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# Assessing the relationship between the abundance and properties of microplastics in water and in mussels

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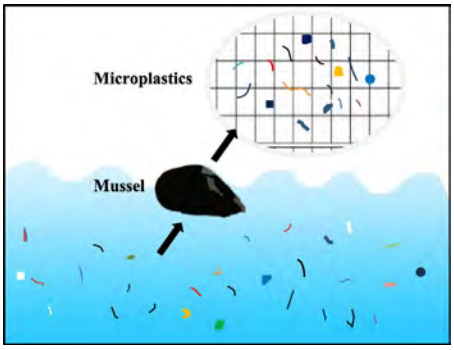
## HIGHLIGHTS

- The abundance of microplastics in mussels depended on those in water.
- Fibers accounted for >60% of the microplastics in field investigations.
- Mussels were more likely to ingest smaller rather than larger microplastics.
- The abundances and types of microplastic ingestion between field and laboratory observations were different.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Microplastic pollution is increasingly becoming a great environmental concern worldwide. Microplastics have been found in many marine organisms as a result of increasing plastic pollution within marine environments. However, the relationship between micoplastics in organisms and their living environment is still relatively poorly understood. In the present study, we investigated microplastic pollution in the water and the mussels (*Mytilus edulis*, *Perna viridis*) at 25 sites along the coastal waters of China. We also, for the first time, conducted an exposure experiment in parallel on the same site using *M. edulis* in the laboratory. A strong positive linear relationship was found between microplastic levels in the water and in the mussels. Fibers were the dominant microplastics. The sizes of microplastics in the mussels were smaller than those in the water. During exposure experiments, the abundance of microbeads was significantly higher than that of fibers, even though the nominal abundance of fibers was eight times that of microbeads. In general, our results supported positive and quantitative correlations of microplastics in mussels and in their surrounding waters and that mussels were more likely to ingest smaller microplastics. Laboratory exposure experiment is a good way to understand the relative impacts of microplastics ingested by marine organisms. However, significant differences in the results between exposure experiments and field investigations indicated that further efforts are needed to simulate the diverse environmentally relevant properties of microplastics.



## 1. Introduction

A microplastic is defined as a plastic particle or fragment smaller than 5 mm (GESAMP, 2015). Microplastics have become a serious

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environmental problem due to their persistence, ubiquity and toxic potential in aquatic environments (Gusmao et al., 2016; Li et al., 2016; Anderson et al., 2017). They are widely distributed throughout the water column, sediments, and even within icebergs (Cole et al., 2011; Wright et al., 2013). The abundances of microplastics has been reported to reach $6.8 \times 10^6$ items $km^{-2}$ in freshwater in China and $1.0 \times 10^5$ in the Southern Ocean (Su et al., 2016; Isobe et al., 2017).

A potential environmental risk of microplastics is their bioavailability to organisms (Wright et al., 2013; Desforges et al., 2015). Microplastics have been found in diverse organisms, including fishes, invertebrates and zooplanktons in field investigations (Li et al., 2016; Nel et al., 2018). Adverse effects on feeding, function, behavior and fecundity have also been observed in test organisms after exposure to microplastics in the laboratory (Cole et al., 2015; Sussarellu et al., 2016; Chen et al., 2017). In previous studies, microplastics have exerted toxic effects on mussels in exposure experiments. A dramatic decrease in phagocytosis and strong lysosomal destabilization were observed in mussels after exposure to 50 mg $mL^{-1}$ polystyrene nanoparticles (Canesi et al., 2015). In another study, the filtering activity of mussels was reduced after exposure to 0.1 g $L^{-1}$ polystyrene microbeads (Wegner et al., 2012).

In general, contaminant levels in organisms are usually closely related to contaminant levels in the surrounding environment. Microplastics exhibit themselves specific physicochemical properties that make the microplastics behave and interact with biota differently from other chemical contaminants (Potthoff et al., 2017). Ingestion is widely accepted as the pathway for animals to uptake microplastics (Browne et al., 2008). A recent study suggested that adherence is another way for animals to uptake microplastics beyond ingestion (Kolandhasamy et al., 2018). This new finding makes one reconsider the bioavailability and accumulation of microplastics within aquatic animals (Kolandhasamy et al., 2018).

There is a lack of combined studies measuring microplastics in both organisms and their living environment; most published studies treated them separately (De Witte et al., 2014; Mathalon and Hill, 2014; Song et al., 2014). In laboratory studies, commercially available microbeads are often used (Browne et al., 2008; Lambert et al., 2017). However, microplastics in the environment represent a mixture of particles and are different from the primary microbeads which have single physicochemical properties (Hu et al., 2016; Su et al., 2016). It is difficult to estimate a relationship between the accumulation of microplastics in organisms and the microplastics in water in the same way that bioconcentration factors are often used for traditional chemical contaminants. Therefore, as for a special emerging contaminant, many basic questions remain to be answered. The relationship between micoplastics in organisms and in their living environment still remains relatively unclear.

Mussels are filter feeders and benthic organisms, which have large geographic distribution and are important species within intertidal ecosystems. Based on these properties, mussels have been successfully used as indicators of marine pollution (Bricker et al., 2014). In recent years, mussels have also been widely used in microplastic studies in field investigations and in laboratory exposure experiments (Von Moos et al., 2012; Farrell and Nelson, 2013; Li et al., 2015; Kolandhasamy et al., 2018). In previous studies, researchers have found widespread microplastic pollution in blue mussels (*Mytilus edulis*) along the coastal waters of China (Li et al., 2016).

In this study, we conducted a large-scale survey of microplastic pollution in mussels and in their surrounding waters along the coastal waters of China. Meanwhile, we conducted exposure experiments with microbeads, fibers and fragments in the laboratory. The accumulation characteristics of microplastics in mussels were analyzed and compared to the results of the field investigation. Our aim was to determine the relationship of microplastics between mussels and their surrounding waters. Furthermore, we also aimed to determine whether a laboratory exposure experiment could reflect the characteristics of microplastics in a field investigation.

## 2. Materials and methods

### 2.1. Sample collection

Two common species of mussels were chosen from the northern (*M. edulis*) and southern (*Perna viridis*) along the coastal waters of China. Mussels and water samples were collected from 25 sites between March 2016 to June 2017 according to the methods of Li et al. (2016). The sampled coastline covers approximately 80% of the total length of the mainland China coastline (Fig. 1). *M. edulis* were collected from 14 sites, and *P. viridis* were collected from 11 sites. Approximately 30 mussels were collected at each site, distributed among six replicates per site. The collected mussels were placed in the aluminum foil bag and stored under ice in the field before being stored at −20 °C in the laboratory. Approximately 5 L of bulk water samples were collected using steel samplers, and three replicates were sampled individually for each sampling site. The exact information for the sites and mussels were also recorded (Table S1; Fig. S1).

### 2.2. Laboratory uptake experiment

The mussels were acclimated to laboratory conditions with aerated artificial seawater at 18 ± 1 °C, 28‰ salinity and a 12 h light-dark photoperiod for five days. Five mussels were randomly grouped into a glass tank with 4 L seawater. Two exposure groups (100 and 1000 particles $L^{-1}$) and one control group were set, with four replicate tanks for each group.

Three types of microplastics (i.e., beads, fragments and fibers) were used in the exposure experiment (Table S2). Beads were ball-like microplastics, and fibers were rod-like and flexible strips. The rest were defined as fragments, which were variable in shape (Yang et al., 2015). Microfibers were prepared manually using scissors. The fragments were purchased from Sigma-Aldrich (China) and dyed in Nile red. The beads were purchased from Thermo Scientific (Rainbow, China). Beads, fragments and fibers were mixed in a ratio of 1:1:8 in filtered water based on the proportion of microplastics observed in the environment (Su et al., 2016). The glass bottles were shaken well until the microparticles were mixed thoroughly. During the five-day exposure, mussels were randomly collected every day from each tank before feeding. After collection, the water, which was already mixed with microplastics, was changed for each tank.

### 2.3. Extraction of microplastics from waters and soft tissue

The bulk water was filtered onto a 20-μm-pore size, 47-mm-diameter nylon membrane filter (Millipore NY2004700) using a vacuum pump according to methods by Su et al. (2016). The substances on the filter were collected into a glass flask using 100 mL of 30% hydrogen peroxide (V/V) to digest organic materials. The flasks were then placed into an oscillation incubator for approximately 72 h. The temperature was kept at 65 °C, and the rotation speed was 80 rpm. The liquid in each bottle was filtered again with a 5-μm-pore size, 47-mm-diameter cellulose nitrate membrane filter (Whatman AE98), which was then covered and stored in the dry glass petri dish for further observation.

The analysis of microplastics in mussels followed previous methods for bivalves (Li et al., 2015). In brief, after the weight and shell length were measured, the soft tissues were removed and weighed (Table S1). The tissues of 2–5 mussels were pooled as a replicate; six replicates were in each field site and four replicates for each exposure group. Approximately 200 mL of 30% hydrogen peroxide was added to each bottle for digestion in an oscillation incubator.

### 2.4. Flotation and filtration of microplastics with saline (NaCl) solution

A concentrated saline solution (1.2 g $mL^{-1}$) was used to separate the microplastics via flotation (Li et al., 2015). NaCl is the most common

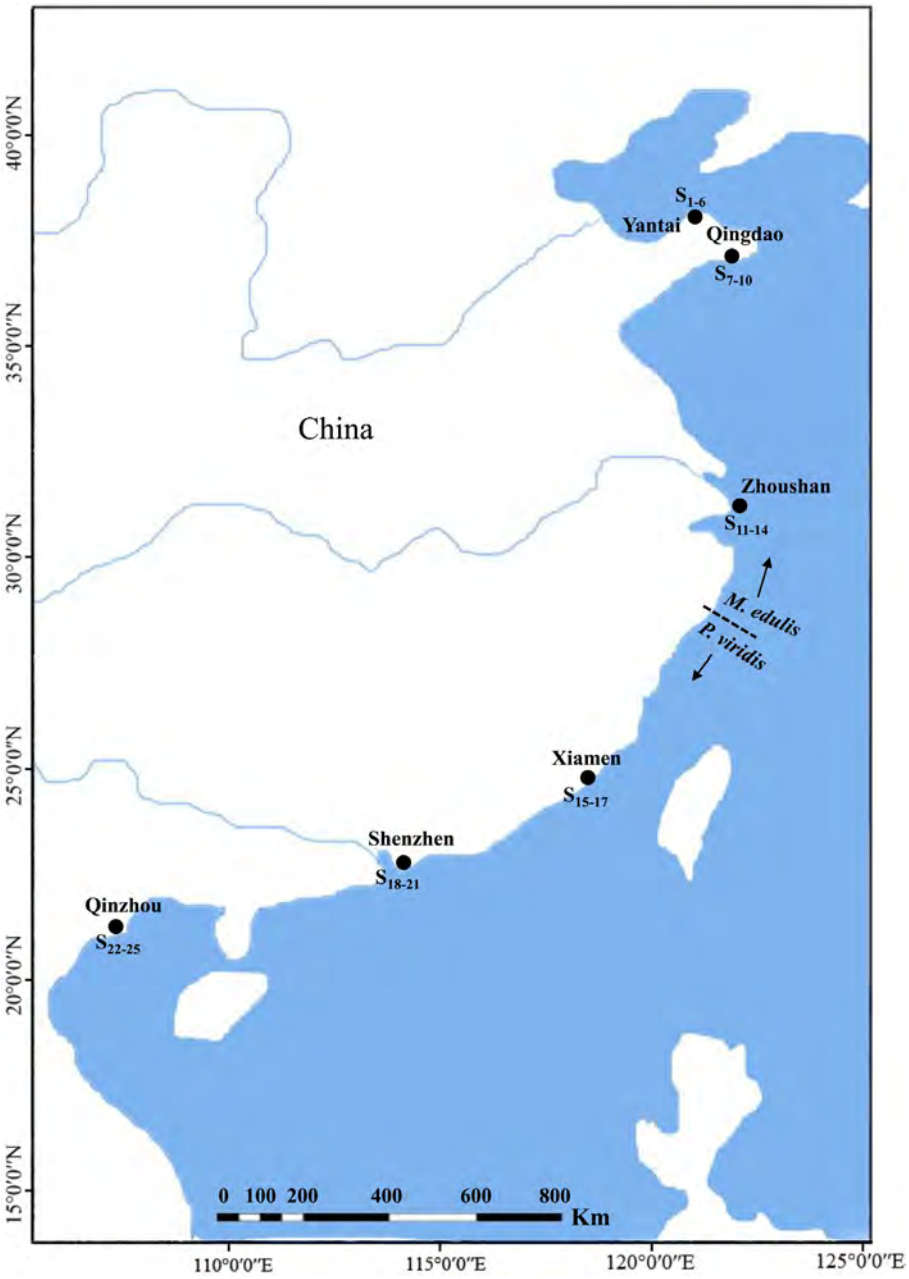


**Fig. 1.** Sampling sites along the coastal waters of China.

chemical used in the floating separation of microplastics because it is ecologically friendly and cheap. Approximately 800 mL of filtered saturated NaCl solution was added to each bottle of digested mussel tissue. The liquid was mixed, and the bottles stood overnight. The overlying water was directly filtered over a 5-μm-pore size, 47-mm-diameter filter using a vacuum pump. The filter was placed into a glass petri dish with a cover for further analysis. To avoid contamination, all of the liquid (freshwater, salt water and hydrogen peroxide) was filtered with 1-mm filter paper prior to use. All of the containers and beakers were previously rinsed three times with filtered water.

### 2.5. Observation and validation of microplastics

The filters were observed under a Carl Zeiss Discovery V8 Stereo microscope (MicroImaging GmbH, Göttingen, Germany), and images were taken using an AxioCam digital camera (Li et al., 2015, 2016). For further validation, 570 microplastics were randomly selected from mussel samples (306 particles) and water samples (264 particles). A number of common and undeterminable particles were detected. LUMOS (Bruker) microscopy (ATR mode) was used to verify these suspected items. All data were measured at a resolution of 4 $cm^{-1}$ with a 32-s scan time. All spectra were compared with a database from Bruker to verify the polymer type (Su et al., 2016). In particular, rayon is an organic cellulose-based polymer widely used in cloth. Synthetic additives have been added to the products of rayon, though the base polymer is derived from biomass. Therefore, rayon was classified as a microplastic, as reported in previous studies.

### 2.6. Data analysis

Data analysis was conducted using SPSS version 22 (SPSS, Inc., Chicago, IL, USA), GraphPad Prism 5 and Origin 9. The difference of quantities of microplastics between mussels and water was determined by one-way analysis of variance (ANOVA) followed by Tukey's HSD test (in

the case of homogeneous variances). Pearson's coefficient was chosen, and the significance level was set at 0.05 and 0.01. The density curve and the empirical cumulative distribution curve were used to compare the size distribution of microplastics in different fractions. Kolmogorov-Smirnov test was applied to test the difference between one distribution to the known distribution or between any two distributions.

## 3. Results

### 3.1. Microplastics in water and mussels in the field

Sample contamination was well prevented during collection and processing. Only 0.4 ± 0.5 items filter$^{-1}$ of microplastics was found in the blanks for the field samples, and no microplastics were found in the blanks for the laboratory samples. The number of microplastics in waters varied from 0.68 to 6.44 items L$^{-1}$ (Fig. 2a). The abundance of microplastics in mussels varied from 1.52 to 5.36 items g$^{-1}$ (wet weight) and from 0.77 to 8.22 items individual$^{-1}$ (Fig. 2b, c). A positive linear correlation was observed between the abundance of microplastics in the surrounding water and in the mussels, regardless of whether a single species or both species were used ($p < 0.05$) (Fig. 3).

The size distribution of microplastics showed little variation in mussels from different sites and mainly distributed from 0.25 to 1 mm, with a proportion of 57–79% in water and 48–76% in mussels (Fig. S2a, b). The density curve analysis showed that the patterns of size distribution were similar to each other in mussels and in water (Fig. 4a), but the sizes of microplastics in mussels were smaller than those in water ($p < 0.001$, $p = 8.8 \times 10^{-8}$) (Fig. 4b).

The most common type of microplastics was fiber, followed by fragment and bead (Fig. 5a, b). Fibers accounted for >80% of the total microplastics in water from 23 sites and in mussels from 18 sites (Fig. S2c, d). Compositions of microplastics showed consistency in mussels and in water. The most common composition of microplastics was polyester (PET), followed by rayon, PE, PVC and PP. PET accounted for 77% in water and 74% in mussels (Fig. 5c, d).

### 3.2. Microplastics in water and mussels in the laboratory

In the exposure experiment, the accumulation of microplastics was observed in all mussels. During five exposure days, the abundance of microplastics in mussels was significantly higher in the high concentration treatment group than that in low concentration treatment group ($p < 0.05$) (Fig.6). The abundance of fibers was significantly higher than that of beads at the fourth and the fifth day in the low concentration treatment group ($p < 0.05$). In the high concentration group, however, the abundance of beads in the mussels was very close to that of fibers, even though the initial concentration of fibers in the water was much higher than that of beads (Fig. 6).

### 3.3. The differences between field investigations and laboratory experiments

A comparison between field and laboratory results suggested that the nominal concentrations of microplastics in water were much higher than microplastic levels found in the field (Fig. 7a). However, the average abundance of microplastics in mussels by weight was higher in the field than in the laboratory (Fig. 7b); this trend was reversed when the abundance was calculated by individual (Fig. 7c).

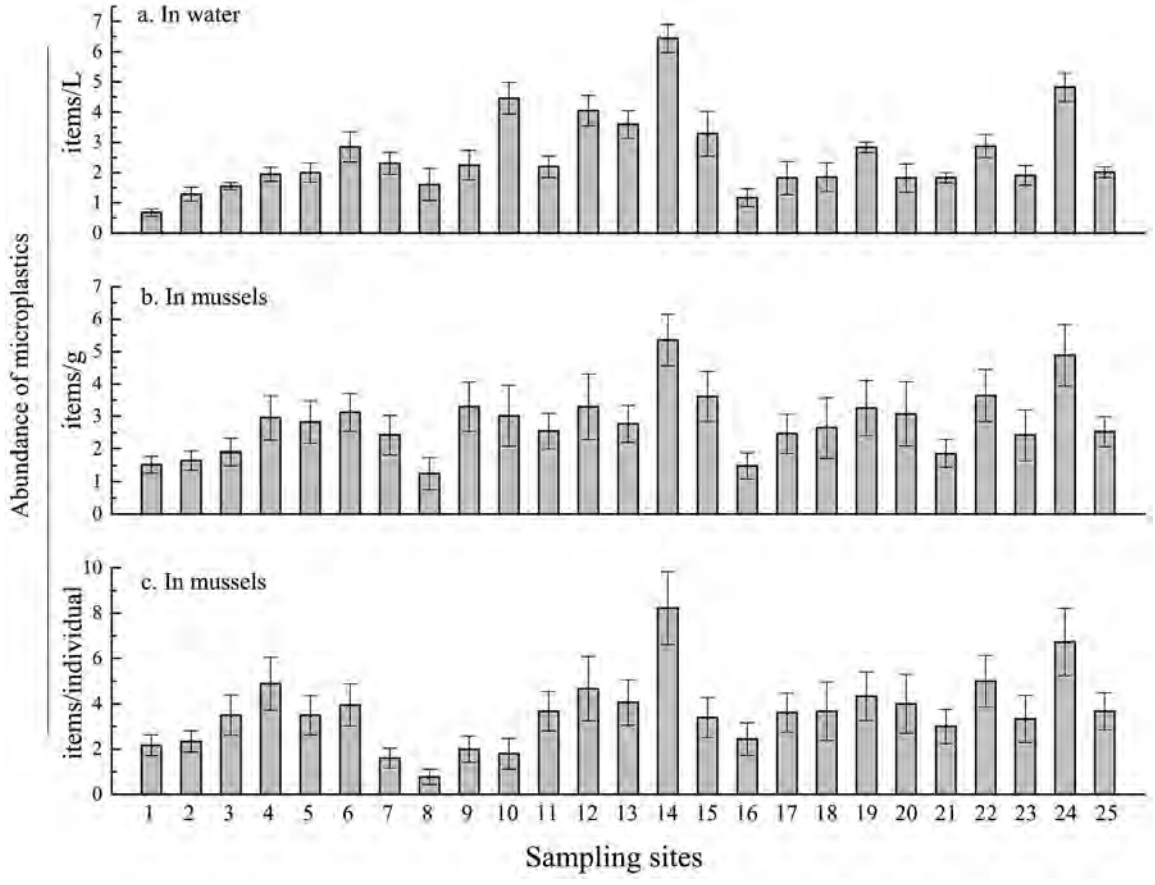


**Fig. 2.** Abundance of microplastics in water (a) and in mussels (b, c). Three replicates were collected for water at each site (n = 3). Six replicates were collected for mussels at each site (n = 6), and 3–5 individuals were pooled per replicate.

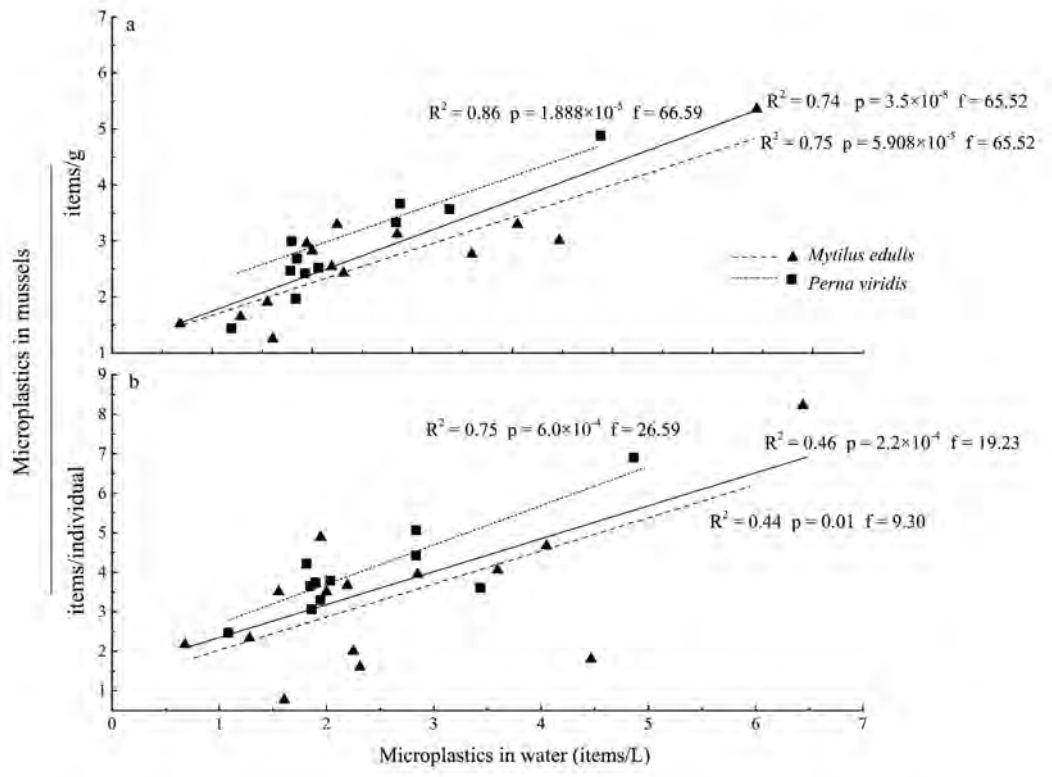


**Fig. 3.** Correlation between the abundance of microplastics in water and in mussels in terms of items $g^{-1}$ (a) and items $individual^{-1}$ (b). The solid lines represent all mussels, and the dotted lines represent *M. edulis* and *P. viridis* separately.

## 4. Discussion

### *4.1. The relationship between microplastics in mussels and in their living waters*

In the present study, we found a positive relationship between microplastic levels in mussels and in waters. The size distributions and the proportion of microplastic morphotypes were similar in mussels and in waters. We also found that mussels were likely to uptake smaller size fractions of microplastics from their surrounding waters. In this investigation, we could only count the microplastics remaining in the body of mussels, which is usually determined by a balance between intake and elimination. Previous studies have suggested that smaller sizes of microplastics are easily ingested and accumulate in the tissues of an organism (Browne et al., 2008). Furthermore, smaller sizes of microplastics also show greater toxicity to organisms like *Copepoda* adults and offspring (Lee et al., 2013). Therefore, great attention should be paid to the size effects of microplastics ingested by mussels, especially for those microplastics in smaller size classes.

China has a long coastline, and no one mussel species is common to all Chinese coastal regions. *M. edulis* and *P. viridis* are complementary to each other in their distributions and nearly cover the whole coastline of China. Both species belong to the order *Mytiloida* and share similar characteristics. Therefore, we selected these two species as representatives thoroughly along China's coastline. Our results also suggested that microplastic contamination was widespread in both species. A strong positive correlation was observed for the abundance of microplastics in water and in mussels even though the two species were considered together, indicating that it is appropriate to use these two species together for our later analyses.

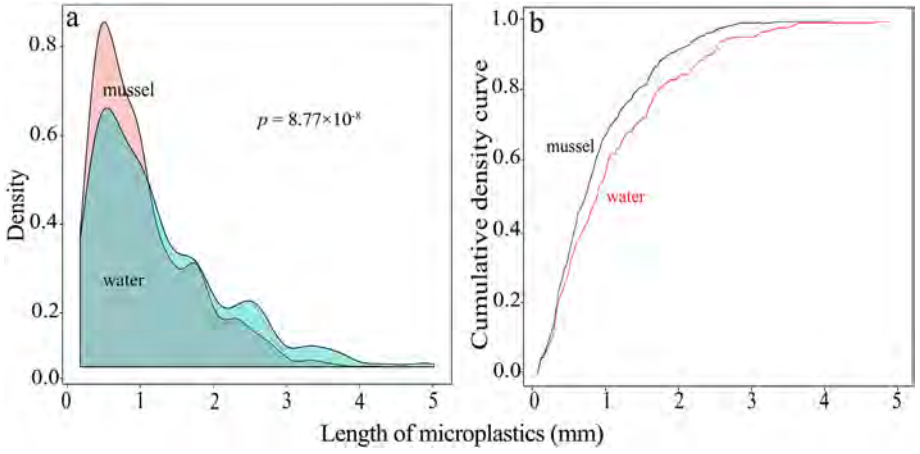


**Fig. 4.** The density curve (a) and cumulative density curve (b) of microplastic sizes in water and in mussels.

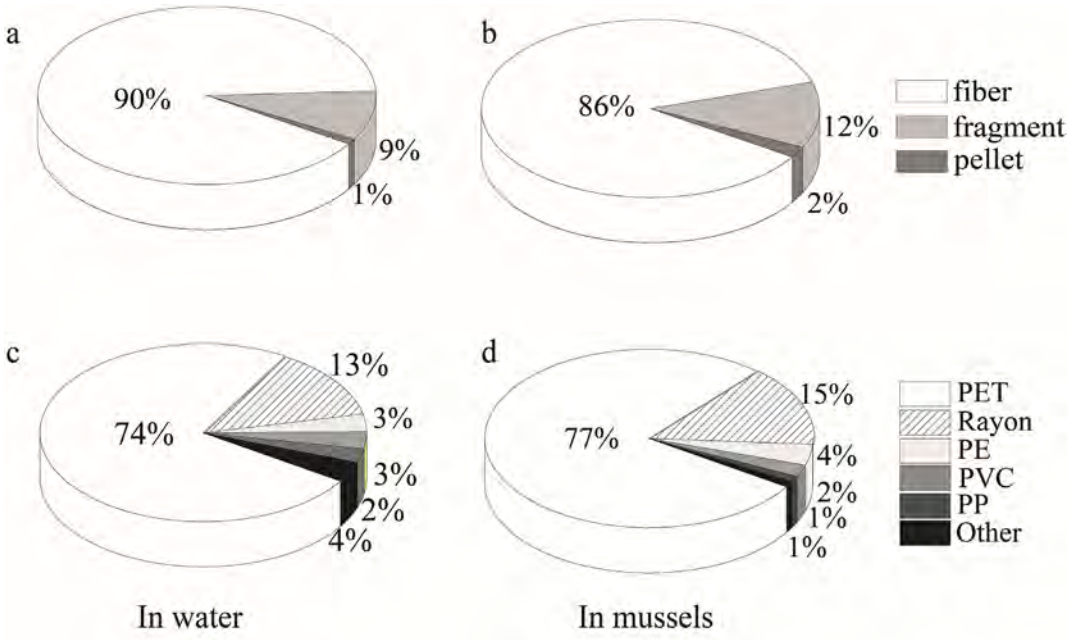


**Fig. 5.** The shapes (a, b) and compositions (c, d) of microplastics in water and in mussels. Three replicates were collected for water at each site (n = 3). Six replicates were collected for mussels at each site (n = 6), and 3–5 individuals were pooled per replicate.

In brief, in the field investigation, the correlations of microplastics in mussels and in surrounding waters included not only the abundance of microplastics but also the dominant features of microplastics, namely sizes, shapes and compositions. The abundance of microplastics in mussels has been found to be closely related to human activity (Li et al., 2016). Our present study provides further evidence that mussels can be used as an indicator of microplastic pollution in coastal waters.

### *4.2. The comparison between field investigations and laboratory experiments*

Exposure experiments are regarded as effective methods to study the uptake, accumulation and toxicity of contaminants. The ingestion and biological effects of microplastics have also been tested in many previous studies (Van Cauwenberghe et al., 2015; Hall et al., 2015;

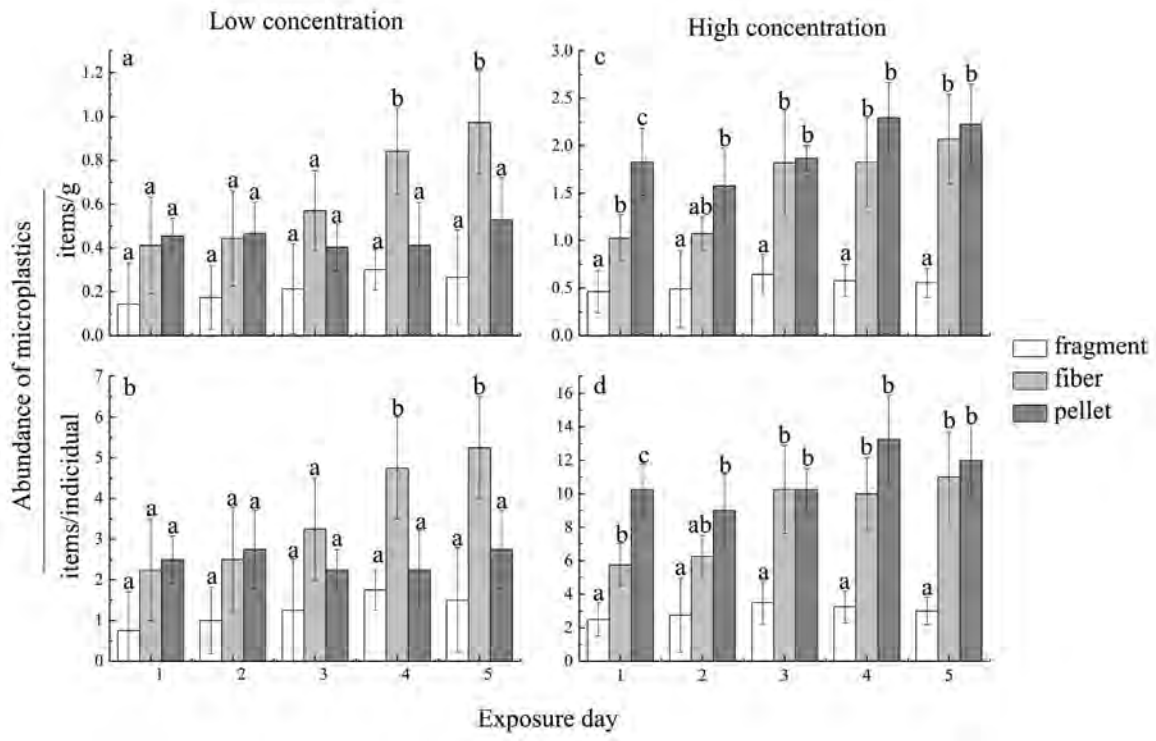


**Fig. 6.** Abundance of microplastics ingested by mussels in the low concentration group (a, b) and the high concentration group (c, d). Each value represents the mean ± SD of four replicates (n = 4). One-way analysis of variance (ANOVA) was used to determine significant differences among the microplastic abundance in different shapes. Groups that do not share the same letter above the bars are significantly different ($p < 0.05$).

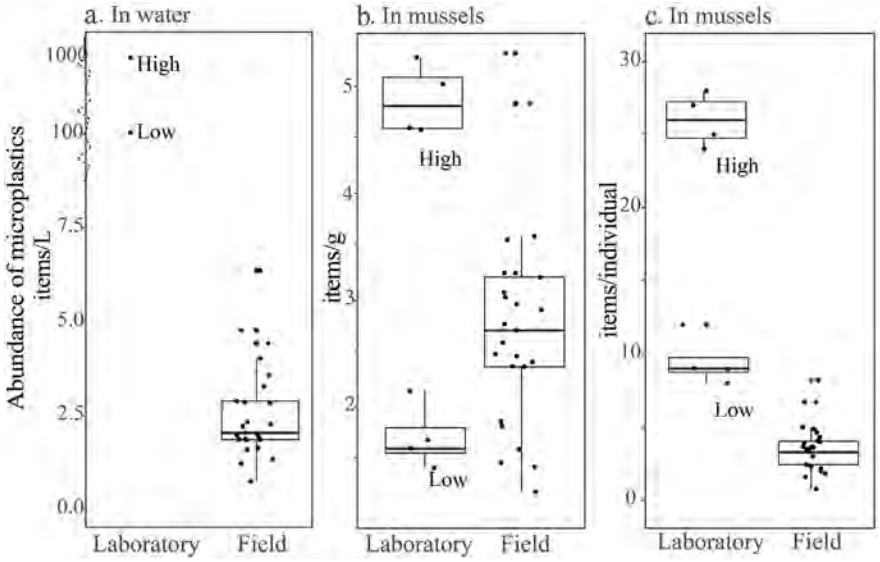


**Fig. 7.** Comparison of microplastic abundance in the exposure experiment on the fifth day and field investigation in water (a) and in mussels (b, c). In the box plots, lines indicate upper quartile, median, and lower quartile, and dots show the individual observations.

Avio et al., 2015; Hu et al., 2016). Considering the limitations imposed by the common approach of using a single type of microbead, we used microplastics of three different shapes to simulate the microplastic types observed in the real environments in the present study.

Our results revealed parallels between the exposure experiments and the field investigation with respect to size distributions. Mussels ingested more microplastics of smaller sizes both in the laboratory and in the field. However, the proportions of the three main shapes of microplastics in mussels differed greatly between the field investigation and the exposure experiments. This occurred even though the nominal amounts added to the water in the exposure experiment were set based on previous results from the field. This result suggested that beads were more easily ingested by mussels in laboratory conditions. Because beads were smaller than fibers, they could be transferred and accumulated in the digestive system and the haemolymph (Browne et al., 2008; von Moos et al., 2012).

There are still some differences between field investigations and exposure experiments. These differences indicated that the present exposure experiments using microplastics were different from the real conditions of microplastics in the real environment even though some relationship between microplastic in mussels and in exposure water could be observed. Several factors might lead to such differences. First, microplastics in the environments own diverse physicochemical properties such as shapes, sizes, colors, compositions and additives (Lambert et al., 2017). It is difficult to use several types of microplastics to emulate the mixture present in the environment. Second, the accumulation of microplastics is a long period in the real environment, but exposure experiments usually last for a short time. Therefore, great efforts are needed to simulate and use microplastics with environmentally relevant properties in future exposure studies.

## 5. Conclusions

In light of the field investigation and laboratory studies, a quantitative correlation existed between microplastics in mussels and in their surrounding waters, but mussels are more likely to ingest smaller microplastics from the water. In addition, the present study further proved that mussels can be useful tools to indicate microplastics in the marine environment. The significant differences in the results between exposure experiments and field investigations indicated that great efforts are needed to simulate diverse environmentally relevant properties of microplastics in laboratory research.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2017.11.284.

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