Environmental Pollution 245 (2019) 1071–1078

Contents lists available at ScienceDirect

Environmental Pollution

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

Microplastic ingestion by the farmed sea cucumber *Apostichopus japonicus* in China☆

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ARTICLE INFO

Article history:
Received 31 August 2018
Received in revised form
18 November 2018
Accepted 26 November 2018
Available online 28 November 2018

Keywords:
Sea cucumber
Microplastics ingestion
Deposit feeder
Coelomic fluid
Sediment

ABSTRACT

Microplastic ingestion by the farmed sea cucumber is undocumented. Microplastics were isolated from the sea cucumber *Apostichopus japonicus* that was collected from eight farms along the Bohai Sea and the Yellow Sea in China. To examine microplastic ingestion, the intestines were isolated, digested and then subjected to the floatation test. The microplastic abundance in the sediment ranged from 20 to 1040 particles kg^{-1} of dry sediment, while the ingested microplastics ranged from 0 to 30 particles $intestine^{-1}$. After filtering the coelomic fluid, the extracted microplastics from the coelomic fluid ranged from 0 to 19 particles $animal^{-1}$. Thus, we speculated that microplastics may transfer to the coelomic fluid of sea cucumber. The ingested microplastics did not correlate with the animal body weight but was site dependent, suggesting that sea cucumber may serve as sentinel for microplastic pollution monitoring in the sediment. The microplastics were identified by Fourier transform infrared micro spectroscopy, and the polymer types were mainly cellophane, polyester, and polyethylene terephthalate. This study revealed that, microplastics widely existed in sea cucumber farms, and that sea cucumbers ingest microplastics as suitable with their mouth open. Moreover, the microplastics might transfer to the coelomic fluid of the sea cucumber. Further investigations are needed to assess the chronic effect of the microplastics on the growth and physiological status of the sea cucumber.

1. Introduction

Microplastics (MPs), which are usually defined as plastic particles smaller than 5 mm in diameter (Arthur et al., 2009), are derived mainly from large plastics (Costa et al., 2010; Andrady, 2011) or enter the environment, with a microscopic size, from many sources such as cosmetics products (Fendall and Sewell, 2009) or from washing clothes (Browne et al., 2011). MP waste has become a global issue that exists in water and sediment (reviewed by Cole et al., 2011; Van Cauwenberghe et al., 2015a). The components of some MPs are considered hazardous, such as the monomer of polyvinyl chloride (Lithner et al., 2011). Nevertheless, MPs are observed to adsorb organic (Graham and Thompson, 2009) or inorganic contaminants (Ashton et al., 2010) from the surroundings. Such MPs can be consumed, causing physical harm (Wright et al., 2013a) by, for instance, blocking or harming the gut. Additionally, some ingested MPs reduce the feeding ability (Wright et al., 2013b), the growth rate (Besseling et al., 2014), fecundity (Lee et al., 2013; Sussarellu et al., 2016) and disturb the immune system (Von Moos et al., 2012; Avio et al., 2015) in many species. On the other hand, some ingested MPs do not affect the growth or the survival of *Gammarus pulex* (Weber et al., 2018) or the physiology of the mussels *Perna perna* (Santana et al., 2018).

☆ This paper has been recommended for acceptance by Maria Cristina Fossi.

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https://doi.org/10.1016/j.envpol.2018.11.083
0269-7491/

The sea cucumber *Apostichopus japonicus* (*A. japonicus*), one of the most preferred sea foods in Asia (Choo, 2008), is known around the world, especially in China, for its nutritional and pharmaceutical values (Yang et al., 2015b). Additionally, sea cucumber plays a crucial role in the marine ecosystem through feeding and bioturbation. Sea cucumbers decrease the organic matter content and algal bloom (Michio et al., 2002), buffer the effect of ocean acidification on coral reef (Schneider et al., 2015) and regenerate nutrients in the marine ecosystem (MacTavish et al., 2012).

A. japonicus sea cucumbers are non-selective feeders that ingest the sediment to extract organic matter and microorganisms (Xu et al., 2015), and thus, they are easily exposed to MPs in the sediment due to their feeding behaviour. Understanding MP uptake from the habitat is essential to evaluate the impact of MPs on such an important taxon. Up to now, few studies have paid attention to MP uptake in sea cucumbers. In the laboratory, the ingestion of MPs by some sea cucumber species, including *Holothuria grisea*, *Cucumaria frondosa*, *Holothuria floridana*, and *Thyonella gemmate* is reported (Graham and Thompson, 2009). Although microfibre ingestion is reported in the sea cucumber collected from the deep sea (Taylor et al., 2016), the characterization of MP ingestion by sea cucumber in the wild is not well studied, including the MP number and size. To our knowledge, this is the first field study characterizing MP ingestion by sea cucumber in shallow water.

The aims of the current study were as follows: 1) Characterize MP ingestion by the sea cucumber *A. japonicus* 2) Examine the size selection of the MPs by the sea cucumber, 3) Observe the possibility of MP transfer in the sea cucumber; and 4) Investigate the relationship between the MP concentration in the sediment and the MP uptake by sea cucumber. Additionally, the correlation between the number of MPs ingested and body weight of sea cucumber was analysed to assess the effect of the MP on the growth of the sea cucumber.

2. Materials and methods

2.1. Sediment and sea cucumber sampling

Samples were collected during 30/November/2017 to 29/May/2018, with the exception of the winter months (December–February), from eight sites, representing the major farming sites of sea cucumber in China, and of which, four sites were located in the Yellow Sea, and the other four sites were located in the Bohai Sea (Fig. 1) (Table S1).

The sediment samples and sea cucumber animals were collected randomly from each site, with five replicates. Most of the samples were taken from the cultured ponds, with the exception of two sites (Tangshan and Rizhao), which were marine ranching sites. Marine ranching is farming in a suitable area in the open ocean. The sediment was collected using a Van Veen grab and was transferred in aluminium bags to the freezer at −20 °C. The sea cucumber animals (n = 5) were collected by divers and were dissected directly at each site to separate the intestines and coelomic fluid. The tissues were stored in 250 mL clean glass bottles and were then transferred to the lab in an ice box (Fig. S1).

2.2. MP extraction from the sediment

The sediment from each site was carefully homogenized under a clean hood and was dried at 60 °C for 72 h, resulting in a constant weight. Two hundred and 50 g of dried sediment was separated into five replicates, and the flotation method was conducted (Thompson et al., 2004), with little modifications. Two hundred millilitres of a saturated NaCl solution ($\rho = 1.20$ g mL^{-1}) was stirred with each 50 g of sediment for 1 min. After about 5 min of settlement, the supernatant was transferred to a clean glass beaker, paying attention to the adhesion of any particles to the walls of the beakers. The saturated solution was added to the sediment, and it was transferred three times to recover more MPs from the sediment. To degrade the organic matter and calcium floats, 5 mL of H_2O_2 (30%) was added, reaching a concentration of 0.73% of the supernatant. After 24 h of sedimentation, the clean supernatant was filtered through 8 μm glass microfibre filter paper under vacuum filtration, and then, the filter paper was placed in a new petri dish for later inspection by a microscope. Three identical blanks were set up, using the floatation test, for background correction.

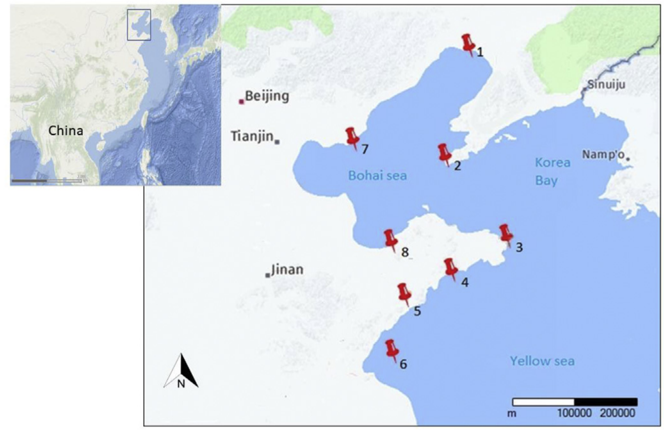

Fig. 1. Sampling location of the farms in the Bohai Sea and the Yellow Sea. Site number (S1–S8).
(S1: Panshan, Panjin, Liaoning), (S2: Lvshunkou, Dalian), (S3: Rongcheng, Weihai), (S4: Haiyang, Yantai), (S5: Chengyang, Qingdao), (S6: Qianshan Island, Rizhao), (S7: Tangshan), (S8: Laizhou, Yantai).

2.3. MP extraction from the intestines of the sea cucumbers

The isolated intestines from the animals were transferred to a clean glass beaker, and 20 mL of KOH (10%) was added for digesting the tissue (Karami et al., 2017). We also assessed whether of KOH (10%) affected the morphological characteristics of the MPs (SI). After 24 h of digestion at 40 °C, the digestion solution was carefully transferred and was filtered through an 8 μm glass microfibre filter. A separation test was done using the residue, as described above, without adding H_2O_2 (30%) this time, and it was repeated three times to increase the recovery. Three identical blanks were set up, using the floatation test or KOH digestion, for background correction.

2.4. MP extraction from the coelomic fluid of the sea cucumbers

The coelomic fluid was extracted by making an opening on the posterior side of the sea cucumber that let the fluid flow freely into the bottle. Three bottles of ultrapure water were left open during the experiment, as controls, in each site to detect any airborne contamination. Once the samples reached the lab, the coelomic fluid and the ultrapure water from the blanks in the glass bottles were directly filtered under a clean hood through an 8 μm glass microfibre filter paper with vacuum filtration, and then, the filter paper was placed in a new petri dish.

2.5. Prevent contamination by airborne MPs

To prevent contamination during dissection, the animals were washed carefully with filtered water together with the dissection

tools, and the nitrile-coated gloves were rubbed and washed with filtered water after each dissection. Moreover, all the procedures were conducted under a clean hood with air flow including the preparation of the sediment, the filtration, the preparation of the intestines for digestion. Every piece of glass equipment (including the 250 mL bottles and the glass beakers) was washed twice with filtered water and with ultra-pure water before using it directly. All the chemical solutions were filtered first through 8 μm glass microfibre filters before use. Coats made of cottons and nitrile-coated gloves were worn during all the experimental procedures.

2.6. MPs inspection by microscopy

A dissecting microscope was used to examine the 8 μm glass filter paper. The filter was examined horizontally and vertically, and the observed MPs were isolated to a new filter in groups, depending on colour and shape. The MPs were identified according to colour homogeneity, by how they could not be cracked by tweezers or needles, and by how they did not have any tissue structure (Hidalgo-Ruz et al., 2012). After isolating the MPs under a dissecting microscope, a fluorescence microscope (OLYMPUS IX51) was used to image the microplastics. The size of each microplastic particle was calculated later using Digimizer software (MedCalc Software bvba, Ostend, Belgium).

2.7. Identification of the polymer type

The MPs isolated from each section (sediment, intestines, and coelomic fluid) at each site were identified separately. In general, the total amount of the microplastics was analysed at each site. However, if the total amount exceeded a considerable number (>75), at least 25% of the total amount was selected randomly in each site (Table S2). A Nicolet™ iN10 infrared microscope (Thermo Fisher Scientific, USA) was used to distinguish the polymer type of 1261 microplastics particles from the sediment, intestines and coelomic fluid. The apparatus was supplied with a mercury cadmium telluride (MCT) sensor and an ultra-fast motorized phase. The MCT sensor was cooled using liquid nitrogen, in which the MPs were identified. The spectrum of the particle was within 650–4000 cm^{-1}. The aperture was adjusted using knife-edges to 150 × 150 μm. The recorded spectra were examined using the OMNIC library, and only matches over 70% were accepted, which resulted in a total of 126 particles that were not identified as MPs.

2.8. Statistical analysis

SPSS Statistics 20.0 statistical software (SPSS Inc, Chicago, IL) was employed to conduct the Kruskal-Wallis H test to compare the MP abundance in the sediment, intestines, and coelomic fluid between the sites. When a significant difference was found, the Mann–Whitney test was employed for comparisons with a significance level of 0.05. Additionally, Pearson correlation coefficient was used to investigate the relationships between the variables.

3. Results

3.1. MPs abundance in the sediments of the sea cucumber farms

No MPs were detected in the blank replicates. A total of 687 particles was isolated from all the sites in the range of 1–52 MP per 50 g of the sediment. The mean abundance of the MPs in the eight sites was sequenced as follows: S8 (46.8 ± 4.81) > S5 (38.8 ± 7.72) > S4 (23 ± 7) > S3 (8.4 ± 1.14) > S6 (7.6 ± 3.13) > S1 (5.8 ± 3.96) > S7 (4.2 ± 2.16) > S2 (2.8 ± 1.30) per 50 g (Fig. 2). The MPs in the sediment showed a significantly higher abundance at S8 and S5 than the other sites ($P < 0.05$) (Fig. S3).

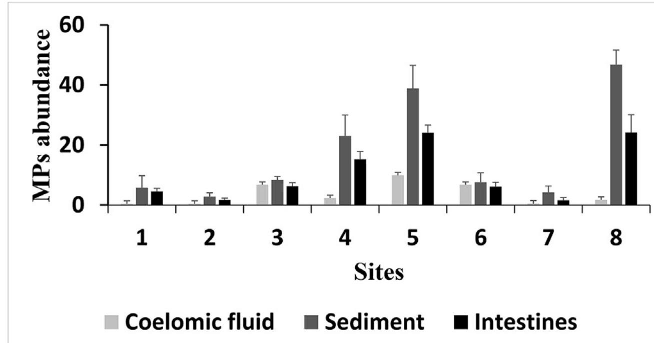

Fig. 2. Average MP abundances in the sediments (MP per 50 g sediment), the intestines and the coelomic fluid from different farms of the sea cucumber.

Microfibres were the most abundant shape in the sediment from all the sites (97%) followed by fragments and films (3%). The MPs were mainly blue in colour, accounting for 48% of the total, followed by transparent at 27%, black at 14%, red at 5%, purple at 5% and brown at 1%. Small MPs (SMPs ≤ 1 mm) were the most abundant, at 82%, while large MPs (LMPs:1–5 mm) only made up 18% of the total (Fig. 3). The width of the isolated microplastics had a maximum value of 281 μm. Several types of polymers were identified from a total of 300 randomly selected particles, and of which 30 particles were not identified as MPs. Their frequencies were arranged as follows: cellophane > polyester > polyethylene terephthalate > polyethylene > polypropylene > polyamide > polyvinyl acetate > polyacrylonitrile (Fig. 4).

3.2. MP ingestion by sea cucumbers

The digestion method had no effect on the shape or the colour of the homemade MPs with a diameter of ca. 80 μm (Fig. S6). The average sea cucumber body weight ranged from 53 g to 144 g. A total of 2030 particles were extracted from 200 sea cucumber animals without detecting MPs in the blank replicates from the digestion or the isolation processes. The mean ingestion numbers from the animals from each site were sequenced as follows: S8 (24.2 ± 5.90) > S5 (24.08 ± 2.57) > S4 (15.24 ± 2.60) > S3 (6.28 ± 1.20) > S6 (6.08 ± 1.54) > S1 (4.6 ± 0.97) > S2 (1.68 ± 0.64) > S7 (1.56 ± 0.96) $intestines^{-1}$ (Fig. 2) which is nearly the same sequence of the MPs abundance in the sediment. A high MP abundance was observed at S8 and S5, which were significantly higher than of the other sites ($P < 0.05$) (Fig. S4). As the number of MPs in the sediment increased, the MPs from the intestines increased correspondingly (Pearson: $r = 0.767$). Microfibres were the major shape of the extracted MPs, with a percentage of 91% followed by fragments (8%) with a maximum width of 55 μm. SMPs were the most abundant (81%), while LMPs constituted only 19% (Fig. 5). A blue colour constituted 59% of the total followed by transparent at 24%, black at 6%, red at 5%, purple at 4%, brown at 1%, and green at 1%. The polymer types were reported from 668 randomly selected particles, and of which 72 particles were not identified as MPs. Polymer types were sequenced as follows: cellophane > polyester > polyethylene terephthalate > polyethylene > polypropylene > polyvinyl acetate > polyacrylonitrile (Fig. 6).

3.3. MPs isolated from the coelomic fluid of the sea cucumbers

A total of 620 particles was extracted from the coelomic fluid of

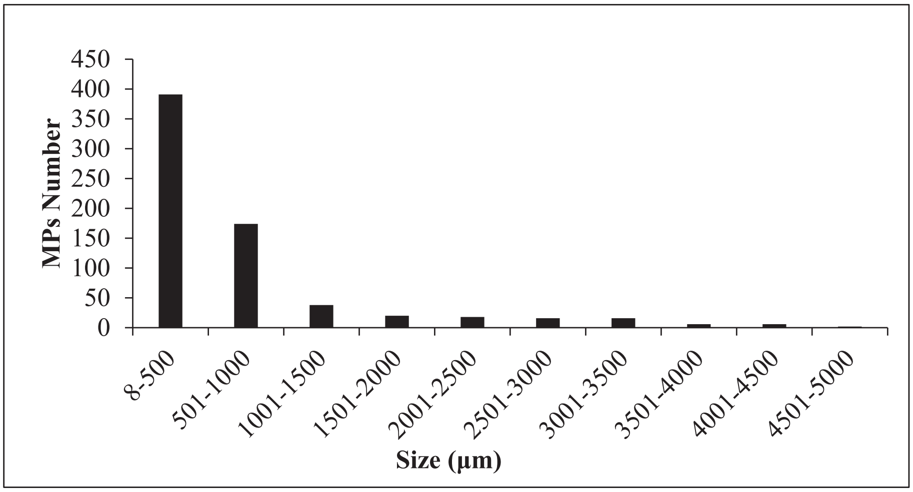

Fig. 3. Length distribution of the total MP observed from the sediment.

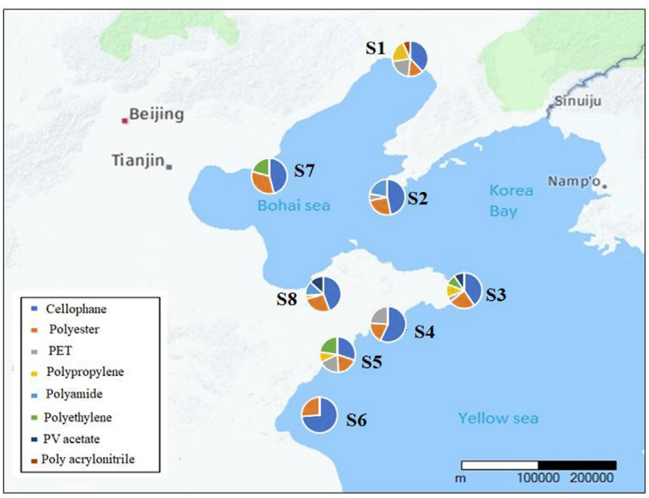

Fig. 4. MP type composition observed from the sediment.

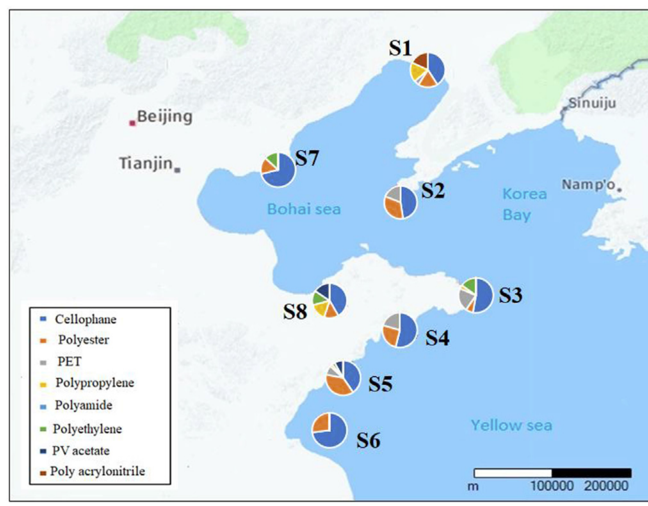

Fig. 6. MP type composition observed from the intestines.

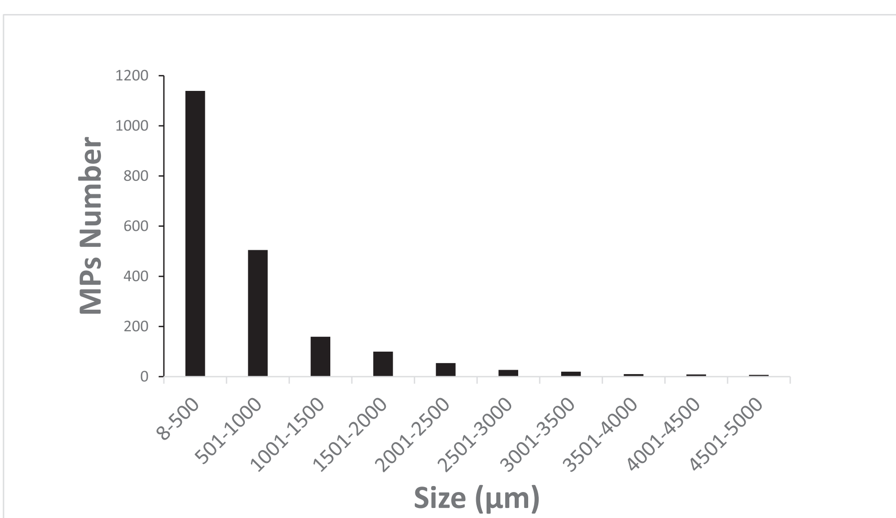

Fig. 5. Length distribution of the total MP observed from the intestines.

the sea cucumbers, and their means were sequenced as follows: S5 (9.88 ± 6.81) > S6 (6.72 ± 1.79), S3 (6.72 ± 1.50) > S4 (2.28 ± 0.78) > S8 (1.72 ± 0.58) > S7 (0.48 ± 0.27) > S1 (0.4 ± 0.31), S2 (0.4 ± 0.37) animal^{-1} (Fig. 2), which did not follow the exact order of the MP concentration in the sediment or in the intestines. The sites S1, S2, and S7 had significantly lower MPs abundances in the coelomic fluid than in those of the other sites ($P < 0.05$) (Fig. S5). The extracted MPs were only microfibres, with a maximum width of 20 μm. SMPs were the dominant size and accounted for 84% of the total, while the LMPs accounted for 16% (Fig. 7). The most abundant colour was blue at 67% followed by transparent at 13%, red at 7%, purple at 6%, black at 5% and brown at 2%. The polymer type composition was examined from 293 randomly selected particles, and of which 24 particles were not identified as MPs. The types had the following order: cellophane > polyethylene terephthalate > polyester > polyacrylonitrile > polypropylene (Fig. 8). The extracted MPs from the coelomic fluid showed a moderate relationship with the isolated MPs from the intestines (Pearson: $r = 0.476$).

4. Discussion

4.1. MP abundance in the sediments of sea cucumber farms

The sea cucumber *A. japonicus* in ponds or marine ranching feeds on the sediment. The sediments were collected from sea cucumber farms located in the Bohai Sea and the Yellow Sea in China. A high MP abundance was noticed at S8 in the Bohai Sea, which had intensive farming activities, such as stock enhancement and fishery production management. In addition, the high amount of the microplastics in S8 may refer to the high pollution in the Laizhou Bay. Laizhou Bay, which accounts for 10% of the Bohai Sea, receives discharging from more than 23 rivers, including the second longest river (Yellow River) in China. These rivers receive wastewater from industrial, agriculture and urban activities, which discharge, in turn, into the Laizhou Bay (Zhang et al., 2012). Nevertheless, industrial zones located along the Laizhou Bay, together with urban sprawl, discharge wastewater to the Laizhou Bay (Wang et al., 2007). In the Yellow Sea, S5 had the highest MP abundance, which may refer to the pollution in the water supply from the Jiaozhou Bay in Qingdao. Qingdao is known for its high population density, reaching to approximately 9 million people, whose activities are reported to contaminate the Jiaozhou Bay together with the input from polluted rivers (Shen, 2001; Zhang et al., 2006; Deng et al., 2010).

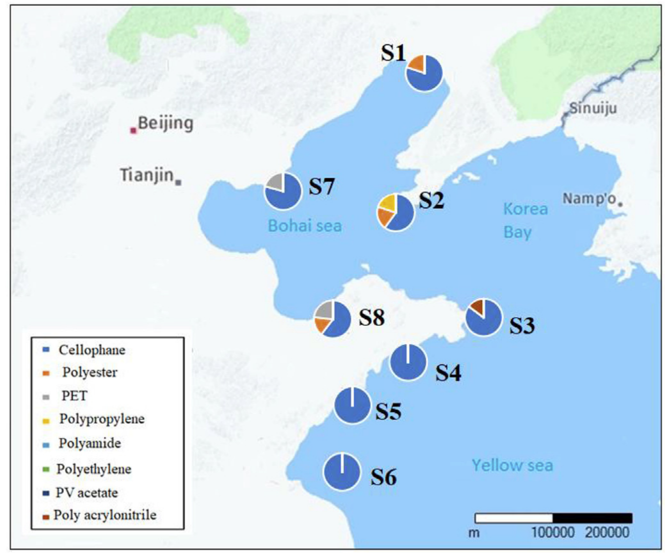

Fig. 8. MP type composition observed from the coelomic fluid.

MPs were found in the sediment of all the sea cucumber farms, with a range of 20–1040 MP kg^{-1} and an average of 343.6 MP kg^{-1} and were mainly in the form of microfibres. Similarly, microfibres were the dominant shape in the habitat of some sea cucumber species in the eastern coast of USA (Graham and Thompson, 2009). The MP abundance in this study is comparable with those observed in the habitat of the worm *Tubifex tubifex* in Irwell river in the United Kingdom (56–2543 MP kg^{-1}) (Hurley et al., 2017) and was less than the habitat of mussels and worms in Halifax Harbor in Canada (average 2000–8000 MP kg^{-1}) (Mathalon and Hill, 2014). However, the abundance was higher than that of the worm *Arenicola marina* habitat along the Dutch, Belgian, and French shorelines (6 MP kg^{-1}), which are mainly a microfiber shape (Van Cauwenberghe et al., 2015b) and higher than that of the mussle habitat in the North Sea coast with an average of 48 MP kg^{-1} (Karlsson et al., 2017). The abundance of the MPs detected in this study was also higher than those observed in the Northern Yellow Sea, the Southern Yellow Sea and the Bohai Sea (123.6 and 72, 171.8 MP kg^{-1} respectively) (Zhao et al., 2018) and in the Changjiang Estuary (121 MP kg^{-1}) in China (Peng et al., 2017). Nevertheless, the MP abundance in our study was less than those observed at a sand beach in the south coastal line of China (6923 MP kg^{-1}) (Qiu

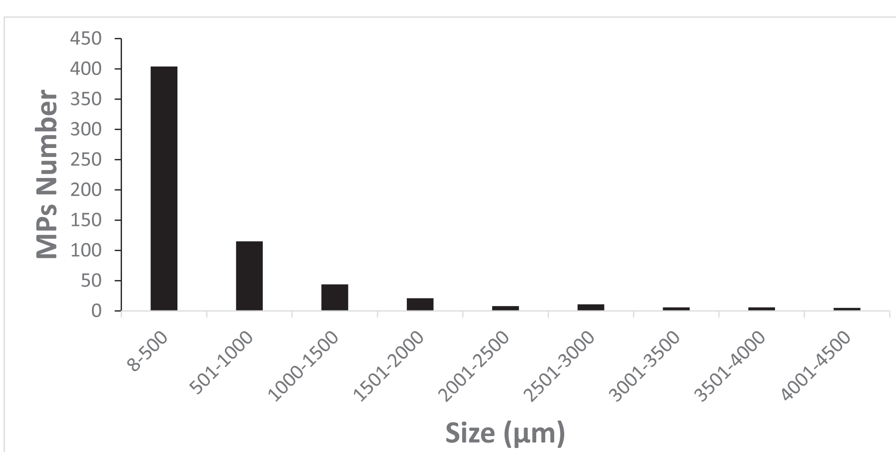

Fig. 7. Length distribution of the total MP observed from the coelomic fluid.

et al., 2015). Our study sites were located in the coastal areas, which are easily affected by land-based pollution and aquaculture activity, and thus, they are more polluted than the offshore areas and some less disturbed shorelines. However, the MP pollution level in the sea cucumber habitats was less than those of the reported harbours and sand beaches, which are affected by frequent shipping or tourism activities. Moreover, the extraction method used in our study might underestimate the MP abundances in the sediments if there were some dense polymers, such as PVC or Polytetrafluorethylene (PTFE) (Coppock et al., 2017). Thus, a different extraction method used in another study might also contribute to the observed differences between different sites.

4.2. MPs ingestion by sea cucumbers

The ingestion of MPs by the farmed sea cucumber was not surprising, as other sea cucumber species ingest them in lab experiments. Sea cucumber *A. japonicus* ingested more microfibre in the field than of the sea cucumber *Cucumaria frondosa* and *Thyonella gemmate* in the lab experiment. However, *A. japonicus* ingested less microfiber than *Holothuria floridana* and *Holothuria grisea.* Furthermore, Sea cucumber *A. japonicus* ingested less fragments than other sea cucumber species and no MP pellets were detected in sea cucumber *A. japonicus* (Graham and Thompson, 2009) (Table 1). Additionally, MP ingestion is reported for many bottom feeders such as *Arenicola marina* (Besseling et al., 2012), *Perinereis aibuhitensis* (Leung and Chan, 2017), *Marenzelleria* spp (Setälä et al., 2016) and *Nephrops norvegicus* (Murray and Cowie, 2011).

The amount of the sediment in the intestines of the sea cucumber correlates positively with the weight of the animals (Xu et al., 2015). In the current study, there was a high correlation between the amount of the MPs in the intestines and the amount of the MPs in the sediment (Pearson: $r = 0.767$). However, there was no correlation between sea cucumber body weight and the number of the ingested MPs (Pearson, $r = 0.135$). Furthermore, the length of the MP in the current study was almost in a same percentage between the intestines of the sea cucumber and the sediment. This indicates that once the MPs exist in the sediment, sea cucumber will ingest them. Indeed, the sea cucumber *A. japonisus* would rather select the organic-rich sediment, since the organic matter in their intestines is higher than of the sediments (Xu et al., 2015). The MP ingestion rate was site dependent, which was similarly to other experiments. From the coast line of China, wild and farmed mussels contain MPs, with an average of 4 MP animals^{-1} (Li et al., 2016). While in Germany and France, the MP ingestion by the mussels was an average of 0.36 MP g^{-1} (Van Cauwenberghe and Janssen, 2014). In Nova Scotia, Canada, the farmed and wild mussels ingest MP with an average of 178–126 MP animal^{-1} respectively (Mathalon and Hill, 2014). Additionally, MP concentration varies among fish species collected from different locations. Fish contain 1.90 MP animals^{-1} in southwest Plymouth of United Kingdom (Lusher et al., 2013), while only 1.0 MP animals^{-1} is found in fish from the North Sea (Foekema et al., 2013).

The sea cucumber *A. japonicus* is a deposit feeder that exploits its tentacles to bring the sediment into its mouth. The sea cucumber *A. japonicus* shows preference of grain size that ranges from 1 μm to 80 μm (Zhao and Yang, 2010). Additionally, sea cucumber shows a significant low growth, since the sand or mud size increases from 8 μm to 0.5 mm (Shi et al., 2015). In the current study, the sea cucumber *A. japonicus* showed a particle ingestion preference for the MPs based on the width. The extracted MPs from the intestines had a maximum width of 55 μm, indicating that, for ingestion, it was a suitable diameter for the mouth. While the MPs from the sediment had a maximum width of 281 μm (Fig. S7). Similarly, other sea cucumber species select the size of the ingested MPs as long as MPs were fit into their mouth or the tentacles were able to grasp them. The sea cucumbers in the laboratory ingest particles that are less than 0.5 mm more than larger size categories (Graham and Thompson, 2009). Microplastic ingestion is also size dependent in other species of deposit feeders such as Tubifex worms which show a size selectivity of less than 63 μm (Hurley et al., 2017). In addition, zooplankton ingest MPs that are the size of their prey (Desforges et al., 2015).

4.3. MPs isolated from the coelomic fluid of sea cucumber

The coelomic fluid in the sea cucumbers serves as a nutrient, waste, and gas transporter. MPs were isolated from the coelomic fluid, without observing pollution in the control treatment in any site. Thus, we speculated that MPs may be transferred to the coelomic fluid of the sea cucumber (Fig. S8). One possible scenario is the uptake of the MPs by the gastrointestinal epithelium. The translocation of MPs (3 and 9.6 μm) is observed from the gut to the circular system of the invertebrate *Mytilus edulis* (Browne et al., 2008). Moreover, the translocation of substances across the gut is observed in humans with a particle size of 0.16–150 μm, in rabbits (0.1–10 μm), in rats (0.03–40 μm) and in dogs (3–100 μm) (Hussain et al., 2001). However, the isolated MPs from the coelomic fluid were generally larger than 20 μm, which were not easily transferred from the intestine. Thus, the MPs might be transferred from the respiratory tree epithelium to the coelomic fluid. It is shown that the sea water enters from the anus to the respiratory trees allowing the gas exchange by diffusion (Gao et al., 2015). Additionally, the respiratory tree of sea cucumbers is wide enough to include particles from the surrounding water. For example, the crab *Pinnotheres haling*, with a carapace width of less than 6 mm, were found inside the respiratory tree of the sea cucumber *Holothuria scabra*, at the same time the width of the respiratory tree was increased three to five times for the host. Furthermore, the fish *Encheliophis gracilis* was found in the coelomic fluid of the sea cucumber (Hamel et al., 1999). However, this hypothesis needs further confirmation.

4.4. Polymer types identification of the MPs

In the current study, the saturated NaCl (density = 1.2 g cm^{-3})

Table 1
Comparison of the ingested MPs by different sea cucumber species.

Microplastics	Laboratory study [a]				Field study [b]
	Holothuria floridana	*Holothuria grisea*	*Cucumaria frondosa*	*Thyonella gemmata*	*Apostichopus japonicus*
fibers	0–517	0–200	0–22	0–26	0–30
fragments	0–77	0–134	0–27	0–8	0–3
pellets	0	0–34	0–2	0	0

[a] (Graham and Thompson, 2009). Direct counting the defecated MPs under microscope.
[b] Current study. Counting MPs after digesting the intestines and density separation.

extraction method was used to separate the different polymers. This method has its limitations compared with the $ZnCl_2$ or NaI extraction, because some dense polymers, such as PVC and PTFE, cannot be recovered effectively (Coppock et al., 2017). However, the dense polymer PET (density = 1.39–1.44 g cm^{-3}) was identified in our study; although, its density was larger than saturated NaCl. Similar results are also found in several studies by using the method of NaCl extraction (Qiu et al., 2015; Zhao et al., 2018; Peng et al., 2017; Graca et al., 2017).

Our study demonstrated that, cellophane was the most abundant polymer in all the farms from the intestines, coelomic fluid, and sediment. Cellophane is widely used in packaging that is combined with some polymers and in cigarette wrappings. The identified cellophane may originate from degrading cellophane films in the environment or fibreglass products. Cellophane is considered an MP in previous studies and is among the most common MP that is identified from the sea salts in China (Woodall et al., 2014; Yang et al., 2015a). Only polyamide was not identified in the intestines but was identified in the sediment which cannot conclude the polymer type selection for sea cucumber. Because MP ingestion may be linked to the MP abundance in the selected food by sea cucumbers. The coelomic fluid contained limited types of polymers, which may indicate another supposed pathway of the MPs. An example of each identified polymer is given in (Fig. S9).

In conclusion, MPs were widely detected in the habitat, the intestines and coelomic fluid of the sea cucumber *A. japonicus*. A high correlation between the number of MPs in the sea cucumbers and the number of MPs in the sediment of their habitat suggested that the sea cucumber might be used as a bioindicator of microplastic pollution in the sediment. Although only the body wall of the sea cucumber is consumed by humans, the potential health risk by consuming sea cucumber needs further investigation, because the pollutants might transfer with the MPs. Moreover, further research is needed to evaluate the chronic effect of MP pollution on the sea cucumber, due to their ecological and economical values.

Acknowledgement

We highly appreciate the people who helped in the field work including Dr. Xiutang Yuan, Dr. Kun Xing, Dr. Xu Min, Jie Feng, Kui Ding, Xiaoshang Ru, Xiaoke Xin, Xinyuan Yang, Jia Teng, Wen Ran together with the divers and farmers.

This research was supported by the National Natural Science Foundation of China (No. 41576122, 41776161), the Agricultural Seed Project of Shandong Province (2016LZGC032), the Youth Innovation Promotion Association CAS (2016196), the Taishan Scholars Program (Distinguished Taishan Scholars), Creative Team Project of the Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology (no. LMEES-CTSP-2018-1).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2018.11.083.

References

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605.
Arthur, C., Baker, J., Bamford, H. (Eds.), 2009. Proceedings of the International Research Workshop on the Occurrence, Effects and Fate of Microplastic Marine Debris. Sept 9-11, 2008. NOAA Technical Memorandum NOS-OR&R-30. https://marinedebris.noaa.gov/sites/default/files/publications-files/TM_NOS-ORR_30.pdf.
Ashton, K., Holmes, L., Turner, A., 2010. Association of metals with plastic production pellets in the marine environment. Mar. Pollut. Bull. 60, 2050–2055.
Avio, C.G., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., d'Errico, G., et al., 2015. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environ. Pollut. 198, 211–222.
Besseling, E., Wang, B., Lürling, M., Koelmans, A.A., 2014. Nanoplastic affects growth of *S. obliquus* and reproduction of *D. magna*. Environ. Sci. Technol. 48, 12336–12343.
Besseling, E., Wegner, A., Foekema, E.M., Van Den Heuvel-Greve, M.J., Koelmans, A.A., 2012. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). Environ. Sci. Technol. 47, 593–600.
Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., et al., 2011. Accumulation of microplastic on shorelines worldwide: sources and sinks. Environ. Sci. Technol. 45, 9175–9179.
Browne, M.A., Dissanayake, A., Galloway, T.S., Lowe, D.M., Thompson, R.C., 2008. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L). Environ. Sci. Technol. 42, 5026–5031.
Choo, P.S., 2008. Population status, fisheries and trade of sea cucumbers in Asia. In: Toral-Granda, V., Lovatelli, A., Vasconcellos, M. (Eds.), Sea Cucumbers. A Global Review of Fisheries and Trade. FAO, Rome, pp. 81–118. FAO Fisheries and Aquaculture Technical Paper. No. 516.
Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62, 2588–2597.
Coppock, R.L., Cole, M., Lindeque, P.K., Queirós, A.M., Galloway, T.S., 2017. A small-scale, portable method for extracting microplastics from marine sediments. Environ. Pollut. 230, 829–837.
Costa, M.F., Do Sul, J.A.I., Silva-Cavalcanti, J.S., Araújo, M.C.B., Spengler, Â., Tourinho, P.S., 2010. On the importance of size of plastic fragments and pellets on the strandline: a snapshot of a Brazilian beach. Environ. Monit. Assess. 168, 299–304.
Deng, B., Zhang, J., Zhang, G., Zhou, J., 2010. Enhanced anthropogenic heavy metal dispersal from tidal disturbance in the Jiaozhou Bay, North China. Environ. Monit. Assess. 161, 349–358.
Desforges, J.P.W., Galbraith, M., Ross, P.S., 2015. Ingestion of microplastics by zooplankton in the northeast pacific ocean. Arch. Environ. Contam. Toxicol. 69, 320–330.
Fendall, L.S., Sewell, M.A., 2009. Contributing to marine pollution by washing your face: microplastics in facial cleansers. Mar. Pollut. Bull. 58, 1225–1228.
Foekema, E.M., Gruijter, C.D., Mergia, M.T., Franeker, J.A.V., Murk, A.T.J., Koelmans, A.A., 2013. Plastic in North Sea fish. Environ. Sci. Technol. 47, 8818–8824.
Gao, F., Yang, H., 2015. Chapter 4. Anatomy. In: Yang, H., Hamel, J.-F., Mercier, A. (Eds.), The Sea Cucumber *Apostichopus Japonicus*.History, Biology and Aquaculture. Academic Press, pp. 53–76. https://doi.org/10.1016/B978-0-12-799953-1.00004-0.
Graham, E.R., Thompson, J.T., 2009. Deposit-and suspension-feeding sea cucumbers (Echinodermata) ingest plastic fragments. J. Exp. Mar. Biol. Ecol. 368, 22–29.
Graca, B., Szewc, K., Zakrzewska, D., Dołęga, A., Szczerbowskaboruchowska, M., 2017. Sources and fate of microplastics in marine and beach sediments of the southern baltic sea—a preliminary study. Environ. Sci. Pollut. Res. 24 (8), 7650–7661.
Hamel, J.-F., Ng, P.K., Mercier, A., 1999. Life cycle of the pea crab *Pinnotheres halingi* sp. nov., an obligate symbiont of the sea cucumber *Holothuria scabra* Jaeger. Ophelia 50, 149–175.
Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ. Sci. Technol. 46, 3060–3075.
Hurley, R.R., Woodward, J.C., Rothwell, J.J., 2017. Ingestion of microplastics by freshwater tubifex worms. Environ. Sci. Technol. 51, 12844–12851.
Hussain, N., Jaitley, V., Florence, A.T., 2001. Recent advances in the understanding of uptake of microparticulates across the gastrointestinal lymphatics. Adv. Drug Deliv. Rev. 50, 107–142.
Karami, A., Golieskardi, A., Choo, C.K., Romano, N., Ho, Y.B., Salamatinia, B., 2017. A high-performance protocol for extraction of microplastics in fish. Sci. Total Environ. 578, 485–494.
Karlsson, T.M., Vethaak, A.D., Almroth, B.C., Ariese, F., Van, V.M., Hassellöv, M., et al., 2017. Screening for microplastics in sediment, water, marine invertebrates and fish: method development and microplastic accumulation. Mar. Pollut. Bull. 122 (1–2), 403.
Lee, K.W., Shim, W.J., Kwon, O.Y., Kang, J.H., 2013. Size-dependent effects of micro polystyrene particles in the marine copepod *Tigriopus japonicus*. Environ. Sci. Technol. 47, 11278–11283.
Leung, J., Chan, K., 2017. Microplastics reduced posterior segment regeneration rate of the polychaete *Perinereis aibuhitensis*. Mar. Pollut. Bull. 129, 782–786.
Li, J., Qu, X., Su, L., Zhang, W., Yang, D., Kolandhasamy, P., et al., 2016. Microplastics in mussels along the coastal waters of China. Environ. Pollut. 214, 177–184.
Lithner, D., Larsson, A., Dave, G., 2011. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Sci. Total Environ. 409, 3309–3324.
Lusher, A.L., Mchugh, M., Thompson, R.C., 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Mar. Pollut. Bull. 67, 94–99.
MacTavish, T., Stenton-Dozey, J., Vopel, K., Savage, C., 2012. Deposit-feeding sea cucumbers enhance mineralization and nutrient cycling in organically-enriched coastal sediments. PloS One 7, e50031.
Mathalon, A., Hill, P., 2014. Microplastic fibers in the intertidal ecosystem

surrounding Halifax harbor, nova Scotia. Mar. Pollut. Bull. 81, 69–79.
Michio, K., Kengo, K., Yasunori, K., Hitoshi, M., Takayuki, Y., Hideaki, Y., et al., 2002. Effects of deposit feeder *stichopus japonicus* on algal bloom and organic matter contents of bottom sediments of the enclosed sea. Mar. Pollut. Bull. 47, 118–125.
Murray, F., Cowie, P.R., 2011. Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). Mar. Pollut. Bull. 62, 1207–1217.
Peng, G., Zhu, B., Yang, D., Su, L., Shi, H., Li, D., 2017. Microplastics in sediments of the Changjiang estuary, China. Environ. Pollut. 225, 283–290.
Qiu, Q., Peng, J., Yu, X., Chen, F., Wang, J., Dong, F., 2015. Occurrence of microplastics in the coastal marine environment: first observation on sediment of China. Mar. Pollut. Bull. 98, 274–280.
Santana, M.F.M., Moreira, F.T., Pereira, C.D.S., et al., 2018. Arch. Environ. Contam. Toxicol. 74, 594.
Schneider, K., Silverman, J., Woolsey, E., Eriksson, H., Byrne, M., Caldeira, K., 2015. Potential influence of sea cucumbers on coral reef caco3 budget: a case study at one tree reef. J. Geophys. Res. Biogeosci. 116 (G4).
Setälä, O., Norkko, J., Lehtiniemi, M., 2016. Feeding type affects microplastic ingestion in a coastal invertebrate community. Mar. Pollut. Bull. 102, 95–101.
Shen, Z.L., 2001. Historical changes in nutrient structure and its influences on phytoplantkon composition in Jiaozhou Bay. Estuar. Coast. Shelf Sci. 52, 211–224.
Shi, C., Dong, S., Wang, F., Gao, Q., Tian, X., 2015. Effects of the sizes of mud or sand particles in feed on growth and energy budgets of young sea cucumber (*Apostichopus japonicus*). Aquaculture 440, 6–11.
Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E., et al., 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. Proc. Natl. Acad. Sci. U. S. A 113, 2430.
Taylor, M.L., Gwinnett, C., Robinson, L.F., Woodall, L.C., 2016. Plastic microfibre ingestion by deep-sea organisms. Sci. Rep.-UK 6, 33997.
Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W., et al., 2004. Lost at sea: where is all the plastic? Science 304, 838-838.
Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human consumption. Environ. Pollut. 193, 65–70.
Van Cauwenberghe, L., Devriese, L., Galgani, F., Robbens, J., Janssen, C.R., 2015a. Microplastics in sediments: a review of techniques, occurrence and effects. Mar. Environ. Res. 111, 5–17.
Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B., Janssen, C.R., 2015b. Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. Environ. Pollut. 199, 10–17.
Von, M.N., Burkhardt-Holm, P., Köhler, A., 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. Environ. Sci. Technol. 46, 11327.
Wang, L., Lee, F.S., Wang, X., Yin, Y., Li, J., 2007. Chemical characteristics and source implications of petroleum hydrocarbon contaminants in the sediments near major drainage outfalls along the coastal of Laizhou Bay, Bohai Sea, China. Environ. Monit. Assess. 125, 229–237.
Weber, A., Scherer, C., Brennholt, N., Reifferscheid, G., Wagner, M., 2018. Pet microplastics do not negatively affect the survival, development, metabolism and feeding activity of the freshwater invertebrate gammarus pulex. Environ. Pollut. 234, 181.
Woodall, L.C., Sanchezvidal, A., Canals, M., Paterson, G.L., Coppock, R., Sleight, V., et al., 2014. The deep sea is a major sink for microplastic debris. Roy. Soc. Open Sci. 1, 140317.
Wright, S.L., Thompson, R.C., Galloway, T.S., 2013a. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492.
Wright, S.L., Rowe, D., Thompson, R.C., Galloway, T.S., 2013b. Microplastic ingestion decreases energy reserves in marine worms. Curr. Biol. 23, R1031–R1033.
Xu, Q., Hamel, J.-F., Mercier, A., 2015. Feeding, Digestion, Nutritional Physiology, and Bioenergetics Developments in Aquaculture and Fisheries Science, vol. 39. Elsevier, pp. 153–175.
Yang, D., Shi, H., Li, L., Li, J., Jabeen, K., Kolandhasamy, P., 2015a. Microplastic pollution in table salts from China. Environ. Sci. Technol. 49, 13622.
Yang, H., Hamel, J.-F., Mercier, A., 2015b. The Sea Cucumber *Apostichopus Japonicus*: History, Biology and Aquaculture, vol. 39. Academic Press.
Zhang, G., Zhang, J., Xu, J., Zhang, F., 2006. Distributions, sources and atmospheric fluxes of nitrous oxide in Jiaozhou Bay. Estuar. Coast Shelf Sci. 68, 557–566.
Zhang, R., Zhang, G., Zheng, Q., Tang, J., Chen, Y., Xu, W., et al., 2012. Occurrence and risks of antibiotics in the Laizhou Bay, China: impacts of river discharge. Ecotoxicol. Environ. Saf. 80, 208–215.
Zhao, J., Ran, W., Teng, J., Liu, Y., Liu, H., Yin, X., et al., 2018. Microplastic pollution in sediments from the Bohai Sea and the Yellow Sea, China. Sci. Total Environ., s 640–641, 637–645.
Zhao, P., Yang, H., 2010. Selectivity of particle size by sea cucumber *Apostichopus japonicus* in different culture systems. Mar. Sci. 34, 11–16.