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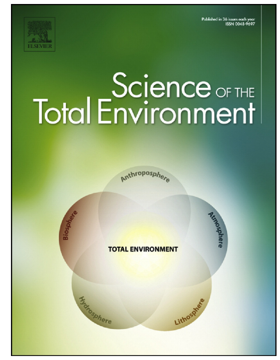

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Microplastics in four bivalve species and basis for using bivalves as bioindicators of microplastic pollution

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Abstract

Microplastics in bivalves have caused widespread concern due to their potential health risk to humans. In this study, microplastics in the digestive systems of four locally cultured bivalve species (scallop *Chlamys farreri*, mussel *Mytilus galloprovincialis*, oyster *Crassostrea gigas*, and clam *Ruditapes philippinarum*) in Qingdao, China, were analyzed and detected in 233 out of 290 bivalve samples (80%) over four seasons. The microplastic abundance in four species of bivalves ranged between 0.5 and 3.3 items/individual or 0.3 and 20.4 items/g wet weight digestive system, with significant species-specific and region-specific differences but no season-specific differences. Microfiber was the most predominant shape of all microplastics found. Eighteen types of polymer with diameters between 7 and 5000 μm were identified by μ-FT-IR (505 of 587 suspected items identified as microplastics) with polyvinyl chloride (PVC) and rayon being the most abundant ones. Bivalves collected in summer contained more larger-sized microplastics. *R. philippinarum* accumulated more smaller-sized microplastics and showed different microplastic features compared with the other three species of bivalves. By comparing and analyzing the microplastic polymer types between each bivalve species and the ambient environment, microplastic in clam can best reflect the variability of microplastic polymer types in sediment among different areas. Mussels can reflect the variability of microplastic polymer types in water to an extent. Therefore, clam and mussel are recommended to serve as bioindicators for microplastic pollution in the sediment and water, respectively. The occurrence of microplastics pollution in bivalves worldwide is wide, and bivalves can act as the transporter of microplastics to humans. Our results suggest that bivalves have an important role as environmental bioindicators and the pollution of microplastics in bivalves needs attention.

Keywords: Microplastics; Bivalves; Abundance; Polymer; Bioindicator

1. Introduction

The large-scale production and use of synthetic plastics date back to the 1950s (Geyer et al., 2017). Mismanaged plastic wastes discarded into the environment have been considered an emerging pollutant. Discarded plastic wastes are gradually broken into smaller particles under the combined actions of physical abrasion and ultraviolet radiation (Fu et al., 2020). Presently, microplastic (< 5 mm) pollution is no longer a special national or local phenomenon, but a global phenomenon. Marine microplastics have been well documented after the concept was first proposed in 2004 by Thompson et al. (2004). Microplastic contamination has even been detected in the deep ocean sediments (Van Cauwenberghe et al., 2013) and remote polar areas (Bergmann et al., 2019). Additionally, studies have found microplastic occurrence across trophic levels, including zooplankton (Md Amin et al., 2020), coral (Ding et al., 2019), bivalves (Sfriso et al., 2020), shrimp (Hossain et al., 2020), fish (Park et al., 2020), bird (Avery-Gomm et al., 2018), and whale (Zhu et al., 2019).

Given the widespread distribution of microplastics in the environment and the wide range of effects on organisms, it is necessary to develop long-term monitoring programs for microplastic contamination in different components, including water, sediment, and biota (Li et al., 2019; Xu et al., 2020). Microplastic abundance in water and sediment is heterogeneous and can be easily affected by winds, tides, currents, bioturbation, and many other factors (Li et al., 2019). Thus, evidence for biological monitoring in environmental matrices should be considered in comprehensive assessments of the time-averaged influence and potential adverse effects of microplastics on individuals and ecosystems (Cho et al., 2021). Bivalves have been extensively utilized as bioindicators to monitor environmental pollution due to their particular characteristics, such as broad distribution, easy accessibility, fixation living, and high tolerance to a wide range of ambient conditions (Li et al., 2019). They are also considered as one of the most impacted groups by microplastic in the ambient environment (Ward et al., 2019a). Previous studies have used bivalves, such as mussel and Asian clam, to assess the load of microplastics in the environment (Su et al., 2018; Li et al., 2019; Kazour et al., 2020). Despite increasing numbers of monitoring studies using bivalves to detect microplastic pollution (Bråte et al., 2018; Li et al., 2019; Su et al., 2019; Cho et al., 2021), information on the correlation between the microplastic distribution patterns in bivalves and the ambient environment has not been fully clarified.

Furthermore, bivalves are essential seafood for humans, therefore, microplastic ingestion by bivalves is of particular concern given the transfer of microplastics through human consumption. One study focusing on the microplastic pollution of bivalves in South Korea revealed that shellfish consumption was an important route of microplastic exposure to humans (Cho et al., 2019). Understanding the bioaccumulation of microplastics in bivalves is crucial to assess the potential risk to the marine ecosystem, especially for human health. Bivalves maybe good bioindicators for microplastic monitoring and assessment of human exposure to microplastics through shellfish intake. However, there are also many voices against the use of bivalves as bioindicators of microplastic pollution (Ward et al., 2019a; Ward et al., 2019b).

Qingdao is a typical coastal port city for tourism and mariculture in China. It includes a typical semi-closed bay (Jiaozhou Bay) and is adjacent to the Yellow Sea. Previous studies showed that the sediments in the Jiaozhou Bay and the Yellow Sea were polluted by microplastics, especially in the sea area close to the shoreline (Zhang et al., 2019a; Zheng et al., 2019). Yet, there is little information on microplastic pollution in locally cultured bivalves in Qingdao, China (Ding et al., 2018a; Ding et al., 2018b; Ding et al., 2020). Therefore, building on our previous work investigating the microplastic distribution in the digestive system of shellfish in Qingdao, China (Ding et al., 2018a; Ding et al., 2018b; Ding et al., 2020), we have conducted a survey on microplastic distribution in four species of bivalves sampled from two locally cultured areas across four seasons, and analyzed the relationship between microplastics in bivalves and the surrounding environment combined with the literature data. This survey is to explore whether the features of microplastics ingested by bivalves are related to the differences in species, seasons, and sampling regions. If we can find a connection, can these differences provide a basis for using bivalves as bioindicators of microplastic pollution? Additionally, is there any other basis to support the selection of bioindicators? Since current controversy remains over using bivalves as bioindicators of microplastic pollution (Li et al., 2019; Ward et al., 2019a; Ward et al., 2019b), our research can provide new insights into the bioindicator selection.

Here, the combination of field-survey and literature review is to test the following hypotheses that (1) the features of microplastics, including abundance, shape, size, color, and polymer type, in bivalves is related to the species, season, and sampling region, and (2) the species, seasonal, or regional differences in microplastic features enable using bivalves as bioindicators of microplastic pollution.

2. Materials and methods

2.1. Sampling strategy

In this study, we assigned two typical areas as sampling regions: one is Huangdao (HD) to the west of Jiaozhou Bay, and the other is Shazikou (SZK) to the east of Jiaozhou Bay (**Fig. 1**). Both HD and SZK have a long-term mariculture history for bivalves, but the population density, fishery output, tourist activities, and the direction of ocean currents are different at these two sites. For the detailed features of these two regions, please refer to Supplementary Methods. Four species of bivalves were purchased alive from the local fishery market of HD and SZK for this study: scallop *Chlamys farreri*, mussel *Mytilus galloprovincialis*, oyster *Crassostrea gigas*, and clam *Ruditapes philippinarum*. The sampling periods were November (autumn) in 2017, and February (winter), May (spring), and August (summer) in 2018. Due to season limitation, *M. galloprovincialis* was absent in winter from the two regions, as well as in summer from SZK. To ensure the accuracy of the results, *R. philippinarum* was selected with no sand spitting. Only locally cultivated bivalves were purchased, and bivalves in different seasons were obtained from the same suppliers. Each bivalve species was individually wrapped in aluminum foil and then sealed in a zip-lock bag before being transferred to the laboratory. The collected bivalves were immediately frozen and stored at -20 °C before microplastic analysis.

2.2. Isolation of microplastics in the digestive system of bivalves

For each species, 10 individuals were selected for study based on similar basic physical parameters, including bivalve length and total body wet weight (**Table S1**). Microplastic extraction from the digestive system of bivalves followed our established method (Ding et al., 2018a). In brief, the digestive system of bivalve was carefully dissected out and weighed (**Table S1**). The individual digestive system was transferred into a conical flask (250 mL) and 100 mL of 10% potassium hydroxide (KOH) was added. Then the conical flask was covered immediately with aluminum foil, sonicated for 5 min, and placed in an oscillation incubator (60 °C, 90 rpm, and for no more than 24 h). Once digested, the solution was filtered onto Whatman GF/F glass fiber membrane (0.7 μm pore size with a 47 mm diameter) without cooling, and the filter membrane was placed in Petri dishes and dried for further observation.

2.3. Observation and identification of microplastics

2.3.1. Observation

A visual inspection was first carried out to quantify and screen the suspected microplastics based on their characteristics. All particles suspected to be microplastics were observed, photographed, and marked under a stereo-microscope (Nikon SMZ1270, Japan) with a Nikon Ds-Ri2 digital camera. The size of microplastics was measured using the image-processing NIS-Elements software, and then the sizes, shapes, and colors of microplastics were recorded. The shapes of microplastics were categorized as fiber, fragment, film, and granule. To measure the sizes of microplastics, the fibrous microplastics were measured along their actual length, whereas the fragmented, filmy, and granular ones were measured to the longest axis (Ding et al., 2018a). Additionally, microplastics were categorized in size ranges: < 100 μm, 100–200 μm, 200–300 μm, 300–400 μm, 400–500 μm, 500–1000 μm, 1000–1500 μm, 1500–2000 μm, 2000–2500 μm, 2500–3000 μm, 3000–3500 μm, 3500–4000 μm, 4000–4500 μm, and 4500–5000 μm. In the current study, the transparent and white microplastics were grouped as colorless, and the other colors of microplastics were grouped as colored.

2.3.2. Identification

All marked items were confirmed with a PerkinElmer Spectrum Spotlight 400 micro-Fourier transform infrared spectroscope (μ-FT-IR; PerkinElmer Inc., U. S. A.) based on our previous protocol (Ding et al., 2018b). The attenuated total reflection (ATR) mode was used and the germanium (Ge) crystal on the ATR imaging attachment was in direct contact with the microplastics. The spectra were acquired from a spectral resolution of 8 cm^{-1} and a spatial resolution of 6.25 μm (highest spatial resolution is 1.56 μm), and the spectral range was set from 4000 to 750 cm^{-1} with 16 coscans for each measurement. Therefore, the diameter of microplastics down to 6.25 µm could be identified in this study. The resulting spectra were compared with a database from Sadltler to confirm the polymers, and the spectra matching higher than 70% were reliable and accepted as microplastics (Su et al., 2020). Non-plastics were removed from the microplastic counts, and the number of microplastics was recalculated.

2.4. Quality control

Common measures such as washing glass containers, wearing cotton lab and nitrile gloves, filtering solutions, and etc. were taken to prevent external contaminations as Ding et al. (2020) described. The microplastic identification was conducted in a closed lab, and the stereo-microscope was covered with a glass cover. In between the particle verification, the Ge crystal surface was wiped with dust-free paper containing alcohol. To account for procedural contaminations, blanks with the same volume of 10% KOH but no tissues were performed simultaneously during sample processing procedures. Additionally, a blank membrane was directly exposed to the air for correcting the airborne pollution when the suspected microplastics were marked and identified under stereo-microscope and µ-FT-IR. Results showed that one or two fibrous cellophane and rayon were detected on 4 out of 8 blank membranes. The blank results were qualitatively considered in the interpretation but were not subtracted from the experimental result because the average number of microplastics in the blanks did not exceed 1.

2.5. Data collection

To further evaluate the use of bivalve species as microplastic pollution bioindicators on a large area scale, a literature review combined with research data of this study was used to determine the relationship of microplastics in bivalves and water or sediment. The data used in this study were collected from the Web of Science by the end of December 2020. The keywords used in the literature search were microplastics, bivalves, shellfish, scallop, mussel, oyster, clam, and polymer type. The studies selected for inclusion reported microplastic polymer types in biological samples (i.e. scallop, mussel, oyster, or clam), and/or the polymer types in the surrounding water or sediment. The data of proportions or numbers of microplastics and polymer types were obtained from tables when available; if necessary, software Plot Digitizer was used to extract data from figures. To unify the data, microplastic polymer types reported as percentages of all particles were converted into numbers. If the microplastic polymer types in the water or sediments were not reported in the literature, a further search of the literature from the Web of Science on the microplastic polymer types in water or sediments where the bivalves were collected was conducted. Data on microplastic pollution in the scallop was not used as very limited data was available. Eventually, a total of 10 publications focusing on the polymer type of microplastics in clams (Cho et al., 2019; Su et al., 2016; Su et al., 2018), mussels (Cho et al., 2021; Li et al., 2018b; Qu et al., 2018), oysters (Cho et al., 2021; Li et al., 2018a; Teng et al., 2019), and the surrounding water (Fan et al., 2019) or sediment (Jang et al., 2020) were analyzed. Additionally, available data concerning microplastic polymer types in seawater or sediment in Qingdao (Jiaozhou Bay), China was reported by Zheng et al. (2019). The sample collection was conducted in

November 2017 (Zheng et al., 2019), therefore, data in our study concerning bivalves sampled in November 2017 was used to analyze the relationship of microplastic polymer types between bivalves and seawater or sediment in Qingdao, China. To further provide the basis for using bivalves as bioindicators for microplastic pollution, we also summarised the available data concerning the microplastic pollution in bivalves (see Supplementary Methods).

2.6. Data analysis

Data analysis was processed using Microsoft Excel 2016, and SPSS 24.0. Normality of data set was tested with Shapiro-Wilk test. Then, non-parametric tests were used if the data were not normally distributed. Mann–Whitney *U* test was used to analyze the differences between the two groups, and the differences among multiple groups were accessed with the Kruskal–Wallis test followed by multiple comparisons. Significant differences were represented as $p < 0.05$ and $p < 0.01$ levels.

The microplastic abundances in bivalves were expressed as both the microplastics per individual (unit: items/individual) and microplastics per gram based on the wet weight of the digestive system (unit: items/g). Differences of microplastic features in bivalves among species, seasons, and sampling regions were analyzed using principal component analysis (PCA). The microplastic features (abundance (items/individual and items/g), shape, size, color, and polymer type) were the variables used in PCA. The species differences discriminated by PCA were tested by permutational multivariate analysis of variance (PERMANOVA). The research data of this study combined with the literature data were used for PCA to determine the relationship between the polymer types of microplastics in each bivalve species and the surrounding environment. Simpson diversity index (Simpson, 1949) was calculated to compare the diversity of microplastic polymer types between studies. Figures were drawn by Surfer 11, Origin 2019b, and R 3.5.1 software.

3. Results

3.1. Abundance of microplastics in bivalves

Microplastics were widely distributed in the studied bivalves with an 80% detection rate (233 out of 290 bivalve samples). The average microplastic abundance in four species of bivalves over four seasons in different areas ranged from 0.5 to 3.3 items/individual and from 0.3 to 20.1 items/g (wet weight of the digestive system) (**Fig. S1, Table S1**). In this study, we divided all data of microplastic abundance based on different species, seasons, and sampling regions for discussion. Focusing on the microplastic abundance in four species of bivalves (**Fig. 2A and B**), *R. philippinarum* (average: 1.2–3.2 items/individual, 4.5–20.1 items/g) contained a significantly higher abundance of microplastics than *Ch. farreri* (average: 0.5–2.9 items/individual, 0.4–3.4 items/g) and *M. galloprovincialis* (0.8–2.1 items/individual, 1.6–2.6 items/g) either by items per individual ($p < 0.05$, Kruskal–Wallis test) or items per gram ($p < 0.01$, Kruskal–Wallis test). The microplastic abundance in *Cr. gigas* (1.2–3.3 items/individual, 0.3–3.0 items/g) was significantly lower than that in *R. philippinarum* by items per gram ($p < 0.01$, Kruskal–Wallis test), but this difference was not statistically significant based on items per individual. Additionally, when emphasizing the microplastic abundance in bivalves collected in different seasons

(**Fig. 2C and D**), the overall seasonal difference of microplastic abundance in four bivalve species was not statistically significant either by items per individual or per gram. When comparing regional differences of microplastic abundance in bivalves (**Fig. 2E and F**), region-specific differences both in microplastic abundance per individual ($p < 0.05$, Mann–Whitney *U* test) and per gram ($p < 0.05$, Mann–Whitney *U* test) were detected in bivalves. Overall, bivalves sampled from SZK (0.9–3.3 items/individual, 0.6–20.1 items/g) contained more microplastics than those from HD (0.5–2.1 items/individual, 0.3–9.6 items/g).

3.2. Morphology of microplastics in bivalves

Microplastics were observed in bivalves with various shapes, sizes, and colors. Fiber, fragment, film, and granule were observed in bivalves, accounting for 45%, 23%, 28%, and 4% of all particles, respectively. Fiber was the most dominant shape of microplastics and significantly more abundant than the other shapes ($p < 0.01$, Mann–Whitney *U* test). The size of microplastics in four bivalve species ranged from 7 to 5000 μm, with an average size of 1145 μm (**Fig. 3A**). Overall, the number of microplastics decreased with increasing size. The size range of < 500 μm represented the most particles ($p < 0.01$, Mann–Whitney *U* test), accounting for more than 36% of all particles. The size range of < 100 μm accounted for 47% of particles < 500 μm. Additionally, the microplastic size order of different shapes was as follows: film (2211±866 μm) > fiber (1044±757 μm) > granule (354±493 μm) > fragment (172±288 μm). Moreover, the wet weight of the bivalves digestive system and the longest microplastic size were found to have a moderate correlation ($R^2 = 0.5847$, $p = 0.003$)

(**Fig. S2**). Meanwhile, these microplastics were colorful, including transparent (36%), blue (29%), black (15%), white (7%), gray (4%), red (3%), pink (3%), and etc. (3%). A significantly higher level of transparent and blue microplastics was found in bivalves than other colors (p < 0.01, Mann–Whitney U test); furthermore, colorless microplastics were significantly abundant than other colored microplastics (p < 0.01, Mann–Whitney U test).

3.3. Material composition

Of the total suspected 587 items in bivalves, 505 (86%) were verified as microplastics through μ-FT-IR analysis. Eighteen polymer types were confirmed, with polyvinyl chloride (PVC), rayon, cellophane, polyester, chlorinated polyethylene (CPE), polyethylene terephthalate (PET), polyvinylidene fluoride (PVDF), and polyvinylidene chloride–polyethylene (PVDC–PE), contributing between 23% (PVC) and 2% (PVDC–PE) to the total measured microplastic composition. Polyamide (nylon, 1%), polyvinyl ester (PVE, 1%), polyethylene (PE, 1%), polyetherimide (PEI, 0.6%), polyvinylidene chloride–polyacrylonitrile (PVDC–PAN, 0.4%) occurred rarely in bivalves (**Fig. 4**). There was a significant proportion of PVC and rayon in bivalves than other polymer types (p < 0.01, Mann–Whitney U test). The microscope images and IR spectra of the top four abundant microplastics are shown in **Fig. 4**.

3.4. Species, season, and regional differences in relation to features of microplastics in bivalves

The density curve analysis showed that *Cr. gigas* and *R. philippinarum* shared similar microplastics size distribution, and they ingested smaller microplastics in comparison with those in *Ch. farreri* and *M. galloprovincialis* (**Fig. 3B and Fig. S3A**). The sizes of microplastics in *Cr. gigas* ($p < 0.01$, Kruskal–Wallis test) and *R. philippinarum* ($p < 0.05$, Kruskal–Wallis test) were significantly smaller than those in *M. galloprovincialis*. Microplastic size density was similar between bivalves in autumn and winter or spring (**Fig. 3C and Fig. S3B**). However, bivalves in summer contained a lower proportion of smaller microplastics compared with those in the other three seasons. As for microplastic size between bivalves from different regions, kernel density estimation indicated that bivalves from SZK contained smaller microplastics than those from HD ($p < 0.01$, Mann–Whitney *U* test) (**Fig. 3D and Fig. S3C**).

The microplastic polymer type and shape composition varied greatly among the bivalve species. The most abundant microplastic in *Ch. farreri* was rayon films, but rayon fibers dominated in *M. galloprovincialis* (**Fig. S4A and B**). Polyester fragments followed closely by PVC films dominated in both *Cr. gigas* and *R. philippinarum* (**Fig. S4C and D**). Additionally, the predominant shape and type of microplastics in bivalves sampled in autumn, winter, spring, and summer was rayon film, cellophane fiber, polyester fragment, and PVC film, respectively (**Fig. S5)**. PVC film was the most predominant in bivalves from HD, but the prevalent polymer in bivalves from SZK was polyester fragments (**Fig. S6**). Representative images of microplastics with prevalent shapes and polymer types are shown in **Fig. S7**. Transparent microplastics were the most abundant in *Ch. farreri*, *M. galloprovincialis*, and *R. philippinarum*, while the dominant color found in *Cr. gigas* was blue (**Fig. S3D**). Transparent microplastics were predominant in bivalves collected in autumn and summer, whereas bivalves in winter ingested more blue microplastics. Bivalves in summer contained an almost equal amount of blue and transparent microplastics (**Fig. S3E**). The proportion of transparent microplastics in bivalves from HD was higher than those from SZK, while more blue microplastics were in bivalves from SZK than those from HD (**Fig. S3F**).

PCA was applied to determine the relationships between microplastic features in bivalves among different species, seasons, and regions (**Fig. 5A**). The increasing distance indicates highly diverse microplastic features among bivalve species. The first principal component axis (PC1) in the PCA plot accounted for 31.34% of the total variance, which divided the bivalves in sampling area HD (to the left of the plot) from those in sampling area SZK (to the right of the plot). Clam *R. philippinarum* samples were separated from the other three species of bivalves on the second principal component axis (PC2), accounting for 12.21% of the total variance. The microplastic features in clam *R. philippinarum* clustered in the gray ellipse in the PCA plot were significantly different from the other three species of bivalves (PERMANOVA, $p < 0.01$). Overall, the difference of microplastic features in bivalves was not obvious among different seasons but showed some differences between sampling areas and species.

3.5. The relationship between microplastic polymer types in bivalves and the surrounding environment

According to the above results, we further analyzed the distribution pattern of microplastics in bivalves and the surrounding environment (water or sediment) using the PCA based on the literature data and our research data. PCA based on the polymer types showed that samples including bivalves and the surrounding environments from different sampling areas were respectively clustered into different groups. PC1 and PC2 explained > 57% of the total variance on the PCA plots (**Fig. 5B and C**; **Fig. S8**). It would be reasonable to use the microplastic polymer types to compare the relationship between microplastics in bivalves and the surrounding environment. Based on PCA results, we found a significant positive correlation between microplastic polymer types in the clam and sediment samples in South Korea, Taihu Lake, Yangtze River, and Qingdao, China (**Fig. 5B**). There was a closer relationship between microplastic polymer types in mussels and water than that in oysters and water (**Fig. 5C and Fig. S8**).

4. Discussions

4.1. Comparison of microplastic abundance in bivalves

This study provided a report of microplastics in bivalves over four seasons. This adds to the mounting evidence that microplastic contamination is widespread in marine organisms. In a study of microplastics in the four species of bivalves from South Korea, the average abundance of microplastics in each bivalve species was in line with our study when calculating the microplastic abundance using individual-based unit (microplastic particles per individual) (Cho et al., 2019). When the unit of these values was converted to items per gram of whole soft tissue, the similarity between the microplastic abundance disappeared (Cho et al., 2019). The potential reason for the difference was that the digestive system in bivalves has a small mass but was where microplastics mainly accumulated. This finding demonstrated that ingestion was the primary pathway for microplastics entering the bivalves.

The abundance of microplastics in clam *R. philippinarum* was significantly higher than those in the other three species of bivalves. *R. philippinarum* is a sediment-dwelling bivalve, while *Ch. farreri*, *M. galloprovincialis*, and *Cr. gigas* are all water-dwelling bivalves. Sediment act as a sink of microplastics due to the sinking of negatively buoyant microplastics and the interactions between microplastics and marine life (e.g., egested fecal pellets, and plankton-formed aggregation) (Cho et al., 2021). Hence, *R. philippinarum*, which feeds on suspended particles in the pore water of sediments, could ingest more microplastics than the water-dwelling bivalves. Furthermore, microplastics in bivalves showed no seasonal variations. In a previous Jiaozhou Bay seawater study, the seasonal variation in the microplastic abundance was also not significant (Liu et al., 2020). No significant seasonal difference in microplastic abundance might be related to the surrounding environmental conditions where bivalves live. Additionally, the abundance of microplastics in bivalves sampled from SZK was significantly higher than those collected from HD. There is a very limited data set for regional comparison of microplastic abundance in the seawater or sediment along the coast of Qingdao, China. Gao et al. (2020) reported that the nearshore current direction was different along the coast of Qingdao, which might influence the migration and accumulation of microplastics in the seawater. Therefore, the discrepancies referred above need further investigation in future studies.

4.2. Variations of microplastic features in bivalves

Fiber (45%) was the most prevalent shape of microplastics observed in bivalves, followed by film (28%) and fragment (23%). Fibrous microplastics were widely detected in bivalves among most field investigations (Li et al., 2019; Ding et al., 2020). The percentage of filmy microplastics was relatively high in bivalves in this study compared with other regions, including the Xiamen market in China (10%, Fang et al., 2019) and the Persian Gulf (14%, Naji et al., 2018). An Asian clam study in Yangtze River found that the proportion of filmy microplastics reached 10%–20% of particles across some sampling sites (Su et al., 2018). Films in bivalves in this study were mainly comprised of transparent PVC, which is extensively used in agriculture as plastic mulch film (Cheng et al., 2020). The broken PVC plastic film can be washed into the river and eventually into the ocean. Furthermore, the higher proportion of filmy and fragmented microplastics in bivalves might be ascribed to their non-selective filter-feeding habits (Moore, 2008; Fang et al., 2019; Su et al., 2019). Colorless (transparent and white) was the most common color of microplastics in bivalves in this study, which was similar to the result of Korean bivalves (Jang et al., 2020; Cho et al., 2021).

In our study, the size of microplastics in bivalves down to 7 µm was detected using ATR-µ-FT-IR, which can in-situ detect microplastics. Currently, some studies failed to detect a lower size owing to the methodology limitations (**Table S2**). The order of mean microplastic size in bivalves collected from different seasons was as following: summer (1313 μm) > spring (1202 μm) > winter (1148 μm) > autumn (915 μm). Microplastics observed in summer (August) and spring (May) had larger sizes relative to other seasons. These two seasons are usually the flood season and with high tourist activities. This finding fits well with a previous publication that detected more larger-sized microplastics in the seawater of the South Yellow Sea in April and August than those in January (Jiang et al., 2020). Furthermore, more meso-plastics were also found in summer than in other seasons in the seawater of Jiaozhou Bay (Liu et al., 2020). Most of the microplastic emissions occurred during the rainy season between May to October in East Asia (Lebreton et al., 2017). Therefore, it demonstrated that during the rainy seasons, the larger-sized microplastics might enter the ocean from the land by the surface runoff. Bivalves collected in spring and summer contained more longer-sized microplastics, which might have a strong relationship with the rainfall. Additionally, microplastics in bivalves from SZK were smaller in size compared with those from HD, indicating that the microplastic sizes in the bivalve aquaculture environment in SZK were relatively small. In this study, the smallest average microplastic size was found in *R. philippinarum* (1022 μm) in comparison with the other three bivalve species, which was consistent with a study that also found the average microplastic size was the smallest in the clam (Wu et al., 2020). Cho et al. (2021) reported that more non-fiber particles smaller than 300 μm were in clam than those in oyster/mussel. Our previous study revealed that the features of microplastics including size in the sediment-dwelling bivalves were different from those in the water-dwelling bivalves (Ding et al., 2020).

In terms of microplastic polymer type, the composition differed among different bivalve species in this study, as well as among various recent studies. Rayon was the most abundant polymer in *Ch. farreri*. *M. galloprovincialis* contained an equal amount of rayon and PVC, whereas a relatively high proportion of PVC was observed both in *Cr. gigas* and *R. philippinarum*. There are a couple of possible reasons for the high proportions of PVC and rayon found in this study. First, the production of PVC was among the top three in global plastic production (Geyer et al., 2017; Sendra et al., 2021). PVC products are wildly used in the aquaculture industry, such as aquaculture buoys and PVC tubes, because of their stabilization, acid and alkali resistance, excellent thermal insulation property, and low price. Most of the bivalves sold in the fishery market were farmed in Qingdao coastal water by raft culture and bottom sowing culture, which commonly used PVC buoys and tubes. An Argentina study also found a high concentration of PVC microplastics in the mussel *Mytilus chilensis* (Pérez et al., 2020). Additionally, the source of rayon was likely the breakdown of the clothing or hygiene products, which were discharged into the marine environment with sewage (Ding et al., 2018b). Zhang et al. (2019b) reported rayon was the most abundant polymer type in the surface sediments from the North Yellow Sea. Second, oyster, mussel, and scallop are cultured in the water column below the surface water (Cho et al., 2019). PVC and rayon are all denser polymers (PVC 1.38 g/cm^3 and rayon 1.70–1.80 g/cm^3). Therefore, they have more opportunities to encounter bivalves than the buoyant microplastics in the aquaculture environment. Third, the percentage of FT-IR identification of suspected microplastics could also contribute to the difference in the polymer composition between various studies. Randomly selecting and analyzing suspected microplastics were adopted by many researchers (Catarino et al., 2018; Cho et al., 2019; Deng et al., 2020). This increased the uncertainty of the results, especially in small biological individuals. One study has shown that randomly selecting and identifying the suspected microplastics in a small sample size would be insufficient for estimating microplastic pollution in a large population (Su et al., 2019). In our study, the microplastics diversity index (Simpson diversity index: 0.85) was higher than the data in Chinese coastal mussel (0.70) and oyster (0.74) (Li et al., 2016; Teng et al., 2019), which might be due to the different percentage of FT-IR detection. Therefore, we recommend a 100% FT-IR validation after visual identification whenever possible. Even though this is highly recommended, instrument availability and time costs should also be considered. ATR-μ-FT-IR, which allows directly scanning microplastics on the membranes, has the benefit of rapid analysis with a simple sample pretreatment. Additionally, optimizing instrument parameters, such as the spectral resolution and the scan times, can also maximize detection efficiency. The use of the optimized ATR-μ-FT-IR method was recommended to meet the detection requirements of a large number of microplastics (Ding et al., 2018b). The overall result of polymer type distribution in this study was consistent with our previous study, which also found that rayon was the main polymer type (Ding et al., 2020).

4.3. Bivalves as bioindicators of microplastic pollution

Ingesting microplastics by *R. philippinarum* was significantly different from the other three species of bivalves. This finding implied that the habitat environment greatly influenced the ingestion of microplastics in bivalves, which was similar to previous work by Ding et al. (2020). Additionally, two studies focusing on microplastics in different fishes also reported that habitat was an important factor involved in microplastic ingestion (Feng et al., 2019; Su et al., 2019). A previous study has highlighted using Asian clam as a bioindicator to monitor microplastic pollution in the freshwater system, especially for sediments (Su et al., 2018). In our study, species-specific differences in microplastic features presented in *R. philippinarum* might provide the basis for supporting clam as a bioindicator to monitor microplastic pollution in the sediment. The close relationship between microplastic polymer types in clam and sediment further indicates that clam can serve as a bioindicator to monitor the spatial distribution pattern of microplastic polymer types in the sediment. Moreover, it seems mussels are more appropriate than oysters to reflect microplastic polymer types in the water. One study has proposed using mussel as a bioindicator for monitoring microplastic pollution in water (Li et al., 2019).

It is indisputable that there was a wide occurrence of microplastics in bivalves from all over the world (available data from 22 countries). The abundance of microplastics in bivalves between different studies ranged from 0 to 259 items/g (**Table S2**). Bivalves from areas with intensive human activities contained a higher number of microplastics than those with fewer human activities (Li et al., 2016). Previous studies of field-collected microplastics and bivalves revealed a significant quantitative correlation between the abundance of microplastics in bivalves and the surrounding environment (Qu et al., 2018; Su et al., 2018). As discussed above, there were many indications that bivalves could reflect the microplastic pollution in the environment.

In the studied bivalves, microplastics smaller than 1000 μm made up over half (53%) of the total particles, and the size range of 1000–2000 μm accounted for 27% of the total particles. The size of most microplastics in bivalves was similar to that of diatoms (2–1000 μm) ingested by bivalves, indicating bivalves selectively ingest certain size ranges of particles. A large body of evidence demonstrated that bivalves could rapidly sort particles depend upon the physical and chemical characteristics of particles (Ward et al., 2019a; Ward et al., 2019b). Additionally, the variability of chlorophyll *a* and temperature in the ambient seawater could affect the ingestion of microplastics by bivalves (Stamataki et al., 2020). These factors might be the limitations of bivalves as bioindicators for monitoring microplastic pollution. Currently, the proposition of using bivalves as bioindicators of microplastic pollution in the environment is still under discussion.

However, bivalve consumption is a nonnegligible pathway for human exposure to microplastics (evidence see Supplementary Results; Table S3). They can act as the transporter of microplastics into the marine food web and humans. Two Korean studies also reported that shellfish consumption was a potential route of microplastic exposure for humans (Cho et al., 2019; Cho et al., 2021). Therefore, the microplastic pollution in bivalves is closely connected to human health, which further provides the basis for using bivalves as bioindicators of microplastic contamination. Hence, bivalves might be a good bioindicator to monitor microplastic pollution in the environment until a better bioindicator is proposed. But when using bivalves to evaluate the microplastic pollution status, environmental and biological factors including feeding mechanisms of bivalves should be considered in future monitoring programs.

However, worth noticing is that the results obtained from the literature data have some limitations, due to limited available studies, limited spatial coverage in each country, and different analytical methods. First, limited studies were available, thus, the microplastic data cannot cover the overall pollution level of microplastics in each country. Second, different analytical methods including sample digestion and microplastic identification can influence the results of microplastics in bivalves between studies. For example, acid digestion procedures might underestimate the microplastic pollution level as this method (e.g., HNO_3) could destroy the pH-sensitive polymers (Catarino et al., 2017). Therefore, if bivalves are used as bioindicators for microplastic pollution monitoring in the future, effective and economical methods for large-scale monitoring programs still need more constructive work (Li et al., 2019). First, the standard and optimized methods should be established and adopted. Second, monitoring based on established standard methods should be regularly conducted regionally or globally to provide more comparable data. Third, the environmental and biological factors should be considered when building future monitoring programs.

5. Conclusions

The study investigated the distribution and characteristics of microplastic in four species of bivalves over four seasons at two local sites. Significant differences in microplastic abundance existed among different species and regions but not among different seasons. The sizes of microplastics in bivalves were affected by different seasons, sampling regions, and habitats. PCA results demonstrate that habitat is one of the factors to be considered when studying microplastic pollution and selecting bioindicators. Based on PCA results on the relationship between microplastic polymer types in each bivalve species and the surrounding environment, we recommend using clams and mussels as microplastic pollution bioindicators in sediment and water, respectively. Combined with the consideration of the wide distribution of microplastics in bivalves and their close correlation with human health, we propose using bivalves as bioindicators for microplastic monitoring in the future monitoring program. More attention and study are needed for microplastic pollution in seafood.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix B. Supplementary data

Supplementary data related to this article can be found in the attached materials.

References

Avery-Gomm, S., Provencher, J.F., Liboiron, M., Poon, F.E., Smith, P.A., 2017. Plastic pollution in the Labrador Sea: an assessment using the seabird northern fulmar *Fulmarus glacialis* as a biological monitoring species. Mar. Pollut. Bull. 127, 817–822.

Bergmann, M., Mützel, S., Primpke, S., Tekman, M.B., Trachsel, J., Gerdts, G., 2019. White and wonderful? Microplastics prevail in snow from the Alps to the Arctic. Sci. Adv. 5, eaax1157.

Bråte, I.L.N., Hurley, R., Iversen, K., Beyer, J., Thomas, K.V., Steindal, C.C., Green, N.W., Olsen, M., Lusher, A., 2018. *Mytilus* spp. as sentinels for monitoring microplastic pollution in Norwegian coastal waters: A qualitative and quantitative study. Environ. Pollut. 243, 383-393.

Catarino, A.I., Macchia, V., Sanderson, W.G., Thompson, R.C., Henry, T.B., 2018. Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal. Environ. Pollut. 237, 675–684.

Catarino, A.I., Thompson, R., Sanderson, W., Henry, T.B., 2017. Development and optimization of a standard method for extraction of microplastics in mussels by enzyme digestion of soft tissues. Environ. Toxicol. Chem. 36, 947-951.

Cheng, Y., Zhu, L., Song, W., Jiang, C., Li, B., Du, Z., Wang, J., Wang, J., Li, D., Zhang, K., 2020. Combined effects of mulch film-derived microplastics and atrazine on oxidative stress and gene expression in earthworm (*Eisenia fetida*). Sci. Total Environ. 746, 141280.

Cho, Y., Shim, W.J., Jang, M., Han, G.M., Hong, S.H., 2019. Abundance and characteristics of microplastics in market bivalves from South Korea. Environ. Pollut. 245, 1107–1116.

Cho, Y., Shim, W.J., Jang, M., Han, G.M., Hong, S.H., 2021. Nationwide monitoring of microplastics in bivalves from the coastal environment of Korea. Environ. Pollut. 270, 116175.

Dai, Z., Zhang, H., Zhou, Q., Tian, Y., Chen, T., Tu, C., Fu, C., Luo, Y., 2018. Occurrence of microplastics in the water column and sediment in an inland sea affected by intensive anthropogenic activities. Environ. Pollut. 242B, 1557–1565.

Deng, H., Wei, R., Luo, W., Hu, L., Li, B., Di, Y.N., Shi, H., 2020. Microplastic pollution in water and sediment in a textile industrial area. Environ. Pollut. 258, 113658.

Ding, J., Jiang, F., Li, J., Wang, Z., Sun, C., Wang, Z., Fu, L., Ding, N.X., He, C., 2019. Microplastics in the coral reef systems from Xisha Islands of South China Sea. Environ. Sci. Technol. 53, 8036–8046.

Ding, J., Li, J., Sun, C., He, C., Jiang, F., Gao, F., Zheng, L., 2018a. Separation and identification of microplastics in digestive system of bivalves. Chin. J. Anal. Chem. 46, 690–697.

Ding, J., Li, J., Sun, C., Jiang, F., He, C., Zhang, M., Ju, P., Ding, N.X., 2020. An examination of the occurrence and potential risks of microplastics across various shellfish. Sci. Total Environ. 739, 139887.

Ding, J., Li, J., Sun, C., Jiang, F., Ju, P., Qu, L., Zheng, Y., He, C., 2018b. Detection of microplastics in local marine organisms using a multi-technology system. Anal. Methods-U.K. 11, 78–87.

Fang, C., Zheng, R., Chen, H., Hong, F., Lin, L., Lin, H., Guo, H., Bailey, C., Segner, H., Mu, J., Bo, J., 2019. Comparison of microplastic contamination in fish and bivalves from two major cities in Fujian province, China and the implications for human health. Aquaculture 512, 734322.

Fan, Y., Zheng, K., Zhu, Z., Chen, G., Peng, X., 2019. Distribution, sedimentary record, and persistence of microplastics in the Pearl River catchment, China. Environ. Pollut. 251, 862-870.

Feng, Z., Zhang, T., Li, Y., He, X., Wang, R., Xu, J., Gao, G., 2019. The accumulation of microplastics in fish from an important fish farm and mariculture area, Haizhou Bay, China. Sci. Total Environ. 696, 133948.

Fu, Z., Chen, G., Wang, W., Wang, J., 2020. Microplastic pollution research methodologies, abundance, characteristics and risk assessments for aquatic biota in China. Environ. Pollut. 266, 115098.

Gao, F., Li, J., Hu, J., Li, X., Sun, C., 2020. Occurrence of microplastics carried on *Ulva prolifera* from the Yellow Sea, China. Case Studies in Chemical and Environmental Engineering, 2, 100054.

Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3, e1700782.

Hossain, M.S., Rahman, M.S., Uddin, M.N., Sharifuzzaman, S.M., Chowdhury, S.R., Sarker, S., Nawaz Chowdhury, M.S., 2020. Microplastic contamination in Penaeid shrimp from the Northern Bay of Bengal. Chemosphere 238, 124688.

Jang, M., Shim, W.J., Cho, Y., Han, G.M., Song, Y.K., Hong, S.H., 2020. A close relationship between microplastic contamination and coastal area use pattern. Water Res. 171, 115400.

Jiang, Y., Zhao, Y., Wang, X., Yang, F., Chen, M., Wang, J., 2020. Characterization of microplastics in the surface seawater of the South Yellow Sea as affected by season. Sci. Total Environ. 724, 138375.

Kazour, M., Amara, R., 2020. Is blue mussel caging an efficient method for monitoring environmental microplastics pollution? Sci. Total Environ. 710, 135649.

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.

Li, H., Ma, L., Lin, L., Ni, Z., Xu, X., Shi, H., Yan, Y., Zheng, G., Rittschof, D., 2018a. Microplastics in oysters *Saccostrea cucullata* along the Pearl River estuary, China. Environ. Pollut. 236, 619-625.

Li, J., Green, C., Reynolds, A., Shi, H., Rotchell, J.M., 2018b. Microplastics in mussels sampled from coastal waters and supermarkets in the United Kingdom. Environ. Pollut. 241, 35–44.

Li, J., Lusher, A.L., Rotchell, J.M., Deudero, S., Turra, A., Bråte, I.L.N., Sun, C., Shahadat Hossain, M., Li, Q., Kolandhasamy, P., Shi, H., 2019. Using mussel as a global bioindicator of coastal microplastic pollution. Environ. Pollut. 244, 522–533.

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.

Liu, T., Zhao, Y., Zhu, M., Liang, J., Zheng, S., Sun, X., 2020. Seasonal variation of micro- and meso-plastics in the seawater of Jiaozhou Bay, the Yellow Sea. Mar. Pollut. Bull. 152, 110922.

Md Amin, R., Sohaimi, E.S., Anuar, S.T., Bachok, Z., 2020. Microplastic ingestion by zooplankton in Terengganu coastal waters, southern South China Sea. Mar. Pollut. Bull. 150, 110616.

Moore, C.J., 2008. Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. Environ. Res. 108, 131-139.

Naji, A., Nuri, M., Vethaak, A.D., 2018. Microplastics contamination in molluscs from the northern part of the Persian Gulf. Environ. Pollut. 235, 113-120.

Park, T., Lee, S., Lee, M., Lee, J., Lee, S., Zoh, K., 2020. Occurrence of microplastics in the Han River and riverine fish in South Korea. Sci. Total Environ. 708, 134535.

Pérez, A.F., Ojeda, M., Rimondino, G.N., Chiesa, I.L., Di Mauro, R., Boy, C.C., Calcagno, J.A., 2020. First report of microplastics presence in the mussel *Mytilus chilensis* from Ushuaia Bay (Beagle Channel, Tierra del Fuego, Argentina). Mar. Pollut. Bull. 161, 111753.

Qu, X., Su, L., Li, H., Liang, M., Shi, H., 2018. Assessing the relationship between the abundance and properties of microplastics in water and in mussels. Sci. Total Environ. 621, 679-686.

Sendra, M., Sparaventi, E., Novoa, B., Figueras, A., 2021. An overview of the internalization and effects of microplastics and nanoplastics as pollutants of emerging concern in bivalves. Sci. Total Environ. 753, 142024.

Sfriso, A.A., Tomio, Y., Rosso, B., Gambaro, A., Sfriso, A., Corami, F., Rastelli, E., Corinaldesi, C., Mistri, M., Munari, C., 2020. Microplastic accumulation in benthic invertebrates in Terra Nova Bay (Ross Sea, Antarctica). Environ. Int. 137, 105587.

Simpson, E., 1949. Measurement of diversity. Nature 163, 688.

Stamataki, N., Hatzonikolakis, Y., Tsiaras, K., Tsangaris, C., Petihakis, G., Sofianos, S., Triantafyllou, G., 2020. Modelling mussel (*Mytilus spp*.) microplastic accumulation. Ocean Science 16, 927-949.

Su, L., Cai, H., Kolandhasamy, P., Wu, C., Rochman, C.M., Shi, H., 2018. Using the Asian clam as an indicator of microplastic pollution in freshwater ecosystems. Environ. Pollut. 234, 347–355.

Su, L., Deng, H., Li, B., Chen, Q., Pettigrove, V., Wu, C., Shi, H., 2019. The occurrence of microplastic in specific organs in commercially caught fishes from coast and estuary area of east China. J. Hazard. Mater. 365, 716-724.

Su, L., Sharp, S.M., Pettigrove, V.J., Craig, N.J., Nan, B., Du, F., Shi, H., 2020. Superimposed microplastic pollution in a coastal metropolis. Water Res. 168, 115140.

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.

Teng, J., Wang, Q., Ran, W., Wu, D., Liu, Y., Sun, S., Liu, H., Cao, R., Zhao, J., 2019. Microplastic in cultured oysters from different coastal areas of China. Sci. Total Environ. 653, 1282–1292.

Thompson, Richard, C., Olsen, Ylva, Mitchell, Richard, P., Davis, Anthony, Rowland, Steven, J., 2004. Lost at sea: where is all the plastic? Science. 304, 838–838.

Van Cauwenberghe, L., Vanreusel, A., Mees, J., Janssen, C.R., 2013. Microplastic pollution in deep-sea sediments. Environ. Pollut. 182, 495–499.

van Franeker, J.A., Blaize, C., Danielsen, J., Fairclough, K., Gollan, J., Guse, N., Hansen, P., Heubeck, M., Jensen, J., Le Guillou, G., Olsen, B., Olsen, K., 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.

Ward, J.E., Zhao, S., Holohan, B.A., Mladinich, K.M., Griffin, T.W., Wozniak, J., Shumway, S.E., 2019a. Selective ingestion and egestion of plastic particles by the blue mussel (*Mytilus edulis*) and eastern oyster (*Crassostrea virginica*): implications for using bivalves as bioindicators of microplastic pollution. Environ. Sci. Technol. 53, 8776–8784.

Ward, J.E., Rosa, M., Shumway, S.E., 2019b. Capture, ingestion, and egestion of microplastics by suspension-feeding bivalves: a 40-year history. Anthropocene Coasts 2, 39–49.

Wu, F., Wang, Y., Leung, J.Y.S., Huang, W., Zeng, J., Tang, Y., Chen, J., Shi, A., Yu, X., Xu, X., Zhang, H., Cao, L., 2020. Accumulation of microplastics in typical commercial aquatic species: a case study at a productive aquaculture site in China. Sci. Total Environ. 708, 135432.

Xu, X.Y., Wong, C.Y., Tam, N.F.Y., Liu, H.M., Cheung, S.G., 2020. Barnacles as potential bioindicator of microplastic pollution in Hong Kong. Mar. Pollut. Bull. 154, 111081.

Zhang, C., Zhou, H., Cui, Y., Wang, C., Li, Y., Zhang, D., 2019a. Microplastics in offshore sediment in the Yellow Sea and East China Sea, China. Environ. Pollut. 244, 827–833.

Zhang, B., Wu, D., Yang, X., Teng, J., Liu, Y., Zhang, C., Zhao, J., Yin, X., You, L., Liu, Y., Wang, Q., 2019b. Microplastic pollution in the surface sediments collected from Sishili Bay, North Yellow Sea, China. Mar. Pollut. Bull. 141, 9–15.

Zheng, Y., Li, J., Cao, W., Liu, X., Jiang, F., Ding, J., Yin, X., Sun, C., 2019. Distribution characteristics of microplastics in the seawater and sediment: a case study in Jiaozhou Bay, China. Sci. Total Environ. 674, 27–35.

Zhou, Q., Tu, C., Fu, C., Li, Y., Zhang, H., Xiong, K., Zhao, X., Li, L., Waniek, J.J., Luo, Y., 2020. Characteristics and distribution of microplastics in the coastal mangrove sediments of China. Sci. Total Environ. 703, 134807.

Zhu, J., Yu, X., Zhang, Q., Li, Y., Tan, S., Li, D., Yang, Z., Wang, J., 2019. Cetaceans and microplastics: first report of microplastic ingestion by a coastal delphinid, *Sousa chinensis*. Sci. Total Environ. 659, 649–654.

Figure Captions

Fig. 1. Locations of sampling regions. HD represents Huangdao in Qingdao, China, and SZK represents Shazikou in Qingdao, China.

Fig. 2. Abundance of microplastics in bivalve samples among different species (A, B), seasons (C, D), and regions (E, F) by items/individual and items/g. Kruskal–Wallis test followed by multiple comparisons was used to determine the differences of microplastic abundance in different species and seasons. Mann–Whitney *U* test was used to test the difference of microplastic abundance between sampling regions. Letters above the bar indicate the result of comparisons of microplastic abundance; the bars that do not share the same letter are significantly different ($p < 0.05$).

Fig. 3. Size of microplastics in this study. A. Size distribution of total microplastics in all bivalve samples. The dashed line represents the measured mean size. B, C, and D depicted the Kernel density estimation of microplastic size concerning different bivalve species (B), different seasons (C), and different regions (D).

Fig. 4. Polymer composition, IR spectra, and microscope images of microplastics in the bivalves. PVC: polyvinyl chloride; CPE: chlorinated polyethylene; PET: polyethylene terephthalate; PVDF: polyvinylidene fluoride. Scale bar = 100 μm or 200 μm in the right images.

Fig. 5. (A) Principal component analysis (PCA) of microplastic distribution patterns in four species of bivalves over four seasons in HD and SZK based on microplastic abundance (items/individual, and items/g), shape, color, size, and polymer type. Different geometric shapes

and different colors in the plot represent sampling seasons and regions. The light-coral and cyan shapes represent bivalves sampled from HD and SZK, respectively. The gray ellipse represents the community cluster of *R. philippinarum* separated from the other three species of bivalves. (B and C) PCA of the distribution pattern of microplastic polymer types between each bivalve species and the surrounding environment: (B) clam and sediment; (C) mussel and water. The gray arrows represented the polymer types of microplastics. PS: polystyrene; PE: polyethylene; PP: polypropylene; PEVA: polyethylene vinyl acetate; PC: polycarbonate; PVC: polyvinyl chloride; PEI: polyetherimide; CPE: chlorinated polyethylene; PET: polyethylene terephthalate; PA: polyamide; PES: Polyester, terephthalic acid; PAN: polyacrylonitrile; PU: polyurethane; POM: polyoxymethylene; PVA: polyvinyl acetate; PDMS: polydimethylsiloxane.

Credit Author Statement

Jinfeng Ding: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Software, Writing-Original Draft, Writing - Review & Editing, Visualization.

Chengjun Sun: Validation, Data curation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

Changfei He: Data curation, Software.

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Fengmin Li: Writing - Review & Editing, Project administration, Funding acquisition.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

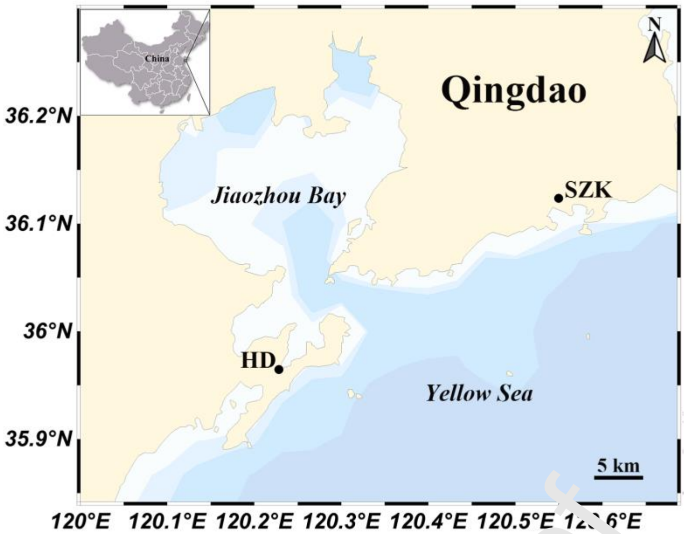


Fig. 1. Locations of sampling regions. HD represents Huangdao in Qingdao, China, and SZK represents Shazikou in Qingdao, China.

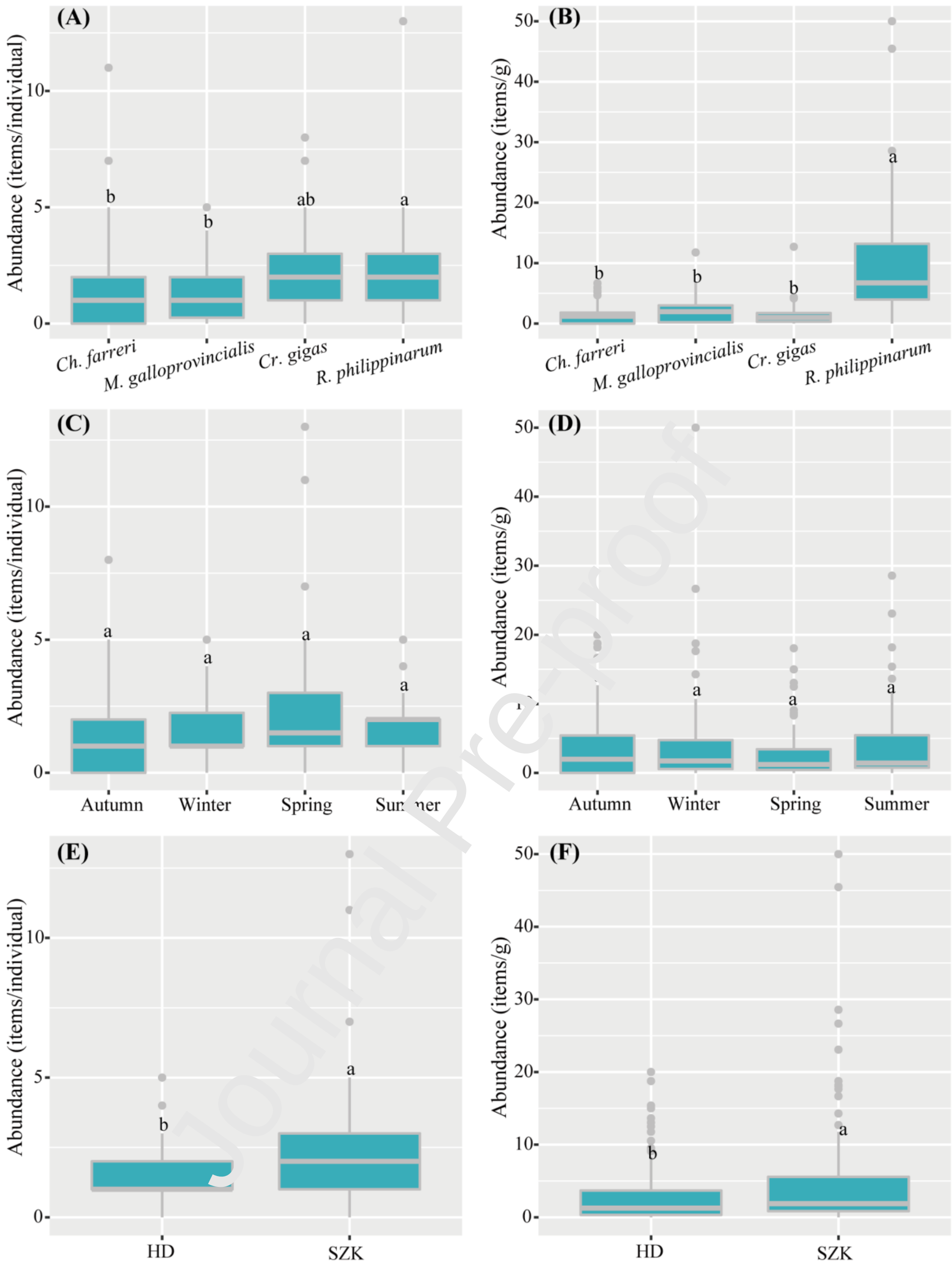


Fig. 2. Abundance of microplastics in bivalve samples among different species (A, B), seasons (C, D), and regions (E, F) by items/individual and items/g. Kruskal–Wallis test followed by multiple comparisons was used to determine the differences of microplastic abundance in different species and seasons. Mann–Whitney *U* test was used to test the difference of microplastic abundance between sampling regions. Letters above the bar indicate the result of comparisons of microplastic abundance; the bars that do not share the same letter are significantly different ($p < 0.05$).

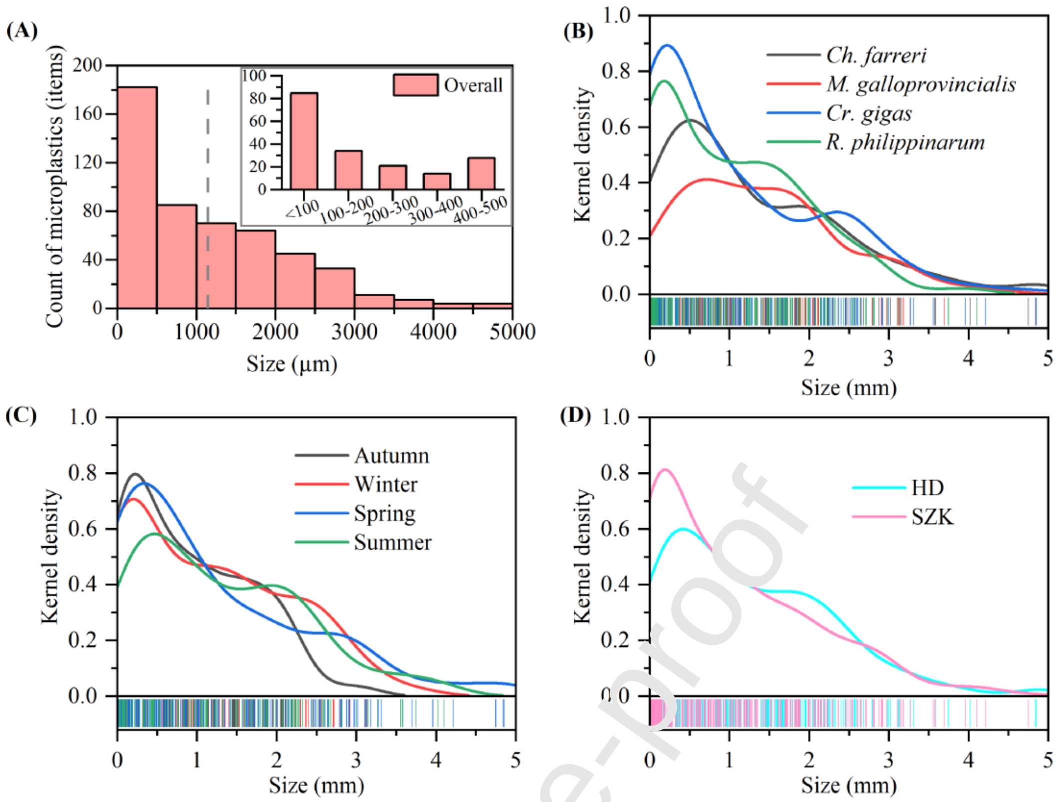


Fig. 3. Size of microplastics in this study. A. Size distribution of total microplastics in all bivalve samples. The dashed line represents the measured mean size. B, C, and D depicted the Kernel density estimation of microplastic size concerning different bivalve species (B), different seasons (C), and different regions (D).

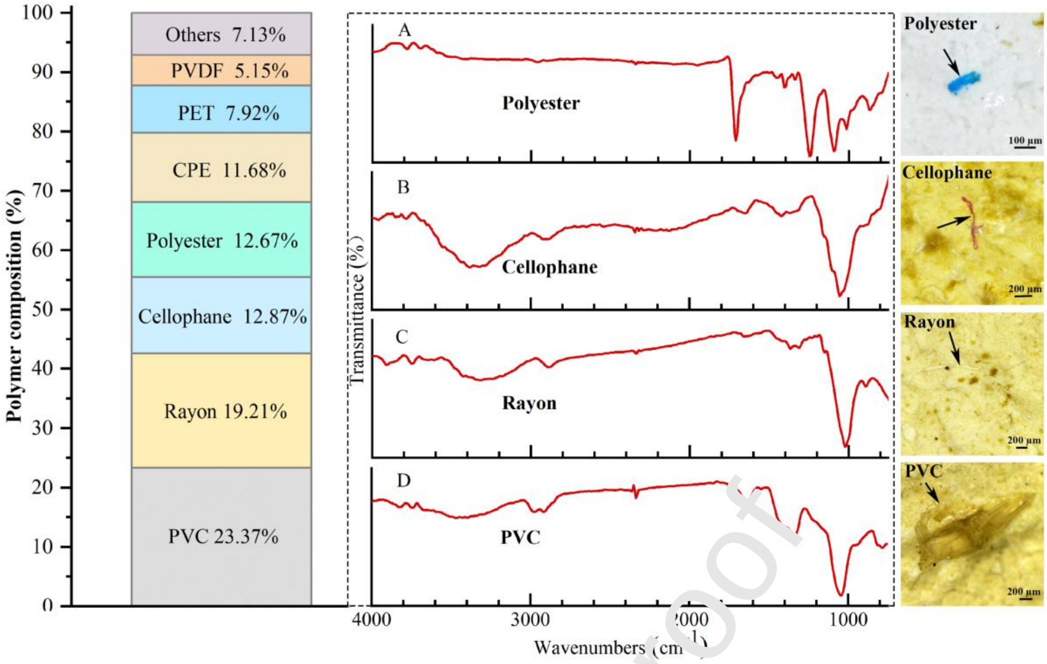


Fig. 4. Polymer composition, IR spectra, and microscope images of microplastics in the bivalves. PVC: polyvinyl chloride; CPE: chlorinated polyethylene; PET: polyethylene terephthalate; PVDF: polyvinylidene fluoride. Scale bar = 100 μm or 200 μm in the right images.

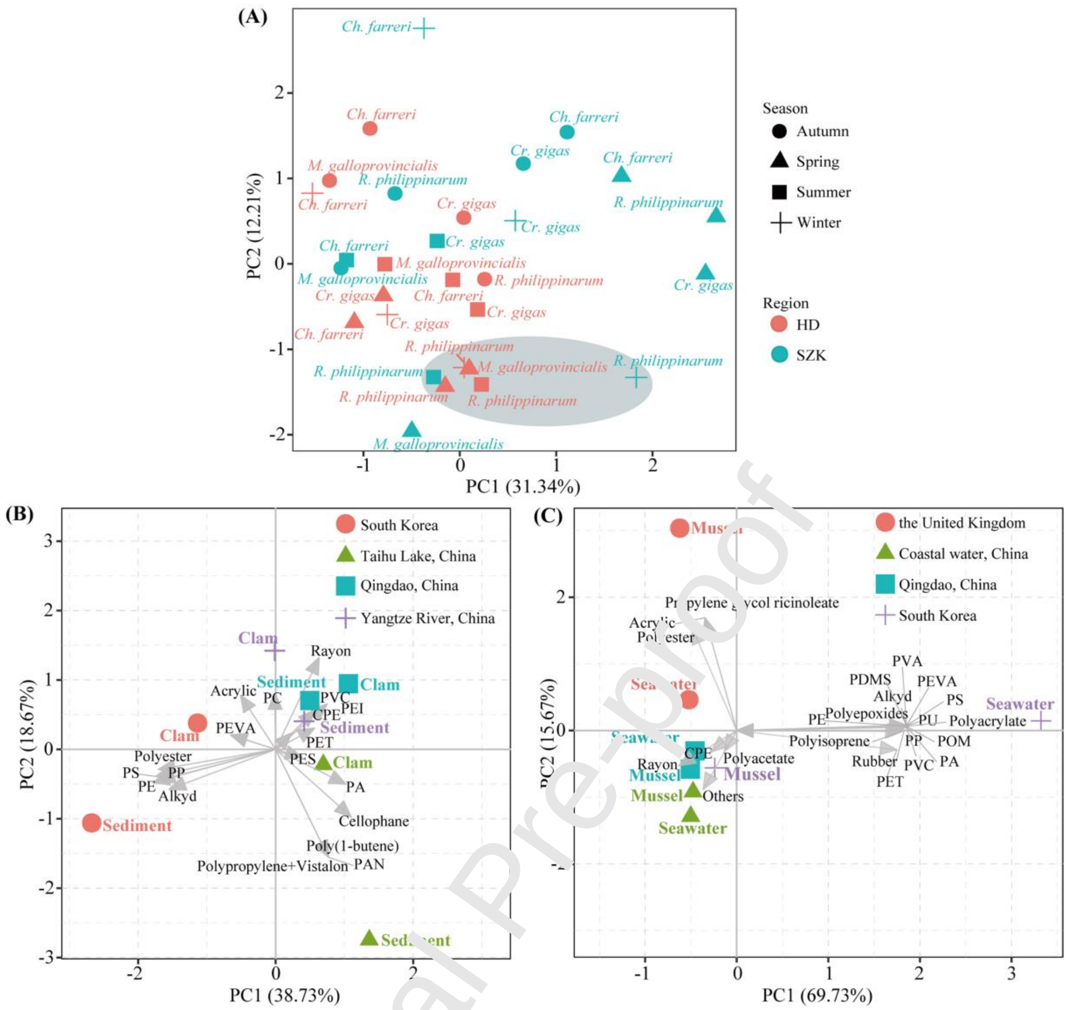
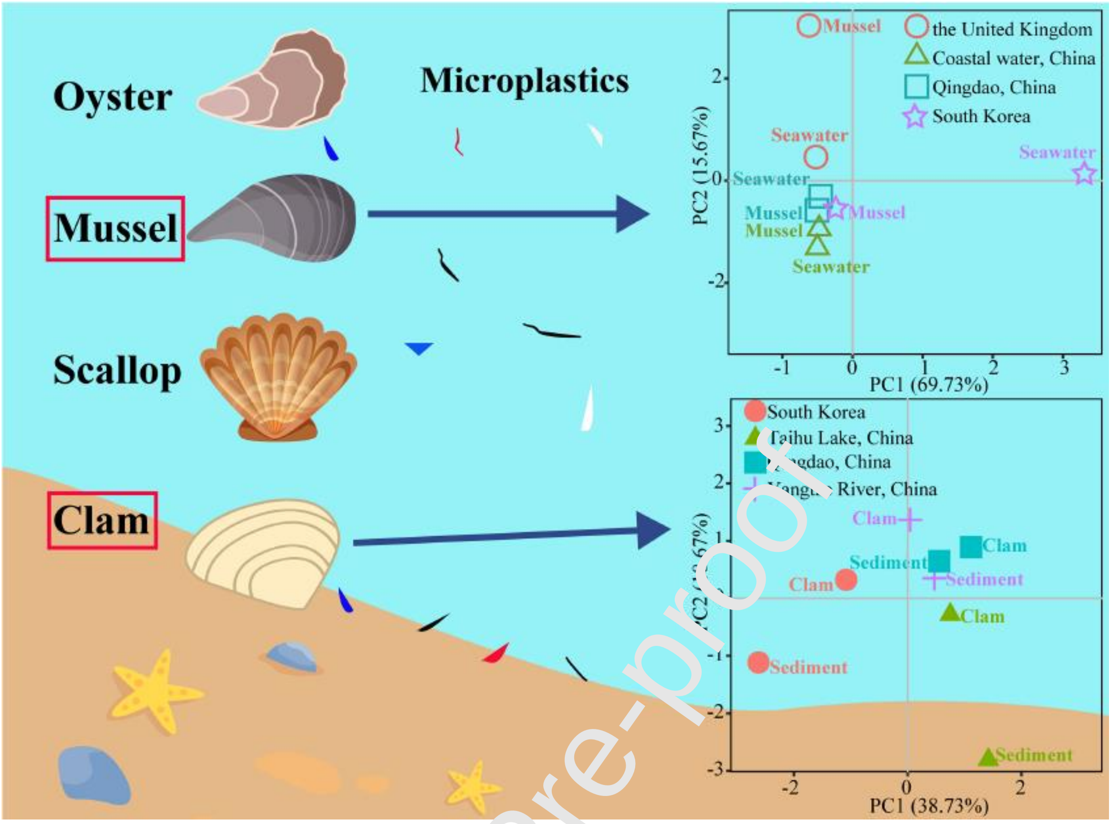


Fig. 5. (A) Principal component analysis (PCA) of microplastic distribution patterns in four species of bivalves over four seasons in HD and SZK based on microplastic abundance (items/individual, and items/g), shape, color, size, and polymer type. Different geometric shapes and different colors in the plot represent sampling seasons and regions. The light-coral and cyan shapes represent bivalves sampled from HD and SZK, respectively. The gray ellipse represents the community cluster of *R. philippinarum* separated from the other three species of bivalves. (B and C) PCA of the distribution pattern of microplastic polymer types between each bivalve species and the surrounding environment: (B) clam and sediment; (C) mussel and water. The gray arrows represented the polymer types of microplastics. PS: polystyrene; PE: polyethylene; PP: polypropylene; PEVA: polyethylene vinyl acetate; PC: polycarbonate; PVC: polyvinyl chloride; PEI: polyetherimide; CPE: chlorinated polyethylene; PET: polyethylene terephthalate; PA: polyamide; PES: Polyester, terephthalic acid; PAN: polyacrylonitrile; PU: polyurethane; POM: polyoxymethylene; PVA: polyvinyl acetate; PDMS: polydimethylsiloxane.

Graphical abstract



Highlights:

- Microplastic abundance in four species of bivalves showed no seasonal variations.
- Microplastic features in bivalves showed regional and species differences.
- Bivalves can act as the transporter of microplastics to humans.
- The use of bivalves as bioindicators of microplastic pollution is suggested.