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Microplastic ingestion reduces energy intake in the clam *Atactodea striata*

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ABSTRACT

The effects of microplastic concentrations (10 items l^{-1} and 1000 items l^{-1}) on the physiological responses of *Atactodea striata* (clearance rate, absorption efficiency, respiration rate) were investigated. The fates of ingested microplastics and the efficiency of depuration in removing ingested microplastics were also studied. *A. striata* ingested microplastics and the clearance rate was reduced at high concentration of microplastics. Since the respiration rate and absorption efficiency remained unchanged in exposed *A. striata*, reduction in the clearance rate would reduce the energy intake. Ingestion and retention of microplastics in the body were further limited by the production of pseudofaeces and faeces, and depuration in clean water, resulting in a very small amount of microplastics stored in the body of the clam.

1. Introduction

Plastics are polymers that have been extensively used and revolutionized our lives in the last few decades. However, plastic debris is commonly encountered in the marine environment and threatens marine life through entanglement, ingestion and suffocation (Gregory, 2009). In recent years, microplastic pollution in the marine environment has become a major concern (Thompson et al., 2004) and now it is known that the problem occurs in oceans worldwide (Barnes et al., 2009). Microplastics are generated either from consumer products such as facial cleansers or from the breakdown of meso- and macroplastic debris. These polymer particles, generally of <5 mm in size, can be transported vast distances in the oceans and deposited on the shore when being caught in nearshore currents (Cole et al., 2011).

Through laboratory and field studies, various animal groups with different feeding modes and occupying different habitats have demonstrated ingestion of microplastics. They include crustaceans (Murray and Cowie, 2011; Watts et al., 2014; Setälä et al., 2016), lugworms (Thompson et al., 2004), polychaetes (Setälä et al., 2016), sea cucumbers (Graham and Thompson, 2009), barnacles and bivalves (Thompson et al., 2004; Mathalon and Hill, 2014; Green, 2016; Setälä et al., 2016; Sussarellu et al., 2016). Among various feeding modes, ingestion of microplastics is the highest in filter feeders (Setälä et al., 2016), possibly owing to their efficient water processing mechanism. For example, in a laboratory experiment using 10 μm polystyrene beads at a concentration of 5 beads ml^{-1}, 90% of the filter-feeding bivalve individuals contained beads, as compared with 0 to 20% of the individuals in other taxa with different feeding modes.

Ingestion of microplastics has a wide ranging effects on marine invertebrates including inflammation (Browne et al., 2008), reduction of survival, growth and fecundity (Lee et al., 2013; Besseling et al., 2014; Cole et al., 2015), depletion of energy reserve (Wright et al., 2013), and retardation of larval development (Sussarellu et al., 2016).

Filter-feeding bivalves create feeding current by ciliary movement and particles collected on the gills are ingested (Ruppert and Barnes, 1994). It is, however, not a passive process as particle selection occurs primarily on gills and labial palps depending on the size, shape, nutritive value or chemical nature of the particles (Ward et al., 1997; Arapov et al., 2010). Unwanted particles will be moulded into a mucous ball and deposited near the entrance of the inhalant siphon as pseudofaeces for later disposal (Garrido et al., 2012). This process is effective in removing inorganic or less nutritious detrital matters in bivalves. Various studies have investigated the fate of microplastics in bivalves after they enter the digestive system. No accumulation of microplastics was shown in the gut of Pacific oyster *Crassostrea gigas*, suggesting a high potential of egestion (Sussarellu et al., 2016). Studies on mussels, however, have demonstrated the uptake of microplastics through endocytosis by digestive epithelial cells (von Moos et al., 2012) and translocation to haemolymph (Browne et al., 2008; Avio et al., 2015).

Hong Kong has a population of 7.3 million people (Census and Statistics Department, 2016), and generated plastic wastes at an amount of 1866 tons daily in 2013 (HKEPD, 2015). Through the stormwater drainage system and littering on the shores, plastic wastes are commonly found on local coastal habitats. Another major source of plastic wastes is through riverine input from the third largest river in China, the Pearl River, where several densely populated cites are located

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along with an estimated population of 60 million (http://gd.sina.com.cn/news/m/2014-05-22/0539101433.html). Microplastic pollution did not receive any public's concern in Hong Kong until >150 tons of virgin plastic pellets spilled into the sea when Hong Kong was hit by a typhoon in July 2012, and >35 beaches were contaminated. In a survey of 25 beaches along the Hong Kong coastline, the mean abundance of microplastics was 5595 items m^{-2}, which is higher than international averages, with 92% being represented by expanded polystyrene (EPS). This indicates that Hong Kong is a hotspot of marine plastic pollution (Fok and Cheung, 2015). *Atactodea striata* is a small sturdy clam commonly found in Hong Kong at the top of the beach with coarse sand (Morton and Morton, 1983). As a filter feeder, accumulation of microplastics through ingestion in *A. striata* not only affects the species but would affect higher consumers and the whole community through trophic transfer. We investigated the physiological changes in *A. striata* following the exposure to a mixture of microalgae and microplastics (polystyrene) in the laboratory and hypothesized that feeding rate and energy intake would decrease, although most of the microplastics could be removed through pseudofaeces and faeces production. The results can provide useful information in assessing how polystyrene, the most dominant microplastics in Hong Kong waters, affects the coastal community.

2. Materials and methods

2.1. Collection and maintenance of *Atactodea striata*

Individuals of *Atactodea striata* (shell length: 3.5 cm–4 cm) were obtained from a fish market between September and November 2014 and 30 individuals were selected for each replicate. In the laboratory, ten clams were cultured in each replicate of each of the three treatments in a tank with 3000 ml filtered seawater (salinity: 32 psu, temperature: 21–23 °C) for 2 weeks during the experiment. Clams were kept in recirculating water with a filtration system and fed with the green alga *Dunaliella tertiolecta* at a concentration of about $150 \times 10^7 \pm 5 \times 10^{-7}$ cells l^{-1} daily. To standardize the hunger level, all clams were starved for 2 days before the start of the experiments.

The experiment included three treatments. In Treatment 1, clams were fed with 50 ml of green alga *Dunaliella tertiolecta* at a concentration of about $150 \times 10^7 \pm 5 \times 10^7$ cells l^{-1} daily except on Saturday and Sunday. Before feeding, water in the tank was renewed to remove wastes and the filtration system was stopped. For Treatments 2 and 3, in addition to the algae, clams were offered microplastics at a concentration of 10 items l^{-1} and 1000 items l^{-1}, respectively. Each tank was equipped with air supply to ensure that the algae and microplastics mixed well and suspended freely in water.

2.2. Preparation of microplastics

The experiment included three treatments: two microplastic concentrations (10 items l^{-1} and 1000 items l^{-1}) and a control without microplastics. Plastic polystyrene was used in the experiment because it was the dominant type of microplastics in Hong Kong waters (Fok and Cheung, 2015). Microgranules of polystyrene with size between 63 μm and 250 μm were prepared by grinding polystyrene and a sieve was used to collect the desired size fraction. Microplastics were soaked in seawater for one week before use. In contrast to the study of Graham and Thompson (2009) in which natural seawater was used, artificial seawater was used in the present study.

2.3. Clearance rate

Clearance rates of *Atactodea striata* under different microplastic concentrations were measured on Day 1, Day 5 and Day 10. Three clams were randomly selected from each replicate of each treatment and transferred to a glass container with 225 ml of filtered seawater (salinity: 32 psu, temperature 21–23 °C). The clams were allowed to adapt to the environment for 5 min before adding 75 ml of *Dunaliella tertiolecta* or mixtures with *D. tertiolecta* and either 10 items l^{-1} or 1000 items l^{-1} of microplastics to the containers. A control with the algal solution only was used to ensure the change in the algal concentration was caused by the clams only. The initial concentration of *D. tertiolecta* in each container was ca. $65 \times 10^7 \pm 15 \times 10^7$ cells l^{-1}. After the addition of *D. tertiolecta*, three water samples were collected 10 min later and the number of *D. tertiolecta* counted using a hemocytometer (1 ml/1 μl) under a microscope. The clearance rate of the clams (cells ind^{-1} h^{-1}) was calculated using the following equation:

$$CR = V[(\ln C0 - \ln Ct)]/nt$$

where

CR: clearance rate (ml min^{-1}),

V: volume of the water in the beaker (ml),

C0: initial concentration of alga in the beaker with clams (cells ml^{-1}),

Ct: final concentration of alga in the beaker with clams (cells ml^{-1}),

n: number of individuals of *Atactodea striata* in each beaker,

t: duration of the measurement (min).

2.4. Absorption efficiency

The absorption efficiency (AE) of *Atactodea striata* under different microplastic concentrations was measured on Day 1, Day 5 and Day 10. AE represents the efficiency organic matter is absorbed from the ingested matters. It is determined by measuring the organic content of the alga *D. tertiolecta* and that of the faeces produced by the clams. At the end of the feeding experiment, three clams were maintained in the containers with oxygen supply and faeces were collected 2–2.5 h later for each treatment using a dropper. Bivalve faeces can be distinguished from pseudofaeces by different appearance, as faeces are usually more tightly packed whereas pseudofaeces are loosely held by mucus (Wong and Cheung, 2003). The collected faeces were placed on the filter paper. Five samples of algal solutions without microplastics were prepared as replicates and the organic content of the alga was determined by the ignition method. The organic content of the faeces collected was determined by hydrogen peroxide digestion as the ignition method would burn away the microplastics. The amount of microplastics in the faeces was counted and removed before the determination of organic content. AE was calculated as follows:

$$AE = (F - E)/[(1 - E) \times F]$$

where AE = absorption efficiency (%), F = ash free dry weight: dry weight ratio in the food, and E = difference in dry weight before and after hydrogen peroxide digestion: dry weight before hydrogen peroxide digestion ratio in the faeces.

2.5. Microplastics in faeces and pseudofaeces

Quantities of microplastics in the faeces and pseudofaeces produced by *Atactodea striata* under different microplastic concentrations were measured on Day 1, Day 5 and Day 10 after the feeding experiment. Three clams from each replicate were maintained in separate containers and all faeces and pseudofaeces were collected separately 2–2.5 h later using a dropper and counted under a microscope.

2.6. Respiration rate

On Day 1, Day 5 and Day 10, the respiration rate (R) was determined before the feeding experiment by measuring the amount of oxygen consumed by a clam in 60 min. Each clam was put in an experimental chamber containing 125 ml of fresh seawater (at 22 °C) with dissolved