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Science of the Total Environment

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https://doi.org/10.1016/j.scitotenv.2020.140815

Science of the Total Environment 745 (2020) 140815

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

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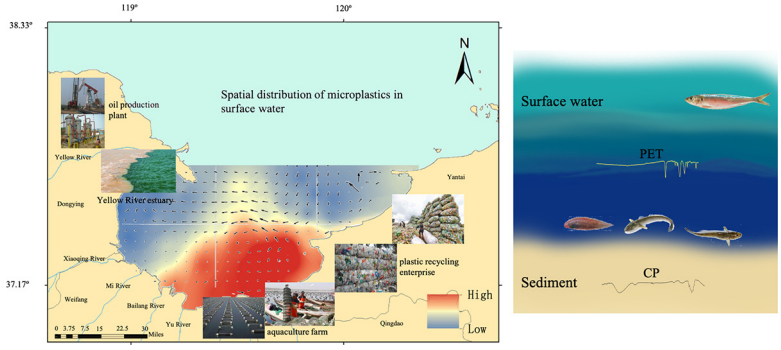
Jia Teng [a,b,c], Jianmin Zhao [a,b], Chen Zhang [b], Bo Cheng [d,e], Albert A. Koelmans [e], Di Wu [a,b], Meng Gao [a], Xiyan Sun [b], Yongliang Liu [b], Qing Wang [a,b,*]

[a] Research and Development Center for Efficient Utilization of Coastal Bioresources, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, PR China
[b] Muping Coastal Environment Research Station, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, PR China
[c] University of Chinese Academy of Sciences, Beijing 100049, PR China
[d] Chinese Academy of Fishery Sciences, No. 150, Qingta, Yongding Road, Beijing 100141, PR China
[e] Aquatic Ecology and Water Quality Management group, Wageningen University, P.O. Box 47, 6700 AA Wageningen, the Netherlands

HIGHLIGHTS

- MPs were pervasively distributed and mainly in the form of fibers in Laizhou Bay.
- The MPs characteristics were different among surface water, sediment and biota.
- MPs distribution in the Laizhou-Weifang area was mainly affected by ocean current.
- The possible transfer mechanism between different environmental media was suggested.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 April 2020
Received in revised form 6 July 2020
Accepted 6 July 2020
Available online 12 July 2020

Editor: Damia Barcelo

Keywords:
Microplastic
Yellow River Estuary
Sediment
Surface water
Fish
Multi-media distribution

ABSTRACT

Microplastic contamination is attracting increasing attention worldwide. In this study, the patterns of microplastic contamination in surface water and sediment from 58 sites, and living fish from 31 sites were investigated in a semi-closed bay (Laizhou Bay, China). Microplastics in Laizhou Bay were pervasively distributed, particularly in the form of fibers. Microplastic abundance exhibited no significant differences among regions in either surface waters or sediments, indicating multiple sources of microplastics pollution in the bay. Spatial hotspot (Getis-Ord Gi*) analysis demonstrated that microplastic pollution was mainly concentrated in the Laizhou-Weifang area, which in turn was mainly affected by ocean current dynamics. Although the spatial distribution of microplastics in sediments was different from surface water, it was also affected by geology, hydrogeology, and anthropogenic activities. The most common polymer in the surface waters was polyethylene terephthalate (PET), while cellophane (CP) was the most frequently observed polymer in sediment, suggesting different sinking behaviors of these microplastics. The proportion of low-density microplastics (PE and PP) in surface water was approximately 19.9%, but these microplastics accounted for only approximately 1.7% in the sediment, suggesting that low-density microplastic particles preferentially migrate to open sea. There were significant differences in shape, size and polymer type of the microplastics among surface water, sediment and biota ($p < 0.05$). Cluster analysis suggested that the Gudong, Yellow River Estuary and Laizhou-Weifang regions are three sources of microplastics, which might originate from river input, plastic recycling and marine raft aquaculture. Furthermore, microplastic particle diversity was greater in sediment at offshore sites, suggesting that these sites receive microplastics from multiple sources. Our results characterize the microplastic pollution pattern, clarify the

* Corresponding author at: Research and Development Center for Efficient Utilization of Coastal Bioresources, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, PR China.
E-mail address: qingwang@yic.ac.cn (Q. Wang).

https://doi.org/10.1016/j.scitotenv.2020.140815

possible transfer mechanisms between different environmental media, and will provide important information for risk evaluation and pollution control in this area.

1. Introduction

Microplastics (plastic particles with a size < 5 mm) (Andrady, 2011) are ubiquitous in the marine environment, even in the subtropical gyres (Law et al., 2010), mid-ocean islands (Ivar et al., 2009) and the Arctic (Lusher et al., 2015). Ingestion is widely recognized as a pathway for aquatic animals to acquire microplastics (Markic et al., 2019). Microplastics can be ingested by a wide diversity of marine organisms, including plankton, bivalves, fish, and mammals (Desforges et al., 2015; Steer et al., 2017; Lusher et al., 2018; Teng et al., 2019; Markic et al., 2019), which may adversely affect their health (Watts et al., 2015; Nadal et al., 2016; Kolandhasamy et al., 2018). False satiation, loss of energy, alteration of hormone levels, growth inhibition, and delayed maturity have been observed (Chae and An, 2017; Galloway et al., 2017). In addition to the physical harm caused to marine organisms, microplastics are sometimes believed to act as vectors for other pollutants (Zhang et al., 2015; Brennecke et al., 2016; Foulon et al., 2016; Wu et al., 2017) or to leach additive chemicals from manufacture (Li et al., 2016; Hermabessiere et al., 2017). When aquatic organisms ingest contaminated microplastics, these contaminants and microplastics can enter the food web and then be exposed to the human body through the consumption of seafood (Diepens and Koelmans, 2018).

Because microplastic particles can settle, alone or as part of aggregated particles, the seafloor is widely considered to be a vital sink. In addition, seawater and sediments are the major habitat environments for marine biota. As a result, a growing number of studies have investigated the difference in microplastic abundance among different habitats. There was no correlation between microplastic abundance in surface water and sediment samples of Poyang Lake (Yuan et al., 2019), Antuã River (Rodrigues et al., 2018) and the Three Gorges Reservoir (Di and Wang, 2018). This can be attributed to water area features, flow velocity, biofouling and the specific density of microplastics. In addition, there were differences between them due to the differences in the microplastic residence times in surface water and sediment in Hiroshima Bay (Sagawa et al., 2018). Therefore, sampling only one medium does not accurately describe how much or what type of pollution exists in the entire region. Previous research has found that microplastic levels in water samples were positively correlated with microplastics in corresponding mussel samples (Qu et al., 2018), and there is also research showing a positive correlation between microplastic concentrations in sediment and in benthic invertebrates (Redondo-Hasselerharm et al., 2018). To date, there has been little systematic study of microplastic pollution in sea water, sediments and marine biota from one aquatic system, concurrently. Moreover, few studies have addressed possible transfer mechanisms of microplastic between different environmental media and biota.

Bay areas are particularly susceptible to plastic pollution due to their proximity to microplastic sources. High human activity has intensified the risk of microplastic pollution in bay areas (Mathalon and Hill, 2014). In addition, the hydrodynamics in the bay area are complex, and the abundance and distribution of microplastics are greatly affected by wind and tidal currents (Zheng et al., 2019; Ramírez-Álvarez et al., 2020). Laizhou Bay is a typical semi-enclosed inner sea and is one of the three major bays in the inner Bohai Sea (Zhang et al., 2012), making up 10% of the total sea area (Wang et al., 2007). In addition, there are many rivers flowing into Laizhou Bay, including China's second largest river, the Yellow River, and more than 10 other rivers. There are large areas of tidal flat and many rivers flowing in and carry rich organic matter in Laizhou Bay, providing a habitat for a variety of fish and shellfish (Yang et al., 2016). Laizhou Bay is on the verge of Dongying, Weifang and Yantai cities, with a total population of approximately 20 million across all three of the major cities. Around the bay, rapid urbanization and industrialization have led to large inputs of various pollutants, including those from more than 2000 private enterprises engaged in the recycling of waste plastics (annual recycling amount > 150 million tons) (Hongxin, 2010), large-scale raft aquaculture areas (41,157 hm^2) and greenhouse vegetable cultivation bases (34,000 hm^2), which have rapidly deteriorated the quality of the local environment.

To date, several researchers have reported on microplastic contamination in surface water and sediments in China, such as Jiaozhou Bay (Zheng et al., 2019), Poyang Lake (Yuan et al., 2019), Qin River (Zhang et al., 2020), Maozhou River (Wu et al., 2019), Haikou Bay (Qi et al., 2020). However, there is little research investigating the levels of microplastic pollution in marine media and biota concurrently, analyzing the potential links of microplastic characteristics in the different compartments. Therefore, the specific aims of this study were to: (1) determine the characteristics and spatial distribution of microplastics in surface seawater, sediments and fish of Laizhou Bay; (2) investigate the relationships between microplastic concentrations across water, sediment and fish, and (3) discuss the potential sources of microplastics. To achieve this aim we did high quality assurance and quality control (QA/QC) measurements and ocean current simulations. Furthermore, we also applied high-dimensional k-sample comparison analysis, cluster analysis and microplastic diversity index to answer the questions.

2. Materials and methods

2.1. Sample collection

Sampling was conducted in August 2017. Surface water and sediment samples were collected from 58 sites in Laizhou Bay, while fish samples (*Sardinella zunasi*, *Cynoglossus joyneri*, *Acanthogobius ommaturus* and *Chaeturichthys stigmatias*) were collected from 31 sites in Laizhou Bay (Fig. 1). These sites were distributed as evenly as possible in the bay. Based on various geographical features of Laizhou Bay, the sampling sites were further divided into eight areas (R1 - western periphery of the Laizhou Bay, R2 - near shore of the Yellow River estuary, R3 - far shore of the Yellow River estuary, R4 - far shore of Dongying, R5 - near shore of Dongying, R6 – Weifang, R7 - eastern periphery of the Laizhou Bay, R8 - Laizhou). The sample sites were fixed and recorded using a GPS (Garmin 62sc, Garmin Ltd., China). Samples in surface seawater were collected using a trawl net (1 m wide × 15.8 cm vertical opening, 3 m long, 333 μm mesh) at a speed of 5.0 knots for 10 min in each transect. In order to reduce the impact of the sampling boat on the water flow through the nets, the manta trawl was vertically connected to the boat's hull with a rod and kept at the windward side of the hull. The sample water volume was calculated from the cross-sectional area of water passing through the net (m^2), multiplied by flow velocity (m/s) and the sampling time (t). When a trawl was completed, the Manta trawl net was thoroughly rinsed with seawater and filtered using a Whatman GF/B glass microfiber filter (pore size 1.0 μm), to ensure that all visible material moved down to the cod end. The cod end was detached and its content was thoroughly rinsed with Milli-Q water into a glass bottle for laboratory analysis. Between each sample, the Manta net was back washed with sea water, and the cod end was washed with deionized water in an effort to limit cross-contamination between sampling locations. At the same site, three sediment samples were collected from the middle and both sides of the

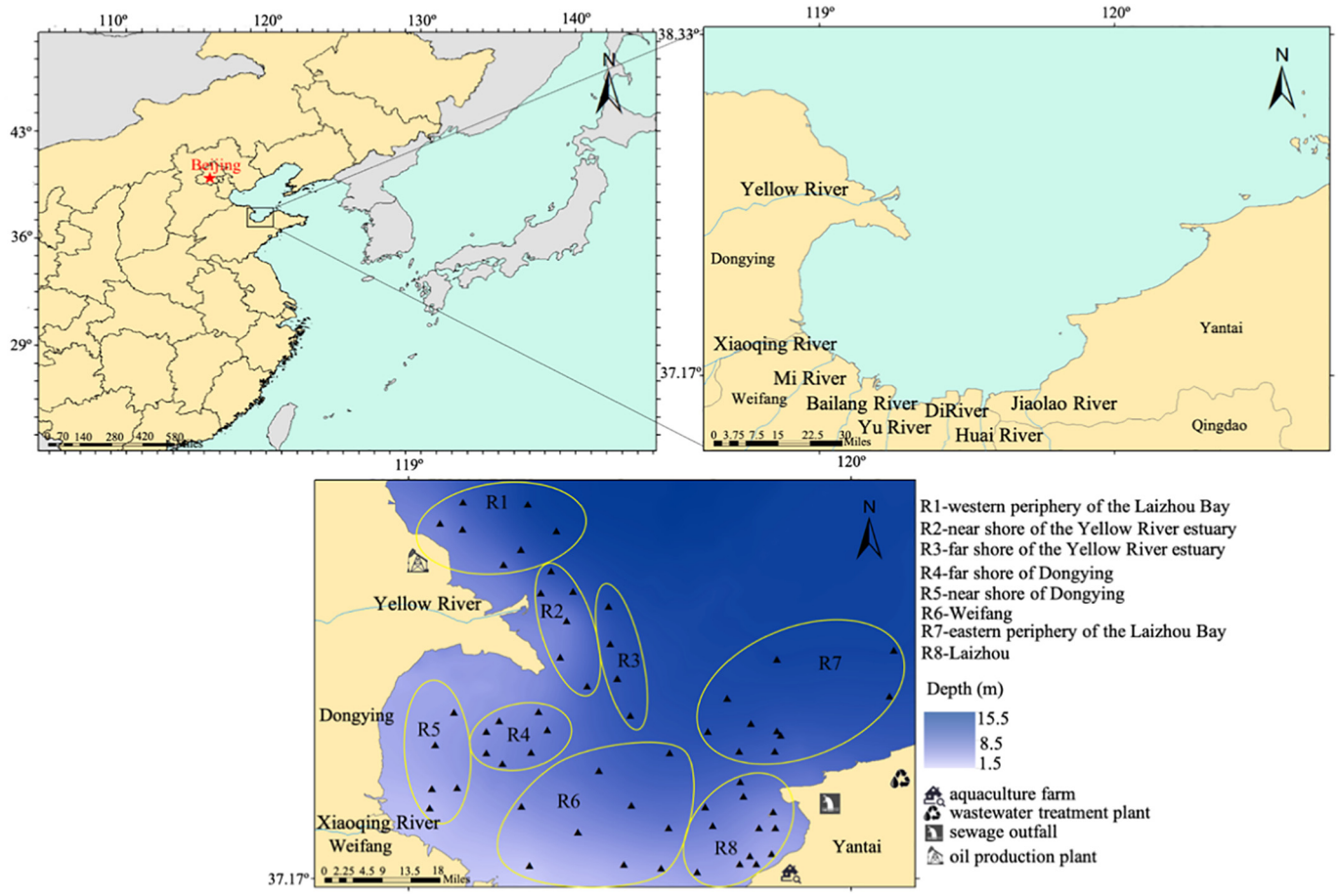


Fig. 1. Surface water, sediments and organism sampling sites in Laizhou Bay. Black triangle: surface water and sediment; red dot: organism. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

boat using a Van Veen grab (area = 0.05 m^2, height = 20 cm). The grab was put into the sea from the boat via a winch. Portions of more than 500 g sediment from the top 5 cm layer of the grab were collected by a stainless steel shovel and stored in an aluminum foil bag. All sediment samples were refrigerated at −20 °C prior to analysis. Contact with plastic materials was avoided at all times to prevent contamination. All of the sampling tools were cleaned between each site. Fish were caught by one-boat bottom trawl fisheries (30.6 m vertical opening, 20 mm mesh). At each site, sampling was carried out by towing for 1 h at a speed of 3 knots. When trawling, the mesh opening width is about 8 m. Fish samples were randomly selected from commercially important fishes that included both pelagic fish and benthic fish, and stored in aluminum foil in a − 20 °C freezer until further examination. All experimental materials were pre-rinsed with filtered seawater or Milli-Q water in the field.

2.2. Microplastic extraction

2.2.1. Sediment

Extraction of microplastic particles was performed in accordance with the density separation procedure reported by Thompson and colleagues (Thompson et al., 2004), with some minor modifications. All sediment samples were transferred into a glass beaker and dried at 60 °C for 72 h to yield a constant weight. Subsequently, 50 g of each sediment sample was transferred to a glass beaker and mixed with 200 mL of prefiltered saturated salt solution (NaCl with ρ = 1.20 g/mL). The resulting mixture was stirred for 1 min using a stainless steel trowel and was allowed to settle for 5 min. After 5 min of settling, the overlying water was carefully transferred to another glass beaker. This isolation procedure was repeated three times for each glass beaker to increase the recovery rate. Subsequently, to reduce interference with the plastic identification, 5 mL of 30% H_2O_2 was added to 200 mL salt solution to degrade the organic matter (Zhao et al., 2018). After 24 h of sedimentation, the clean supernatant was vacuum filtrated through a Whatman GF/B glass microfiber filter (pore size 1.0 μm). The recovery rate of this method was 92.5% as demonstrated earlier by Zhao et al. (2018).

2.2.2. Surface water

In the laboratory, the large debris in the water samples was first screened with a 5 mm steel-wire sieve and discarded. Water passing through the sieve was collected and passed through a 300 μm metal sieve. The particulates were then resuspended using Milli-Q water in a glass beaker and oxidized using 30% H_2O_2 for 24 h (Nuelle et al., 2014) to digest biogenic materials. Subsequently, the mixed solution was vacuum-filtered as in the sediment procedure.

2.2.3. Fish

The fishes were taken out of the freezer, thawed for 1 h to measure their length and weight. A fresh, moist filter paper was placed on the table in the working area and the viscus tissue (stomach and intestine) was dissected from the fishes. The instruments and dishes to were cleaned and inspected before each tissue was processed. Then the tissue was placed into a labeled 500 mL glass beaker and approximately 180 mL of 10% (m/v) KOH and 20 mL of 30% H_2O_2 was added to digest the tissue. The beakers were covered with aluminum foil and placed in an oven at 60 °C for 24–48 h, depending on the digestion effect on

the soft tissues, and agitated once every 8 h. When there was no visible organic residue and the solution was clear, the digestion was considered complete. This method was based on Munno et al. (2018), and was reported to have a recovery higher than 98%. The mixed solutions were then vacuum filtered over 1.0 μm glass microfiber filters (Whatman GF/B). Three glass beakers were combined as one replicate and six replicates were prepared for each site.

2.3. Microplastic analysis and polymer identification

Filters were examined under a stereoscopic microscope (Olympus, SZX10, Japan). Microplastic particles were visually identified according to the artificial colors, smooth edges, uniform width and lack of biological features and structure in accordance with Hidalgo-Ruz et al. (2012) protocol. Suspected microplastics on filters were enumerated and categorized on the basis of four shapes, including fibers, fragments, plastic films and particles. Additionally, based on the largest dimension (length), every particle was assigned to one of twelve distinct size classes: 1–100, 101–300, 301–500, 501–1000, 1001–1500, 1501–2000, 2001–2500, 2501–3000, 3001–3500, 3501–4000, 4001–4500 and 4501–5000 μm. A total of 800 (30.7% of all particles), 1350 (30.9% of all particles), 300 (33.1% of all particles) and 120 (36.6% of all particles) microplastics were randomly selected from surface water, sediment, pelagic fish and benthic fish samples for compositional analysis using a micro-Fourier Transformed Infrared Spectroscope (μ-FTIR) (Nicolet™ iN10, Thermo Fisher Scientific, USA) in transmittance mode. The obtained spectra were compared with the OMNIC standard spectra libraries of Hummel Polymer Sample Library and Polymer Laminate Films (Jabeen et al., 2017), and the type of microplastics was determined when the match rate was higher than 70% (see SI for details).

2.4. Quality assurance and quality criteria (QA/QC)

Following recently published QA/QC criteria, strict control measures were implemented (Hermsen et al., 2018; Koelmans et al., 2019). All of the sampling tools were cleaned between each site, and the trawl was rinsed three times before the next sampling to reduce cross-contamination. To avoid potentially artificial and airborne plastic contamination, all apparatus components were rinsed carefully with ultrapure water and wrapped tightly in aluminum foil. All of the experiment tools were rinsed three times with Milli-Q water and then dried before the experiments in a clean bench. During the entire process of sample collection and laboratory analysis, cotton laboratory coats and latex gloves were always worn. Samples were operated in a laminar flow cabinet and covered with aluminum foil during digestion and when not in use. To monitor air contamination, three empty glass beakers were opened during both surface water and sediment sampling. Similarly, three procedural blanks were run at all steps of the sample analysis using only reagents and excluding the sample itself. Each procedural blank beaker was then rinsed with Milli-Q water followed by vacuum filtration using 1.0 μm glass microfiber filters followed by quantification of background contamination. According to the quantitative scoring system by Hermsen et al. (2018), a good method score was obtained (accumulated reliability score was 16 out of 20).

2.5. Ocean current simulation

The Regional Ocean Modeling System (ROMS, UCLA version) was used to simulate currents in Laizhou Bay in the period from June to August 2017. ROMS is a three-dimensional, free surface, terrain-following numerical model, for which a complete description can be found in Shchepetkin and McWilliams (2005). For the present study, a 1-km horizontal grid resolution and 12 vertically stretching layers were used, while the minimum water depth was set at 0.1 m. The boundary condition and initial fields for the ROMS were derived from the outputs of the HYbrid Coordinate Ocean Model (HYCOM, GLBa0.08/expt_90.6). Tidal data used to force the coupling system was obtained from the tidal database of the Advanced Circulation Model (ADCIRC) with nine tidal constituents (K1, O1, Q1, M2, S2, N2, K2, M4, M6). The wind and air pressure forcing were achieved by using NCEP-CFSR (National Centers for Environmental Prediction Climate Forecast System Reanalysis) hourly time series data.

2.6. Data analysis

In this study, 1.4 ± 0.5 particles/sample (n = 3) were detected in the blank samples and all the microplastics in blank samples were fibers. The average number of microplastic fibers in the blank sample was subtracted when calculating the abundance of the fibers in each field sample. For other microplastic types no contamination was found and therefore no blank correction was needed. In addition, microplastic abundance was corrected based on the non-microplastic particles for which no polymer type was identified by μ-FT-IR according to the corresponding shape. The abundance of microplastics in water was calculated by dividing the microplastics number by the sample water volume and was expressed as the number of microplastics per cubic meter. Microplastic concentrations in sediment samples were expressed as the number of microplastics per kilogram of dry sediment. The concentration of microplastics in fish was expressed as the number of microplastics per gram of wet weight or per individual. All statistical analyses were performed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). All values are reported as the mean ± SD. The nonparametric Kruskal-Wallis test was applied for multiple comparisons of the plastic concentrations at each area. High-dimensional k-sample comparison analysis was used to determine the relationship between fish and environment (water and sediment) microplastic characteristics. This analysis is based on high-dimensional exploratory analysis as proposed by Wang (2019). Spearman rank correlation was used to determine the relationship between surface water and sediment microplastic concentration levels. According to the previous method, the data were subjected to cluster analysis (Zhang et al., 2019), and the log-linearized particle size distribution was assessed using standard linear regression analysis (Kooi and Koelmans, 2019). Principal component analysis (PCA) was performed with R Studio (R Studio Version 1.2.1335, © 2009–2019 RStudio, Inc.) to describe the principal components (PC) of primary microplastics size. The Getis-Ord Gi* was used to confirm the high and low value areas for the spatial distribution of microplastics. According to the method of T. Wang et al. (2019), the microplastic diversity index (D′(MP)) for each station is calculated as follows:

$$D_{1-D'}(MP) = 1 - \sum_i^S q^2 \; AND \; q^2 = \left(\frac{n_i}{N}\right)^2$$

where i is the sample number; n_i is the number of individuals in the i-th microplastic type; N is the total number of microplastic particles; S is the total number of microplastic types, and q is the relative abundance of each type in a microplastic combination (T. Wang et al., 2019).

3. Results

3.1. Microplastics abundance

Microplastics were detected in all the surface seawater and sediment samples, with a total of 2602 particles and 4374 particles, respectively. The abundance in surface water samples varied from 0.1 to 6.7 particles/m^3 with an average of 1.7 ± 1.5 particles/m^3. In sediment samples, the average abundance of microplastics was 461.6 ± 167.0 particles/kg d.w. with a range from 193 to 1053 particles/kg (Fig. 2). Four fish species were collected in the Laizhou Bay (Table 1). *Chaeturichthys stigmatias* had the highest ingestion rate and *Sardinella zunasi* had the lowest ingestion rates. The four fish species were grouped as pelagic fish and benthic fish. Microplastic contamination in fishes is widespread

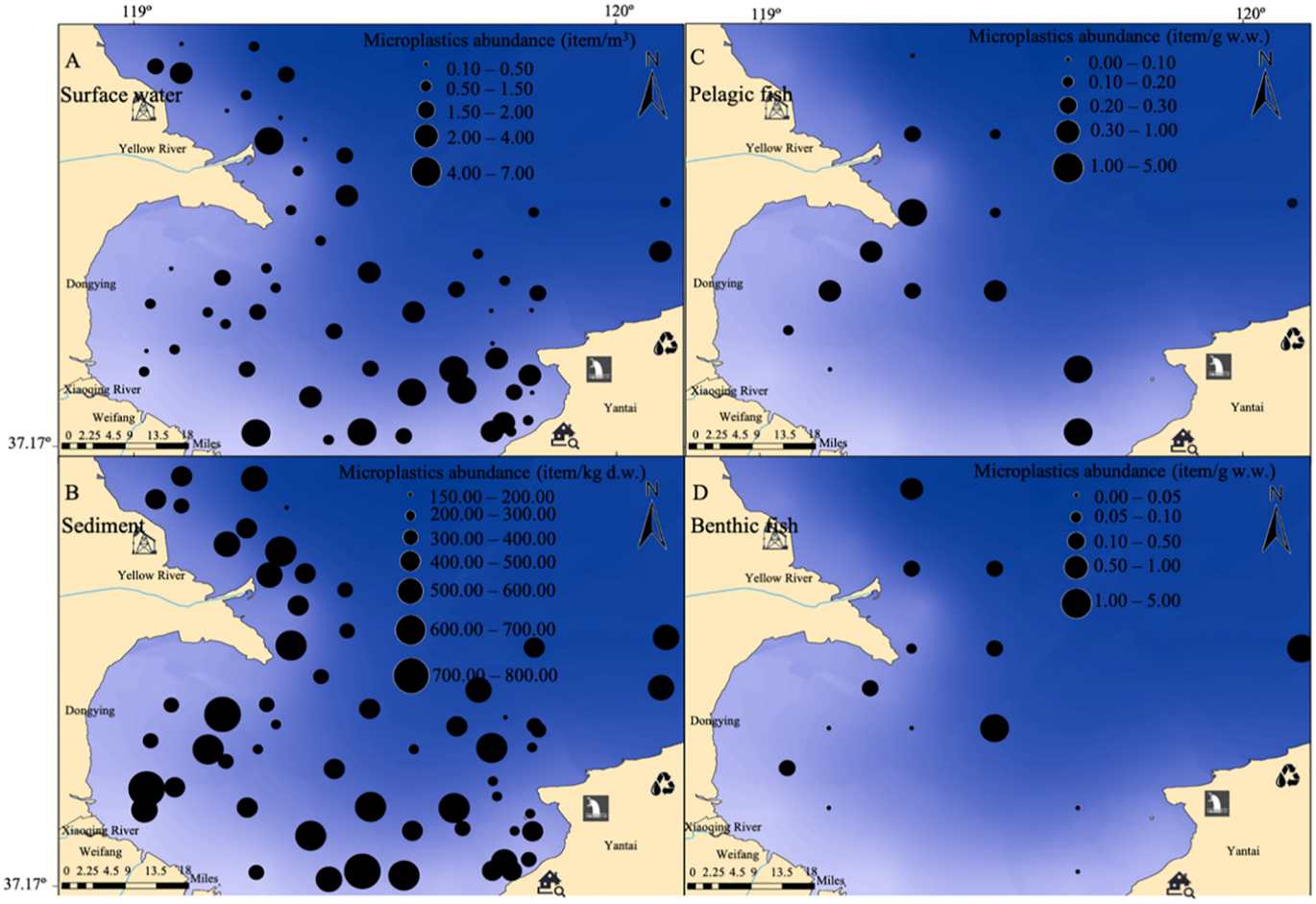


Fig. 2. Abundance of microplastics in surface water (A), sediments (B), pelagic fish (C) and benthic fish (D) at each site on the map.

in Laizhou Bay. 78.8% and 91.5% of the fish individuals of pelagic fish (*Sardinella zunasi*) and benthic fish (*Cynoglossus joyneri*, *Acanthogobius ommaturus* and *Chaeturichthys stigmatias*) contained MP, respectively. The average abundance of microplastics in fish was 2.8 ± 1.9 particles/individual (range 0–6.2) or 0.8 ± 1.4 particles/g w.w. (range 0–4.9) for pelagic fish. For benthic fish, the average abundance of microplastics in fish was 3.5 ± 1.2 particles/individual (range 0–8.0) or 0.7 ± 0.9 particles/g w.w. (range 0–1.7). A detailed overview of microplastics occurrence in fish is provided in Table 1.

3.2. Microplastic characteristics

The surface water, sediment and biota samples collected from the research area had similar microplastic compositions in terms of shape. Fibers were dominant component in surface water, sediment, pelagic fish and benthic fish samples, accounting for 96.08%, 94.10%, 99.02% and 97.91% of the total microplastics, respectively (Fig. 3). Furthermore, the proportion of fibers and films in the sediment (3.59%) was higher than that in the surface water (2.44%). In fishes, only a few fragments were found, while films and particles were not found. The average microplastics size was 1660.0 ± 1310.4 μm (size range: 336.2 to 4997.7 μm), 876.8 ± 1027.5 μm (size range: 28.3 to 4933.0 μm), 1063.7 ± 873.3 μm (size range: 60.1 to 4913.9 μm) and 1069.3 ± 934.9 μm (size range: 94.1 to 4842.9 μm) in the surface water, sediment, pelagic fish and benthic fish, respectively. The particle size of microplastics was subjected to standard linear regression analysis according to the method of Kooi and Koelmans (2019). As shown in Fig. 4C, the microplastic concentration in the surface water and sediment decreased with increasing particle size. In addition, the fitted trend lines for the particle size of microplastics in surface water ($y = -0.793x + 3.57$) and sediments ($y = -0.746x + 3.07$) have similar slopes on the log-log scale. From the data in Fig. 4D, it is apparent that the size range of microplastics ingested by fish was less than that of the water and sediment.

3.3. Microplastic composition

The identified microplastics accounted for more than 30% of the total detected microplastics, and the success rate of identification was over 90%. Typical spectra of the selected microplastics are shown in Fig. S1.

Table 1
Percentage of individuals with ingested microplastics and their corresponding levels of microplastic ingestion in analyzed fish collected from the Laizhou Bay.

Species	Habitat	Percentage microplastic ingestion (%)	Mean microplastics per g w.w. (SD)	Mean microplastics per fish (SD)
Sardinella zunasi	Bathypelagic	78.8	0.77 (1.42)	2.84 (1.93)
Cynoglossus joyneri	Benthopelagic	80.0	0.20 (0.16)	2.16 (1.35)
Acanthogobius ommaturus	Benthopelagic	94.4	1.75 (1.15)	3.87 (0.99)
Chaeturichthys stigmatias	Benthopelagic	100	0.17 (0.16)	4.44 (1.93)

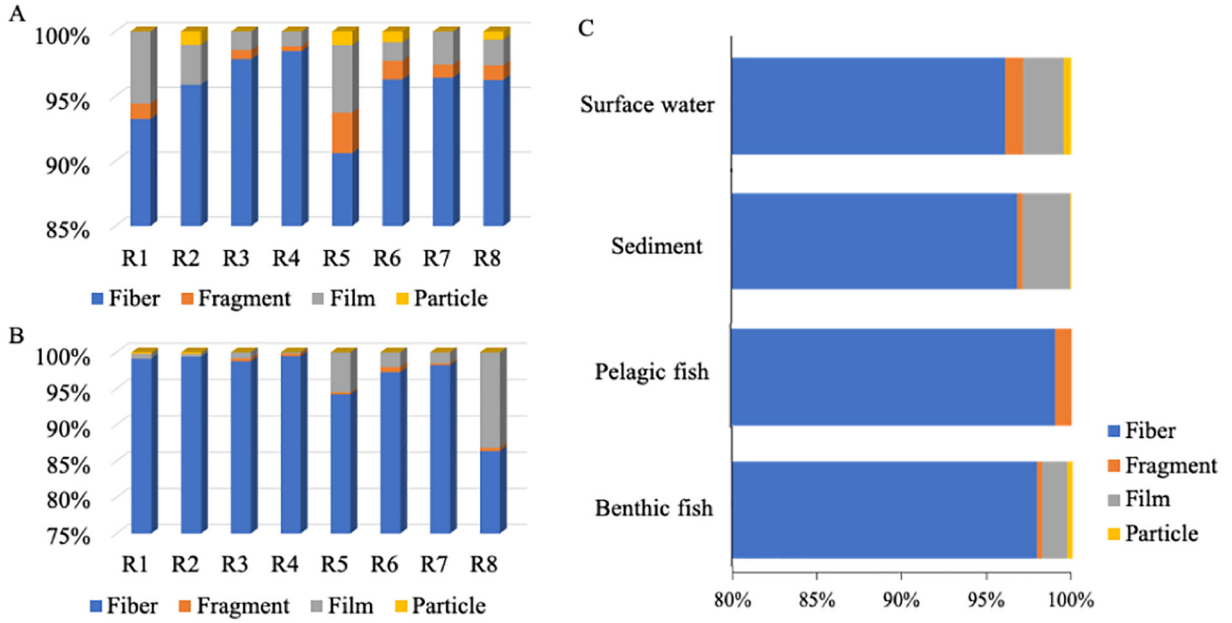


Fig. 3. Percentages of shape distribution of total microplastics in surface water (A) and sediments (B) in 8 areas. R1 - western periphery of the Laizhou Bay, R2 - near shore of the Yellow River estuary, R3 - far shore of the Yellow River estuary, R4 - far shore of Dongying, R5 - near shore of Dongying, R6 – Weifang, R7 - eastern periphery of the Laizhou Bay, R8 – Laizhou. Size distribution as percentages of the total microplastics in surface water, sediments and organisms (C).

For surface water and sediment samples, there were obvious differences in the chemical compositions of the microplastics (Fig. 5). In surface water samples, nine different plastic types were determined, including polyethylene terephthalate (PET), cellophane (CP), polypropylene (PP), polyacrylonitrile (PAN), polyethylene (PE), polyvinyl acetate (PVAc), polyamide (PA), polystyrene (PS), and polyphenylene oxide (PPO). PET was the dominant type (accounting for 32.8%), followed by CP (27.8%) and PP (14.5%). However, in sediment samples, ten polymer

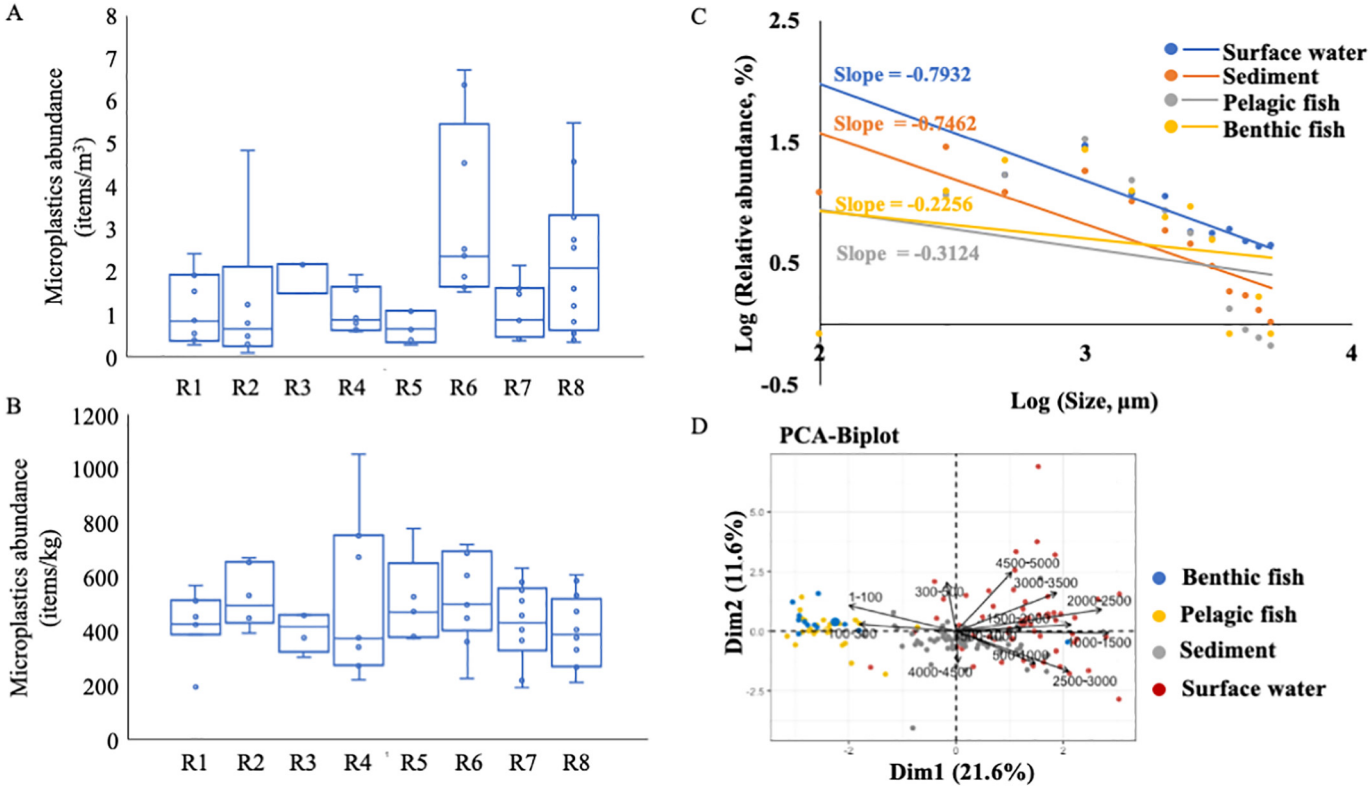


Fig. 4. Box–whisker plot for microplastic abundance of the surface water (A) and sediment (B) in Laizhou Bay. The box plots represent the 1st and 3rd quartiles separated by the median. The blue dot indicates the abundance value of each site in the corresponding region. R1 - western periphery of the Laizhou Bay, R2 - near shore of the Yellow River estuary, R3 - far shore of the Yellow River estuary, R4 - far shore of Dongying, R5 - near shore of Dongying, R6 – Weifang, R7 - eastern periphery of the Laizhou Bay, R8 – Laizhou. Relative microplastic abundance for different particle sizes in surface water, sediments and organisms (C). Principle component analysis (PCA) biplot for microplastic size range at all sampling stations (D). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

types were identified, including CP, PET, PP, PVAc, polyphthalamide (PPA), PA, PAN, PE, polyethylene glycol adipate (PEA) and polyvinyl chloride (PVC). The most common type in the sediments was CP, accounting for 85.4%, followed by PET (8.9%). The most common chemical composition of the plastic collected from fishes was also CP, followed by PET (Fig. 5). The percentages of CP and PET in pelagic fish were 61.0% and 29.0%, respectively, and the percentages in benthic fish were 78.0% and 18.3%, respectively. In addition, PP (6.0%), PA (2.4%) and PAN (1.6%) were found in the pelagic fish, while PVAc (2.5%) and PP (1.3%) were found in the benthic fish. As expected, more dense microplastics were more abundant in sediment and in benthic fish.

3.4. Microplastic distribution analysis

A Spearman rank correlation analysis indicated that there was no significant relationship between microplastic abundance in surface waters and the sediments (Fig. S2). Getis-Ord Gi* analysis demonstrated that microplastic pollution hotspots in surface water are mainly distributed in the Laizhou-Weifang area (R6 and R8 areas) (Fig. S3), although no statistically significant differences were shown among microplastic abundance of the eight regions (Fig. 4). The k-sample GLP statistic along with component-wise *p*-value for each comparison was calculated using a high-dimensional k-sample comparison algorithm (Table 2). The comparisons indicated significant differences in the characteristics (shape, size and polymer type) of the microplastics among the surface water, sediments, and biota ($p < 0.05$). However, the p-value of the single component suggested similarity in shape, size and polymer type character of the microplastics in the benthic fish samples to those in the surface water, sediments and the pelagic fish. Cluster analysis by microplastic shape, particle size and color suggested that sampling sites can be distinguished into three main clusters: Gudong, Yellow River Estuary and Laizhou-Weifang region (Fig. S4). This result was similar to that of microplastic contamination hotspots in sediments (Fig. S3).

4. Discussion

4.1. Microplastics in surface water and sediment

4.1.1. Spatial distribution characteristics

Our study has shown that microplastics were distributed unevenly in surface water of the Laizhou Bay. This result was determined by emission and transportation processes. For example, R8 station is located in the Longkou-Laizhou section with a dense population, where frequent human activities such as industrial production, aquaculture and shipping will have caused intensive emissions of microplastics. The specific

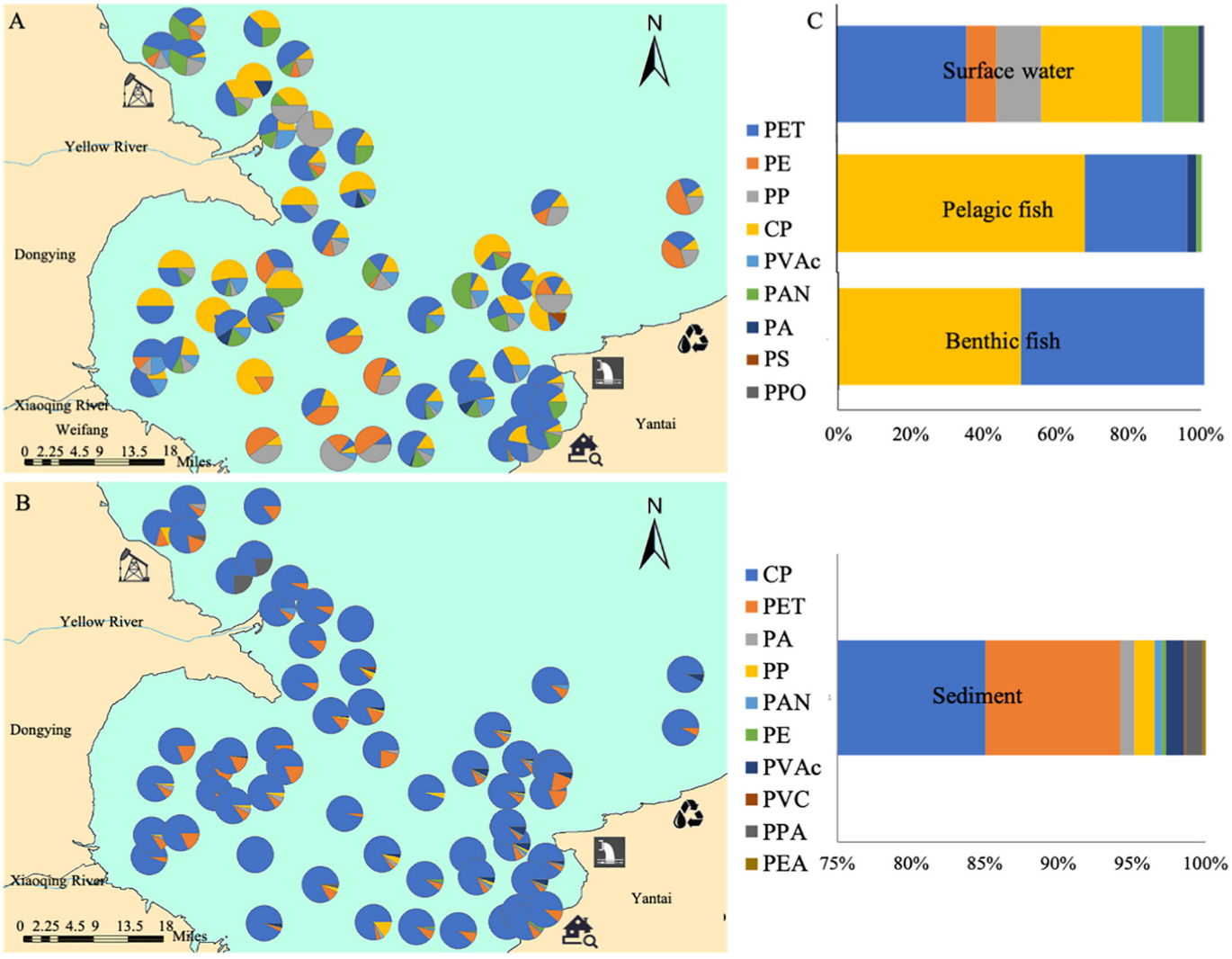


Fig. 5. Spatial distribution of microplastic polymer types detected from surface water (A) and sediment (B) of Laizhou Bay. Different colors represent different polymer types. Percentages of polymer types among the total microplastics in surface water, sediments and organism (C).

Table 2
GLP multivariate k-sample test for microplastic of surface water, sediment, pelagic fish and benthic fish. The table shows the output of our GLP algorithm. The overall statistic provides the global k-sample confirmatory test, while the individual "components" give exploratory insights into how the multivariate distributions are different. The significant components based on *p*-values adjusted for multiple comparisons are marked with an asterisk '*'.

Component	GLP	*p*-Value
Surface water*	0.320	3.14×10^{-23}
Sediment*	0.060	4.06×10^{-3}
Pelagic fish*	2.020	3.72×10^{-168}
Benthic fish	0.023	0.431
Overall	0.901	4.09×10^{-72}

features of topography and currents were also considerable reasons for the microplastic accumulation in Laizhou-Weifang (Bergmann et al., 2016; Christiansen et al., 2016; Li et al., 2018), which had hampered the transport of microplastics from southeast of Laizhou Bay (R6 and R8) to the outer seas, leading to an enrichment of microplastic in this area. Meanwhile, R6 station is located in a sheltered bay with relatively shallow slopes (Claessens et al., 2011; Cluzard et al., 2015; Vianello et al., 2013), which might lead to greater microplastics retention. As shown in Fig. S5, after July, the currents in Laizhou Bay decreased rapidly, while alongshore, eastward currents dominated the south coast of Laizhou Bay. These may carry a large number of near-shore derived microplastics to southeast of Laizhou Bay. In addition, due to the blockage of the peninsula terrain of north Taiping Bay, the direction of currents near the peninsula switched from eastward to northward, causing attenuation of the exchange of water between the southern and the northern area of Taiping Bay, as well as enrichment of microplastics in Taiping Bay. In contrast, the southwest of Laizhou Bay was influenced by westward currents derived from the center of Laizhou Bay, while its water body came from the outer area of the bay. Consequently, microplastic abundance in surface water in the western area is similar to that in the outside area of the bay, however, remains much lower than in the eastern area.

In contrast to the surface, the spatial pattern of microplastic distribution in the sediments was different, but also affected by geology, hydrogeology, and anthropogenic activities. The microplastic level in sediments was greater in the western part of Laizhou Bay than in the eastern part. In addition, the stations with high microplastic pollution levels in the west were mainly detected around the Yellow River Estuary. A possible explanation for this might be that the Yellow River, as the second largest river in China, is a significant input source of coastal microplastic pollution, in addition to human activities (Klein et al., 2015; Lebreton et al., 2017). Since the near-shore water temperature was higher than the far-shore in Laizhou Bay, the center of warm water indicated the convergence area of the near-shore water. Also, the locations of warm water center in June and August were highly consistent with microplastic pollution hotspots in sediments and surface water (Figs. S3 and S6). This is because although microplastic abundance in sediments and surface water both were controlled by emission and transportation processes, the spatial pattern of microplastic distribution in sediments reflected continuous accumulation under the long-term influence of human activities and annual mean currents, and in surface water it was dominated by short-term emission and monthly-mean currents. The interannual characteristics of hydrological processes in Laizhou Bay were similar to monthly characteristics in June, and quite different from those in August (Figs. S5 and S6). The annual average current along the southern coast of Laizhou Bay was westward, and the annual convergence center of the near-shore water was essentially located on the westward, which both shifted to eastward in August. This may lead to longer residence times of near-shore water with high levels of microplastics in southwest of Laizhou Bay than in southeast, and result in the difference between spatial distribution of microplastics in sediments and surface water.

We also found that surface water microplastic concentrations do not correlate with sediment concentrations from the same station. This result may reflect differences in floating and sinking plastics across each station, and denser polymers were more abundant in sediment samples. Furthermore, differential biofouling may also result in the varying floating and sinking of low-density plastics (Barnes et al., 2009; Browne et al., 2010; Cózar et al., 2014). The lack of correlation between microplastic concentration in sediment and in surface water may also be explained by different residence times of microplastics in each environment. Therefore, the spatial distribution pattern of microplastic diversity observed in the sediments and surface water further supports that microplastics in the surface water only represent the short-term influences of hydrological environment and human emissions. The microplastic diversity increased in sediments but not in surface water at offshore sites (Fig. S7). A possible explanation for this result is that compared to nearshore sites, the microplastics in sediment at offshore sites are mainly delivered through ocean currents and atmospheric deposition. Therefore, the multiple sources might result in higher diversity in distant sea areas (Cole et al., 2011; Dris et al., 2015; Desforges et al., 2014; T. Wang et al., 2018; W. Wang et al., 2018).

4.1.2. Microplastic shape

Fiber was the dominant particle shape in both the surface water and the sediment samples collected from the Laizhou Bay, which was consistent with other studies (Wright et al., 2013; Martin et al., 2017; Di and Wang, 2018; Lin et al., 2018; T. Wang et al., 2018). Furthermore, the higher ratio of the number of fibers and films in the sediment may be explained by the fact that fibers and films have a high ratio of surface area to volume. This property enables microplastics to have a higher rate of aggregation/biofouling, causing them to sink faster than larger plastic fragments on relatively shorter time scales (Ryan, 2015). Fiber can originate from fishing activities, heavy marine traffic and textiles (Claessens et al., 2011; Napper and Thompson, 2016). Wastewater may also be an important source of microplastics (Browne et al., 2011; Isobe, 2016; Sutton et al., 2016). In contrast to the sediment samples, the surface water samples contained microbeads (classified as particles), although in relatively small quantities. Because microbeads are unlikely to be formed by natural degradation of large plastic waste (Isobe, 2016), they were possibly derived from personal care (hand or facial cleansers) and cosmetic products (Isobe, 2016; Fendall and Sewell, 2009; Cheung and Fok, 2016). The presence of microbeads is consistent with previous results for surface water in the Bohai Sea (W. Zhang et al., 2017), the East China Sea and Yangtze Estuary (Zhao et al., 2014; Peng et al., 2017). The fragmentation of plastic bags is probably a source of film microplastics in surface water samples (Sruthy and Ramasamy, 2017), and plastic film that is widely used in agriculture in China may also have contributed to the film microplastic pollution in this sea area. The presence of more PE and PP in the surface water in this study also confirmed the point. PE and PP were employed as plastic films and extensively applied as plastic mulches in agriculture (Zhang and Liu, 2018; Zhang et al., 2018; J. Wang et al., 2019).

4.1.3. Microplastic composition

CP is an organic cellulose-based polymer which is used in food packaging and as a release agent in the production of rubber and fiberglass products. CP has also been detected in some previous environmental samples (Peng et al., 2017; Mohsen et al., 2019). PET is a raw material for spinning polyester fibers, which is an important fabric and one of the main products related to wastewater treatment plant (WWTP) pollution (McCormick et al., 2014; Yang, 2017). PP is widely used in food packaging film, milk boxes, plastic bags (Siracusa et al., 2008), fishing nets and ropes, which are expected to influence water samples. However, density is not the only factor affecting the distribution of microplastics in aquatic ecosystems. Biofouling, attached biomass, temperature, and storms can also play a role (Andrady, 2011; Ballent et al., 2013; Zhao et al., 2015). Thus, despite having a density lower than

freshwater, PP and PE can be submerged and be found in sediments as well (Corcoran et al., 2015; Ballent et al., 2016; K. Zhang et al., 2017). In summary, due to the difference in residence time of microplastics in surface water and sediments, the distribution of microplastics in sediments was more regular and the content of low-density plastics was less.

4.1.4. Source analysis of microplastics

There were three main sources of microplastics in Laizhou Bay, that is, Gudong, the Yellow River Estuary and Laizhou-Weifang region. The Gudong area has oil-production plants and the Yellow River input can provide a large number of microplastics. The Laizhou-Weifang region has up to 2000 private enterprises engaged in the recycling of waste plastics (annual recycling amount > 150 million tons) and 41,157 hm^2 of marine raft farming area. In addition, the polymer type can also attest to the above conclusion. In the present study, we found that in the sediments, the Gudong area had unique PPA and PEA microplastics, and PE microplastics only existed in the Laizhou-Weifang region. Both PPA and PEA polymers are oil-resistant and heat-resistant and are used to make equipment for oil-extraction operations, while PE has the advantages of flexibility, impact resistance and anti-aging and is commonly used as a rope for raft aquaculture. Moreover, a high proportion of PE was found in the surface water of the Laizhou-Weifang region. In addition, in the Laizhou-Weifang region, the microplastic characteristics of different sites have almost the same characteristics, suggesting that the microplastics can move frequently between regions to achieve similar microplastic compositions. Therefore, cluster analysis may be a useful tool for elucidating the sources of microplastics.

4.2. Microplastics in fishes

The ingestion rates of microplastics in the four fish species were above 75%. High values of ingestion rates of microplastics were also found in the Thames estuary, where 71% of flounders *Platichthys flesus* have ingested microplastics (McGoran et al., 2017). Some studies have reported high microplastic occurrence percentages of up to 100% in South America (Pazos et al., 2017). The occurrence and absence of microplastic ingestion was associated with the sample sizes, analytical methods used for processing samples, species-specific and location-specific (Markic et al., 2019). The benthic fish seem to show higher microplastic ingestion frequencies and greater numbers of microplastics per individual than pelagic fish, especially the benthic species *Chaeturichthys stigmatias* (100% incidence), although the difference was not statistically significant ($p > 0.05$). It has been shown that feeding type could influence the ingestion of microplastics (Setälä et al., 2016; Mizraji et al., 2017). *Cynoglossus joyneri* feeds on detritus, while *Chaeturichthys stigmatias* feeds on crustaceans (Xu, 2019). Many studies have shown that microplastics can be ingested by these demersal organisms (Devriese et al., 2015; Carreras-Colom et al., 2018), and thus enter the upper trophic levels. Therefore, the trophic transfer of microplastics from crustaceans (as food) to *Chaeturichthys stigmatias* might have occurred, which leads to a high ingestion rate of microplastics. A recent study found that demersal bigeye sculpin showed a significantly larger value of ingested MPs compared to pelagic polar cod (Morgana et al., 2018). However, this outcome is contrary to that of Güven et al. (2017) and Anastasopoulou et al. (2018), who found that pelagic fish ingested more microplastics than benthic fish. This pattern probably is related to the type of fish and the degree of contamination at the sampling site. Due to such variability, it would not be sound to draw firm conclusions regarding the patterns in the occurrence of microplastic ingestion. There was no apparent relationship between feeding strategy and occurrence of microplastic ingestion in fish (Markic et al., 2019).

4.3. Association of the microplastics derived from environmental samples and fishes

Contrary to expectations, in this study no significant correlation was found among the surface water, sediments, and biota. One possible explanation for this result is that the uneven distribution of plastics in marine environments leads to large differences between water, sediment and fish samples (standard deviations for all three samples are relatively high) (Moreira et al., 2016). Additionally, ingestion of microplastics by fishes can be affected by various factors, such as environmental microplastic concentrations and foraging behavior (Ryan et al., 2016). Microplastics can be ingested directly by fishes (confusing plastic with prey) or indirectly by eating prey contaminated with microplastics. Benthic fish acquires most microplastics from macroinvertebrates and the water. In addition, the uptake and ingestion of microplastics depend on the characteristics of the microplastics and the life history strategy of the species (Van Franeker et al., 2011). Only microplastics of appropriate shape, density and size can be ingested by organisms (Rochman et al., 2017; Redondo-Hasselerharm et al., 2018). The size of the microplastics ingested by pelagic and benthic fish was mostly between 500 and 1000 μm, which might be related to the size of the fish mouth opening. Furthermore, Watts et al. (2015) showed that microplastics ingested by certain organisms would be broken down as the microplastics pass through the gut. However, the reason for the similarity of microplastic characteristics in the benthic fish samples to those in the surface water, sediments and the pelagic fish is not clear but it may be associated with the feeding processes of benthic fish. Microplastics in sediments could be transferred to fish by the macro-invertebrate preys, which in turn ingest substances from the suspended sediments (Hickey et al., 1995). The resuspension of sediment may also transfer microplastics to the upper water layer, where substances could be ingested by benthic fish directly. On the other hand, defecations of the benthic filter-feeders enable the sinking of microplastics from the water to sediment, which may alter the microplastics compositions between water and sediments (Galloway et al., 2017). Collectively, these factors may contribute to the observed similarity between the characteristics of microplastic in benthic fish samples and the environment in this study.

5. Conclusions

Most of the industrial and urban development in Laizhou Bay is located in coastal areas, and raw sewage is often discharged into coastal waters (Wang et al., 2007). This study is the first to simultaneously investigate microplastics contamination in surface water, sediments and organisms in Laizhou Bay. Our results reveal that the hydrological processes are the major causes for the difference between spatial distribution of microplastics in sediments and surface water, and indicate that the sediment can better reflect the microplastic pollution in certain areas than the surface water.

CRediT authorship contribution statement

Jia Teng: Writing - original draft, Visualization, Methodology, Investigation, Formal analysis. **Jianmin Zhao:** Supervision, Resources. **Chen Zhang:** Investigation, Formal analysis, Visualization. **Bo Cheng:** Formal analysis. **Albert A. Koelmans:** Writing - review & editing. **Di Wu:** Investigation, Validation. **Meng Gao:** Formal analysis. **Xiyan Sun:** Investigation. **Yongliang Liu:** Investigation. **Qing Wang:** Conceptualization, Resources, Writing - review & editing, Supervision.

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.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (No. 41576122), the Strategic Priority Research Program of the Chinese Academy of Sciences (No. XDA23050303), the Youth Innovation Promotion Association, CAS (2016196).

Appendix A. Supplementary data

Detailed information about microscope inspection and identification of microplastics and quality assessment system. The seven figures depict the μ-FT-IR spectrum of microplastics found in samples of surface water, sediments and organisms in Laizhou Bay, the correlation between the abundance of microplastics in surface water and in sediments, the spatial distribution of microplastics in surface water and sediment of the simulation of spatial patterns, a tree diagram of cluster analysis in surface water and sediment for each site as well as surface water and sediment for 8 areas, the June, July and August mean currents and surface temperature in Laizhou Bay and the microplastic diversity indices ($D_{1-D}{}'$ (MP)) for surface water and sediments samples from the Laizhou Bay. A table depicts relative abundance, percentages and density of identified microplastics. Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.140815.

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