



# Ingestion of microplastics by natural zooplankton groups in the northern South China Sea



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## ABSTRACT

The ingestion of microplastics by five natural zooplankton groups in the northern South China Sea was studied for the first time and two types of sampling nets (505  $\mu\text{m}$  and 160  $\mu\text{m}$  in mesh size) were compared. The microplastics were detected in zooplankton sampled from 16 stations, with the fibrous microplastics accounting for the largest proportion (70%). The main component of the found microplastics was polyester. The average length of the microplastics was 125  $\mu\text{m}$  and 167  $\mu\text{m}$  for Nets I and II, respectively. The encounter rates of microplastics/zooplankton increased with trophic levels. The average encounter rate of microplastics/zooplankton was 5%, 15%, 34%, 49%, and 120% for Net I, and 8%, 21%, 47%, 60%, and 143% for Net II for copepods, chaetognaths, jellyfish, shrimp, and fish larvae, respectively. The average abundance of microplastics that were ingested by zooplankton was 4.1 pieces/ $\text{m}^3$  for Net I and 131.5 pieces/ $\text{m}^3$  for Net II.

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## 1. Introduction

The widespread occurrence and accumulation of plastic waste in the environment has become a growing global concern over the last decade (Lonnstedt and Eklov, 2016). Microplastics, the small-sized plastic fragments of <5 mm in size (Moore, 2008), have recently drawn increasing attention due to its high abundance in sea water (Ng and Obbard, 2006) and bioavailability to organisms throughout the food-web (Cole et al., 2011). The sources of these microplastics are extensive, mainly including raw materials used in the plastic industry, plastic particles, plastic debris from the large plastics degraded by various physical processes, additives from common daily items, and polishing material used in the industrial production (Fendall and Sewell, 2009; Thompson et al., 2009). In the past years, most studies have focused on quantifying microplastics abundance in the marine environment (Hidalgo-Ruz et al., 2012). The studies mentioned above covered the coastal area of different countries, the open ocean, and the deep sea (Barnes et al., 2009; Van Cauwenberghé et al., 2013; Law and Thompson, 2014), providing useful information on the background concentrations of microplastics in various environments.

Microplastics can affect marine organisms physically by blocking the alimentary tract upon ingestion (Cole et al., 2013), and chemically by toxic pollutants contained in or absorbed by the plastics (Rochman et

al., 2013). Laboratory experiments have indicated that a variety of invertebrates ingest microplastics, including planktonic organisms such as copepods, cladocerans, salps and larval fish (Brown and Heseltine, 1968; Wilson, 1973; Frost, 1977; Kremer and Madin, 1992; Cole et al., 2013; Lonnstedt and Eklov, 2016), and benthos such as lugworms, amphipods, barnacles, holothurians, and blue mussels (Thompson et al., 2004; Browne et al., 2008; Graham and Thompson, 2009; von Moos et al., 2012; Wegner et al., 2012). The majority of the laboratory experiments have revealed the impact of microplastics on the feeding, growth, physiological function, immune system, and the development of the relevant marine biota. In natural ecosystems, microplastic fibers have been reported in Norway lobster (Murray and Cowie, 2011) and Chinese mitten crab (Wójcik-Fudalewska et al., 2016), pelagic and demersal fish (Lusher et al., 2013), mesopelagic fish (Boerger et al., 2010), gooseneck barnacles (Goldstein and Goodwin, 2013), bivalves (Van Cauwenberghé and Janssen, 2014), and zooplankton (Frias et al., 2014; Desforges et al., 2015). The ingestion and accumulation of microplastics in a wide range of marine species ranging from zooplankton to bivalves, crustaceans, and fish indicate the potential for microplastics to accumulate in the marine food chain (Vandermeersch et al., 2015). However, information on the ingestion of microplastics by natural populations and their possible accumulation is scanty (Lusher et al., 2013).

In pelagic ecosystems, zooplankton links the primary producers and the higher trophic levels, thus playing an important role in the marine food web. The ingestion of microplastics by zooplankton serves as an important link to the marine food web, transferring these materials to higher trophic levels along the food chain. It is thus essential to

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understand the ingestion and transfer of microplastics by different groups of zooplankton in natural sea water in order to lay a foundation for the ecological risk assessment of microplastics in natural ecosystems.

The coastal area of China is a hotspot for microplastic pollution (Zhao et al., 2014). Both the diluted water from the Pearl River Estuary and the waters of the South China coast have affected the northern part of the South China Sea. China's annual fishing output in the South China Sea is about  $3 \times 10^6$  t, and the northern South China Sea is an important fishery ground (Wang et al., 2015). The impact of microplastics on the fishery resources through the transfer of zooplankton is thus of prime concern. To study the ingestion and transfer of microplastics in marine zooplankton in the natural ecosystem, the present study, for the first time, investigated the ingestion of microplastics by five groups of zooplankton in the coastal area of China, which include copepods, chaetognaths, jellyfish, shrimps, and fish larvae. The purpose of this research was to: 1) identify the characteristics of microplastics that are ingested by different groups of zooplankton, 2) determine the encounter rates between microplastics and zooplankton, 3) estimate the abundance of microplastics ingested by zooplankton, 4) discuss the transfer of microplastics between trophic levels in the northern South China Sea.

## 2. Materials and methods

### 2.1. Study area

The study area was the northern part of the continental slope of the South China Sea, which encompassed the continental shelf, slope, and deep water area. Samples were collected in June 2015 using the research vessel, Nan Feng. The sampling stations used in the present study are shown in Fig. 1.

### 2.2. Sampling

The zooplankton samples were collected by vertical tows using conical plankton nets from a depth of 200 m, or 10 m off the seabed when the depth was  $<200$  m, to the surface. The nets were designed according to the Specifications for the Oceanographic Survey of China (2007). Two types of nets were used to compare the ingested microplastics collected by nets with different mesh sizes. Net I was 145 cm in length, 50 cm in the inner diameter of the net mouth, and 505  $\mu\text{m}$  in mesh size. Net II was 140 cm in length, 31.6 cm in the inner diameter of the net mouth, and 160  $\mu\text{m}$  in mesh size. The zooplankton samples were preserved immediately after collection in a formaldehyde solution (final concentration 5%). All samples were split into two equal parts. One part was used for zooplankton abundance analysis, and the other was utilized for microplastics analysis.

### 2.3. Zooplankton analysis

The zooplankton samples were analyzed with a ZooScan digital imaging system, which was developed in the Laboratory of Oceanography of Villefranche (LOV) (Gorsky et al., 2010). The zooplankton samples were split into suitable fractions with a Motoda Plankton Splitter (Motoda, 1959) until the subsample was dilute enough to allow separation of all organisms in the scanning tray. The samples that were collected using Nets I and II were digitized with the ZooScan at 2400 dpi and 4800 dpi resolution, respectively. 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 zooplankton was manually validated to ensure the correct classification of the zooplankton groups. The abundance of each group was determined based on the zooplankton abundance per net, which was divided

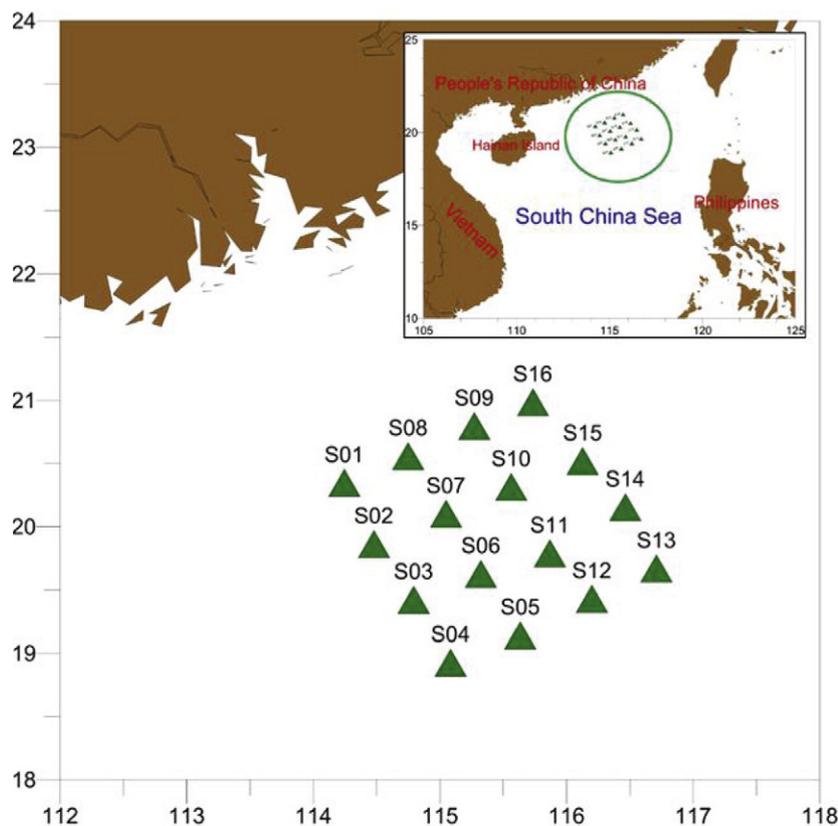


Fig. 1. Sampling stations in the northern part of the South China Sea.

by the volume of filtered seawater. Based on the composition of the zooplankton in the studied area, five predominant groups, including copepods, chaetognaths, jellyfish, shrimps (Euphausia and other species of shrimp), and fish larvae were selected for microplastics analysis based on their importance in the marine food web of the South China Sea. The percentage of the five groups accounted for 80% of the total zooplankton abundance or biovolume.

#### 2.4. Microplastics analysis

Each sample was checked at 6–12 $\times$  magnification under a stereomicroscope (Stemi SV11, ZEISS, Shanghai, China). In each station, 50 copepods, 20 chaetognaths, 10 jellyfishes, 10 shrimps, and several fish larvae (all fish larvae in a sample) were picked out from the samples under a stereomicroscope. The selected individuals were rinsed with deionized water several times. Each group was placed in a 20-mL scintillation vial. To destroy their body tissue and then examine the remaining material for microplastic particles, the 100% HNO<sub>3</sub> digestion method described by Desforges et al. (2015) was performed. A 100% HNO<sub>3</sub> solution was poured into the scintillation vials until the samples were submerged. After 3 h of digestion in a water bath at approximately 80 °C, the samples were filtered through 0.45- $\mu$ m mixed-cellulose ester filter papers, and the filter papers were examined under a stereomicroscope for completeness of digestion as well as the presence of microplastics. Several blanks (HNO<sub>3</sub> in an empty vial) were run to correct for potential air-borne particle deposition in the laboratory, and no contamination of blanks was observed during the experiments.

When microplastics were detected, the particles were counted, and images of each microplastic particle were captured using an AxioCam HRc (ZEISS) that was connected to a stereomicroscope. The length of

each microplastic was measured manually using the Image J software. The microplastic particles were analyzed by using the  $\mu$ FT-IR technique to confirm their composition.

#### 2.5. Data analysis

Data analysis was performed using the SPSS software (SPSS 17.0). Independent-samples *t*-test was used to compare differences in encounter rates and the size of the microplastic particles between Nets I and II. One-way ANOVA was used to compare the encounter rates and microplastic size among different zooplankton groups. Plots were created using SigmaPlot 12.5 and Microsoft Excel 2010.

### 3. Results

#### 3.1. Characteristics of the microplastics ingested by zooplankton

Fig. 2 shows that the microplastics that were ingested by zooplankton varied in shape and size, which included fibrous, particles, and other irregular shapes. The length of the microplastic particles ranged from 4  $\mu$ m to 1037  $\mu$ m for Net I, and from 5  $\mu$ m to 2399  $\mu$ m for Net II, and with an average length of 125  $\mu$ m and 167  $\mu$ m, respectively.

The lengths of the microplastic particles ingested by different zooplankton groups are shown in Fig. 3. The average length of the microplastic particles was 0.14, 0.12, 0.11, 0.13, and 0.10 mm for Net I, and 0.15, 0.20, 0.16, 0.19, and 0.09 mm for Net II for copepods, chaetognaths, jellyfish, shrimp, and fish larvae, respectively. No significant differences in the lengths among the five groups using both nets were detected ( $p > 0.05$ ).



Fig. 2. Microplastics ingested by zooplankton in the northern part of the South China Sea.

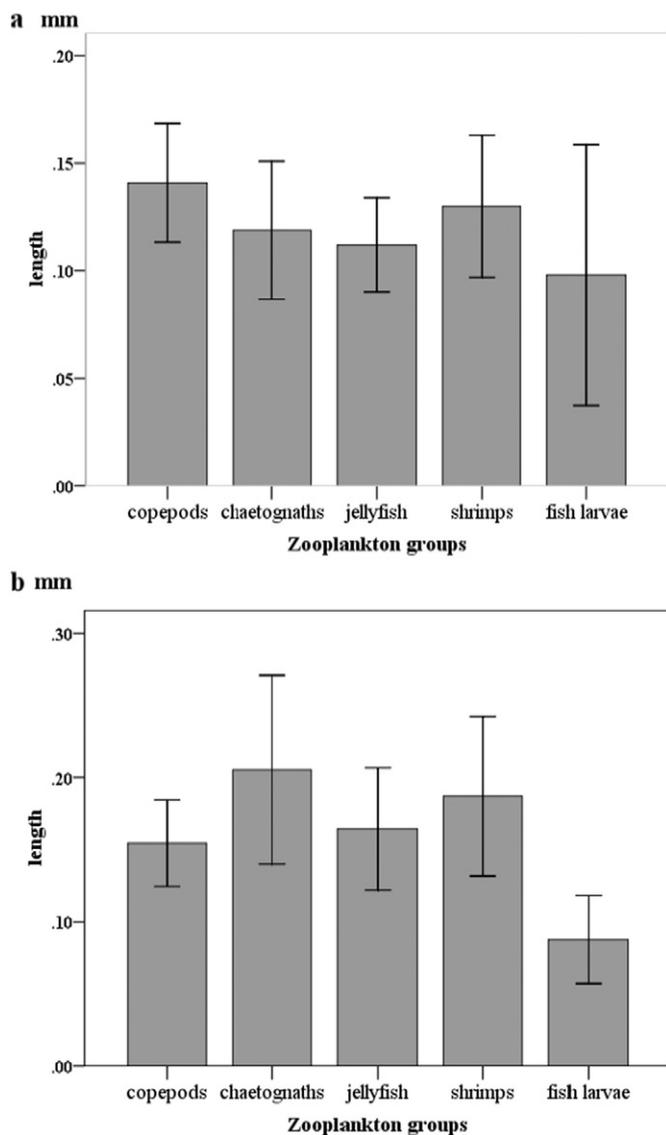


Fig. 3. Size of the microplastics ingested by different zooplankton groups (a. Net I, b. Net II).

The microplastics ingested by the zooplankton collected by using two types of nets showed similar composition. In general, fibrous microplastics accounted for the largest proportion (70%). The percentages of different forms of microplastics varied among various zooplankton groups. The fibrous microplastics accounted for a large proportion in copepods, and showed a decreasing trend from chaetognaths to fish larvae. However, the amount of particle microplastics increased from copepods to fish larvae, from 21% to 58% for Net I and from 24% to 42% for Net II (Fig. 4).  $\mu$ FT-IR analysis indicated that the main component of the fibrous microplastics was polyester (Fig. 5).

### 3.2. The encounter rates between microplastics and zooplankton

The microplastics were detected in the zooplankton from all sampled stations. The range of encounter rates between microplastics and zooplankton for Net I was from 2%–6%, 0–43%, 0–50%, 10%–167%, and 0–200%, with average encounter rates of 5%, 15%, 34%, 49%, and 120% for copepods, chaetognaths, jellyfish, shrimp, and fish larvae, respectively (Fig. 6a). The range of encounter rates between microplastics and zooplankton for Net II was 4%–12%, 5%–35%, 20%–100%, 25%–200%, and 60%–300%, with average encounter rates of 8%, 21%, 47%, 60%, and 143% for the five zooplankton groups, respectively (Fig. 6b).

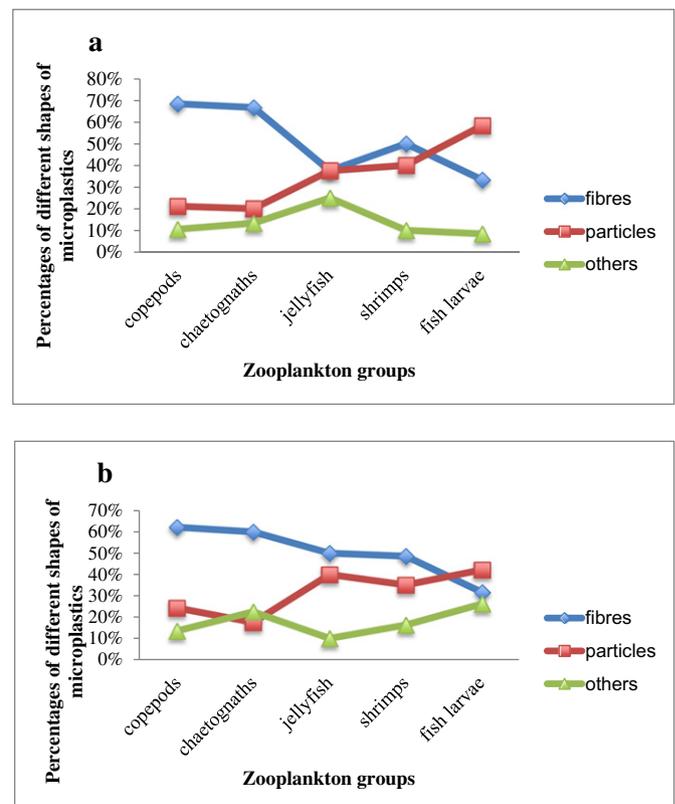


Fig. 4. Percentages of shapes of microplastics (a. Net I, b. Net II).

Comparison of the contents of the two nets indicated that the encounter rates between microplastics and zooplankton of Net II were higher than those of Net I. Comparative analysis of the five zooplankton groups showed that the encounter rates with microplastics increased from copepods to chaetognaths, jellyfish, shrimp, and fish larva. Similar trends were observed in both types of nets, with higher encounter rates at higher trophic levels.

The encounter rates between microplastics and zooplankton varied among stations. Fig. 7 shows that for the Net I zooplankton, the total encounter rates were high in stations 1, 2, 3, 9, and 12, and low in stations 4, 10, and 11. For the Net II zooplankton, the total encounter rates were high in stations 1, 2, 3, 9, 12, and 16, and low in stations 6, 10, and 11. The spatial distribution of the encounter rates was similar for both types of nets.

### 3.3. The abundance of microplastics ingested by zooplankton

By combining the encounter rates and the abundance of the five zooplankton groups, the abundance of the microplastics that were ingested by the predominant groups in the upper 200 m water column was estimated. Fig. 8 shows the abundance of the microplastics ingested by Net I zooplankton was 2.19, 0.67, 0.12, 0.81, and 0.29 pieces/m<sup>3</sup> for copepods, chaetognaths, jellyfish, shrimps, and fish larvae, respectively. The microplastics were mainly ingested by copepods, accounting for 54% of the total number of particles consumed, followed by shrimps, 20%. The microplastics ingested by Net II zooplankton were much higher than those of Net I. The abundance of the ingested microplastics was 103.49, 20.03, 2.83, and 5.16 pieces/m<sup>3</sup> for copepods, chaetognaths, jellyfish, and shrimps, respectively. Similar to the Net I, microplastics were mainly ingested by copepods, accounting for 79% of the total number of particles consumed, followed by chaetognaths, 15%. The abundance of the fish larvae was not estimated due to the extremely low number of organisms captured by using Net II. In contrast with the encounter rate, which increased with trophic levels, the total abundance of the

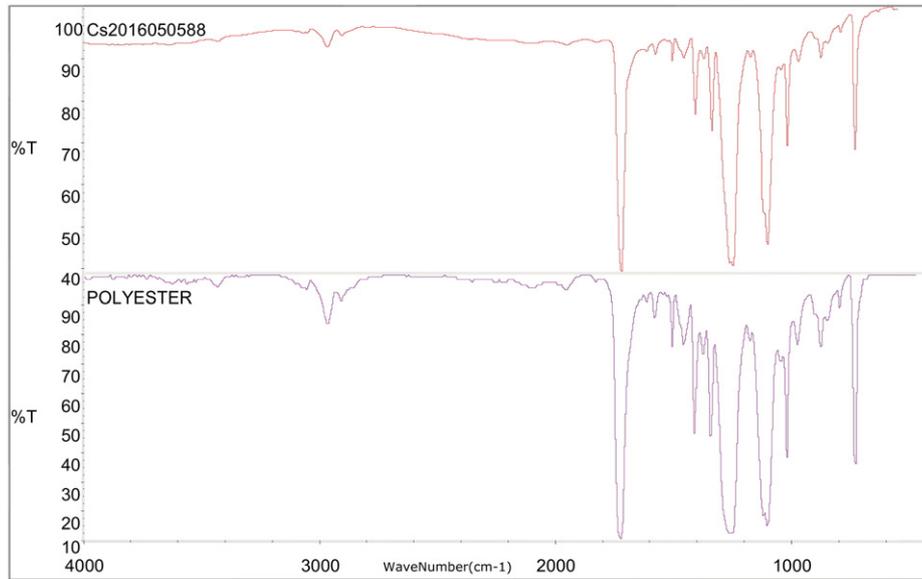


Fig. 5. μFT-IR analysis of the fibrous microplastics.

ingested microplastics decreased with higher trophic levels due to the low abundance of the high trophic level zooplankton such as jellyfish, shrimp, and fish larvae.

The spatial distribution of the ingested microplastics was high in stations 1 and 9–15, and low in stations 2–7 and 16 (Fig. 9). This pattern

was relevant to the high zooplankton abundance in these stations. The average abundance of the ingested microplastics in the studied area was 4.1 pieces/m<sup>3</sup> for Net I and 131.5 pieces/m<sup>3</sup> for Net II, with a difference of as high as 33-fold.

4. Discussion

4.1. The abundance and composition of the ingested microplastics collected by using two types of nets

Comparison of the two sampling nets showed that the total abundance of the ingested microplastics was significantly different (*t*-test, *p* < 0.01). A variety of nets with different mesh sizes, aperture, and length were used by different laboratories to collect suspended microplastics and was reviewed by Hidalgo-Ruz et al. (2012). Inconsistencies in the sampling methods hindered the comparison of microplastics among different areas of various seas. Desforges et al. (2014) suggested that using mesh sizes that were smaller than those currently used in standard neuston nets (300- μm in mesh size) could improve the collection of microplastics in the sea water. The mesh size of Net II used in this research is smaller than the standard neuston net, therefore the result obtained from Net II is a better representation of the actual conditions. A common minimum particle size and a standardized sampling regimen are two study design elements that may improve the comparison of future studies (GESAMP, 2010). The composition and size of the microplastics collected using both nets were relatively similar, with fibrous microplastics identified as the predominant shape. In terms of encounter rates, no significant differences between the two types of nets in the chaetognath, jellyfish, shrimp, and fish larvae groups were detected, and significant changes were observed only in the copepod group (*t*-test, *p* < 0.01). These findings indicated that the characteristics of the microplastics collected by two different nets were essentially similar, whereas the relative abundance of various particles was markedly variable. Taken together, we infer that different nets may still be useful for qualitative comparisons, but not suitable for quantitative comparisons.

4.2. Ingestion of microplastics by natural zooplankton groups

Ingestion of microplastic particles by marine biota has often been studied in the laboratory, and rarely in natural ecosystems. The findings of the present study showed that zooplankton ingest microplastics at all

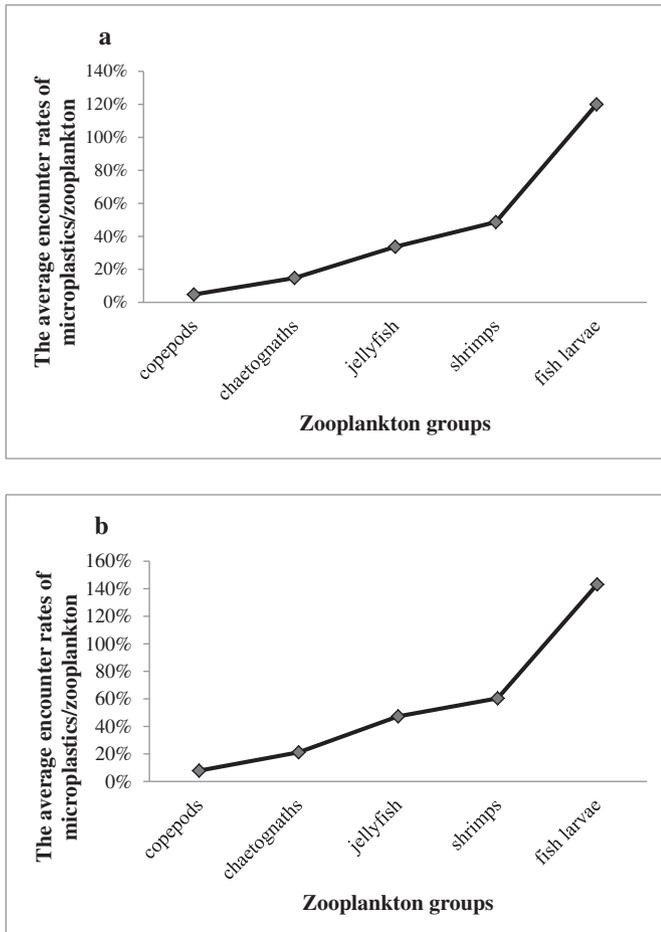


Fig. 6. Average encounter rates between microplastics and zooplankton (a. Net I, b. Net II).

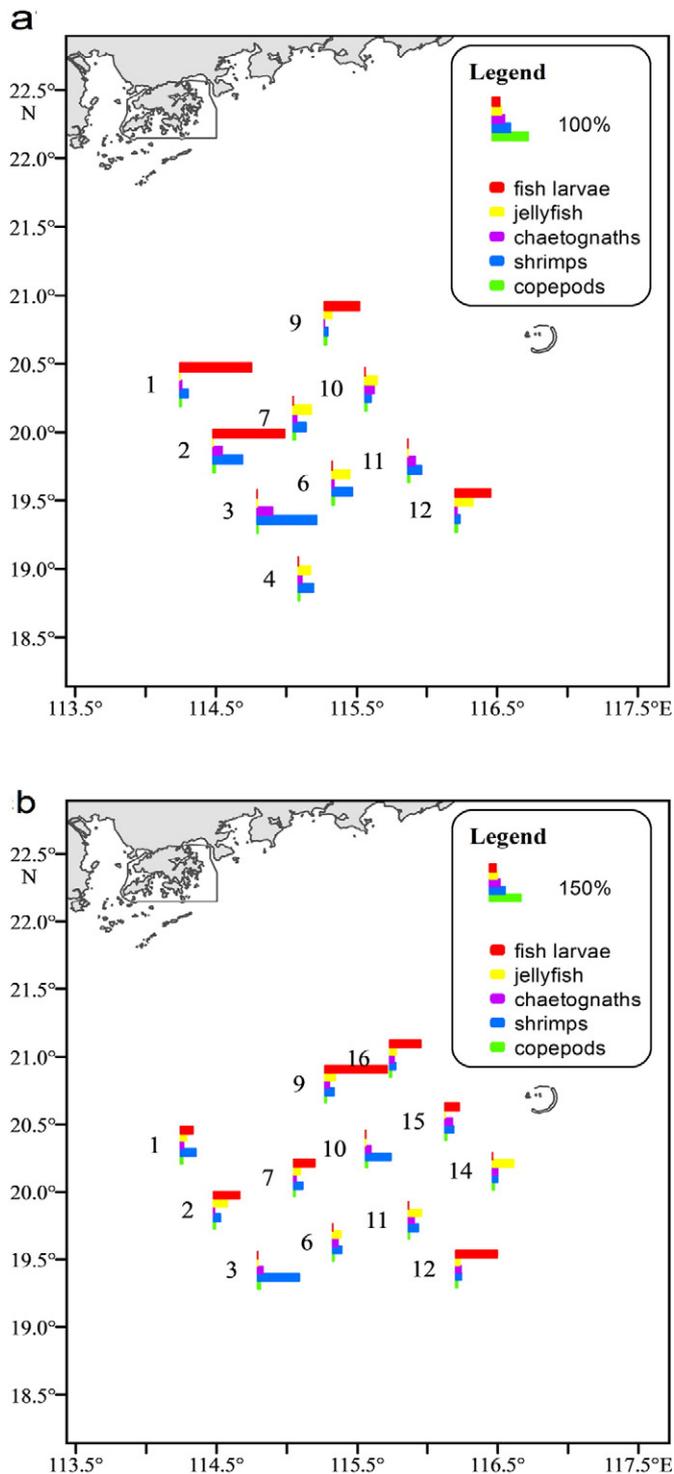


Fig. 7. The spatial distribution of encounter rates in the northern part of the South China Sea (a. Net I, b. Net II).

stations, thereby proving the universality of microplastics ingestion by zooplankton in natural waters. Comparative analysis showed significant differences in encounter rates among the five zooplankton groups. The lowest encounter rate was observed in copepods, whereas the highest was detected in the fish larvae group. This difference may possibly be due to the biological dilution effect (Desforges et al., 2015). In the northern part of the South China Sea, copepods are the predominant zooplankton. In addition, the feeding habits may be also relevant to the observed differences in encounter rates. Natural food for copepods includes phytoplankton, protists, and marine snow/aggregates, whereas

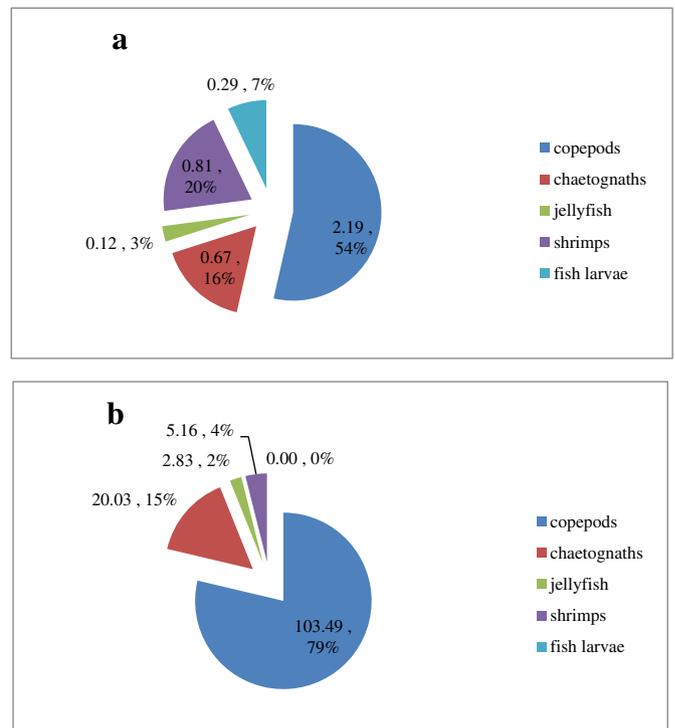
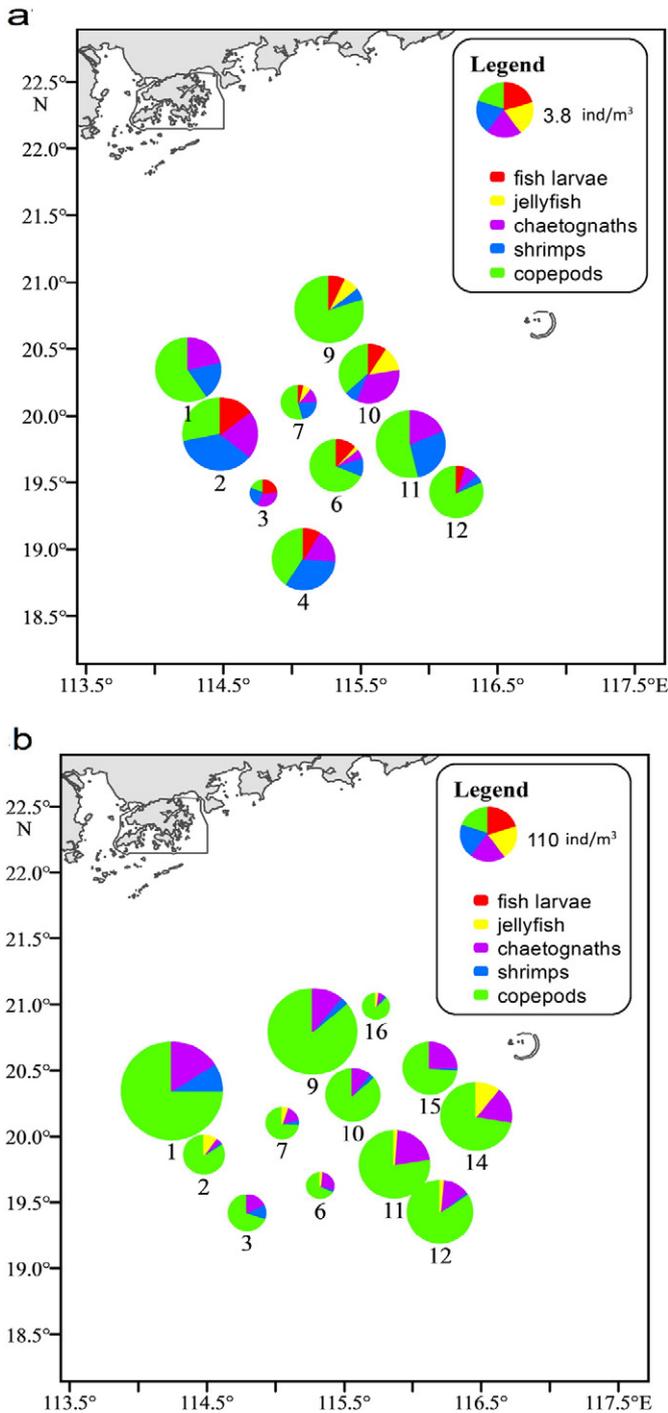


Fig. 8. Percentage of microplastics ingested by different zooplankton groups (a. Net I, b. Net II).

chaetognaths, jellyfish, and fish larvae mainly feed on zooplankton. Carnivorous zooplankton may have a bioaccumulative effect on microplastics, thereby resulting in a higher number of microplastics in these groups than those in copepods.

Comparing the results of the present study with those of Desforges et al. (2015) showed that the encounter rate between microplastics and copepods (5%) in the northern part of the South China Sea was higher than that (2.6%) in the Northeast Pacific. The encounter rate between microplastics and shrimp in the northern part of the South China Sea (50%) was significantly higher than that between microplastics and euphausia in the Northeast Pacific (5.8%). However, the size of microplastics that were ingested by zooplankton in the northern part of the South China Sea (125  $\mu\text{m}$  and 167  $\mu\text{m}$ ) was much smaller than that in the Northeast Pacific (555  $\mu\text{m}$  for copepods and 816  $\mu\text{m}$  for euphausia). This difference may be relevant to the microplastics composition of the sea water, as indicated in the findings in the Northeast Pacific. However, the present study did not monitor the concentration of the <200- $\mu\text{m}$  microplastic particles in the South China Sea because of technical difficulties in visually detecting microplastics within this size range in the sea water.

The potential ecological risk of microplastics in different zooplankton groups has been reported. Cole et al. (2013) studied the impact of microplastic (size range: 1.7–30.6  $\mu\text{m}$ ) ingestion in copepods. The exposure of the copepod *Centropages typicus* to natural assemblages of algae with and without microplastics showed that 7.3- $\mu\text{m}$  microplastic particles (>4000  $\text{mL}^{-1}$ ) significantly decreased algal feeding, thereby indicating that marine microplastics could negatively affect zooplankton function and health. The impact of polystyrene microplastics on the feeding, function, and fecundity of the marine copepod (*Calanus helgolandicus*) has also been tested. Microplastic-exposed copepods underwent energetic depletion over time, and prolonged exposure to polystyrene microplastics (20- $\mu\text{m}$  in size) significantly decreased their reproductive output (Cole et al., 2015). The high encounter rate between microplastics and copepods in the northern part of the South China Sea may also affect the quantity and quality of the copepod population. This in turn may further affect higher trophic levels such as fish.



**Fig. 9.** Spatial distribution of ingested microplastics in the northern part of the South China Sea (a. Net I, b. Net II).

For fish larvae, their encounter rate was much higher than the percentage of adult fish that ingested microplastics. For example, 36.5% of fish in the English Channel were determined to ingest microplastics (Lusher et al., 2013), whereas 35% of the fish in the North Pacific Central Gyre consumed similar particles (Boerger et al., 2010). Lonnstedt and Eklov (2016) showed that the exposure to environmentally relevant concentrations of microplastic polystyrene particles (90-µm in size) inhibited hatching, decreased growth rates, and altered the feeding preferences of the European perch larvae. In the present study, the ratio of ingested microplastics to fish larvae was > 1, thereby indicating a potential threat to the natural fishery resource in this area. Devriese et al. (2015) detected microplastics in brown shrimp from the coastal waters of the

Southern North Sea. Desforges et al. (2015) detected microplastics in euphausia in the Northeast Pacific Ocean. However, no research studies on the ecological impact of microplastics on shrimp have been conducted to date. For chaetognaths and jellyfishes, no report on the ingestion of microplastics in natural sea water has been published, and thus studying the impact of microplastics on these groups is warranted.

**4.3. Factors affecting the abundance of the ingested microplastics in the northern South China Sea**

Land-based human activities are a major source of microplastics that are present in the marine environment (Browne et al., 2010; Browne et al., 2011; Collignon et al., 2012). Browne et al. (2011) showed that disposal of municipal wastewater that was contaminated with fibers from washing clothes is the major source of plastic particles in the UK. A similar conclusion was obtained by Desforges et al. (2014) in the NE Pacific Ocean and Zhao et al. (2014) in the Yangtze River Estuary. The present study showed that the major component of the microplastics is polyester, indicating that the microplastics in the northern part of the South China Sea are also closely related to human activities. The stations with high encounter rates and high abundance of microplastics were influenced by the water from the Pearl River Estuary, as indicated by the results of Lagrangian analysis of surface transport patterns in the northern part of the South China Sea (personal communication, Hu). The hydrodynamic processes in the northern South China Sea are complex, which include upwelling, eddies, water exchange with the coast, the influence of Kuroshio Current, and the open ocean. By zooplankton vertical migration and cross-shelf transport, it is possible to transfer the microplastics into the open ocean and the deep sea. Therefore, the impact of zooplankton-transferred microplastics on the fishery resources should also be considered regardless whether these were derived from the coastal region or the open ocean and deep sea.

**4.4. The transfer of microplastics across the marine food web**

One concern of the impact of microplastics on marine ecosystems is its transfer across marine food web, which may cause negative effects chemically and physically. However, our understanding of the mechanism underlying the transfer of microplastics across the food web in the sea is limited. Statistical analyses performed in the present study showed that the size of microplastics that were ingested by copepods is similar to that of the other four zooplankton groups. Our findings differed from those of previous studies, which showed that larger zooplankton tend to ingest larger microplastics (Desforges et al., 2015). Chaetognaths, jellyfish, and fish larvae are generally carnivorous, feeding on copepods and other zooplankton. Based on the Ecopath model (Deehr et al., 2014), chaetognaths belong to the higher trophic level, situated above copepods, and jellyfish and fish larvae are on a higher level than that of chaetognaths. Carnivorous zooplankton might ingest microplastics directly from sea water due to confusion in identifying their prey. Alternatively, they might ingest microplastics by eating lower trophic level organisms such as copepods that had earlier consumed microplastics. The similarity in size of the ingested microplastics in the five zooplankton groups and the increase in the encounter rate with trophic levels are indicative of the transfer of microplastics along the planktonic food web in this sea area.

The majority of microplastics in copepods were fibrous, and the percentage of the ingested fibrous microplastics decreased with higher trophic levels. These findings indicated that copepods more readily feed on fibrous microplastics, or the residence time of the fibrous microplastics in copepods was relatively longer. It is also possible for carnivorous zooplankton to ingest more granular microplastics from the sea water in addition to its transfer across the food web. It is also possible that the residence time of granular microplastics is prolonged in these zooplankton groups. Food web transfer experiments were performed in the laboratory by offering copepods that were labeled with ingested

microspheres to mysid shrimps, which showed for the first time the potential of plastic microparticle transfer via planktonic organisms from one trophic level (mesozooplankton) to a higher level (macrozooplankton) (Setala et al., 2014). The situation is more complicated in natural sea area. Both the composition of microplastics and the interaction between zooplankton groups are complex. Developing controlled experiments based on the situation of the natural sea area, including experiments using microplastics of different sizes and shapes and various zooplankton groups is thus warranted. Both ingestion and residence time may be utilized as indicators for the assessment of the ecological risk of microplastics in marine organisms.

In summary, a high amount of microplastics was detected in the zooplankton of the northern South China Sea. The encounter rates of microplastics/zooplankton increased with trophic levels, and were significantly higher than that of other reported areas. This phenomenon should thus be immediately addressed and controlled. The northern South China Sea possesses a rich mesopelagic fish resource that is >10 times that of the world's average resource density (Gong et al., 2015). The ingestion of zooplankton by mesopelagic fish may transfer the microplastics to the top predators. It is thus essential to elucidate the consequence of bioaccumulation of microplastics and the possible transfer of the absorbed chemical pollutants within marine food webs, as this may pose an additional threat to higher levels of the food chain, including humans. It is also important to conduct risk assessment of microplastics on the ecosystem, as well as the social and economic levels.

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