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Using the Asian clam as an indicator of microplastic pollution in freshwater ecosystems☆

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ABSTRACT

Bioindicators play an important role in understanding pollution levels, bioavailability and the ecological risks of contaminants. Several bioindicators have been suggested for understanding microplastic in the marine environment. A bioindicator for microplastics in the freshwater environment does not exist. In our previous studies, we found a high frequency of microplastic pollution in the Asian clam (*Corbicula fluminea*) in Taihu Lake, China. In the present study, we conducted a large-scale survey of microplastic pollution in Asian clams, water and sediment from 21 sites in the Middle-Lower Yangtze River Basin from August to October of 2016. The Asian clam was available in all sites, which included diverse freshwater systems such as lakes, rivers and estuaries. Microplastics were found at concentrations ranging from 0.3-4.9 items/g (or 0.4–5.0 items/individual) in clams, 0.5–3.1 items/L in water and 15–160 items/kg in sediment. Microfibers were the most dominant types of microplastics found, accounting for 60–100% in clams across all sampling sites. The size of microplastics ranged from 0.021-4.83 mm, and microplastics in the range of 0.25–1 mm were dominant. The abundance, size distribution and color patterns of microplastics in clams more closely resembled those in sediment than in water. Because microplastic pollution in the Asian clam reflected the variability of microplastic pollution in the freshwater environments, we demonstrated the Asian clam as an bioindicator of microplastic pollution in freshwater systems, particularly for sediments.

1. Introduction

Plastic pollution in the oceans has been an issue of concern since the first report on the subject appeared in the 1970s (Carpenter and Smith, 1972). In recent years, the focus has shifted to small-sized plastic pollutants, called microplastics (plastic items < 5 mm). Global investigations on microplastics have been conducted in a diversity of marine habitats (Cole et al., 2011; Thompson et al., 2004). The occurrence of microplastic pollution has been confirmed in organisms (Gall and Thompson., 2015), water (Van Sebille, 2014) and sediments (Browne et al., 2011) globally. The interactions of microplastics throughout the marine ecosystem have become one of the primary concerns associated with microplastic pollution (Galloway et al., 2017; Wang et al., 2016).

Using field studies, the uptake and ingestion of microplastics has been demonstrated in a wide diversity of marine organisms, including plankton, fish, and mammals (Desforges et al., 2015; Fossi et al., 2014; Wesch et al., 2016). The transfer of microplastics from one trophic level to another has been demonstrated in the laboratory (Setälä et al., 2014; Van Franeker et al., 2011). Animals represent an important transport mechanisms for microplastics in the environment (Clark et al., 2016; Hu et al., 2016). In the oceans, marine vertebrate animals, including fish, seabirds, fin whales and turtles have been suggested as good bioindicator for marine plastic debris due to their life-history strategies (Fossi et al., 2014; Jabeen et al., 2017; Mascarenhas et al., 2004; Provencher et al., 2015). These bioindicators can provide information about microplastic pollution concentrations in their habitats (Wesch et al., 2016).

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Among invertebrates, bivalves are valuable sentinel organisms for indicating levels of different pollutants in the environment (Boening, 1999). They have the ability to concentrate and accumulate pollutants substantially above background environmental levels. Filter feeder organisms act as a trap, accumulating pollutants because of their low excretion rates (Jara-Marini et al., 2013). Such advantages allow the use of bivalves as a tool to biomonitor organic contaminants and metals (Koch et al., 2007). The uptake of microplastics in marine bivalves (e.g. blue mussel) has been well documented (Li et al., 2015, 2016; Van Cauwenberghe and Janssen, 2014). As such, mussels have been proposed as a bioindicator of microplastics. Bivalves make a good bioindicator because of their ability to ingest microplastics, but also because of their relevance to the issue of seafood safety (Rochman et al., 2015). Because humans consume bivalves whole, they are a direct route of exposure via a seafood diet. Although current research cannot provide an accurate dose of microplastics that will pose direct harm to human health, concerns related to microplastic-associated risk to humans is increasing (Seltenrich, 2015). Microplastics may accumulate and cause a potential health risk once they are ingested (Wright and Kelly, 2017). In addition to risk from the physical particle, the chemicals bound to microplastics may be transferred to humans (Browne et al., 2013). Because the level of health risk from microplastics remains unclear, more efforts to address the interaction between microplastics and biota are critical. Measuring the pollutants inside bivalves is a direct way to assess internal exposure levels and to begin to link the bioavailability to effects (Escher and Hermens, 2004).

More recently, researchers have begun to investigate microplastic pollution in freshwater and terrestrial ecosystems which are recognized as a major source and transport pathways of plastics to the ocean (Eerkes-Medrano et al., 2015; Horton et al., 2017; Rillig, 2012). Today, the study of microplastics in freshwater systems remain at an early stage in comparison with the in-depth studies that have been conducted in the marine environment. Microplastic contamination in freshwater and terrestrial environments deserves further investigation and should be considered as a separate issue rather than as supplementary to marine microplastic research.

In our previous study, we found microplastic pollution in a freshwater bivalve, the Asian clam (*Corbicula fluminea*), in all of our sampling sites in Taihu Lake, China (Su et al., 2016). Populations of Asian clams are widely distributed across China and globally. They are also abundant across a diversity of freshwater systems. For the same reasons as stated above for marine bivalves, Asian clams are successfully used to monitor various contaminants (e.g., nanoparticles) and to study toxicological effects of microplastics in the laboratory (Cid et al., 2015; Sousa et al., 2008; Rochman et al., 2017).

A high level of contamination including nitrogen, heavy metals and emerging organic pollutants, have been reported in many parts of the Yangtze River (Chen et al., 2000; Dai et al., 2011; Floehr et al., 2013). An increase in the concentrations of these pollutants has also been reported over decades (Michishita et al., 2012). This area has been polluted for a long period of time. Recently, there have been several studies demonstrating microplastic pollution in fish, water and sediment from the Middle-Lower Yangtze River Basin (Zhang et al., 2017; Zhao et al., 2015). Here, we carried out a large-scale investigation of microplastics in the Middle-Lower Yangtze River Basin sampling Asian clams, water and sediments. The relation of microplastic in the Asian clam to those in water and sediment was also analyzed. Based on our results, we propose that the Asian clam can be used as a bioindicator of microplastic pollution in freshwater systems.

2. Materials and methods

2.1. Survey sites and areas

Our field survey was conducted in the Middle-Lower Yangtze River Basin from August to October 2016 (Fig. 1). Lakes, rivers and estuarine areas in the Yangtze catchments were selected as study areas (S_1-S_{21}). The sampling areas and individual sampling sites were located in urban as well as rural areas, which are impacted by different sources of pollutants. The sources of these pollutants include agriculture, river traffic, industry and tourism. Detailed information on the sampling area is provided in Supplementary Materials Table 1. During sampling, large plastic debris were commonly observed. In addition, Asian clams were successfully acquired in all of the sampling sites.

2.2. Sample collection

Water samples were collected prior to sediments and Asian clams to avoid collecting suspended solids from the bottom of sampling sites. We collected approximately 5 L of water by dipping a steel bucket from a boat. Water was collected from 0-12 cm below the surface, based on the diameter of the bucket. Three samples were collected at each site ($n = 3$). Three samples of sediment were collected at each site ($n = 3$) with a Peterson sampler from the boat (Hosseini Alhashemi et al., 2012). The top 10 cm of sediment was collected. Each replicate contained approximately 2 kg of wet sediment. Three samples of Asian clams were collected at each site using bottom fauna trawls from the boat ($n = 3$). Each replicate consisted of at least 10 living clams of similar sizes. Sediment and water samples were sealed and kept at 4 °C, and the clam samples were kept at −20 °C until further analysis.

2.3. Quality control of experiments

All the containers (glass bottle, aluminum pot and aluminum foil bag) and sampling tools were washed using tap water, which was filtered prior to use (pore size of filter was 0.45 μm). The tools were sealed in an aluminum foil bag and kept clean before using. During the sampling procedure, the tools were prewashed using water *in situ* to avoid contamination. In the laboratory, blanks were run (51 blank samples in total) without water, sediment or clam tissue and were performed simultaneously to correct and evaluate background contamination. Procedural contamination ranged from 0.19 to 0.62 items per treatment group (0–3 particles per sample) for water, clam and sediment samples. All the microplastics in blank samples were microfibers. The background contamination was equal to 4.9–6.9% of the abundance of microplastics in all of the samples. The background contamination was not subtracted from the final results in the current study, but should be taken into consideration for interpretation.

2.4. Isolation of microplastics

A two-step filtration process was used to extract microplastics from the water and sediment samples (Su et al., 2016). Briefly, the volume of water was first recorded and particles in the water were filtered onto nylon net filter using a vacuum system. The pore size of filter was 20 μm (Millipore Nylon NY2004700). Any particles on the filter were washed into a glass flask using 100 mL of hydrogen peroxide (30%, V/V) to digest the organic substances. The flasks were covered and placed in an oscillation incubator at 65 °C and 80 rpm for no more than 72 h. The liquid in the flask was filtered again, and the filter was covered and stored in dry Petri dishes for further observation.