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Microplastics in fishes and their living environments surrounding a plastic production area



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HIGHLIGHTS

GRAPHICAL ABSTRACT

- Microplastics pollution was serious in the plastic production area.
- Pellets and fragments were the main types in the sediment.
- Fragments and pellets were absent from the gut of fish.
- Histopathological damage in fish liver was severe in the plastic production area.



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ABSTRACT

Microplastic-associated risks in freshwater ecosystems have triggered significant concerns in recent years. However, the contribution of plastic production processing to microplastic pollution is largely unknown. The present study investigated microplastic pollution in biotic and abiotic compartments in three sites which are in surrounding area of a plastic industrial colony and a site from a reservoir for drinking water as reference. The abundances of microplastics were 0.4-20.5 items/L in surface water, 44.4-124.7 items/kg (ww) in sediment and 1.9-6.1 items/individual in guts of Hemiculter leucisculus from the industrial area. In contrast, the abundances were much lower levels of 0.1 ± 0.1 items/L in surface water, 0.5 ± 0.2 items/kg (ww) in sediment and 0.2 ± 0.01 items/individual in H. leucisculus in the reference site, respectively. A large quantity of raw pellets were found on the grounds surrounding the plastic factories. The dominant shapes of microplastics found in sediment were fragments (67%), followed by pellets (18%). Unexpectedly, neither fragments nor pellets (> 1 mm) were found in any fish. The organ index of liver in Hemiculter leucisculus, including four types of histopathological changes, was up to 5.5–9.9 in the plastic production area and only 1.6 in the reference site. Our results strongly suggest that microplastic pollution was in high level, and the histopathological damage in fish tissues strongly confirmed the microplastic pollution and ecological response of the plastic production area. Our results also indicate that the feeding types of local fish species might be the reasons leading to the absence of raw pellets or fragments in fish, despite high abundances of microplastics existed in their living environments. Capsule abstract: The plastic production area is a special point source of microplastic in the environments.

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1. Introduction

Plastic has brought great convenience to our daily life. However, mass production and long degradation time of plastics have resulted in prevalent and high environmental concerns (Lebreton and Andrady, 2019). After entering the environments, plastic will break and resolve into small pieces through environmental pressures and natural erosion (Andrady, 2017). These small pieces in the largest dimension of 5 mm are defined as microplastic (Mattsson et al., 2015; Rocha-Santos and Duarte, 2015; Conkle et al., 2018). In recent years, microplastics have been widely found in various environments and biota (Hidalgo-Ruz et al., 2012; Eerkes-Medrano et al., 2015).

Although there are various sources of microplastics, primary and secondary sources are considered as main pathways of microplastics entering the environment, which are closely related to their widespread application and existence (Cole et al., 2011; Wang et al., 2019). Primary sources are defined as the micro-sized plastic particles which are added to the products such as hand cleansers, facial cleansers and toothpastes (Browne et al., 2011; Duis and Coors, 2016), particularly microbeads in personal care products as main primary sources (Napper et al., 2015). Secondary sources refer to those micro-sized plastic particles which come from fragmented macro- and meso- size of plastic items through physical, chemical and biological factors (Cole et al., 2011), such as tyre fragments as one of important secondary sources of microplastics (Kole et al., 2017). Moreover, due to the special shapes of pellets for easier manufacturing, pellets are supposed to mainly come from the plastic processing as primary sources (Fernandino et al., 2015).

Nowadays, plastic pellets have been found not only in surface water but also on beaches all around the world (Eriksen et al., 2013; Fernandino et al., 2015; Fok and Cheung, 2015). However, plastic processing factories use tons of pellets, meaning that primary micro-sized pellets can be released into freshwater systems even to marine environment through production, storage, and transportation (Lebreton et al., 2017). Those pellets can accumulate in sediments by long-term sink or even be ingested by organisms (Rochman, 2018; Karlsson et al., 2018). In addition, leaked pellets can increase the possibility of plastic additives entering into the environment, resulting in potential ecological risks to the local ecosystems (Borges Ramirez et al., 2019). Therefore, it is highly urgent to investigate the occurrence of microplastics in such important point sources of microplastics. Up to date, only one study shows that plastic production plant can leak micro-sized pellets into surrounding areas (Karlsson et al., 2018).

Fish are frequently included in examinations of the occurrence, abundance and distribution of microplastic pollution because they are one of most important food sources for human beings (Dehaut et al., 2019). So far, field investigations on >200 fish species have been conducted and proved that microplastic accumulation in fish bodies is prevalent (Collard et al., 2019). Moreover, extensive exposure experiments have suggested that high concentration of microplastic can lead to adverse effects on fish in laboratory study, such as ingestion of microplastics causes lower hatchability of sea bass, physiological gut distension, and histopathological changes like progressive and inflammatory changes (Pedà et al., 2016; Choi et al., 2018; Jabeen et al., 2018). However, few of these effects have been verified in the field environments, which makes it much harder to assess the potential risk of microplastics in real environments.

Thus, the present study chose to compare a plastic industrial gathering area with an agricultural area as a reference site in Zhejiang province, China. The aim of study was to clarify the distribution and composition of microplastics in freshwater, sediment, and fish surrounding the plastic industrial area, and identify the histopathological alterations and responses to the microplastics in the fish tissues. According to the investigation and historical studies, we also try to find the potential source or pathway of these microplastics in the fish tissues from the high-pollution area. Simultaneously, through a systematic analysis, the study will give a new perspective and help understanding of biomonitor necessity and biological response of the microplastics for environmental management and plastic pollution control.

2. Materials and methods

2.1. Study area

In the plastic production area (P area), there are at least 6 pelletmaking factories and chemical fiber factories. The sampling sites are located 30 km north of Yuyao, China Plastic City, which stands for the main supply and processing sites of Yuyao plastic commodities. The plastic pellet-making factories in this area are mainly small-scale granulation workshops. Here, waste plastics and new materials are reprocessed and granulated into pellets. In comparison, the chemical fiber factories are larger operations, wherein natural or artificial macromolecule materials are used as raw materials to synthesize chemical fibers. The river network in this region is widely distributed and interconnected. The fish within these rivers are in high-demand as a food source for local residents. We also chose a site for reference (R area) in a reservoir which is the source of drinking water for Lin'an city (Fig. 1; Table S1).

2.2. Sample collection

Water, sediment and four fish species (*Hemiculter leucisculus*, *Hypophthalmichthys molitrix*, *Carassius auratus* and *Cyprinus carpio*) (126 individuals in total) were collected from 3 sites in P area and 1 site in R area (Table S2) during June 2016 to May 2018. We sampled about 5 L of surface water (top 0–10 cm) in triplicate with steel buckets and kept in glass bottles for laboratory analysis. Surface sediment samples of top 0–5 cm (about 20 kg in wet sediment) were sampled in triplicate with a clean stainless-steel spatula and pre-sieved through a stainless steel mesh due to the high abundance of raw plastic particles in the sediment. Here, 1 mm screen was selected to filter the sediment in situ instead of the usual methods (Hu et al., 2018). In this way, we can pay more attention to the raw plastic particles and make the follow-up qualitative experiments effective in the laboratory. The sediment samples on the screen were stored in aluminum foil bags at -20 °C.

Fish were captured by nets in this study. We examined the occurrence of microplastic in the digestion tract of *H. leucisculus*, which is a widely distributed and typical freshwater species. Additionally, we also analyzed histopathology alterations on the livers of *H. leucisculus*. Different from large species, the livers of *H. leucisculus* are much smaller and easier to assess for histopathological alterations. Livers were taken out and put into the glass bottoms with 4% formaldehyde (Sinopharm Co., Shanghai, China) in situ. Afterwards, the remainder of each fish was stored at -20 °C and transferred to the laboratory.

Except that, considering the large size of plastic pellets prevalent within this area, we also captured three larger species of fish (*H. molitrix, C. auratus,* and *C. carpio*) in the same niche of the freshwater system and used dissection and visual inspection to determine their intake of plastic pellets and fragments. All fish were stored at -20 °C and transferred to the laboratory.

2.3. Isolation of microplastics

We followed our previous methods to isolate microplastics from water (Su et al., 2016; Hu et al., 2018). In brief, the water samples were filtered through a 20 μ m diameter nylon membrane (Millipore, Burlington, MA, USA, NY2004700) facilitated with a vacuum pump (FY-2C-N, VALUE, China) (Vermaire et al., 2017). The substances on the membranes were then washed into a 250 mL glass flask with 100 mL of 30% H₂O₂ (ν/ν), and the flask was covered with a glass dish as soon as possible. These flasks were transferred to an oscillating incubator (HZ-9612 K, Taicang, China) and organic substances in them were

digested at 65 °C, 80 rpm until the solution was clear. Afterwards, the digestate was filtered through nylon membranes (20 μ m) again, and the membranes were transferred into a glass Petri dishes. The membranes with particles were air dried overnight for following observation. The sediment samples were extracted with the similar method to that of water samples, except that particles on membranes from sediment were filtered with 1 mm stainless steel mesh and then washed into 1 L glass bottles for digestion.

The weight and length of each fish was recorded after defrostation (Table S2). The guts of *H. leucisculus* were also removed and weighed. Approximately 100–200 mL of H_2O_2 (30%, *V*/V) were used for the digestion process. The following filtration steps were similar to those used for water and sediment samples. We transferred the livers of *H. leucisculus* from 4% formaldehyde to the ethyl alcohol for dehydration and paraffin sections. The intestines and stomachs of the three larger fish species were dissected with clean scalpels and forceps for microscope observation.

To prevent contamination, we dressed in 100% cotton laboratory coats during the entire process. All of the liquid solutions, including tap water, and hydrogen peroxide (H_2O_2 , 30%, ν/ν) were filtered (Millipore TMTP04700, filter pore size = 5 µm) before use. Then we washed all containers and devices with filtered water before and after use. Procedural blanks including chemicals and extracted processes were used for water, sediment and fish samples. The results indicate that a contamination rate of <5% was achieved.

2.4. Observation, identification and confirmation of microplastics

Suspected plastic particles on the membrane filters were observed and photographed under a Carl Zeiss Discovery V8 Stereo microscope (Micro Imaging GmbH, Göttingen, Germany). Four types (fibers, fragments, pellets and films) were used to describe the physical appearance of suspected plastic particles by visual assessments.

50% of the water samples (157 items), 50% of the *H. leucisculus* samples (90 items), and 10% of the sediment samples (1350 particles) were randomly selected for polymer confirmation with µ-FT-IR (Nicolet iN 10, Thermo Fisher). Polymer component was measured under the transmission mode. Data were collected at a resolution of 4 cm⁻¹ with a 16-s scan time. Only atmospheric correction was conducted to deduct the effect of carbon dioxide and water. All spectra were matched with our modified database and a quality index ≥70% was accepted (Cai et al., 2019). The number of microplastics reported was recalculated by excluding the verified non-plastic items.

2.5. Histopathological examination of fish liver

The histological sections of each sample were observed, and the condition of damage was recorded with an Olympus BX53 florescent microscope. The photographs were taken using an Olympus DP 80 camera. The histological alterations were evaluated following the method of Bernet et al. (1999) and Saraiva et al. (2015). In brief, these alterations include circulatory, regressive, progressive and inflammatory changes. The organ index, which represents the degree of damage, was obtained by multiplying the sum of the importance factors and scoring the values of all changes found in one fish. The extent of the alterations was assessed by attributing score as described in Pereira et al. (2017) and Pedà et al. (2016). The score was assigned according to the following methods: 0, normal; 1, slight damage; 2, medium damage; 3, pronounce damage; 4, severe damage. The importance factor (from 1 to 3) was evaluated according to the pathological importance.

2.6. Data analysis

The values for different microplastics were calculated using the average of three replicates for water and sediment samples at each site. Statistical analyses were performed using SPSS 23.0, Matlab R 2014a and Origin 9.0 (OriginLab Corp, Northampton, Massachusetts, USA). Because the datasets were not normally distributed, non-parametric methods were used. The Mann-Whitney *U* test was used to compare the difference between two groups. The Kruskal-Wallis test was used to assess the difference among more than two individual groups. The statistical significance was accepted at * = p < .05, ** = p < .01.

3. Results

3.1. The distribution of microplastics in water and sediment samples

The abundances of microplastics varied from 0.4–20.5 items/L in water from the P area and 0.1 \pm 0.1 items/L from the R area. The highest abundance of microplastic in water from P area was found in P2, and lowest in P3. The abundances in P1 and P2 showed the significant differences compared with that in R area (p < .05). The abundances varied from 44.4–124.7 items/kg ww in sediment of P area and 0.5 \pm 0.2 items/kg ww of R area. The highest abundance of microplastics in sediment was found in P3, and the lowest in P1 (Fig. 2). There were significant differences among all sites from P area and that from R area (p < .05). We did not find any relationships on abundances of microplastic between water and sediment.

In water samples, the dominant shape of microplastic was fiber (92.6%), followed by fragments (6.8%) then films (0.6%). Of all the plastics, 46.0% ranged from 0.1 to 1 mm in size, followed by 1–5 mm (45.7%). The dominant color of microplastic was black (40.0%), followed by red (22.9%) (Fig. S1). In sediment samples, however, fragment was the most abundant type, accounting for 67.1% of all the particles. There were also some pellets (18.1%), films (7.3%), fibers (5.0%), and foams (2.4%) in sediment. The dominant color of microplastics in sediment was transparent (28.6%), followed by white (22.9%) (Fig. S1). Unexpectedly, some smaller particles were found to adhere to the surface of other bigger microplastics (e.g., particles>1 mm in size) (Fig. 3).

Of all the 157 selected items from water for FT-IR analysis, 3 particles (1.8%) were identified as non-plastics. The most abundant microplastics were polyester (67.9%), followed by polyethylene terephthalate (PET) fibers (7.6%) and polypropylene (PP) fragments (5.1%). Of all the 1350 selected items from sediment, only 10 items (0.7%) were identified as non-plastics. The dominant type was polypropylene (57.6%), followed by polyethylene (PE) (17.6%) and nylon (15.1%) (Fig. S2).

3.2. The occurrence of microplastics in fish

Microplastics were found in the guts of *H. leucisculus* after digestion. The abundance of microplastics was 1.9-6.1 items/individual (i.e., 2.3–15.8 items/g of digestive tissue) in *H. leucisculus* from P area and 0.2 \pm 0.01 items/individual (i.e., 0.3 \pm 0.1 items/g) from R area (Fig. 4). The highest abundance of microplastic was found at P2. For both units of measurement (items/individual or items/g), there were significant differences among all sites from P area and the reference (p < .05). The dominant shape of microplastics was fiber (82.1%). The size and shape of microplastics were similar between those in water and fish (Fig. 3). The percentages of microplastics with a size <1 mm and 3 mm were 51% and 90% in water samples, and 49% and 95% in fish samples, respectively. The dominant type of microplastic is fiber (86% in fish and 93% in water samples), followed by fragment (14% in fish and 6% in water). Moreover, unexpectedly we did not find any pellets or fragments, which are the dominant local microplastic pollutants, in the guts of C. auratus, C. carpio or H. molitrix using direct observation method.

3.3. Histological changes and evaluation of organ index

The organ index of fish liver from P area (5.53–9.91) was higher than that from R area (1.63 \pm 0.89). There were significant differences among all sites from P area and the reference (**: p < .01). The highest



Fig. 1. Locations of sampling areas and sites (P area: plastic production area; R area: reference).



Fig. 2. Morphology of microplastics detected in water (A) and sediment (B); abundance in water (C) and sediment (D) samples. Letters a, b, c indicate significant differences between different sites (*p* < .05). Error bar is indicated by the whisker.



Fig. 3. Cumulative frequency distribution by size (A) and percentage of microplastics by shape (B) in water, sediment and fish samples from all sites.

organ index of fish liver was found in P2, where the abundance of microplastic also was the highest in water samples. Progressive changes were dominant in the liver in P2 (41.2%) (Fig. 5).

We also analyzed each reaction pattern of organ index separately and compared the score of each part of the four sampling sites (Fig. 6). There were significant differences in the progressive changes and inflammation circulatory disturbances between the P and R area (*: p < .05, **: p < .01).

4. Discussion

4.1. Microplastic pollution level in the water and sediment

In this study, we investigated microplastics in the surface water and sediment samples from a P area and discovered a relatively-high degree of microplastic pollution with special characteristics. The abundance of microplastics in P2 (20.5 ± 5.3 items/L) was higher than that in an influent wastewater treatment work (15.70 ± 5.23 MP·L⁻¹), which has been regarded as a remarkable source of microplastics in water (Murphy et al., 2016). Considering the high percentage of fibers, we supposed that the high microplastic abundance here was due to incoming effluents from the surrounding chemical fiber factories. In contrast with our discovery of fibers accounting for nearly 100% of microplastic pollution at P2, Alam et al. (2019) detected various shapes of MPs in the Ciwalengke River nearby an industrial area, fiber (65%), fragment and foam (35%) for detail. In our study, we did not find foam in water samples because local plastics processing factories mainly use fiber and pellet instead of foams as raw materials.



Fig. 4. Abundance of microplastics in *Hemiculter leucisculus* based on individual and gram. Letters a, b, and c indicate significant differences between different sites (p < .05). Error bar is indicated by whisker.

A high abundance of microplastics was also found in sediments in this study, although there were large error bars in the P area. The main reason of large error bars is that the distribution of microplastics is uneven, unlike traditional soluble pollutants. We used different extraction method (sieving, digestion, and sieving) from other studies (Hanvey et al., 2017). Here, we pre-sieved sediments in situ because of the very high abundances in the surrounding areas of plastic factories. For this reason, most of the fibers passed through 1 mm sieve with filtrate. As a result, there was only 5% of particles in sediment were fibers. However, fiber is the most prevalent type in environmental matrix. For example, fiber accounted for 64% of the total microplastics in small waterbodies and sediment samples (Hu et al., 2018). Therefore, if we consider the sediment moisture, type and size fraction, the total abundance of microplastics should be much higher in sediment than that which was documented in the present study. The dominant shape in sediment was fragment, which possibly came from fragmentation of plastic waste products, followed by, which resulted from nearby workshops and factories. The detected nylon and polypropylene pellets in sediment were similar in morphology to those scattering on the ground nearby the workshops (Fig. S3). Consequently, the P area can be a special source of microplastic to the local environment.

Given that the current operational procedures of plastic processing plants release plastic particles into the environment, it is likely that they will be considered a serious pollution problem for local environments. Leaked plastic pellets on the ground can be flushed into the aquatic environment with artificial or natural factors like heavy rainfall and surface runoff (Wagner et al., 2014; Duis and Coors, 2016; Zhou et al., 2020). Meanwhile, large scale transportation can lead to the



Fig. 5. Microphotographs of *H. leucisculus* liver from the reference site (A) and plastic production area (B) (hep: hepatocytes; pg: pigments; fd: fat droplets). Organ index of fish liver at four sampling sites (C) (**: *p* < .01; error bar is indicated by whisker) and frequency of reaction patterns (%) assigned to the histological change (D) (In: Inflammation; PC: Progressive changes; RC: Regressive changes; CD: Circulatory disturbances).

spreading of these pellets along the river and even into the sea (Lebreton et al., 2017; Karlsson et al., 2018).

4.2. The differences between microplastics in fish and those in the water and sediment

In the present study, microplastics have been clearly found in the gastrointestinal tract of *H. leucisculus*. Compared with quantities reported in previous studies (Lusher et al., 2013; Phillips and Bonner, 2015; Vendel et al., 2017), our result demonstrated that more microplastics were found in the gut of fish from high plastic pollution areas. *H. leucisculus* generally inhabits in the upper layer of the water column and feeds on algae and plant debris. The similarity between the size and shape of microplastics in water and those in fish reflected that the microplastics detected in the fish body mainly came from those in the surface layer of water (Fig. 3).

H. molitrix is a typical herbivore and filter-feeding fish living in the upper-middle layer. Thus, they do not ingest food from sediment, and no raw pellets or fragments were found in their bodies. *C. carpio* and *C. auratus* are omnivorous fish living mainly in the lower layer of water and often search for benthic food sources atop the sediment. This kind of feeding habit provides fish more opportunities to capture or swallow the microplastics in the sediment. Unexpectedly, we did not find any plastic pellets or fragments in these species even from the area with high pellets and fragments in the sediment.

The reasons for missing pellets and fragments in local fish are still unclear. Obviously, high abundances of pellets and fragments have been documented in fish guts in several previous studies. One wellknown example is that 83 particles were found in the gastrointestinal tract of one Myctophum aurolanternatum though the size of the fish was much smaller than those in our investigation (Boerger et al., 2010). Hard pieces of plastic (56%) and fragments of plastic bags (22%) were also found in Galeus melastomus (Anastasopoulou et al., 2013). We supposed that it might be due to different feeding types, including swallowing, filter-feeding and sucking in common. *M. aurolanternatum* mainly relies on swallowing and can swallow food directly without chewing. However, the feeding types of fish we captured were sucking and filter-feeding (Drost and Boogaart, 1986; Radke and Kahl, 2002), and some fish need to grind food through chewing. Previous researches proved that the abundance of microplastic in fish is related to the feeding habits of fish, such as, omnivorous fish ingested more microplastic fibers than herbivorous and carnivorous fish (Mizraji et al., 2017). However, the relationships between the feeding types of fish (swallowing, filter-feeding, sucking, etc.) and characteristics of microplastics (shape, abundance, size) in fish bodies are still not well-understood. Therefore, we need to conduct further research to clarify the exact relationship between feeding types of fish and characteristics of microplastics in fish body through laboratory experiments and field investigations.

4.3. Histological changes of fish liver in plastic production areas

Fish have been applied to elucidate the aquatic behavior of environmental contaminants. Liver was commonly used as a biomarker of pollution (Rodriguez-Ariza et al., 1994; van der Oost et al., 2003; Gül et al., 2004). Our results clearly suggest that the organ index of fish in P area was significantly higher than that in R area. Recent laboratory experiments have shown that microplastics can lead to various histological



Fig. 6. Score value of every reaction pattern of *H. leucisculus* liver at four sampling sites. (The significant differences were compared among the sampling sites in P area and that in R area; *: p < .05, **: p < .01; error bar is indicated by whisker.)

changes, such as passive hyperaemia, dilated sinusoids, hydrophic vacuolization, inflammatory responses and accumulation of fat droplets (Lu et al., 2016; Jabeen et al., 2018). However, these results came from fish exposed to relatively at relative higher exposure concentrations $(2.9 \times 10^5 \text{ particles/mL} \text{ for 5} \mu\text{m} \text{ PS-MPs} \text{ and } 4.5 \times 10^3 \text{ particles/mL} for 20 \,\mu\text{m} \text{ PS-MPs}$ in Lu et al. (2016)). Although the concentrations of microplastic in P area were much lower than those used in the laboratory, on one hand, living in such a highly-polluted environment for a long time may be due cause for the histopathological changes seen in livers of local fish.

On the other hand, there are other compounds than microplastics, such as additives and other pollutants, which could also be responsible for histopathological changes (Benli et al., 2016). Some typical additives, such as phthalates and bisphenol A, can be released from microplastics in the water (Cole et al., 2011). Microplastics can also bind waterborne-pollutants with their large surface area (Karami et al., 2016). Nevertheless, it is still hard to make a strong linkage between the pollutants from plastics and the histological changes in fish due to the complex environmental factors in the field investigations. Therefore, further research under more controlled conditions, combined with field-based approaches and laboratory experiments, are highly needed to answer these questions.

5. Conclusion

In the present study, the concentration of microplastics was higher in P area than that recorded in most of other studies due to the local existence of local plastic processing workshops and chemical fiber factories. Our results strongly suggest that the plastic production area is a special point source of microplastic for environment. Our results also indicate that some fish species can avoid ingesting pellets or fragments, which may be due to their different feeding behaviors, and that *H. leucisculus* in more contaminated areas present more microplastics and higher organ index of liver. Future studies are highly needed to study the effect of different feeding types on ingested microplastics in fish; find the exact reasons leading to the histopathological changes in fish liver in plastic production area.

CRediT authorship contribution statement

Bowen Li:Conceptualization, Methodology, Software, Writing - original draft, Investigation, Formal analysis.**Lei Su:**Conceptualization, Investigation.**Haibo Zhang:**Investigation, Resources.**Hua Deng:** Investigation.**Qiqing Chen:**Writing - review & editing.**Huahong Shi:**Supervision, Writing - review & editing.

Declaration of competing interest

We declare that our research group do not have any conflict of interest such as economic, political interests or national affinities, family or emotional ties, or any other relevant connection or shared interest.

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Appendix A. Supplementary data

The supplementary materials includes additional detailed information about the longitude and latitude of sampling sites (Table S1), and about fish samples (Table S2). Figure S1 and Figure S2 show the colors and compositions of microplastics in water, sediment and fish samples, respectively. Figure S3 shows the photographs of plastic pellets taken in the sampling sites. Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.138662.

References

- Alam, F.C., Sembiring, E., Muntalif, B.S., Suendo, V., 2019. Microplastic distribution in surface water and sediment river around slum and industrial area (case study: Ciwalengke River, Majalaya district, Indonesia). Chemosphere 224, 637–645.
- Anastasopoulou, A., Mytilineou, C., Smith, C.J., Papadopoulou, K.N., 2013. Plastic debris ingested by deep-water fish of the Ionian Sea (eastern Mediterranean). Deep-Sea Res. Pt. I: Oceanographic Research Papers 74, 11–13.
- Andrady, A.L., 2017. The plastic in microplastics: a review. Mar. Pollut. Bull. 119, 12–22. Benli, A.Ç.K., Erkmen, B., Erkoç, F., 2016. Genotoxicity of sub-lethal di-n-butyl phthalate (DBP) in Nile tilapia (*Oreochromis niloticus*). Arch. Ind. Hyg. Toxicol. 67, 25–30.
- Bernet, D., Schmidt, H., Meier, W., Burkhardt Holm, P., Wahli, T., 1999. Histopathology in fish: proposal for a protocol to assess aquatic pollution. J. Fish Dis. 22, 25–34.
- Boerger, C.M., Lattin, G.L., Moore, S.L., Moore, C.J., 2010. Plastic ingestion by planktivorous fishes in the North Pacific central gyre. Mar. Pollut. Bull. 60, 2275–2278.
- Borges Ramirez, M.M., Dzul Caamal, R., Rendón Von Osten, J., 2019. Occurrence and seasonal distribution of microplastics and phthalates in sediments from the urban channel of the Ria and coast of Campeche, Mexico. Sci. Total Environ. 672, 97–105.
- 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.
- Cai, H., Du, F., Li, L., Li, B., Li, J., Shi, H., 2019. A practical approach based on FT-IR spectroscopy for identification of semi-synthetic and natural celluloses in microplastic investigation. Sci. Total Environ. 669, 692–701.
- Choi, J.S., Jung, Y., Hong, N., Hong, S.H., Park, J., 2018. Toxicological effects of irregularly shaped and spherical microplastics in a marine teleost, the sheepshead minnow (*Cyprinodon variegatus*). Mar. Pollut. Bull. 129, 231–240.
- 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.
- Collard, F., Gasperi, J., Gabrielsen, G.W., Tassin, B., 2019. Plastic particle ingestion by wild freshwater fish: a critical review. Environ. Sci. Technol. 53, 12974–12988.
- Conkle, J.L., Báez Del Valle, C.D., Turner, J.W., 2018. Are we underestimating microplastic contamination in aquatic environments? Environ. Manag. 61, 1–8.
- Dehaut, A., Hermabessiere, L., Duflos, G., 2019. Current frontiers and recommendations for the study of microplastics in seafood. TrAC-Trend. Anal. Chem. 116, 346–359.
- Drost, M.R., Boogaart, J.G.M., 1986. The energetics of feeding strikes in larval carp, *Cyprinus carpio*. J. Fish Biol. 29, 371–379.
- Duis, K., Coors, A., 2016. Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environ. Sci. Eur. 28 (1), 2.
- 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., Maximenko, N., Thiel, M., Cummins, A., Lattin, G., Wilson, S., Hafner, J., Zellers, A., Rifman, S., 2013. Plastic pollution in the South Pacific subtropical gyre. Mar. Pollut. Bull. 68, 71–76.
- Fernandino, G., Elliff, C.I., Silva, I.R., Bittencourt, A.C.S.P., 2015. How many pellets are too many? The pellet pollution index as a tool to assess beach pollution by plastic resin pellets in Salvador, Bahia, Brazil. Journal of Integrated Coastal Zone Management 15, 325–332.
- Fok, L., Cheung, P.K., 2015. Hong Kong at the Pearl River estuary: a hotspot of microplastic pollution. Mar. Pollut. Bull. 99, 112–118.
- Gül, Ş., Belge-Kurutaş, E., Yıldız, E., Şahan, A., Doran, F., 2004. Pollution correlated modifications of liver antioxidant systems and histopathology of fish (Cyprinidae) living in Seyhan dam Lake, Turkey. Environ. Int. 30, 605–609.
- Hanvey, J.S., Lewis, P.J., Lavers, J.L., Crosbie, N.D., Pozo, K., Clarke, B.O., 2017. A review of analytical techniques for quantifying microplastics in sediments. Anal. Methods 9, 1369–1383.
- Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ. Sci. Technol. 46, 3060–3075.
- Hu, L., Chernick, M., Hinton, D.E., Shi, H., 2018. Microplastics in small Waterbodies and tadpoles from Yangtze River Delta, China. Environ. Sci. Technol. 52, 8885–8893.

- Jabeen, K., Li, B., Chen, Q., Su, L., Wu, C., Hollert, H., Shi, H., 2018. Effects of virgin microplastics on goldfish (*Carassius auratus*). Chemosphere 213, 323–332.
- Karami, A., Romano, N., Galloway, T., Hamzah, H., 2016. Virgin microplastics cause toxicity and modulate the impacts of phenanthrene on biomarker responses in African catfish (Clarias gariepinus). Environ. Res. 151, 58–70.
- Karlsson, T.M., Arneborg, L., Broström, G., Almroth, B.C., Gipperth, L., Hassellöv, M., 2018. The unaccountability case of plastic pellet pollution. Mar. Pollut. Bull. 129, 52–60.
- Kole, P.J., Löhr, A.J., Van Belleghem, F., Ragas, A., 2017. Wear and tear of Tyres: a stealthy source of microplastics in the environment. Int. J. Env. Res. Pub. He. 14, 1265.
- Lebreton, L., Andrady, A., 2019. Future scenarios of global plastic waste generation and disposal. Palgrave Commun 5, 6.
- Lebreton, L.C.M., van der Zwet, J., Damsteeg, J., Slat, B., Andrady, A., Reisser, J., 2017. River plastic emissions to the world's oceans. Nat. Commun. 8, 15611.Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Ding, L., Ren, H., 2016. Uptake and
- Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Ding, L., Ren, H., 2016. Uptake and accumulation of polystyrene microplastics in Zebrafish (*Danio rerio*) and toxic effects in liver. Environ. Sci. Technol. 50, 4054–4060.
- Lusher, A.L., McHugh, M., Thompson, R.C., 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Mar. Pollut. Bull. 67, 94–99.
- Mattsson, K., Hansson, L.A., Cedervall, T., 2015. Nano-plastics in the aquatic environment. Environ. Sci-Proc. Imp. 17, 1712–1721.
- Mizraji, R., Ahrendt, C., Perez-Venegas, D., Vargas, J., Pulgar, J., Aldana, M., Patricio Ojeda, F., Duarte, C., Galbán-Malagón, C., 2017. Is the feeding type related with the content of microplastics in intertidal fish gut? Mar. Pollut. Bull. 116, 498–500.
- Murphy, F., Ewins, C., Carbonnier, F., Quinn, B., 2016. Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. Environ. Sci. Technol. 50, 5800–5808.
- Napper, I.E., Bakir, A., Rowland, S.J., Thompson, R.C., 2015. Characterisation, quantity and sorptive properties of microplastics extracted from cosmetics. Mar. Pollut. Bull. 99, 178–185.
- Pedà, C., Caccamo, L., Fossi, M.C., Gai, F., Andaloro, F., Genovese, L., Perdichizzi, A., Romeo, T., Maricchiolo, G., 2016. Intestinal alterations in European sea bass Dicentrarchus labrax (Linnaeus, 1758) exposed to microplastics: preliminary results. Environ. Pollut. 212, 251–256.
- Pereira, A., Carvalho, A.P., Cruz, C., Saraiva, A., 2017. Histopathological changes and zootechnical performance in juvenile zebrafish (*Danio rerio*) under chronic exposure to nitrate. Aquaculture 473, 197–205.
- Phillips, M.B., Bonner, T.H., 2015. Occurrence and amount of microplastic ingested by fishes in watersheds of the Gulf of Mexico. Mar. Pollut. Bull. 100, 264–269.
- Radke, R.J., Kahl, U., 2002. Effects of a filter-feeding fish [silver carp, Hypophthalmichthys molitrix (Val.)] on phyto- and zooplankton in a mesotrophic reservoir: results from an enclosure experiment. Freshw. Biol. 47, 2337–2344.
- Rocha-Santos, T., Duarte, A.C., 2015. A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. Trac-Trend Anal. Chem. 65, 47–53.
- Rochman, C.M., 2018. Microplastics research- from sink to source. Science 360, 28-29.
- Rodriguez-Ariza, A., Dorado, G., Navas, J.I., Pueyo, C., Lopez-Barea, J., 1994. Promutagen activation by fish liver as a biomarker of littoral pollution. Environ. Mol. Mutagen. 24, 116–123.
- Saraiva, A., Costa, J., Serrão, J., Cruz, C., Eiras, J.C., 2015. A histology-based fish health assessment of farmed seabass (*Dicentrarchus labrax* L.). Aquaculture 448, 375–381.
- 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.
- van der Oost, R., Beyer, J., Vermeulen, N.P., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57–149.
- Vendel, A.L., Bessa, F., Alves, V.E.N., Amorim, A.L.A., Patrício, J., Palma, A.R.T., 2017. Widespread microplastic ingestion by fish assemblages in tropical estuaries subjected to anthropogenic pressures. Mar. Pollut. Bull. 117, 448–455.
- Vermaire, J.C., Pomeroy, C., Herczegh, S.M., Haggart, O., Murphy, M., 2017. Microplastic abundance and distribution in the open water and sediment of the Ottawa River, Canada, and its tributaries. FACETS 2, 301–314.
- Wagner, M., Scherer, C., Alvarez-Munoz, D., Brennholt, N., Bourrain, X., Buchinger, S., Fries, E., Grosbois, C., Klasmeier, J., Marti, T., Rodriguez-Mozaz, S., Urbatzka, R., Vethaak, A.D., Winther-Nielsen, M., Reifferscheid, G., 2014. Microplastics in freshwater ecosystems: what we know and what we need to know. Environ. Sci. Eur. 26, 12.
- Wang, T., Zou, X., Li, B., Yao, Y., Zang, Z., Li, Y., Yu, W., Wang, W., 2019. Preliminary study of the source apportionment and diversity of microplastics: taking floating microplastics in the South China Sea as an example. Environ. Pollut. 245, 965–974.
- Zhou, B., Wang, J., Zhang, H., Shi, H., Fei, Y., Huang, S., Tong, Y., Wen, D., Luo, Y., Barceló, D., 2020. Microplastics in agricultural soils on the coastal plain of Hangzhou Bay, East China: multiple sources other than plastic mulching film. J. Hazard. Mater. 364, 82–90.