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

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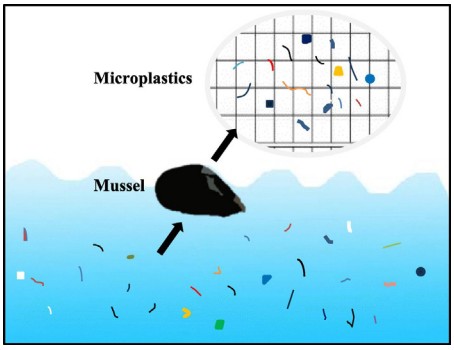
HIGHLIGHTS

- 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 6.8×10^6 items km^{-2} in freshwater in China and 1.0×10^5 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