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

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A B S T R A C T

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.

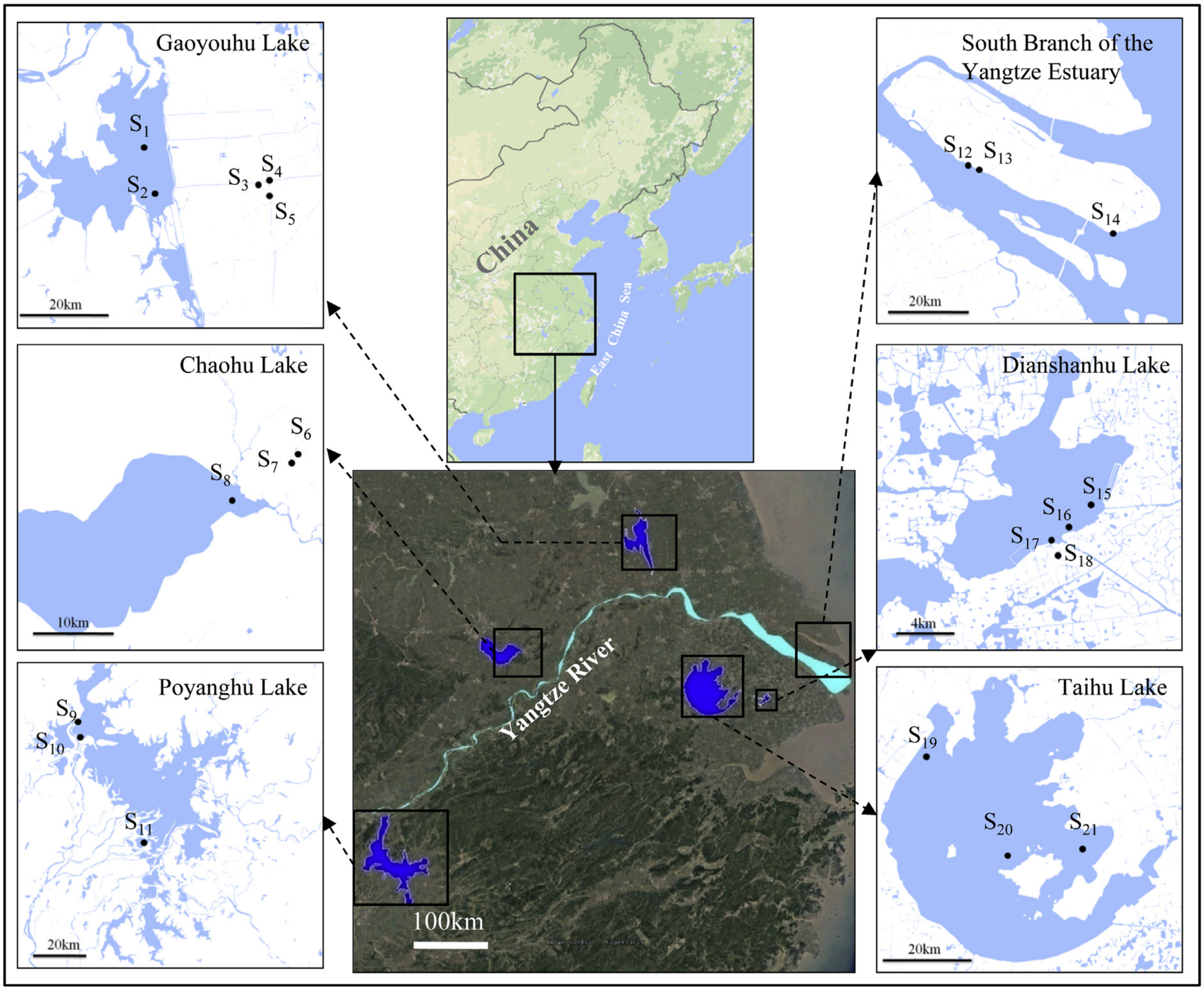

Fig. 1. Locations of sampling areas and sites.

The wet sediment was pooled in an aluminum pot with a cap and dried in an oven at 65 °C. Approximately 300 g of dry sediment was weighed and mixed with saturated sodium chloride solution (1.2 g/mL) at a ratio of 1:2 (V/V) in a 2-L glass container with a 30-cm depth. It should be noted that the use of saturated sodium chloride solution might lead to the loss of some material with densities greater than 1.2 g/mL. The mixture was stirred and settled for 24 h. The supernatant was then transferred onto nylon filters with a 20-µm pore size. All particles on the filter were washed into a glass flask with 100 mL of hydrogen peroxide for digestion. The digestion and filtration processes were performed using the same method for the water samples.

The isolation of microplastics from Asian clams was based on our previous study (Li et al., 2015; Su et al., 2016). Briefly, we recorded the clam's total weight, soft tissues weight and shell length (Supplementary materials Table 1). In the present study, the abundance of microplastics in Asian clam was based on the weight of soft tissue. For each sampling site, three replicates of 2–4 clams were extracted and analyzed. The clams were transferred to a flask and 200 mL of hydrogen peroxide was added to digest them. The flask was then covered and placed in an oscillation incubator at 65 °C and 80 rpm for no more than 72 h. After digestion, the liquid in the flask was filtered, and the filter was covered and stored in dry Petri dishes for further observation.

2.5. Observation and validation of microplastic

All suspected plastic particles on the filters were observed and photographed using a microscope (MicroImaging GmbH, Goottingen, Germany) with 25–80x magnification. We used visual assessments to quantify and sort the suspected microplastics based on their properties (Yang et al., 2015). Visually identified microplastics from water, sediment and clam samples were classified into four groups: fiber, pellet, film and fragment (Su et al., 2016). Fibers were rod-like and flexible strips; pellets were items with a spherical shape; films were very thin and small layers; fragments were incomplete or isolated parts of large plastic debris. The color and size of the microplastics were also measured and recorded in visual assessments.

From the 1303 particles, 150 particles were selected for validation using micro-Fourier Transform Infrared Spectroscopy (µ-FT-IR). To verify the accuracy of our visual identification of microplastics, we randomly selected 1–2 particles from the central area of each filter. The polymer composition was measured under the attenuated total reflection mode of an µ-FT-IR (Bruker, LUMOS). All data were collected 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 (Güven et al., 2017). The spectra matching with a quality index ≥70 were accepted. Finally, the number of microplastics reported was recalculated by excluding the verified non-plastic items.

2.6. Data analysis

A one-way analysis of variance (ANOVA) was used to determine the differences in the quantities of microplastics among individual sampling sites and the distribution of microplastics shape, size and color. To test for multiple comparisons, post-hoc Tukey's HSD test (homogeneous variances) and the Tamhane-Dunnett test were applied (heterogeneous variances). A 0.05 and 0.01 significance level was chosen. A linear regression analysis was used to test whether there was a significant correlation among the abundance of microplastic in clams, water and sediment. The Pearson correlation coefficient determined the goodness of fit and significance of the correlation.

The size distribution of microplastics in different fractions was plotted using a heat map and a cumulative curve to compare different fractions. In the heat map, the depth of gray in a size interval represented the percentage of microplastic in that size interval. The process of degradation in the environment might result in a loss of color. Hence, the transparent and white items in samples were marked as "colorless", and items of other colors were marked as "colored". The digestion of hydrogen peroxide could also have resulted in the bleaching of microplastics and a subsequent overestimation of the number of colorless particles. However, our current digestion process was not strong enough to lead to a complete bleaching of microplastics, and only parts of individual items were discolored (Li et al., 2016). To avoid an overestimation of colorless items, only whole white and transparent items were considered to be "colorless". Principal component analysis was then used to analyze the variance of data and identify the independent principal components (PC). The PC bi-plots were created to describe similar or dissimilar patterns of variance for the colorless and colored group from different samples. The data analysis in the current study was processed using SPSS 22.0 and GraphPad Prism 5.0.

3. Results

Of the 150 randomly selected items, 122 items were confirmed as plastics using μ-FT-IR. As such, the success rate of our visual identification was 81%. We identified fourteen polymer types (Supplementary materials Table 2). The dominant polymer was polyester (33%), followed by polypropylene (19%) and polyethylene (9%). The selected particles represented the most common types of visually identified particles.

3.1. Microplastic pollution in Asian clams

The average abundance of microplastic in clams from each site ranged from 0.3-4.9 items/g and 0.4–5.0 items/individual (Fig. 2). The abundance differed significantly among 21 sampling sites ($p < 0.01$). The lowest abundance of microplastics was found in Poyanghu Lake (S_9) by items/g and in Chaohu Lake (S_8) by items/individual. The highest abundance of microplastics in clams was in S_3 by items/g and in S_5 in Gaoyouhu Lake by items/individual.

For the clams, fibers were most dominant ($p < 0.01$), accounting for 60–100% of particles across all sampling sites (Fig. 3A). The size of microplastics in clam samples ranged from 0.021-4.02 mm, with the 0.25–1 mm size was dominant ($p < 0.01$) (Fig. 3B). Blue and transparent items made up more than 30% of all particles and were significantly more abundant than other colors ($p < 0.05$) (Fig. 3C).

3.2. Microplastic pollution in water and sediment

The average abundance of microplastics in samples across all sites ranged from 0.5-3.1 items/L in water and 15–160 items/kg in sediment (Fig. 4). The microplastic abundance differed significantly

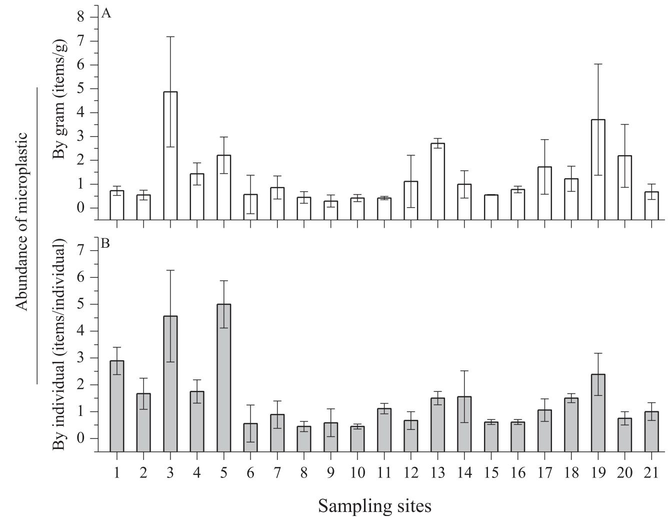

Fig. 2. The abundance of microplastics in the Asian clam by the terms of items/g (A) and items/individual (B).

among the 21 sampling sites ($p < 0.01$). The lowest abundance of microplastics was found in S_{17} in water and in S_{15} in sediment in Dianshanhu Lake, and the highest was found in South Branch of the Yangtze Estuary (S_{13}) in water and Gaoyouhu Lake (S_3) in sediment.

The size of microplastics ranged from 0.022-4.83 mm in water and sediment samples. Fibers were dominant among all types ($p < 0.01$), and particles that were 0.25–1 mm in size dominated most water and sediment samples ($p < 0.01$) (Supplementary materials S1 and 2). Again, the transparent and blue items were most commonly found, and the transparent items dominated in all color classes in water and sediment ($p < 0.01$) (Supplementary materials S1 and 2).

3.3. The relationship of microplastics in clams, water and sediments

Based on the regression analysis, the abundance of microplastics in clams significantly depended on the microplastic pollution in water ($p < 0.05$) and sediment ($p < 0.01$) (Fig. 5A). According to the heat map and the cumulative curve of microplastic size distribution, the microplastics in clams were more closely related to those in sediment (Fig. 5B). The median of microplastics in clams (median = 0.61 mm) was also much closer to sediment (median = 0.53 mm) than to water (median = 0.79 mm). Based on a principal component analysis, the colored and colorless items in clams had a close relationship with those in sediment, and they could be grouped as one category in the first, second and third principal components (Fig. 5C). The dominant type of microplastics in clams, water and sediments was fiber ($p > 0.05$).

4. Discussion

4.1. The level of microplastic pollution in clams, water and sediment

To the best of our knowledge, the current *in situ* work is the first to study microplastics in organisms, water and sediments in freshwater systems on a large spatial scale. Although the sampling areas did cover a large geographic area, it is notable that the Asian clam was available at all 21 sites, which included diverse freshwater systems such as lakes, rivers and estuaries. Overall, we found a wide range of microplastic pollution across the different freshwater systems. We also found variation in microplastic concentrations among different sites within the same lake, river or estuary. The

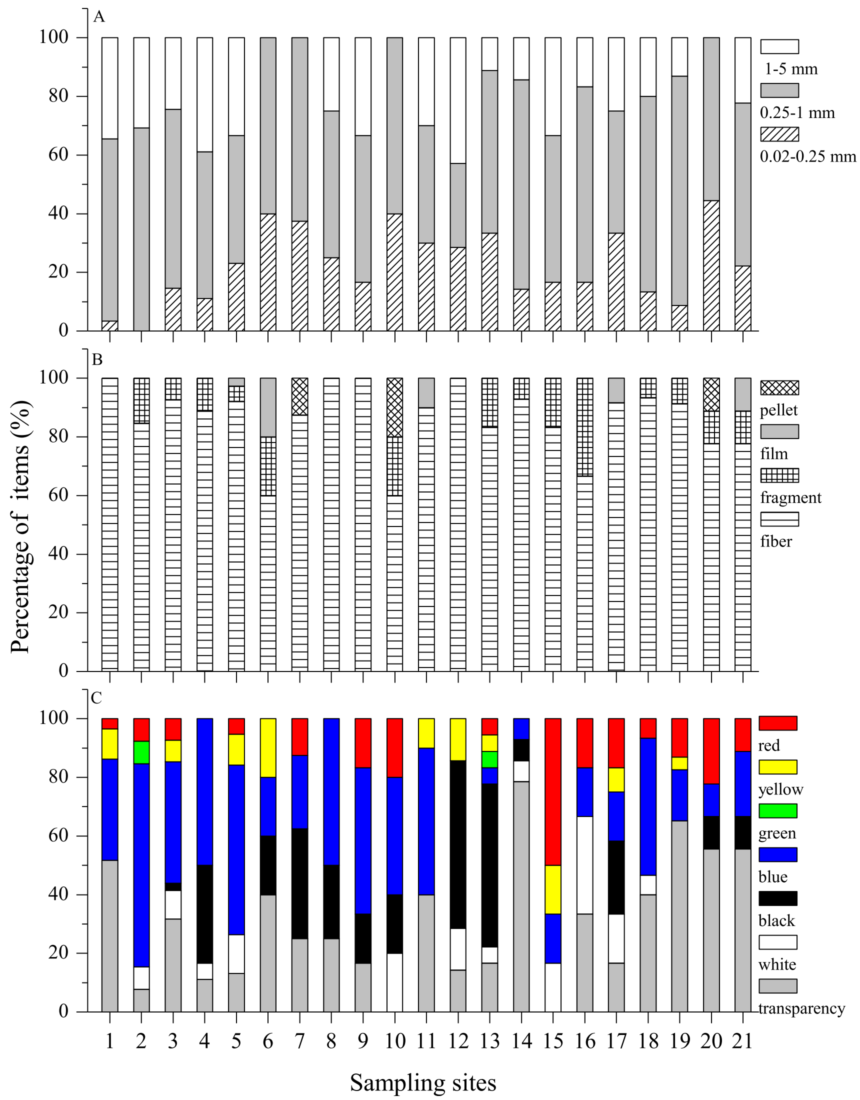

Fig. 3. The distribution of size (A) shape (B) and color (C) in the Asian clam. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

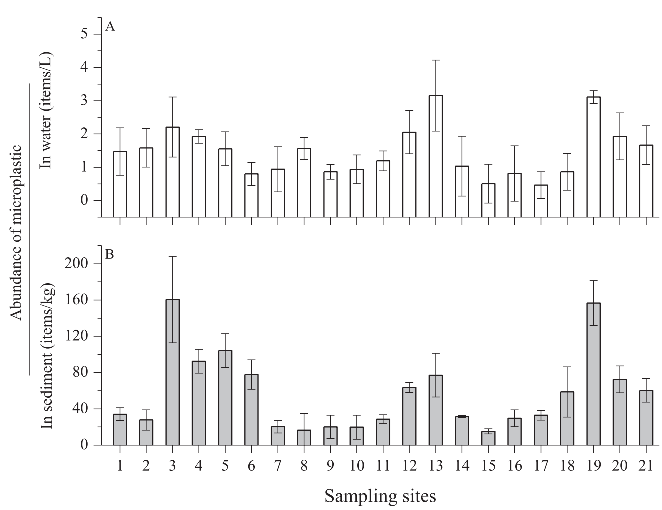

Fig. 4. The abundance of microplastics in water (A) and sediment (B).

concentration of microplastics in clams also varied in accordance with the variation of microplastic concentrations in water and sediment. Because the Asian clam can easily be collected in different freshwater systems and the variance of microplastic concentrations in clams matches environmental concentrations, the Asian clam is appropriate as a bioindicator of microplastic pollution at a large scale.

In our study, we were found microplastics in 61 out of 63 samples of Asian clams (2–4 clams per sample) of clam samples. This frequency of microplastic presence (96%) is much higher than in freshwater fish sampled from China and Switzerland (7.5%–26%) (Faure et al., 2015; Zhang et al., 2017). The concentration of microplastic pollution in Asian clams comparable with those in mussels, providing the possibility of comparing marine and freshwater habitats (Van Cauwenberghe and Janssen, 2014). In addition, the high level of microplastic pollution in Asian clams indicates that freshwater systems are contaminated and that microplastics may be able to transfer through the food chain.

In this study, the levels of microplastic pollution reported for water and sediment fell within the range of microplastic levels in global freshwater systems (Horton et al., 2017). Nevertheless,

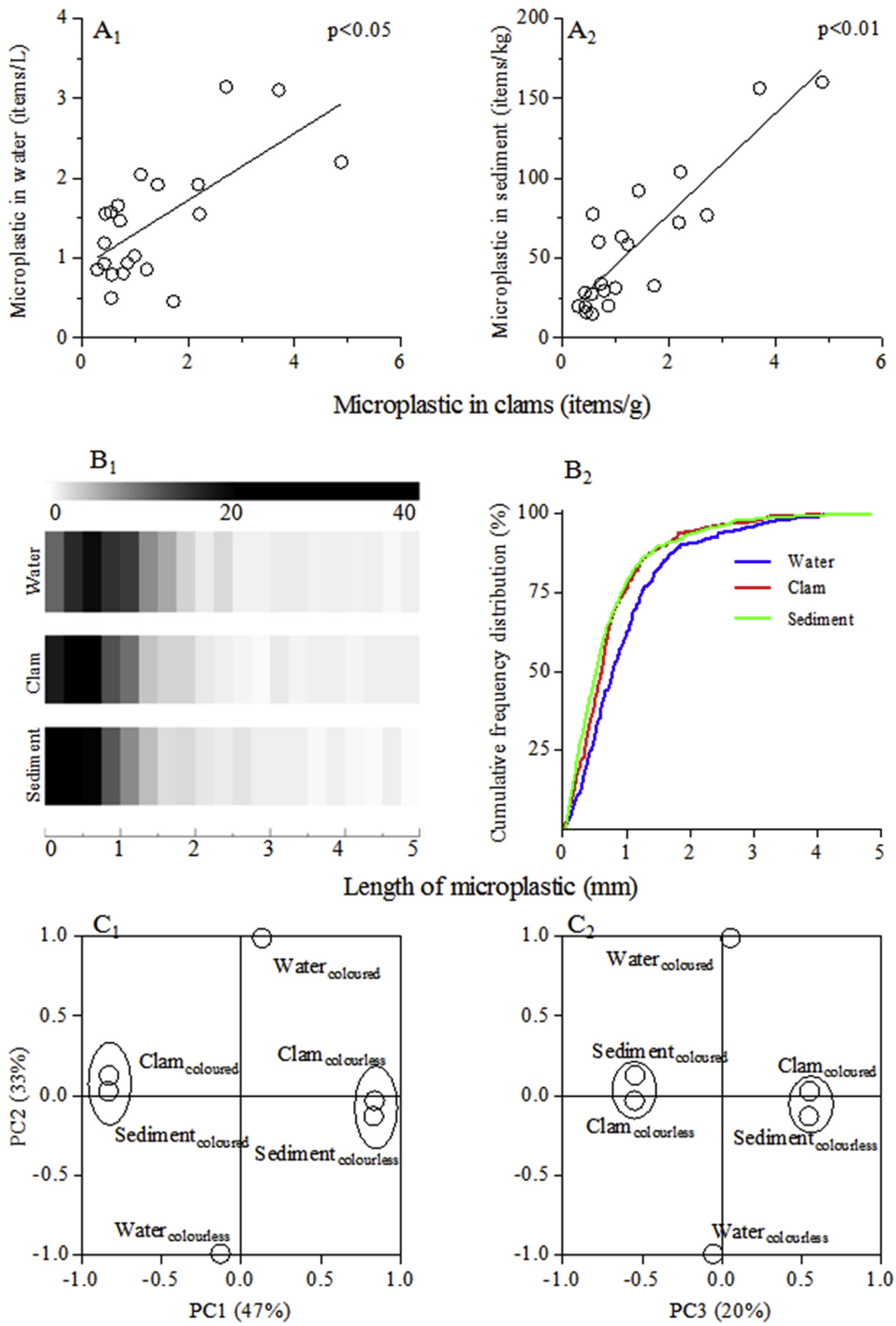

Fig. 5. The relation of microplastics in clams to those in water and sediment. (A) The abundance; (B) The size. The heat map (B_1) and the cumulative curve of frequency (B_2) were used to show the distribution of microplastic sizes; (C) The color. Principle component analysis was used to interpret the microplastic color patterns. Component loadings for the first and second components (C_1) and the second and third component (C_2) were plotted. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

significant variation in microplastic pollution could also be found among different samples from the same lake, river or estuarine area. ($p < 0.05$). Many factors, including the presence of wastewater effluent, hydrological conditions and sedimentation, have been shown to drive microplastic concentrations in freshwater (Eriksen et al., 2013; Free et al., 2014; Vaughan et al., 2017). In the current study, we proposed that the level of industrialization and hydrological conditions should be considered as important contributors to microplastic pollution. First, the level of microplastic pollution in Poyanghu Lake was low. Poyanghu Lake is located in the Middle Yangtze River Valley, which is less developed and has lower levels of anthropogenic activities than the Lower Yangtze River Valley

(Michishita et al., 2012). Second, the sampling sites located in rivers showed higher microplastic pollution levels than those in lakes ($p < 0.05$). There are differences in the hydrological conditions between the river and lake systems. Our findings indicate that the hydrological conditions in river systems may facilitate the retention of microplastics. The rivers in the current study have a smaller volume of water than lakes, which might result in high concentrations of microplastics. In addition, some of the rivers are located in urban areas and receive more wastewater effluent than lakes. The theoretical research conducted by Nizzetto et al. (2016) also indicated that sediments in river catchments are likely hotspots for the deposition of microplastics.

4.2. The relationships between microplastics in clams and in water and sediment

There was a stronger relationship between microplastic concentrations in clams and sediments than in clams and water. In comparison with the microplastics floating on the surface layer of the water, it is reasonable to assume that microplastics accumulating in sediments are more likely to influence benthic filter-feeders. Asian clams filter feed and deposit feed, which may be one reason what we see more closely matches the sediments. The substances associated with suspended sediments can be ingested by benthic filter-feeders because of the process of sediment re-suspension (Hickey et al., 1995). For microplastics, the process of sediment re-suspension could transfer the microplastics in sediment to overlying water which were readily filtered by benthic filter-feeders. On the other hand, benthic filter-feeders are able to draw down microplastics from the water to sediment, which will alter the partitioning of microplastics between water and sediments (Galloway et al., 2017). These factors may help drive the strong correlation between the abundance of microplastics in sediment and clams observed in the present study.

We also found that the size of microplastics in clams was significantly smaller than those in water but similarly sized to those in sediment. This phenomenon may increase concerns about the environmental risks of smaller-sized plastic particles because they are more likely to be taken up by organisms, thus posing a greater risk (Bergami et al., 2016; Besseling et al., 2014). The color patterns of microplastics in clams were also similar to those in sediment in the present study. In particular, the blue microplastic items frequently found in clams have also been reported in field studies of marine fishes (Güven et al., 2017). We found that the clams showed greater retention of fibers. Such patterns might account for the prevalent existence of fibers in all of the samples.

4.3. The Asian clam as an indicator of microplastic pollution in freshwater systems

In the present study, we mapped the spatial patterns of microplastics in Asian clams, along with those in water and sediment. The relationships among the Asian clam, water and sediment with regards to microplastic pollution were also evaluated. These findings suggested that the Asian clam can serve as a bioindicator of microplastic pollution in freshwater systems with several advantages.

First, microplastics in Asian clams represent internal exposure levels of microplastics in benthic organisms in freshwater systems. The measurement of microplastics in clams is a direct way to evaluate the ecological risks imposed by microplastic uptake. In natural conditions, not all of the microplastics in sediment or water are available to specific organisms. In our study, although the microplastics in sediments are more similar to those in clams, there were still differences in size, color and types. It should be noted that the accumulation of microplastics is a bit different from chemical pollutants. The bioavailability of microplastics is largely determined by shape and size rather than their thermodynamic behavior (Wright et al., 2013). The uptake and ingestion of microplastics vary depending on the characteristics of the microplastics and the life-history strategies of the species (Van Franeker et al., 2011). Only those microplastics with the appropriate shape and size can be ingested by organisms (Rochman et al., 2017). Whether or not the particle causes harm, likely depends on exposure concentration, particle type and retention time. In some cases, such process might result in potential damage inside the organisms. (Lu et al., 2016; Watts et al., 2014). The levels and patterns of microplastics in the Asian clam can serve to track the amount and characteristics of microplastics that may be bioavailable to organisms of a similar size. To better understand the potential risks of microplastics, a greater effort to study the impacts of microplastic exposure on organisms in the environment is needed.

Second, the Asian clam is widely distributed and easily collected. The Asian clam is one of the world's most widespread invasive species, and has undergone a massive global range expansion (Pigneur et al., 2014). It dominates the benthic macroinvertebrate community and reaches densities ranging from hundreds to thousands of individuals per square meter (Cai et al., 2012; Paunović et al., 2007). Such advantages ensure that the Asian clam is a reliable subject and is readily available in different habitats. In the present study, approximately 10 individuals in each sampling site were sufficient to reflect the level of microplastic pollution in a large-scale survey. It is also easy and cost-effective to transport the living clams to a laboratory, where they can easily be cultured and used as a model species of ecotoxicology (Spann et al., 2011).

From an ecological perspective, Asian clams can, like other organisms, provide a snapshot of what can be ingested across a large geographic area. In marine systems, the analysis of microplastics in fish, birds and mussels is proposed as a useful method to monitor the microplastics (Wesch et al., 2016). Specimens in freshwater systems are insufficiently available to monitor microplastics. We suggest that in freshwater systems, Asian clams should be considered a valuable monitoring tool. Bottom filter feeders at low trophic levels are critical to understanding the pathways of microplastics in the food chain because these organisms are likely to carry microplastics into the food web (Wright et al., 2013).

Last but not least, measuring microplastics in the Asian clam may be an important step to link microplastic pollution to potential risks for humans. Shellfish are of particular interest to assess human health because the soft tissues of shellfish are usually consumed without the removal of the digestive tract. Any microplastic contained in the soft tissues of shellfish is thus readily transferred to consumers. Furthermore, the potential transfer of plastic additives to other organisms and relevant human exposures through seafood can also increase through microplastic pollution (Seltenrich, 2015). When ingested, microplastics and their associated chemicals may cause a series of inflammatory and immune responses (Wright and Kelly, 2017).

Field studies of microplastic pollution have been conducted in marine mussels to address questions related to microplastic pollution and food safety (Van Cauwenberghe and Janssen, 2014). The Asian clam is a popular shellfish in the human diet, and the aquaculture of the Asian clam in China is widespread, increasing the risks of microplastics through daily consumption. Further, the complete uptake of Asian clam tissues may be a pathway for microplastic ingestion. Though the capita consumption for freshwater shellfish in China was unknown, the amounts of microplastics in Asian clams was similar to mussel in China. As Wright and Kelly (2017) pointed out, microplastics contaminated food for human consumption and potential human health risks should not be ignored. Until now, few studies have quantified the level of microplastics exposure via freshwater shellfish consumption. Therefore, it will be valuable to set the baseline about the microplastic consumption by humans in freshwater system in future.

5. Conclusion

In the present study, Asian clams were available across diverse sites in freshwater lakes, rivers and estuaries. Within these locations, microplastic pollution in the Asian clams reflected the significant variability of microplastic pollution in sediments and water. The abundance, size distribution and color patterns of microplastics in Asian clams were similar to water, but most similar to sediment. Based on our results, we propose the Asian clam an indicator of microplastic pollution in freshwater systems, particularly in sediments.

Notes

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2017.11.075.

References

Bergami, E., Bocci, E., Vannuccini, M.L., Monopoli, M., Salvati, A., Dawson, K.A., Corsi, I., 2016. Nano-sized polystyrene affects feeding, behavior and physiology of brine shrimp *Artemia franciscana* larvae. Ecotox. Environ. Safe 123, 18–25.

Besseling, E., Wang, B., Lurling, M., Koelmans, A.A., 2014. Nanoplastic affects growth of *S. obliquus* and reproduction of *D. magna*. Environ. Sci. Technol. 48, 12336–12343.

Boening, D.W., 1999. An evaluation of bivalves as biomonitors of heavy metals pollution in marine waters. Environ. Monit. Assess. 55, 459–470.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines woldwide: sources and sinks. Environ. Sci. Technol. 45, 9175–9179.

Browne, Mark A., Niven, Stewart J., Galloway, Tamara S., Rowland, Steve J., Thompson, Richard C., 2013. Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity. Curr. Biol. 23, 2388–2392.

Cai, Y., Gong, Z., Qin, B., 2012. Benthic macroinvertebrate community structure in Lake Taihu, China: effects of trophic status, wind-induced disturbance and habitat complexity. J. Gt. Lakes Res. 38, 39–48.

Carpenter, E.J., Smith, K.L., 1972. Plastics on the Sargasso sea surface. Science 175, 1240–1241.

Chen, J., Gao, X., He, D., Xia, X., 2000. Nitrogen contamination in the Yangtze River system, China. J. Hazard. Mater 73, 107–113.

Cid, A., Picado, A., Correia, J.B., Chaves, R., Silva, H., Caldeira, J., de Matos, A.P.A., Diniz, M.S., 2015. Oxidative stress and histological changes following exposure to diamond nanoparticles in the freshwater Asian clam Corbicula fluminea (Müller, 1774). J. Hazard. Mater 284, 27–34.

Clark, J.R., Cole, M., Lindeque, P.K., Fileman, E., Blackford, J., Lewis, C., Lenton, T.M., Galloway, T.S., 2016. Marine microplastic debris: a targeted planfor understanding and quantifying interactions with marine life. Front. Ecol. Environ. 14, 317–324.

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62, 2588–2597.

Dai, Z., Du, J., Zhang, X., Su, N., Li, J., 2011. Variation of riverine material loads and environmental consequences on the Changjiang (Yangtze) Estuary in recent decades (1955–2008). Environ. Sci. Technol. 45, 223–227.

Desforges, J.P.W., Galbraith, M., Ross, P.S., 2015. Ingestion of microplastics by zooplankton in the northeast pacific ocean. Arch. Environ. Con. Tox 69, 320–330.

Eerkes-Medrano, D., Thompson, R.C., Aldridge, D.C., 2015. Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water. Res. 75, 63–82.

Eriksen, M., Mason, S., Wilson, S., Box, C., Zellers, A., Edwards, W., Farley, H., Amato, S., 2013. Microplastic pollution in the surface waters of the laurentian great lakes. Mar. Pollut. Bull. 77, 177–182.

Escher, B.I., Hermens, J.L.M., 2004. Internal exposure: linking bioavailability to effects. Environ. Sci. Technol. 38, 455A–462A.

Faure, F., Demars, C., Wieser, O., Kunz, M., de Alencastro, L.F., 2015. Plastic pollution in Swiss surface waters: nature and concentrations, interaction with pollutants. Environ. Chem. 12, 582–591.

Floehr, T., Xiao, H.X., Scholz-Starke, B., Wu, L.L., Hou, J.L., Yin, D.Q., Zhang, X.W., Ji, R., Yuan, X.Z., Ottermanns, R., Roß-Nickoll, M., Schäffer, A., Hollert, H., 2013. Solution by dilution? A review on the pollution status of the Yangtze River. Environ. Sci. Pollut. Res. 20, 6934–6971.

Fossi, M.C., Coppola, D., Baini, M., Giannetti, M., Guerranti, C., Marsili, L., Panti, C., de Sabata, E., Clo, S., 2014. Large filter feeding marine organisms as indicators of microplastic in the pelagic environment: the case studies of the Mediterranean basking shark (*Cetorhinus maximus*) and fin whale (*Balaenoptera physalus*). Mar. Environ. Res. 100, 17–24.

Free, C.M., Jensen, O.P., Mason, S.A., Eriksen, M., Williamson, N.J., Boldgiv, B., 2014. High-levels of microplastic pollution in a large, remote, mountain lake. Mar. Pollut. Bull. 85, 156–163.

Gall, S.C., Thompson, R.C., 2015. The impact of debris on marine life. Mar. Pollut. Bull. 92, 170–179.

Galloway, T.S., Cole, M., Lewis, C., 2017. Interactions of microplastic debris throughout the marine ecosystem. Nat. Eol. Evol. 1, 1–8.

Güven, O., Gökdağ, K., Jovanović, B., Kıdeyş, A.E., 2017. Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish. Environ. Pollut. 223, 286–294.

Hickey, C.W., Roper, D.S., Holland, P.T., Trower, T.M., 1995. Accumulation of organic contaminants in two sediment-dwelling shellfish with contrasting feeding modes: deposit- (*Macomona liliana*) and filter-feeding (*Austrovenus stutchburyi*). Arch. Environ. Con. Tox 29, 221–231.

Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. Sci. Total. Environ. 586, 127–141.

Hosseini Alhashemi, A., Sekhavatjou, M.S., Hassanzadeh Kiabi, B., Karbassi, A.R., 2012. Bioaccumulation of trace elements in water, sediment, and six fish species from a freshwater wetland. Iran. Microchem. J. 104, 1–6.

Hu, L., Su, L., Xue, Y., Mu, J., Zhu, J., Xu, J., Shi, H., 2016. Uptake, accumulation and elimination of polystyrene microspheres in tadpoles of *Xenopus tropicalis*. Chemosphere 164, 611–617.

Jabeen, K., Su, L., Li, J., Yang, D., Tong, C., Mu, J., Shi, H., 2017. Microplastics and mesoplastics in fish from coastal and fresh waters of China. Environ. Pollut. 221, 141–149.

Jara-Marini, M.E., Tapia-Alcaraz, J.N., Dumer-Gutiérrez, J.A., García-Rico, L., García-Hernández, J., Páez-Osuna, F., 2013. Comparative bioaccumulation of trace metals using six filter feeder organisms in a coastal lagoon ecosystem (of the central-east Gulf of California). Environ. Monit. Assess. 185, 1071–1085.

Koch, I., McPherson, K., Smith, P., Easton, L., Doe, K.G., Reimer, K.J., 2007. Arsenic bioaccessibility and speciation in clams and seaweed from a contaminated marine environment. Mar. Pollut. Bull. 54, 586–594.

Li, J., Yang, D., Li, L., Jabeen, K., Shi, H., 2015. Microplastics in commercial bivalves from China. Environ. Pollut. 207, 190–195.

Li, J., Qu, X., Su, L., Zhang, W., Yang, D., Kolandhasamy, P., Li, D., Shi, H., 2016. Microplastics in mussels along the coastal waters of China. Environ. Pollut. 214, 177–184.

Lu, Y.F., Zhang, Y., Deng, Y.F., Jiang, W., Zhao, Y.P., Geng, J.J., Ding, L.L., Ren, H.Q., 2016. Uptake and accumulation of polystyrene microplastics in zebrafish (*Danio rerio*) and toxic effects in liver. Environ. Sci. Technol. 50, 4054–4060.

Mascarenhas, R., Santos, R., Zeppelini, D., 2004. Plastic debris ingestion by sea turtle in Paraíba. Braz. Mar. Pollut. Bull. 49, 354–355.

Michishita, R., Jiang, Z., Xu, B., 2012. Monitoring two decades of urbanization in the Poyang Lake area, China through spectral unmixing. Remote. Sens. Environ. 117, 3–18.

Nizzetto, L., Bussi, G., Futter, M.N., Butterfield, D., Whitehead, P.G., 2016. A theoretical assessment of microplastic transport in river catchments and their retention by soils and river sediments. Environ. Sci. Proc. Impacts 18, 1050.

Paunović, M., Csányi, B., Knežević, S., Simić, V., Nenadić, D., JakovčevTodorović, D., Stojanović, B., Cakić, P., 2007. Distribution of Asian clams *Corbicula fluminea* (Müller, 1774) and *C. fluminalis* (Müller, 1774) in Serbia. Aquat. Invasions 2, 99–106.

Pigneur, L.M., Falisse, E., Roland, K., Everbecq, E., Deliege, J.F., Smitz, J.S., van Doninck, K., Descy, J.P., 2014. Impact of invasive Asian clams, *Corbicula spp.*, on a large river ecosystem. Freshw. Biol. 59, 573–583.

Provencher, J.F., Bond, A.L., Mallory, M.L., 2015. Marine birds and plastic debris in Canada: a national synthesis and a way forward. Environ. Rev. 23, 1–13.

Rillig, M.C., 2012. Microplastic in eerrestrial ecosystems and the soil? Environ. Sci. Technol. 46, 6453–6454.

Rochman, C.M., Tahir, A., Williams, S.L., Baxa, D.V., Lam, R., Miller, J.T., Teh, F.C., Werorilangi, S., Teh, S.J., 2015. Anthropogenic debris in seafood: plastic debris and fibers from textiles in fish and bivalves sold for human consumption. Sci. Rep. 5, 10.

Rochman, C.M., Parnis, J.M., Browne, M.A., Serrato, S., Reiner, E.J., Robson, M.,

Young, T., Diamond, M.L., Teh, S.J., 2017. Direct and indirect effects of different types of microplastics on freshwater prey (*Corbicula fluminea*) and their predator (*Acipenser transmontanus*). PLoS One 12, e0187664.
Seltenrich, N., 2015. New link in the food chain? Marine plastic pollution and seafood safety. Environ. Health. Perspect. 123, A34–A41.
Setälä, O., Fleming-Lehtinen, V., Lehtiniemi, M., 2014. Ingestion and transfer of microplastics in the planktonic food web. Environ. Pollut. 185, 77–83.
Sousa, R., Antunes, C., Guilhermino, L., 2008. Ecology of the invasive Asian clam *Corbicula fluminea* (Muller, 1774) in aquatic ecosystems: an overview. Ann. Limnol. Int. J. Limnol. 44, 85–94.
Spann, N., Aldridge, D.C., Griffin, J.L., Jones, O.A.H., 2011. Size-dependent effects of low level cadmium and zinc exposure on the metabolome of the Asian clam, *Corbicula fluminea*. Aquat. Toxicol. 105, 589–599.
Su, L., Xue, Y., Li, L., Yang, D., Kolandhasamy, P., Li, D., Shi, H., 2016. Microplastics in Taihu Lake, China. Environ. Pollut. 216, 711–719.
Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at sea: where is all the plastic? Science 304, 838–838.
Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human consumption. Environ. Pollut. 193, 65–70.
Van Franeker, J.A., Blaize, C., Danielsen, J., Fairclough, K., Gollan, J., Guse, N., Hansen, P.-L., Heubeck, M., Jensen, J.-K., Le Guillou, G., Olsen, B., Olsen, K.O., Pedersen, J., Stienen, E.W.M., Turner, D.M., 2011. Monitoring plastic ingestion by the northern fulmar Fulmarus glacialis in the North Sea. Environ. Pollut. 159, 2609–2615.
Van Sebille, Erik, 2014. Plastic in the world's oceans. Sci. Educ. News 63, 39–41.
Vaughan, R., Turner, S.D., Rose, N.L., 2017. Microplastics in the sediments of a UK urban lake. Environ. Pollut. 229, 10–18.
Wang, J., Tan, Z., Peng, J., Qiu, Q., Li, M., 2016. The behaviors of microplastics in the marine environment. Mar. Environ. Res. 113, 7–17.
Watts, A.J.R., Lewis, C., Goodhead, R.M., Beckett, S.J., Moger, J., Tyler, C.R., Galloway, T.S., 2014. Uptake and retention of microplastics by the shore crab *Carcinus manenas*. Environ. Sci. Technol. 48, 8823–8830.
Wesch, C., Bredimus, K., Paulus, M., Klein, R., 2016. Towards the suitable monitoring of ingestion of microplastics by marine biota: a review. Environ. Pollut. 218, 1200–1208.
Wright, S.L., Kelly, F.J., 2017. Plastic and human health: a micro issue? Environ. Sci. Technol. 51, 6634–6647.
Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492.
Yang, D.Q., Shi, H.H., Li, L., Li, J.N., Jabeen, K., Kolandhasamy, P., 2015. Microplastic pollution in table salts from China. Environ. Sci. Technol. 49, 13622–13627.
Zhang, K., Xiong, X., Hu, H., Wu, C., Bi, Y., Wu, Y., Zhou, B., Lam, P.K.S., Liu, J., 2017. Occurrence and characteristics of microplastic pollution in Xiangxi bay of three gorges reservoir, China. Environ. Sci. Technol. 51, 3794–3801.
Zhao, S., Zhu, L., Li, D., 2015. Microplastic in three urban estuaries, China. Environ. Pollut. 206, 597–604.