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Adherence of microplastics to soft tissue of mussels: A novel way to uptake microplastics beyond ingestion☆

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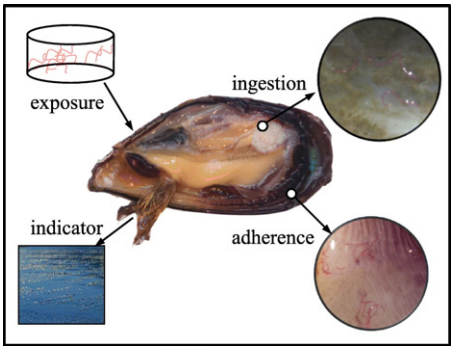
HIGHLIGHTS

- Microplastics were isolated from specific organs of mussels.
- The abundance of microplastic by weight differed in organs of field mussels.
- Microfibers were observed in foot and mantle of mussels in uptake and clearance experiments.
- Adherence contributed about 50% of the microplastic uptake in mussels.
- Adherence is a novel way for animals to uptake microplastics beyond ingestion.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastic pollution is recognized as an emerging threat to aquatic ecosystems. One of the main environmental risks associated with microplastics is their bioavailability to marine organisms. Up to date, ingestion has been widely accepted as the sole way for the animals to uptake microplastics. Nevertheless, microplastics have also been found in some organs which are not involved in the process of ingestion. We hypothesize that the animal might uptake microplastics through adherence in addition to ingestion. To test this hypothesis, we collected mussels from the fishery farms, conducted exposure/clearance experiments and analyzed the accumulation of microplastics in specific organ of mussels. Our studies clearly showed the uptake of microplastic in multiple organs of mussels. In the field investigations, we found that the abundance of microplastic by weight but not by individual showed significant difference among organs, and the intestine contained the highest level of microplastics (9.2 items/g). In the uptake and clearance experiment, the accumulation and retention of microfibers could also be observed in all tested organs of mussels including foot and mantle. Our results strongly suggest that adherence rather than ingestion led to the accumulation of microplastics in those organs which are not involved in ingestion process. To our best knowledge, it is the first time to propose that adherence is a novel way for animals to uptake microplastics beyond ingestion. This new finding makes us rethink about the bioavailability, accumulation and toxicity of microplastics to aquatic animals.

1. Introduction

Microplastics have been recognized as emerging marine pollutants of significant concern, due to their persistence, ubiquity and toxic potential (Engler, 2012; Rochman et al., 2014; Wang et al., 2016). One of

☆ Capsule: Adherence was proved to be a novel way for animals to uptake microplastics beyond ingestion.

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the main environmental risks associated with microplastics is their bioavailability to marine organisms. Because of their small dimensions, microplastics have a similar size range to planktonic organisms and other suspended particles, making them available to an array of marine invertebrates (Wright et al., 2013; Ory et al., 2017). A lot of studies have reported that the animals can uptake microplastics through ingestion. Microplastics have been found in the intestines and stomachs in different species including fishes and birds in the field investigations such as freshwater, marine and terrestrial environments (Jabeen et al., 2017; Zhang et al., 2017). The abundances of microplastics reach 6.8×10^6 items/km^2 in freshwater and 7.6 items/individual in blue mussel (*Mytilus edulis*) in China (Li et al., 2016; Su et al., 2016).

In the laboratory exposure experiments, microbeads have also been found in other organs rather than intestine and stomach. For example, microbeads are not only found in the gills of mussels and crabs but also on the surface of foot of zooplanktons and mussels (Wegner et al., 2012; Setälä et al., 2016; Watts et al., 2016). Gill can be regarded as one of important feeding organs in many species. Foot, however, is not directly related to the feeding process. Therefore, we hypothesize that the animal might uptake and accumulate microplastics through adherence in addition to ingestion.

Mussels are the benthic extensive filter feeding organisms with a selective mechanism of suspension feeding, which leads to accumulation of microplastics, chemical pollutants and microorganisms in mussels (Mathalon and Hill, 2014; Van Cauwenberghe and Janssen, 2014; Paul-Pont et al., 2016; Van Cauwenberghe et al., 2015). Mussels have been widely used for biomonitoring studies in marine environments due to several advantages such as broad geographical distribution, easy accessibility and high tolerance for a considerable range of salinity. Mussels have also been used in microplastics researches including field investigations as well as laboratory exposure experiments (Von Moos et al., 2012; De Witte et al., 2014; Avio et al., 2015; Li et al., 2015, 2016).

The physical ingestion of microplastic by organism leads to blockage of the intestinal tract, inhibition of gastric enzyme secretion, reduction of feeding stimuli, decrease in steroid hormone levels, delay in ovulation and lack of reproduction (Wright et al., 2013; Canesi et al., 2015). Notable histological changes and a strong inflammatory response are observed in mussels after exposure to 2.5 g/L high-density polyethylene (Von Moos et al., 2012). *M. edulis* reduces its filtering activity after exposure to 0.1 g/L polystyrene microbeads (Wegner et al., 2012). Micropolystyrene at 32 μg/L leads to an increase in hemocyte mortality and triggered substantial modulation of cellular oxidative balance in *M. spp.* (Paul-Pont et al., 2016).

The accumulation and potential risks of microplastic are closely related the pathways for the microplastics entering the body of organisms. Therefore, it is critical to clarify the uptake pathways of microplastics in organisms. In the present study, we collected mussels from the fishery farm, conducted exposure/clearance experiments in the laboratory and analyzed the accumulation of microplastics in specific organ of mussels. The aim of the present study was to determine if there was a way for aquatic organisms to uptake microplastics beyond ingestion.

2. Materials and methods

2.1. Sample collection

The blue mussel (*M. edulis*) was collected from a fishery farm area in Zhoushan, Zhejiang, East China Sea. Some specimen were kept in −20 °C immediately for microplastic analysis, the others were cultured for exposure experiments. The total length (cm) and whole body weight (g) of the mussels were measured (Supplementary Table 1). One hundred and twenty-six mussels were totally used throughout the study.

2.2. Laboratory uptake and elimination experiment

The mussels were acclimatized for 5 days in laboratory conditions with aerated artificial seawater at 18 ± 1 °C, 28‰ salinity and a 12 h light-dark illumination regime. The water was filtered through 0.45 μm filter paper and maintained at 18 °C for the exposure experiments. Four mussels were randomly put into a 5 L glass tank with 4 L seawater. Four tanks were set for each group. Two control groups and two exposure groups were used for the exposure experiment. The same experiment was repeated thrice. The man-made microfibers were prepared manually using scissors. The plastics materials were cut into tiny pieces and then mixed with filtered water. The glass bottles were shaked well until the fibers were mixed thoroughly. The solution was mixed well and then filtered through nylon filters. The filtered fibers were transferred to clean bottles to prepare the stock solution 100 mL. Form the stock solution, 5 mL solution with microfibers was filtered using nylon filter. The microfibers were picked up from the filters under a stereomicroscope, and the size ranges of microfibers were measured. The abundance of microplastics was 2000 microfibers/L in the exposure experiment.

Forty-eight hours after exposure, mussels were collected from 2 control tanks and 2 treatment tanks for microplastic analysis. Mussels in the rest 2 control tanks and 2 treatment tanks were rinsed with filtered water three times and transferred into the tanks with clean water and aeration for elimination experiment. Forty-eight hours after elimination, the mussels were collected.

2.3. Dissection of mussel organs

The mussels from the fishery farm and laboratory experiments were washed with filtered water to remove the associated debris and byssal threads. Six replicates with 30 mussels were used for the field samples, and three replicates with 30 mussels were used in laboratory experiments. The organs were dissected according to the method of Avio et al. (2015) with slight modifications. In brief, a small knife was inserted between two valves on the dorsal side, and the anterior adductor muscle was cut to open the valves. The organs were divided based on their functions. Some of them (i.e., gills, intestine, stomach) were closely related to the ingestion process, and the others (i.e., mantle, gonad, adductor and visceral tissue) were not involved in the ingestion process (Fig. 1). The organs were kept in separate clean petri dishes and covered with aluminum foil to avoid contamination. The same organs in each five mussels were pooled together as one replicate.

2.4. Hydrogen peroxide treatment

The isolation of microplastics from mussels followed our previous methods for bivalves (Li et al., 2015). In brief, blank extraction group without tissue was performed simultaneously to correct the potential procedural contamination. All of the liquid (freshwater, saltwater and hydrogen peroxide) was filtered with 1 μm filter paper prior to use. All containers and beakers were rinsed three times with filter water before use to avoid contamination. The organs of mussels were emptied

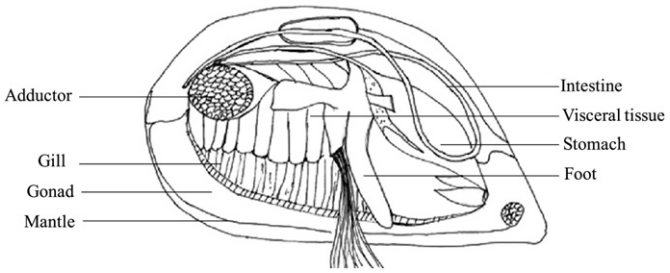

Fig. 1. The specific organs in blue mussel (*Mytilus edulis*).