to the mesh size for collecting MPs from seawater being 500 μm , the small-sized MPs from seawater are neglected in this research. The length of the plastics detected in

seawater ranged from 0.35 to 44.99 mm, with an average length of 3.72 ± 4.70 mm (Fig. 8a). The MP particle size that appeared with the maximum frequency was 1200 μm – indeed, over 82% of the MPs were longer than 1200 μm in seawater. The length of MPs detected in zooplankton ranged from 9.86 to 996.75 μm , with an average of 154.62 ± 152.90 μm (Fig. 8b). Of the MPs in zooplankton, 71.45% were <200 μm and 95.76% were <500 μm in length, suggesting that zooplankton act as the carrier for small sized MPs.

3.4. Chemical composition of MPs in seawater and zooplankton

The chemical compositions of MPs detected in seawater and zooplankton are summarized in Table 1. Six types of MPs were detected in seawater, with low-density plastics polyethylene (PE) and polypropylene (PP) accounting for 55.93% and 32.20% of the total, respectively. The chemical composition of MPs in zooplankton were more varied: 15 types of MP were detected, with organic oxidation polymers (21.88%), poly-octenes (21.88%), and PP (12.5%) being the most abundant. PE accounted for only 4.69% of

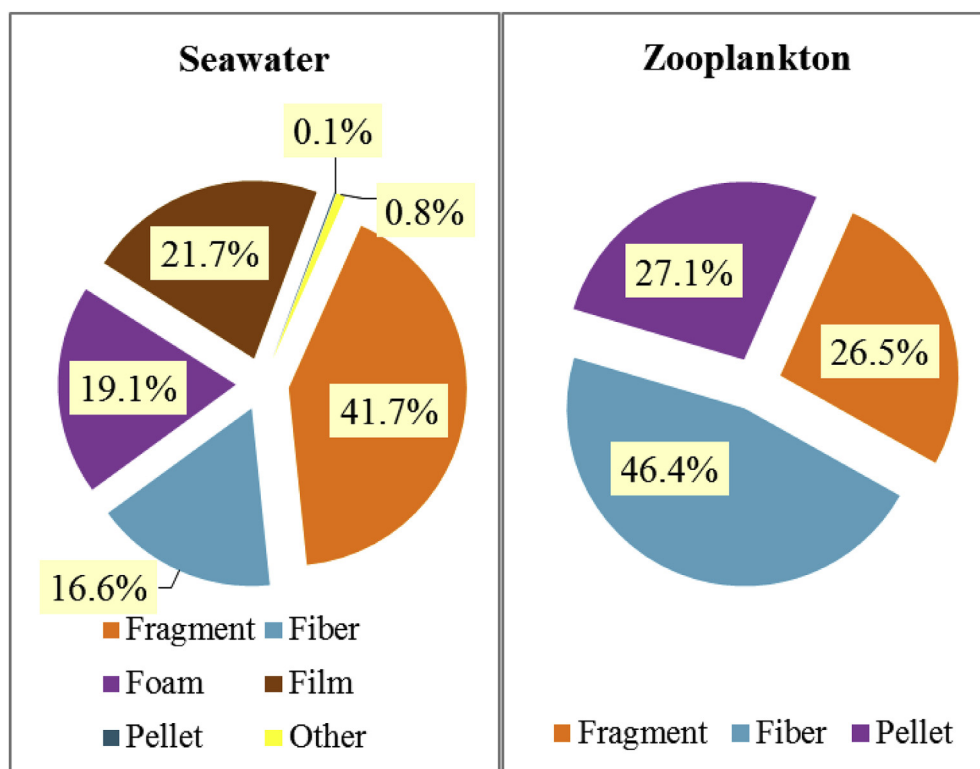


Fig. 6. Shape composition of MPs in seawater and zooplankton.

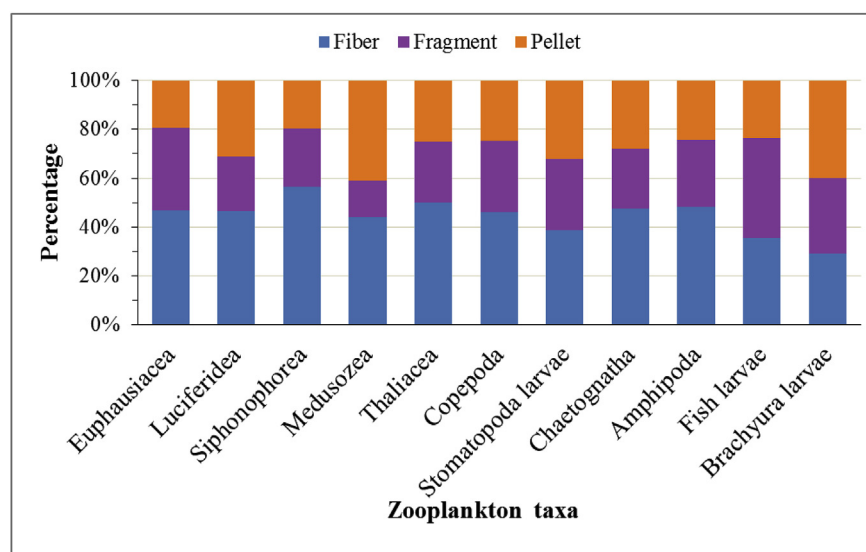


Fig. 7. Shape composition of MPs in seawater and different zooplankton taxa in the Yellow Sea. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

MPs in zooplankton. The proportion of the low-density plastics (i.e. PE and PP combined) in zooplankton was only 17.19%.

4. Discussion

4.1. MP distribution and composition in seawater and zooplankton in the Yellow Sea

Although China was considered a hot spot of plastic debris pollution (Jambeck et al., 2015), the average concentration of MPs in

seawater of the Yellow Sea is only 0.13 piece/m³. This concentration is similar to some reported coastal areas, such as the Mediterranean Sea (Collignon et al., 2012), the East China Sea (Zhao et al., 2014), but far lower than the north pacific area (Moore, 2008; Moore et al., 2001, 2002). Microplastic distribution is a complex issue affected by many factors that include physical, chemical, and biological processes. Most MPs in the Yellow Sea were concentrated in the northernmost part of the sea, which was confirmed by the Global G statistic for spatial autocorrelation (Fig. S6-2). Based on the oceanographic variables, the seawater was divided into three water

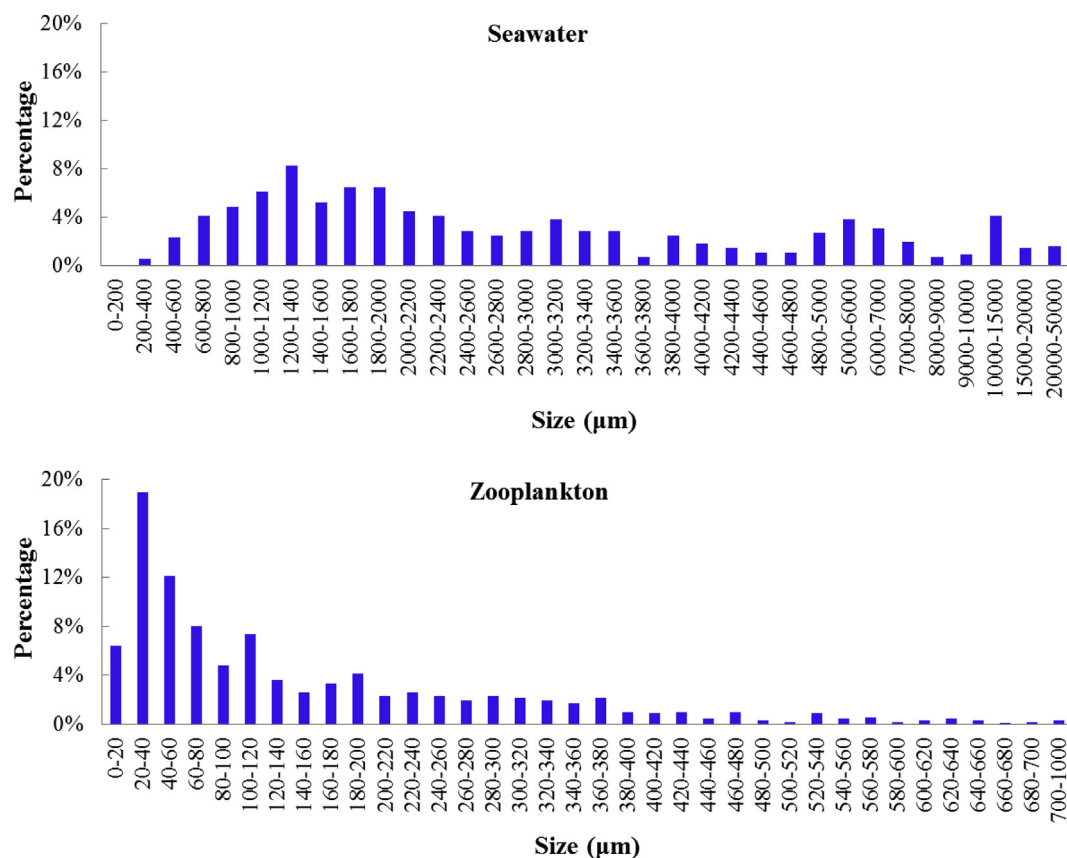


Fig. 8. Size distribution of MPs in seawater and zooplankton.

masses (S6, Fig. S6-1): the Yellow Sea Coastal Water Mass, the Yellow Sea Central Water Mass and the South Yellow Sea High Salinity Water Mass. Although the MPs in the three water masses

Table 1

Main chemical composition of MPs detected in seawater and zooplankton in the Yellow Sea.

MP polymer type	Composition by sample group (%)	
	Zooplankton	Seawater
Organic oxidation polymer	21.88	0
Polyethylene	4.69	55.93
Polyamide (Nylon)	6.25	0
Polymethyl methacrylate	1.56	0
Polypropylene	12.5	32.2
Polybutylene terephthalate	0	0
Poly (1-dodecene)	0	0
Poly (epichlorohydrin)	0	0
Polystyrene	0	6.78
Alkyd resin	7.81	0
Chlorinated polyolefin resin	0	0
Poly (vinyl alcohol)	0	0
Polyethylene glycol terephthalate	1.56	0
Polyurethanes	0	0
Polyamide (Nylon 6.66)	0	1.69
Poly (1-octene)	21.88	0
Poly (vinyl chloride)	4.69	0
Polydimethylsiloxane	3.13	0
Poly (2-butylene)	3.13	0
Poly (2-decyne)	3.13	0
Poly (hydroxyethyl ethylene oxide)	3.13	0
Polyoxyethylene	1.56	0
Poly (methylcaprolactam)	1.56	0
Poly (acrylic acid methylester)	0	1.69
Poly (ethyl acrylate, styrene, acrylamide)	0	1.69

did not have a significant difference in concentration, the MPs in seawater are positively correlated with salinity (S6), indicating that water mass is a critical factor in the distribution of MPs. The overall linear fit is not significant, suggesting that some processes are driving the water mass and the MPs accordingly. The Yellow Sea is mainly affected by two currents, the YSWC and YSCC. We speculate that the primary reason for MP distribution is that this area is affected by the YSCC and the nearby Bohai Sea. The Bohai Sea is denoted as the area with the most serious human activities in China. According to recent findings, the MPs in the Bohai Sea are significantly higher in concentration than the Yellow Sea regions (Zhao et al., 2018). Moreover, the Yellow Sea's summer wind is predominantly south or southeast, which provides favorable conditions for surface MPs accumulation in the northern region. In addition, since the biomass in the north is not high, MPs transferred from seawater to other media through biological processes is not common.

The higher concentrations of MPs around big cities and the Bohai Sea indicate that human activities were a major influence on the distribution in seawater. This was consistent with the pattern observed in other areas (Browne et al., 2011; Desforges et al., 2014). However, the relatively low concentrations indicated that some simultaneous processes played important roles in removing MPs from seawater. These may be physical processes, such as shore deposition, nano-fragmentation, or biological processes, such as predation, biofilm etc., but still need further confirmation from comprehensive research.

By comparison of the zooplankton MPs retention in the three areas (Fig. S6-1), the south is significantly higher than the middle and northern parts of the Yellow Sea. The negative correlation between MP retention in zooplankton and salinity demonstrates that

water mass is associated with zooplankton MPs distribution. The high retention of MPs in zooplankton in the adjacent area of the Yangtze Estuary is mainly due to the high abundance of zooplankton in this area. By comparison with the distribution of zooplankton in the Yellow Sea (S4) and retention of MPs in zooplankton (Fig. 4a), their spatial patterns are similar, indicating that the abundance of zooplankton is a key factor for the retention of MPs. The MPs in different zooplankton groups had inconsistent retention amounts and distribution patterns, indicating that composition of the zooplankton community can also be an important factor that affects the distribution of MPs in zooplankton. Net feeding of MPs was previously reported in mesopelagic fish samples in the Pacific Ocean, but was not high, i.e., approximately 1.4% (Davidson and Asch, 2011). It was possible for zooplankton to ingest MPs in the cod end of the net during sampling, but this needs to be confirmed quantitatively by further research.

It was found that the accumulation of MPs in zooplankton is mainly in the form of fibers and small-sized MPs in the Yellow Sea, indicating that there should be a large number of MPs with similar shape and size in seawater. The small-sized MPs in seawater were mostly ignored in this research due to the mesh size of the sampling net. The technical limitation on the observation of small-sized MPs from seawater is a bottleneck to obtain detailed information on the small-sized MPs of seawater in current research (Burton, 2017). Development of small MP observation technology is urgently needed.

The chemical composition of MPs in seawater of the Yellow Sea is similar with other reported areas (Auta et al., 2017; Avio et al., 2017), with low-density plastics PE and PP absolutely dominating. A total 43% of polymers found in zooplankton were organic oxidation polymers and poly-octenes. The organic oxidation polymers include a series of polymers, such as polyethylene oxide, polypropylene oxide glycol, polyphenylene oxide, etc. This type of plastic has a wide range of applications in chemical industries like papermaking, coatings, inks, textile printing and dyeing, indicating that industries are their main sources. Poly-octene is mainly used in the rubber industry, such as the manufacture of tires, pipes, and various rubber molded products. These plastic types are usually denser than PE and PP, and the retention of these MPs in zooplankton is likely due to the vertical migration of zooplankton, which enables them to ingest MPs throughout the whole water column.

4.2. Marine zooplankton is a large repository of MPs in the Yellow Sea

Our observation of the Yellow Sea shows that, in addition to floating in seawater, MPs were widely present in different zooplankton taxonomic groups. The MP/zooplankton is several times higher in the Yellow Sea compared to other seas. The most abundant zooplankton copepod had a bioaccumulated concentration of 0.03 MP/zooplankton in the northeast Pacific Ocean (Desforges et al., 2015) and 0.05 MP/zooplankton in the northern South China Sea (Sun et al., 2017). The number of MPs in copepod in the Yellow Sea was 3.1 times of the northeast Pacific Ocean, and 1.8 times of the northern South China Sea. Many previous observations of marine MPs have focused solely on MPs floating in seawater or the total MPs in seawater and zooplankton, rather than quantitatively examining MPs retained by different marine organisms. This study demonstrated that, as key components of marine pelagic ecosystems, zooplankton can be a big repository for MPs in the ocean. Only mesozooplankton larger than 500 μm were studied for retention of MPs in this research. The total retention number of MPs for zooplankton collected by a smaller-mesh-sized (170 μm) net could be 33-fold of those collected by a 500 μm net (Sun et al.,

2017), indicating that the actual zooplankton repository of MPs should be much larger than the amount obtained in this study.

Given the high marine biodiversity and biomass of many marine ecosystems, and the reported number of MPs in various marine taxa, such as fish, other nekton, and marine benthos (Lusher et al., 2013; Rist et al., 2016), we believe that the biological repository is likely to be much higher than the zooplankton repository if extrapolated to other marine life. This provides some evidence for the reported missing MPs (Cózar et al., 2014, 2015) which were thought to be transferred by zooplankton to the ocean interior. Considering that the MPs in zooplankton are mainly small-sized MPs, they are usually ignored in routine seawater MPs sampling. With new detection methods to reconstruct the full particle size spectrum of MPs in seawater, it is possible to truly estimate the contribution of zooplankton in the absence of seawater MPs. It is necessary to consider the amount of MPs in seawater and in marine biota separately, to reveal the fate of MPs in the ocean and for a rational ecological risk assessment.

4.3. Retention of MPs in biota can be an index for ecological risk assessment

At present, studies on MPs in the field focus more on the distribution, concentration, and composition of MPs in surface seawater. The MPs observations in the Yellow Sea showed that the MPs in seawater and zooplankton are completely different in concentration, shape, size and chemical composition. This is largely due to the bottleneck in MP observation technique. Most reported MPs collected from seawater were larger than 330 μm . Few attempts have been made to study smaller sized MPs because of methodological challenges (Burton, 2017). Although some new methods, such as Nile Red, were proposed for the detection of 20 μm –1 mm MPs in seawater (Erni-Cassola et al., 2017), they are not widely used. It may be more practical to conduct related controlled experiments, exposure concentration tests, and ecological risk assessments based on the concentration and characteristics of MP retention in marine biota before the technical bottleneck can be resolved.

It was postulated that most MPs debris would be egested, although some MPs debris would remain in the intestinal tracts of zooplankton (Cole et al., 2016). According to Vroom et al. (2017), the majority of tested copepods (94.3%) of *Calanus finmarchicus* that ingested plastics, egested these within 2–4 h, while 5.7% of them egested within 18 h after moving them to seawater without the presence MPs. Rist et al. (2017) found that the complete egestion of MPs by *Daphnia magna* did not occur within 24 h. These results indicate that most ingested MPs exert an influence on zooplankton in very brief time, and only the retention affects zooplankton long term. Therefore, by combining the size, shape, and chemical component of MPs in zooplankton, the retention amounts in different taxonomic groups can be used as a reference value to set the concentration gradient for long term exposure experiments. For short term exposure, it is important to consider the ingestion, retention and egestion rate, or the ratio between the number of MPs ingestion to retention. These parameters are important for determining ecologically relevant metrics (ERMS) of a region. The different values of MP/zooplankton for the observed zooplankton groups indicate that the retention of MPs highly depends on the taxa and region. The above parameters may change with region and taxa, so baseline studies are necessary for different regions.

4.4. Further research on the cycling of MPs in marine ecosystems

The universality of MPs in different marine habitats, and the ingestion, retention, and transfer by zooplankton prove that MPs

are cycled through the entire marine ecosystem. To fully understand this cycling is a long-term task. Understanding the abundance, characteristics, fate, and ecological risk of MPs in the ocean is a prerequisite in order to do so. Future MPs observations should not be limited to seawater and sediments, but should extend to the biological domain.

For the cycling of MPs in a marine ecosystem, it is wise to study the ingestion, retention, and egestion by different zooplankton groups and other marine taxa in different regions by combining controlled experiments and *in situ* observations. This would be crucial for not only MPs simulations, but also for determining the biogeochemical cycles of the complex chemicals absorbed onto MPs. Thus, to reveal the complicated behavior of MPs in the ocean, quantifying the flux of MPs among the various compartments of the cycle is required.

For a rational ecological risk assessment, it is important to investigate the feeding behavior and retention of marine zooplankton and other taxa in different seas, and compare the variability and study the key processes affecting their behavior. Further research on the potential adverse effects from the feeding behavior of marine zooplankton on MPs, including the size, shape, chemical composition, concentration is highly suggested. Aged MPs are encouraged to be used for the experiments considering that the aged MPs are different than the virgin ones (Vroom et al., 2017).

Model simulations are useful tools for guiding field surveys aimed at assessing the magnitude of global marine plastic pollution (Brunner et al., 2016; Sherman and van Seville, 2016). However, the low level of agreement between actual data and model predictions is a major problem (Cózar et al., 2015). We recommend including biological processes in such models. The coupled physical-biological model can be considered for future modeling on marine MPs.

Finally, it is urgent to improve the observation technique of small-sized MPs in various habitats, to better estimates of MPs concentrations in the ocean. Sampling and analysis methods should be standardized globally so that the results of different seas are comparable. More improvement is required to achieve a successful, standard observation and comparison of MPs cycling in various regions.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.envpol.2018.07.014>.

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