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Microplastics in specific tissues of wild sea urchins along the coastal areas of northern China

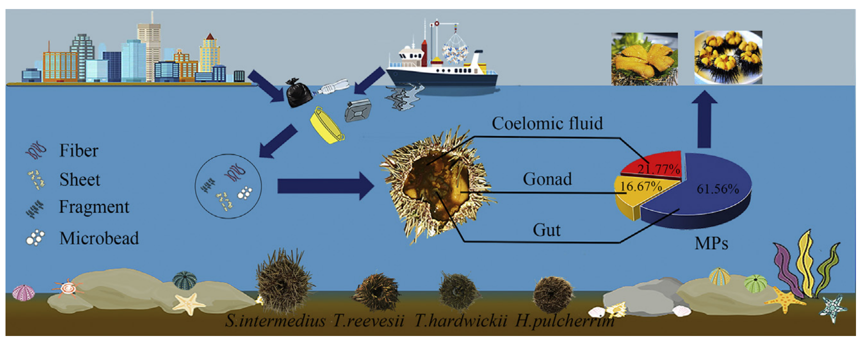
Zhihua Feng [a,c,*], Rui Wang [a], Tao Zhang [a,c], Jiaxuan Wang [a], Wei Huang [d], Ji Li [e], Juntian Xu [a,c], Guang Gao [a,b,*]

[a] *Jiangsu Key Laboratory of Marine Bioresources and Environment, Jiangsu Ocean University, Lianyungang 222005, China*
[b] *State Key Laboratory of Marine Environmental Science & College of Ocean and Earth Sciences, Xiamen University, Xiamen 361005, China*
[c] *Co-Innovation Center of Jiangsu Marine Bio-industry Technology, Jiangsu Ocean University, Lianyungang 222005, China*
[d] *Key Laboratory of Marine Ecosystem and Biogeochemistry, Second Institute of Oceanography, Ministry of Natural Resources, Hangzhou 310012, China*
[e] *School of Oceanography, Shanghai Jiao Tong University, Shanghai 200030, China*

HIGHLIGHTS

- A very high detection rate of MPs was found in wild sea urchins in northern China
- Apart from gut, coelomic fluid and gonads could also accumulate MPs.
- The mean size of MPs in gut was bigger than coelomic fluid and gonads.
- MPs abundance increased with the decrease of anus size and shell diameter.
- MPs abundance was negatively related to gonad index.

GRAPHICAL ABSTRACT



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ABSTRACT

Sea urchins serve as an essential niche for benthic ecosystems and are valuable seafood for humans. However, little is known about the microplastics (MPs) accumulation in sea urchins. Here, we investigated the abundances and characteristics of MPs in specific tissues of wild sea urchins for 12 sites across 2, 900 km of coastlines in northern China. Sea urchins from all sites were detected to have MPs, with a total detection rate of 89.52%. The MPs abundance in sea urchins from all sites ranged from 2.20 ± 1.50 to 10.04 ± 8.46 items/individual or 0.16 ± 0.09 to 2.25 ± 1.68 items/g wet weight. The samples from Dalian were found to have the highest value by individual, and samples from Lianyungang had the highest value by gram. Furthermore, MPs were found in different tissues of sea urchins, i.e., gut, coelomic fluid and gonads. The highest abundance of MPs was found in the gut of sea urchins, followed by coelomic fluid and gonads. The size of MPs ranged from 27 to 4742 μm, and the mean size found in gut was bigger than coelomic fluid and gonads. More interestingly, the MPs abundance increased with the decrease of anus size, shell diameter and gonad index (the wet weight ratio of gonad to total soft tissues). The MPs were dominated by fiber in shape, blue-green in colour and cellophane in composition. The high MPs abundance in sea urchins indicates the potential risks to human as they are consumed in many parts of the world, particularly in Asia and Europe.

* Corresponding authors at: Jiangsu Key Laboratory of Marine Bioresources and Environment, Jiangsu Ocean University, Lianyungang 222005, China; State Key Laboratory of Marine Environmental Science & College of Ocean and Earth Sciences, Xiamen University, Xiamen 361005, China
E-mail addresses: fengzhihua@jou.edu.cn (Z. Feng), guang.gao@xmu.edu.cn (G. Gao).

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1. Introduction

The annual production of plastics in the world reached 348 million tons in 2017 (Plastics Europe, 2018) and the plastic materials account for 80–85% of all marine debris (Auta et al., 2017). Due to the characteristics of degradation-resistant, light weight and low density (Derraik, 2002; Moore, 2008), plastics can be diffused by currents and winds and result in a widespread occurrence in the oceans (Thiel et al., 2003; Cole et al., 2011). Microplastics (MPs) were usually defined as any pieces of plastics ≤ 5 mm in size (Thompson et al., 2004). Compared to large plastics, MPs have smaller sizes and are therefore easier to disperse in various environments (Lusher et al., 2013; Yang et al., 2015; Zhao et al., 2015; Peng et al., 2017, 2018). As a result, MPs are ubiquitous in seawaters and available for various marine organisms in different depths (Lusher et al., 2013).

Previous studies have confirmed the uptake of MPs by marine animals such as fish (Foekema et al., 2013; Abbasi et al., 2018; Feng et al., 2019; Su et al., 2019), marine worms (Besseling et al., 2012), bivalves (Von Moos et al., 2012; Li et al., 2018; Qu et al., 2018) and echinoderm (Mohsen et al., 2019). MPs ingestion can not only cause damages to the cells and tissues of marine organisms, but also pose a potential threat to human health through seafood consumption (Lusher et al., 2013; Jabeen et al., 2017; Su et al., 2019).

As a key marine benthic organism in shallow seas, sea urchin is an important link in trophic chains and plays a vital role in material circulation and energy flow of many coastal ecosystems (Dethiera et al., 2019). Furthermore, the nutritious sea urchin gonads are delicious marine foods with unique flavor and are widely consumed by humans (Archana and Babu, 2016). As a result, about 73,000 metric tons of sea urchins have been harvested and traded worldwide annually (Castilla-Gavilán et al., 2018). The production of sea urchins in China in 2018 reached 8, 844 tons (CFSY, 2019).

Laboratory studies have showed that MPs could affect embryonic development of sea urchins and sea urchins could also break down larger plastic items into smaller particles (Nobre et al., 2015; Messinetti et al., 2018; Porter et al., 2019). Consequently, there is possibility for sea urchins to accumulate MPs in the field. However, little is known about the status of MPs contamination in wild sea urchins, particularly for MPs distribution in different tissues of sea urchins. Especially, sea urchins have unique biological structure with the small anus in the top and the big mouth in the bottom which is close to the sediments. This inverted-funnel structure may facilitate the accumulation of MPs in the body. In this study, we investigate the contamination characteristics and distribution features of MPs in different tissues of sea urchins, including the edible gonad, in the coast of northern China to test this hypothesis.

2. Materials and methods

2.1. Sample collection and preparation

Sea urchin samples were collected at 12 locations (S1-S12) in 6 coastal areas (Dalian, Weihai, Qingdao, Rizhao, Lianyungang, and Yancheng) of northern China through 6 cruise investigations during the period from March to April in 2019 along the coast of northern China (Fig. 1 and Table 1). These areas were chosen because they are the typical habitats for sea urchins in China (Guo et al., 2013). A new Agassiz trawl (2.2 m wide, 0.65 m high, and 4 m long; mesh size 20 mm) was used and the trawling of each site was performed at a speed of approximately 2 knots and lasted for 30–60 min. The trawling depth

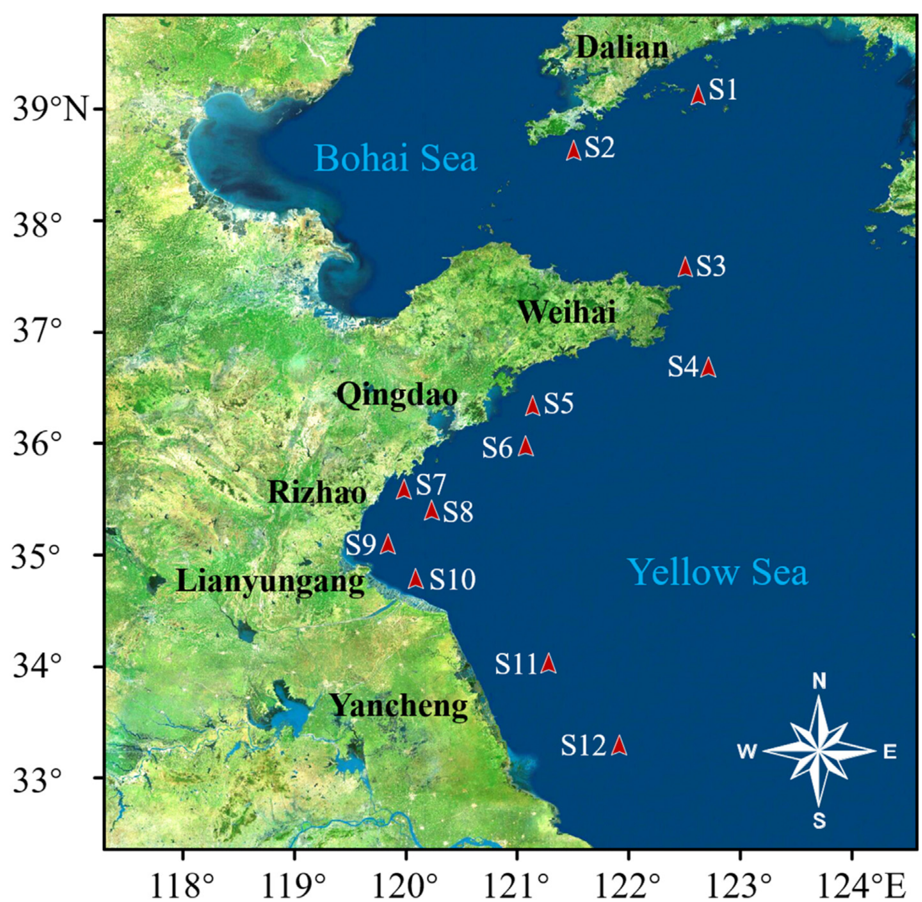

Fig. 1. Geographic position of 12 sampling sites (six areas) along the coastal areas in northern China.

Table 1
A summary of the characteristics of sampling site and sea urchins in this study.

Area	Site	Longitude (°E)	Latitude (°N)	Water depth (m)	Sea urchin number	Weight (g)	Shell diameter (cm)	Anus size (cm)	Mouthpart size (cm)
Dalian	S1	122.6277	39.1035	13	15	98.95 ± 18.62	7.02 ± 0.50	0.66 ± 0.10	1.17 ± 0.31
	S2	121.5080	38.6037	15	11	90.91 ± 22.13	6.71 ± 0.46	0.65 ± 0.05	1.12 ± 0.24
Weihai	S3	122.5101	37.5661	13	16	99.79 ± 47.04	7.67 ± 1.11	0.41 ± 0.10	0.81 ± 0.18
	S4	122.7377	36.6526	16	14	77.02 ± 33.15	7.07 ± 0.79	0.46 ± 0.09	0.76 ± 0.16
Qingdao	S5	121.1694	36.2935	9	20	16.72 ± 8.14	3.67 ± 0.73	0.30 ± 0.05	0.71 ± 0.23
	S6	121.1035	35.9345	11	26	13.75 ± 8.10	3.30 ± 0.55	0.27 ± 0.05	0.66 ± 0.14
Rizhao	S7	119.9928	35.5661	8	13	16.79 ± 8.23	3.61 ± 0.54	0.25 ± 0.05	0.72 ± 0.04
	S8	120.2602	35.3659	10	21	11.66 ± 6.65	3.37 ± 0.26	0.24 ± 0.05	0.72 ± 0.04
Lianyungang	S9	119.8725	34.9861	9	18	8.10 ± 4.63	3.25 ± 0.28	0.18 ± 0.06	0.71 ± 0.18
	S10	120.1171	34.7638	9	12	5.89 ± 1.75	2.89 ± 0.22	0.14 ± 0.04	0.68 ± 0.05
Yancheng	S11	121.3099	33.9993	10	15	8.34 ± 4.59	3.19 ± 0.50	0.27 ± 0.05	0.71 ± 0.13
	S12	121.9426	33.2631	11	29	5.41 ± 1.83	3.22 ± 0.56	0.26 ± 0.04	0.79 ± 0.10
Total					210	32.44 ± 40.47	4.34 ± 1.81	0.32 ± 0.16	0.78 ± 0.22

ranged from 8 to 16 m (Table 1). Following sampling, the sea urchins were quickly sealed within an aluminum foil bag and transferred in a cooler (−5 °C) to the laboratory where they were stored at −20 °C before further processing and analysis. All sea urchin samples were stored for no more than three days before dissection and digestion. To measure MPs abundance and composition in seawater, surface seawater at each site was sampled and processed according to our previous study (Feng et al., 2020).

2.2. Quality assurance and control

A newly purchased trawl net was used to abate the contamination from the net. The caught sea urchins were quickly loaded into the aluminum foil bag to reduce the interference from atmospheric input. Because MPs are ubiquitous in indoor environments (Gasperi et al., 2018), suitable preventive measures were taken to avoid plastic contamination in the laboratory. Air circulation between indoors and outdoors was reduced and the number of operators in the laboratory was minimized. The operators were asked to scrub their hands and forearms thoroughly before operating, and wear cotton lab coats, disposable latex gloves and face masks throughout the whole process of operation. All instruments were cleaned up with 75% alcohol (v/v). All chemical reagents and deionized water were filtered with 2.7 μm glass microfiber filter (Whatman Grade GF/D) before use. Solution preparation, sample digestion and observation of plastics were always conducted in a laminar flow cabinet (SW-CJ-2F, SUJING, China). Sample digestion was conducted in 16 batches (10–16 sea urchins/batch) and five procedural blanks without sea urchin samples were made during every batch of sample processing to determine the degree of microplastic contamination under laboratory conditions.

2.3. Sampling dissection and digestion

After defrosting, the shell of the sea urchin was washed with distilled water to remove any sediment, and the diameter length of individual was measured using a vernier caliper. Each sea urchin was dissected in a metal tray using a scalpel, and dissection began from the mouth of the sea urchin. The outer wall of the sea urchin was carefully cut with tweezers and scissors to prevent damage to the internal tissue. After opening the sea urchin shell, the coelomic fluid, gonad and gut were separated in sequence. The wet weight of these three tissues was determined by a precision electronic balance (BS124S, Sartorius electronic balance, Beijing). The separated tissues were immediately placed into clean beakers and covered with aluminum foil to minimize the risk of contamination. Tissue samples (1–40 g) were digested by 100–200 ml of 10% KOH in oscillation incubators (DKZ-3, Shanghai Yiheng, China) at 40 °C with 60 rpm for 48 h according to the previous studies (Foekema et al., 2013; Karami et al., 2017). Then, the digestion solution was transferred and filtered through a 2.7 μm glass microfiber using a filtration unit with one Büchner funnel (AP-01P, Autoscience, China). And then, the glass microfibers were placed in clean Petri dishes with lids and dried at room temperature for further study. To analyze the relationship between MPs abundance and biological structure feature, anus size and shell diameter were measured by a vernier caliper (WD, 0–150, China). In addition, gonad index and gut index were introduced and calculated, which represent the wet weight ratios of gonad or gut to total soft tissues, respectively.

2.4. Observation and identification of MPs

MPs were categorized according to their size, shape, and colour after observing under a Nikon SMZ 1500 N (Japan) stereo microscope with a charge coupled device (CCD) camera. Because of the large number (1973) of suspected MPs, approximately 20% (300 items from sea urchins and 258 items from seawater) of the total filtered filters in each site were randomly selected and identified by a micro-Fourier Transformed Infrared Spectroscope (μ-FT-IR) (Nicolet iN10, Thermo Fisher Scientific, USA). The identified items account for nearly 30% of the total number. The measurement was conducted using the transmittance mode. The spectral range was set 650 to 4000 cm^{-1} and 64 co-scans were performed at a resolution of 8 cm^{-1}. The pore size ranged from 50 × 50 mm to 150 × 150 mm depending on the particle size. By comparison with standard spectral libraries, OMNIC software was used to identify polymers, and the polymers that matched the reference spectra >70% were identified as plastics (Thompson et al., 2004; Mohsen et al., 2019). The identification of semi-synthetic MPs was performed according to the method proposed by Cai et al. (2019). All data used to calculate MPs abundance were based on the number of plastics observed by visual identification. The data were presented in the forms of both items/individual and items/g fresh weight because the former is the common form for MPs pollution in organisms (Bour et al., 2018; Ory et al., 2018; Cau et al., 2019; Porter et al., 2019; Piarulli et al., 2020) and the latter can eliminate size effect when comparing MPs abundance among sea urchins.

2.5. Data analysis

The software SPSS v.23 was used for data analysis, and the data under every treatment conformed to a normal distribution (Shapiro-Wilk, $P > 0.05$) and the variances could be considered equal (Levene's test, $P > 0.05$). One-way analysis of variance (ANOVA) was conducted to assess the difference of MPs abundance and size in the same sea urchin species and MPs abundance in seawater among different sites. Two-way ANOVA was conducted to assess the effect of tissue type and sampling site on MPs abundance in the same sea urchin species. Least significant difference was conducted for ANOVA post hoc investigation. In addition, curve fitting was conducted to analyze the relationship between the size distribution and the number of MPs. Pearson correlation

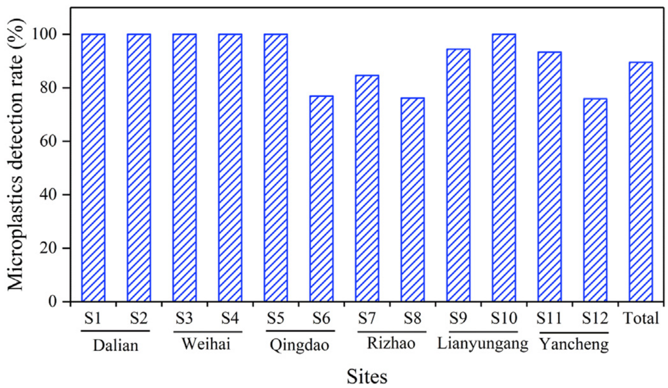

Fig. 2. The microplastic detection rate (%) in the sea urchins at different sites. Total means the total detection rate across all sites in northern China.

coefficient was employed to investigate the relationships between MPs abundance and anus size/shell diameter/gonad index/gut index, between MPs abundance in coelomic fluid and MPs abundance in gonad, between anus size and shell diameter. A confidence interval of 95% was set for all tests.

3. Results

3.1. Characteristics of sea urchins

A total number of 210 sea urchins were caught from 12 sites that locate in the coast of six areas (Dalian, Weihai, Qingdao, Rizhao, Lianyungang, Yancheng) in northern China (Table 1). These sea urchins were identified as four different species, referring to *Strongylocentrotus intermedius* (*S. intermedius*), *Temnopleurus hardwickii* (*T. hardwickii*), *Temnopleurus reevesii* (*T. reevesii*) and *Hemicentrotus pulcherrimus* (*H. pulcherrimus*). The shell diameter of these sea urchins was from 2.48 to 9.16 cm, and the weight ranged from 2.46 to 161.33 g. The

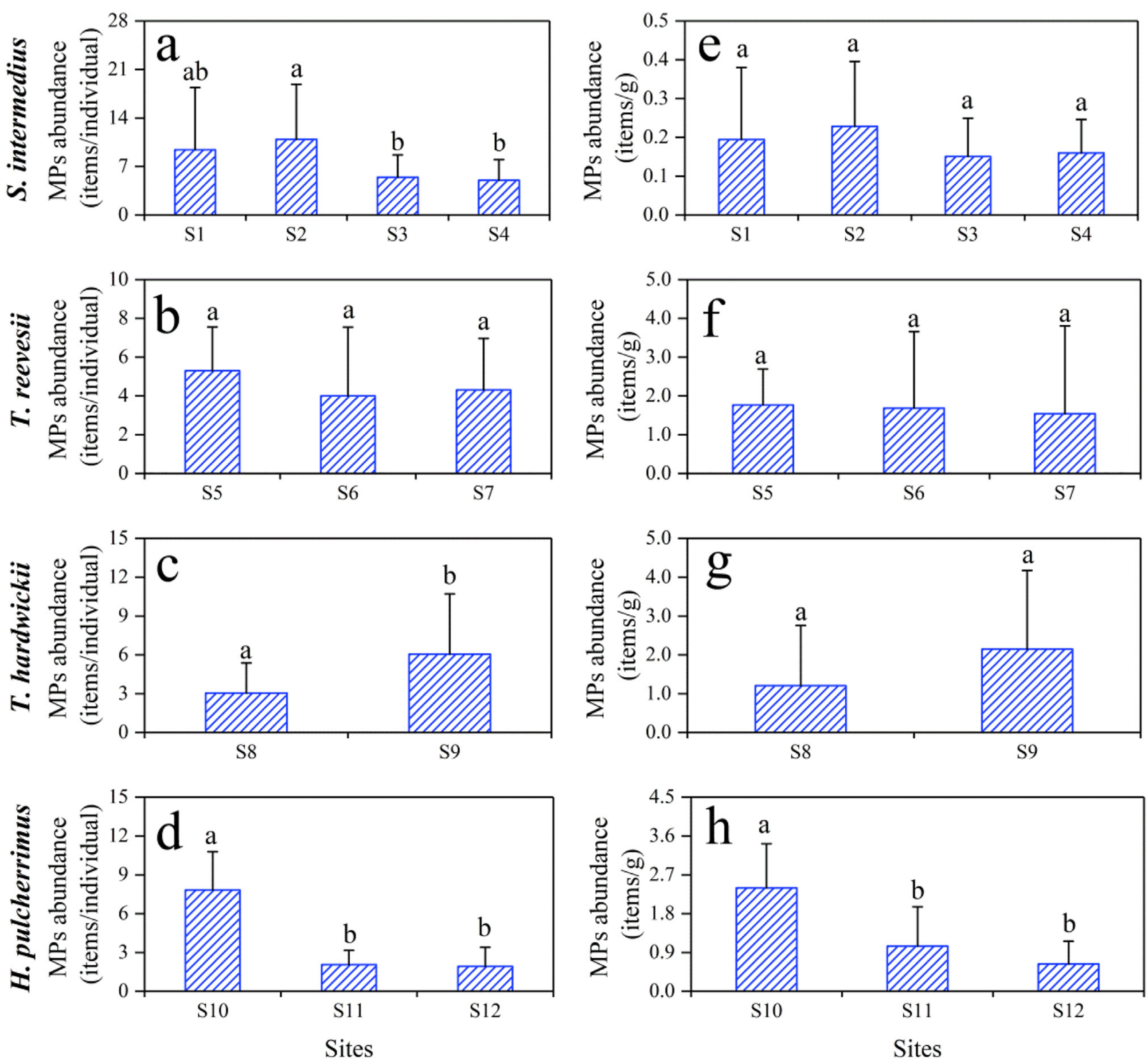

Fig. 3. Abundance of MPs (mean ± SD) in four species of sea urchin from different sites in the Yellow Sea normalized to items per individual (a-d) and items per gram total fresh weight of the three tissues (gonad, coelomic fluid and gut) (e-h). Different letters above the bars indicate significant differences among areas (One-way ANOVA, $P < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sizes for anus and mouthparts also varied with species, with largest in *S. intermedius*.

3.2. MPs abundance of sea urchins in different coastal areas

The potential contamination during the process of dissection and digestion was not taken into account when counting MPs number in the sea urchins because it (0.13 ± 0.15 items/batch) was negligible compared to the MPs levels in the samples (18–158 items/batch and 10–16 sea urchins/batch). A total of 1038 MPs were isolated from 210 sea urchins, and sea urchins from all sites were found to have MPs (Fig. 2). MPs were found in all sea urchins at sites of Dalian and Weihai. The lowest detection rate was from S12 of Yancheng, which also reached 75.86% (Fig. 2). The total detection rate for the coastal areas in northern China was 89.52%.

MPs abundance in four species of sea urchins was shown in Fig. 3. When normalized to items/individual, *S. intermedius* at sites of S1 and S2 had higher MPs abundance compared to those at sites of S3 and S4 (Fig. 3a). There were no significant differences for MPs abundance in *T. reevesii* among sites (Fig. 3b). *T. hardwickii* at site of S8 had lower MPs abundance than that at site of S9 (Fig. 3b). The highest MPs abundance for *H. pulcherrimus* was found at site of S10 (Fig. 3c). In terms of items/g, there were no significant differences for *S. intermedius* among different sites (Fig. 3e) and the same pattern also occurred for *T. reevesii* (Fig. 3f) and *T. hardwickii* (Fig. 3g). On the other hand, *H. pulcherrimus* at site of S10 still had highest MPs abundance compared to those at sites of S11 and S12 (Fig. 3h). The differences in MPs

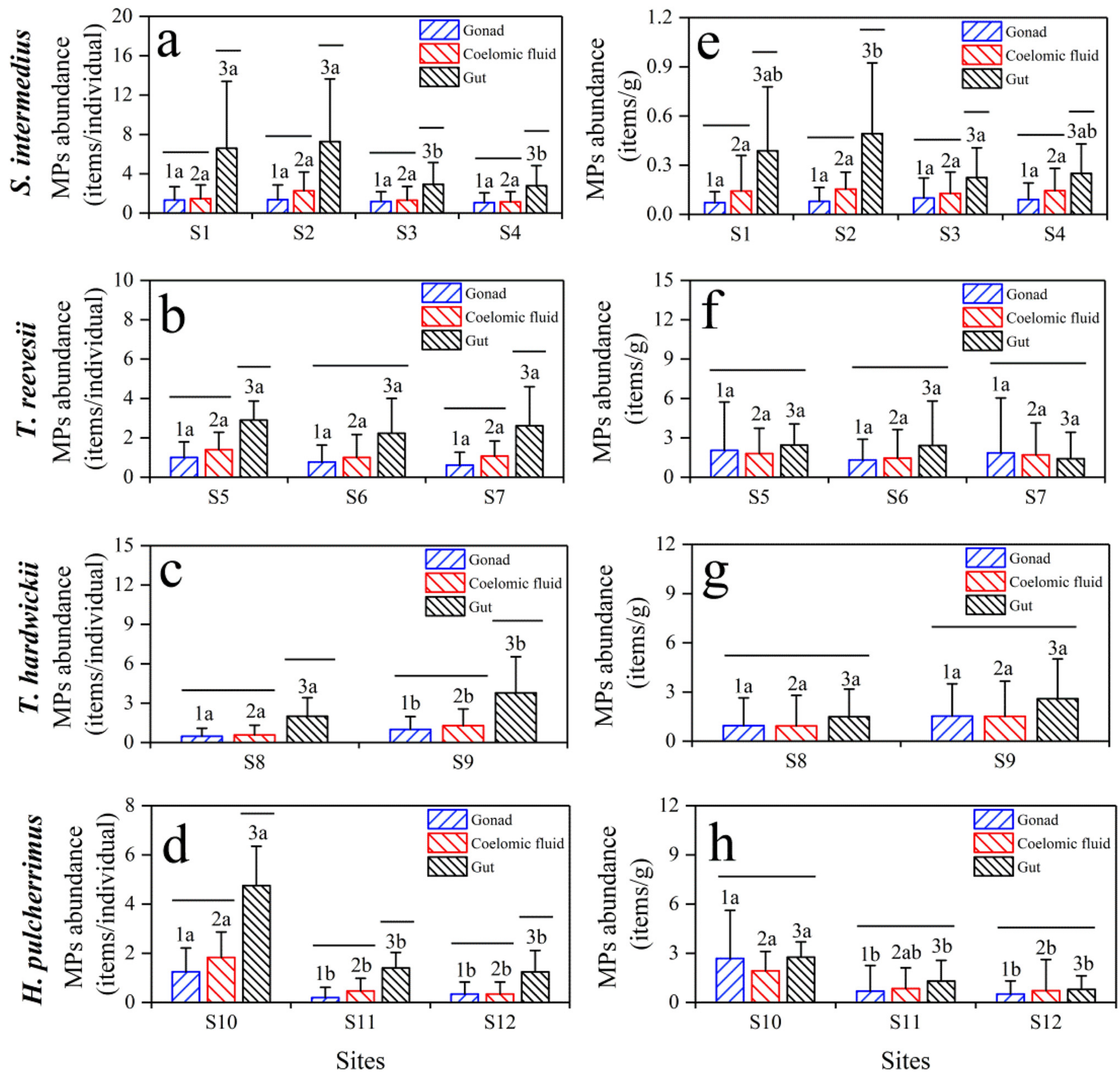

Fig. 4. Abundance of MPs (mean ± SD) in different tissues (gonad, coelomic fluid and gut) of four species of sea urchins from different sites in the Yellow Sea normalized to items per individual (a) and items per gram wet weight of the three tissues (gonad, coelomic fluid and gut) (b). Short lines indicate significant differences among tissues in each area. Different letters above error bars indicate significant differences among areas in each tissue, and 1, 2 and 3 before letters represent the differences for gonad, coelomic fluid and gut respectively (Two-way ANOVA, $P < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Two-way analysis of variance for the effects of tissue and site on MPs abundance in sea urchins. Tissue*site means the interactive effect of tissue and site, df means degree of freedom, F means the value of F statistic, and Sig. means *p*-value.

Species	Source	MPs abundance (items/individual)			MPs abundance (items/g)		
		df	F	Sig.	df	F	Sig.
S. intermedius	Tissue	2	25.909	<0.001	2	22.652	<0.001
	Site	3	4.297	0.006	3	1.540	0.206
	Tissue*Site	6	2.389	0.031	6	1.646	0.138
	Error	156			156		
T. reevesii	Tissue	2	34.714	<0.001	2	0.419	0.658
	Site	2	2.357	0.098	2	0.450	0.638
	Tissue*Sits	4	0.308	0.873	4	0.436	0.783
	Error	168			168		
T. hardwickii	Tissue	2	26.439	<0.001	2	2.210	0.114
	Site	1	14.040	<0.001	1	4.341	0.040
	Tissue*Site	2	2.137	0.123	2	0.227	0.797
	Error	111			111		
H. pulcherrimus	Tissue	2	81.393	<0.001	2	1.264	0.285
	Site	2	89.014	<0.001	2	19.630	<0.001
	Tissue*Site	4	13.873	<0.001	4	0.615	0.652
	Error	159			159		

abundance among different species of sea urchins were not compared because they were from different sites.

3.3. MPs abundance in different species of sea urchins

MPs levels in three types of tissues (gonad, coelomic fluid and gut) of sea urchins were further analyzed (Fig. 4). When expressed as items/individual, two-way ANOVA showed that both tissue type and sampling site affected the MPs abundance in *S. intermedius* and these two factors had an interactive effect (Table 2). Compared to gonad and coelomic fluid, gut had higher MPs abundance for each site; for gonad and coelomic fluid, there were no significant differences among sites while gut at S1 and S2 had higher MPs abundance (Fig. 4a). For *T. reevesii*, only tissue type affected MPs abundance (Table 2). Among the three tissues, gut had highest MPs abundance for each site and the difference between gonad and coelomic fluid was not significant (Fig. 4b). Both tissue type and sampling site affected the MPs abundance in *T. hardwickii* but no interactive effects were found (Table 2); gut still had highest MPs abundance for each site and S9 had higher values for each tissue compared to S8 (Fig. 4c). Both tissue type and sampling site affected the MPs abundance in *H. pulcherrimus* and these two factors had an interactive effect (Table 2); gut had highest MPs abundance for each site and S10 had the highest value for each tissue compared to S11 and S12 (Fig. 4d). In terms of items/g, only tissue type affected the MPs abundance in *S. intermedius* (Table 2) and gut had higher MPs abundance for each site (Fig. 4e). For *T. reevesii*, neither tissue type nor sampling site affected the MPs abundance (Table 2). Only sampling site affected MPs abundance in *T. hardwickii* (Table 2) although the differences between S8 and S9 for each tissue type were not significant (Fig. 4g). The sampling site also affected MPs abundance in *H. pulcherrimus* (Table 2) and S10 had the highest value for each tissue type compared to S11 and S12 (Fig. 4h).

3.4. Size, morphotype, colour and material of MPs in sea urchins

The mean MPs sizes in four species of sea urchins among different sites were compared while no significant differences were found for each species among different sites (Fig. 5a-d). When scanning the MPs in all sea urchins, it was found that the size of MPs ranged from 27 to 4742 μm (mesh size of the filter is 2.7 μm), with the smallest found in the gonad of *S. intermedius* and the largest found in the gut of *H. pulcherrimus*. The MPs abundance exponentially decreased with the increase of MPs size ($R^2 = 0.9967$, $P < 0.001$). The size of 27–1000 μm contributed 60.02% of the total MPs, while the proportion of MPs from 2501 to 5000 μm was only 7.32% (Fig. 5e).

Shape proportions of MPs in four species of sea urchins at different sites were shown in Fig. 6. Fiber is the dominate form for each species at each site. *S. intermedius* ingested fiber (92.91%), fragment (4.96%) and sheet (2.13%) at S1, and fiber (97.50%), microbead (1.67%) and fragment (0.83%) at S2, while all MPs were fiber for S3 and S4. The proportion of fiber ingested by *T. reevesii* also increased from 77.36% to 96.43% when sampling site changed from S5 to S7. *T. hardwickii* ingested only fiber (95.31%) and fragment (4.69%) at S8 while they were fiber (91.74%), fragment (1.83%), sheet (5.50%) and microbead (0.92%) at S9. *H. pulcherrimus* ingested fiber (91.49%), sheet (5.32%) and microbead (3.19%) at S10 while only fiber and fragment at S11 and S12.

Colour proportions of MPs in four species of sea urchins at different sites were also calculated (Fig. 7). Five colours of MPs were found for *S. intermedius* at each site and blue-green and black-gray were the dominant forms, which account for 43.26% and 36.88% for S1, 36.67% and 36.67% for S2, 45.98% and 44.83% for S3, and 54.29% and 40.00% for S4, respectively. *T. reevesii* also ingested five colours of MPs at S5 and S6 with blue-green, black-gray and red-pink dominant while it only ingested three colours (blue-green, black-gray, and white-tranparent) in S7. *T. hardwickii* ingested four colours of MPs at both S8 and S9 where blue-green and black-gray were still the most common. The patterns for *H. pulcherrimus* at sites of S10 and S11 were similar to *T. hardwickii* while it expented to five colours at S12 and red-pink had a noticable proportion (16.07%).

Size and morphotype distribution in different tissues of sea urchins were also analyzed (Fig. 8). Compared to gonad (482 ± 300 μm) and coelomic fluid (679 ± 384 μm), the size of MPs in gut was bigger (1299 ± 969 μm) ($P < 0.05$). Meanwhile, gut had the most diverse MPs morphotype, including fiber, fragment, sheet and microbead, while coelomic fluid had only fiber and fragment MPs and all MPs in gonad were fiber.

Apart from morphotype and colour, the polymer types of MPs in the sea urchins were also identified (Fig. 9a, b). Three hundreds of items were randomly selected from 1038 potential MPs for composition identification, and 79 items were confirmed as non-polymer type. Ten polymer types were found in total. The proportion of MPs material was ranked as follows: cellophane (CP, 36.65%) > polyethylene terephthalate/polyester (PET/Polyester, 16.29%) > polyethylene (PE, 14.03%) > polypropylene (PP, 13.12%) > poly (propylene: ethylene) (PP-PE, 7.69%) > polyamide (PA, 4.07%) > rayon (RY, 3.17%) > polyacrylonitrile (PAN, 2.71%) > polyurethane (PU, 1.36%) > poly (vinylacetate-ethylene) (PVA-PE, 0.90%).

3.5. Relationship between MPs abundance and biological characteristics

To explore the effect of physiological structure on MPs accumulation, relationship between MPs abundance and biological feature was investigated (Fig. 10). It was found that MPs abundance was negatively related to anus size ($r = -0.455$, $P < 0.001$), shell diameter ($r = -0.487$, $P < 0.001$) and gonad index (the weight ratio of gonad to the total soft tissue, $r = -0.415$, $P < 0.001$) but positively related to gut index (the weight ratio of gut to the total soft tissue, $r = 0.313$, $P < 0.001$). In addition, MPs abundance in coelomic fluid and gonad had a positive relationship ($r = 0.540$, $P < 0.001$), and anus size increased with shell diameter ($r = 0.830$, $P < 0.001$).

3.6. MPs in seawater

MPs abundance in seawater was also investigated (Fig. 11a). S1 (1.90 ± 0.15 items/L) and S2 (1.93 ± 0.19 items/L) had the highest value, followed by S9 (1.16 ± 0.11 items/L) and S10 (1.20 ± 0.09 items/L), indicating that Dalian and Lianyungang were the most MPs polluted areas. Sites of S3-S6 and S11-S12 had medium MPs abundance, and the lowest MPs abundance was found in S7 (0.33 ± 0.12 items/L)

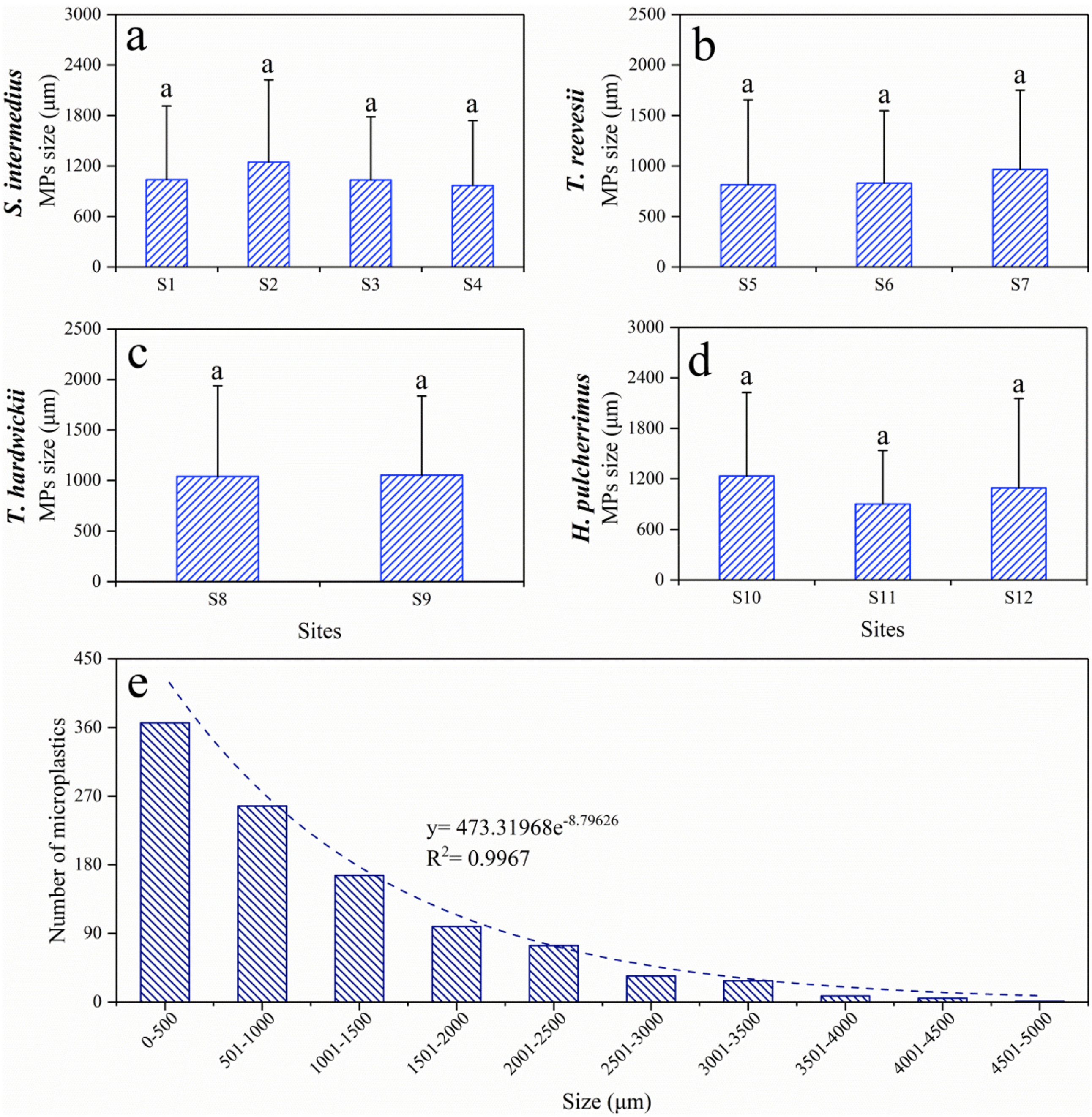

Fig. 5. Mean MPs size (± SD) for four species of sea urchins from different sites (a-d) in the Yellow Sea and the size distribution of the MPs in all sea urchins samples (e). Different letters above the bars indicate significant differences among sites (One-way ANOVA, $P < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and S8 (0.31 ± 0.05 items/L). This pattern of MPs abundance in seawater was consistent with the MPs abundance (items/individual) in sea urchins within the same species.

A number of 258 items were randomly selected from 935 potential MPs for composition identification, and 197 items were confirmed as polymer types. There were 14 polymer types in total (Fig. 11b), led by polyethylene (PE, 24.36%), polyethylene terephthalate/polyester (PET/Polyester, 17.77%) and cellophane (CP, 14.72%). The other polymer types were ranked as follows: polystyrene (PS, 11.17%) > polypropylene (PP, 9.14%) > polyamide (PA, 6.60%) > polyvinyl chloride (PVC, 5.58%) > poly (propylene:ethylene) (PP-PE, 2.54%) > rayon (RY, 2.03%) > polyacrylonitrile (PAN, 1.52%) > polyether polyurethane (PEU, 1.52%) > polyethylene ethyl acrylate copolymer (PE-EA, 1.52%) > polyurethane (PU, 1.02%) > acrylonitrile butadiene styrene copolymer (ABS, 0.51%).

4. Discussion

4.1. MPs pollution level of sea urchins in coastal areas of northern China

China is one of the major sea urchin harvesting countries and an important market. This is the first reported investigation on sea urchin MPs contamination in China. The high MPs detection rate (89.52%) indicates that the seabed of coastal areas of northern China is commonly contaminated by MPs. The average MPs abundance in sea urchins soft tissues was 4.94 ± 4.64 items/individual

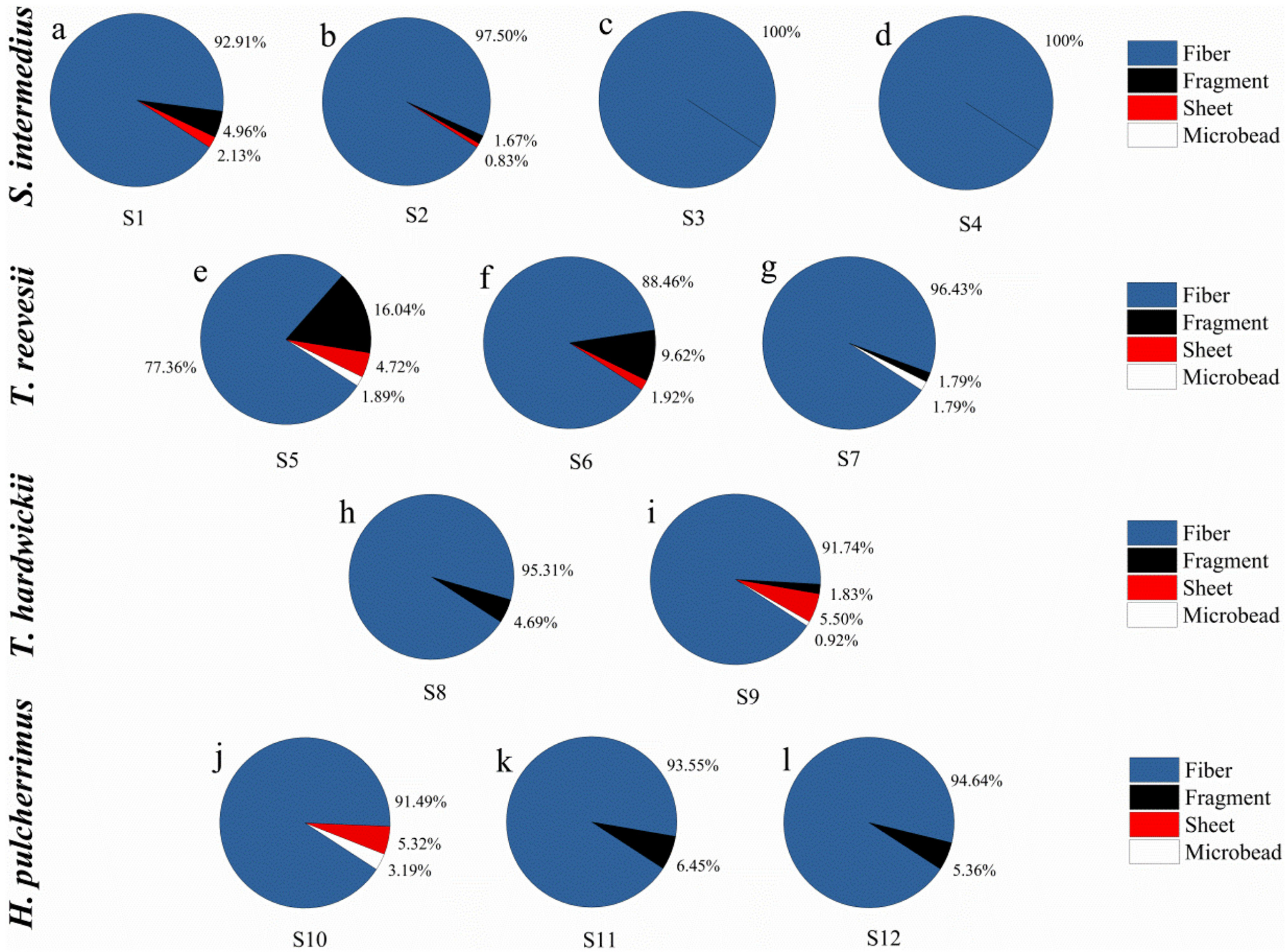

Fig. 6. Shape distribution of MPs in four species of sea urchin from different sites in the Yellow Sea. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(1.12 ± 1.45 items/g), which is much higher than heart urchins (1.1 items/individual) from Oslofjord Norway, comparable with that observed in mussels (4.0 items/individual, 2.2 items/g) and oyster (2.93 items/individual, 0.62 items/g) from coastal areas of China (Table 3). These results indicated that the level of MPs contamination in sea urchins in the northern coastal areas of China was more serious than that in Norwegian waters (Bour et al., 2018). On the other hand, the abundance of MPs in the total soft tissue of sea urchins was lower than that in starfish, sand shrimp and decorator crab, and MPs abundance in the gut of sea urchins was also lower than that in most sea cucumbers collected also from the Yellow Sea (Table 3). This may be due to species differences and feeding mode. For instance, sea urchins feed mainly on algae while sea cucumbers consume sediments that usually accumulate more MPs (Zhu et al., 2018).

The MPs detection rates and abundances in sea urchins collected from different coastal areas of the Yellow Sea showed significant differences, indicating significant spatial variability. Dalian and Lianyungang had higher MPs detection rates and MPs abundances in sea urchins for the same species. The MPs abundance in sea urchins should be related to MPs pollution in seawater because our data showed that Dalian and Lianyungang had higher MPs abundance in seawater compared to other areas. Shipping and mariculture have been proven to be important MPs sources (Carney Almroth and Eggert, 2019; Feng et al., 2020), and Dalian and Lianyungang are two important ports and mariculture areas in China with intensive human activities (Zhao et al., 2012; Gao et al., 2019; Xu et al., 2019), which may lead to the higher MPs pollution in seawater and sea urchins.

4.2. Characteristics of MPs pollution in sea urchins

Among the MPs with different sizes observed in this study, the size <1000 μm was the most abundant. Small-sized MPs were the most frequently observed particles in many studies (Peng et al., 2017; Zhao et al., 2018; Feng et al., 2019). It has been reported that the small-size MPs had the higher possibility to be ingested by marine organisms and then accumulated in the tissues (Browne et al., 2008). Lee et al. (2013) found that small-sized MPs showed extremely high toxic effects on the organs of copepod adult and offspring. In regard to particle shape in the sea urchins, the majority of MPs was fiber, which is a consistent result with that in sediment samples in the Bohai Sea and Yellow Sea (Zhang et al., 2019). Fiber has been found as the most common shape in the South Yellow Sea, because of the high usage rate of plastics fishing tools such as fishing nets, gears and ropes (Browne et al., 2008; Feng et al., 2019). Furthermore, a large number of studies have shown that fiber may be the most abundant shape of MPs in the oceans (Ivar do Sul and Costa, 2014; Woodall et al., 2014; Frias et al., 2016; Taylor et al., 2016). In terms of materials, CP, PET and PE were three dominant polymer types in the sea urchins. These three types also led the polymer composition of MPs in seawater. A large number

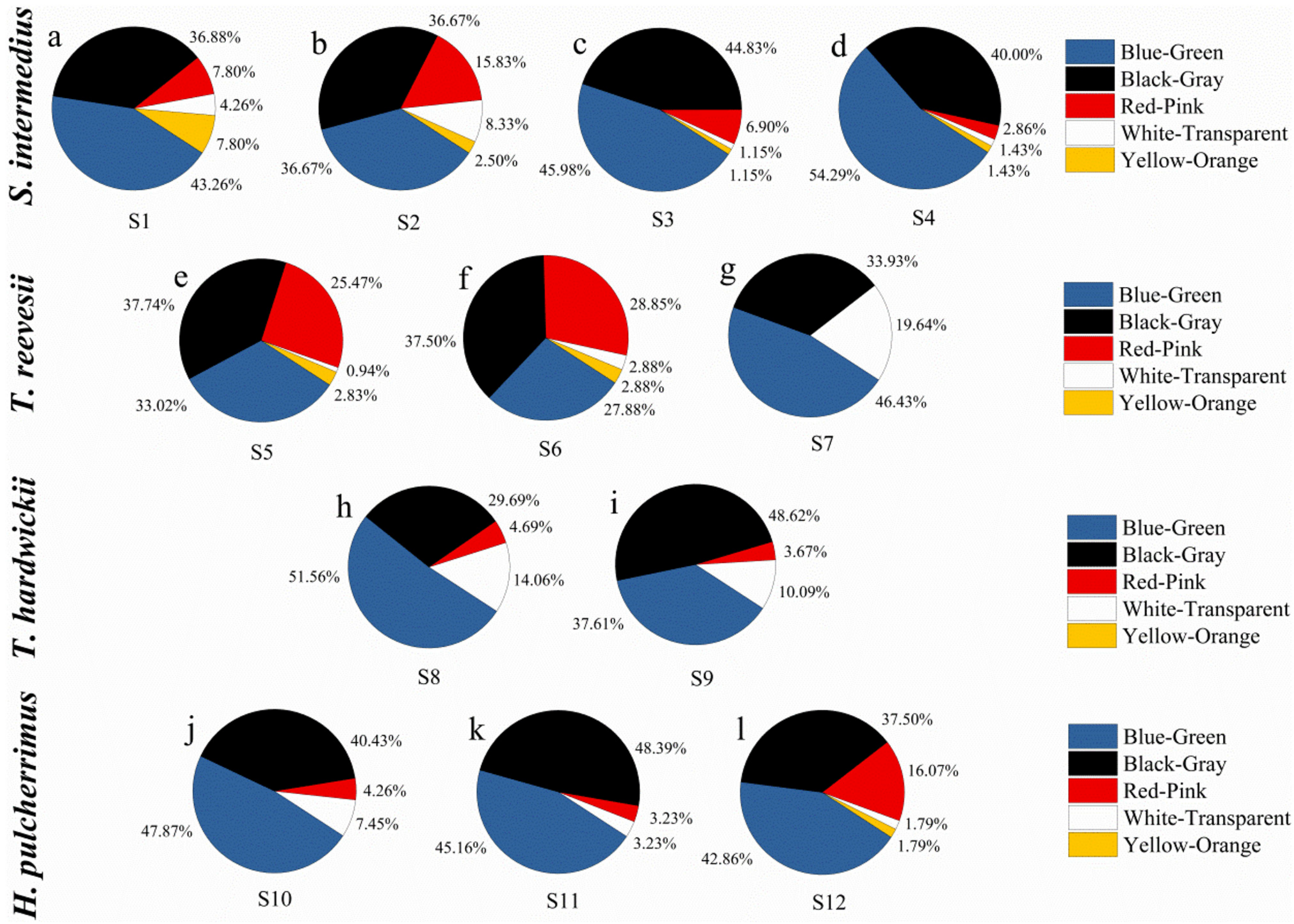

Fig. 7. Colour distribution of MPs in four species of sea urchin from different sites in the Yellow Sea. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of studies have also reported high proportions of these three types in marine environment and organisms (Jabeen et al., 2017; Zhu et al., 2018; Feng et al., 2019; Mohsen et al., 2019; Teng et al., 2019; Zhang et al., 2019).

4.3. MPs in three tissues of sea urchins

Bour et al. (2018) investigated the MPs in the whole soft tissues of heart urchins in the inner Oslofjord, Norway. However, to the best of

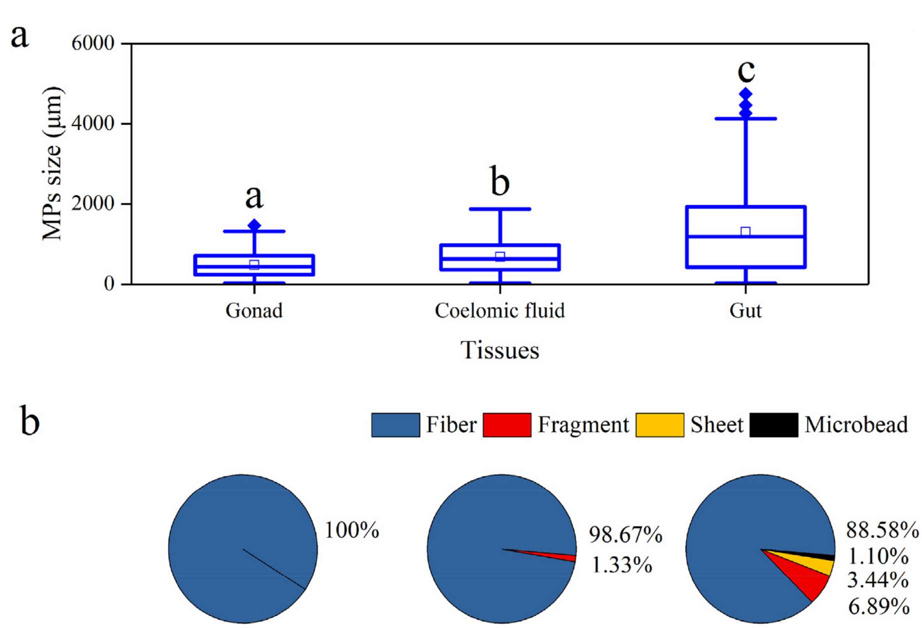

Fig. 8. Size (a) and shape(b) distribution of the MPs observed in three tissues of sea urchins.

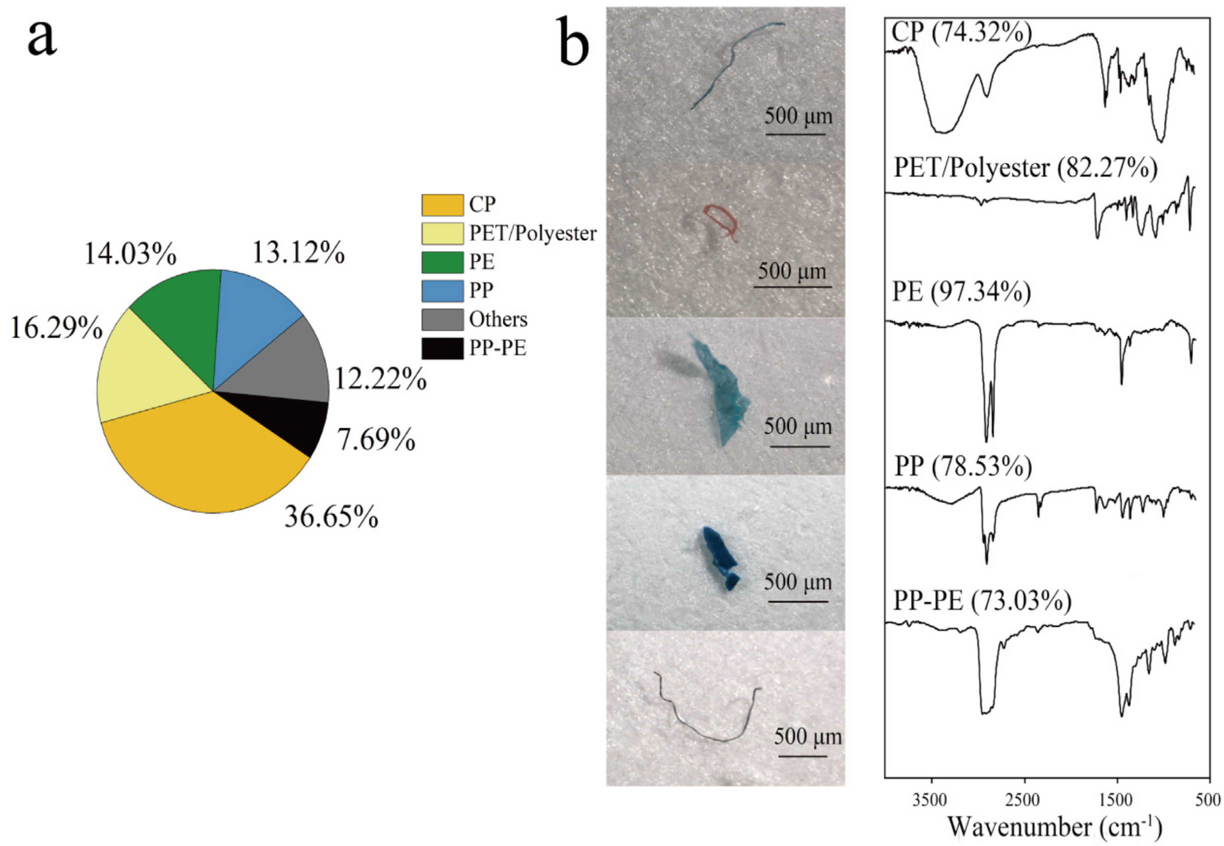

Fig. 9. Type distribution (a) and material spectra (b) identified by μ-FTIR microscope of the MPs observed in sea urchins. The proportions <5% were classified as others, including polyamide (PA, 4.07%), rayon (RY, 3.17%), polyacrylonitrile (PAN, 2.71%), polyurethane (PU, 1.36%), poly (vinylacetate-ethylene) (PVA-PE, 0.90%).

our knowledge, nothing is known about the MPs distribution in different tissues of sea urchins. In this study, we found that gut accumulated more MPs compared to coelomic fluid and gonad. The positive relationship between MPs abundance in total soft tissues and gut index also suggests that gut is the main organ that accumulates MPs for sea urchins. This finding was consistent with the study on sea cucumber collected also from the Yellow Sea (Mohsen et al., 2019). On the other hand, MPs abundance was in negative correlation with shell diameter, anus size and gonad index. This indicates that young individuals could accumulate relatively more MPs compared to older ones. Moore and McPherson (1965) showed that young sea urchins feed three times as fast as older ones. This could explain the finding in the present study. In addition, smaller anus may prevent the MPs discharge from the body.

Interestingly, MPs with size larger than 2000 μm were not detected in coelomic fluid and gonad while there were larger MPs (up to 4742 μm) in gut. There is no doubt that the MPs in gut was from ingestion via mouthpart. Then where were the MPs in coelomic fluid and gonad from? It has been suggested that only nanoparticles could permeate across enterocyte (Hussain et al., 2001; Wang et al., 2019b). In this study, the MPs with the smallest size isolated from the coelomic fluid and gonad was 27 μm, which seems impossible to transfer from the intestine. Thus, we speculate that the MPs in the coelomic fluid may be transferred from the peristomal gill and tube feet, which were two important respiratory organs (Leddy and Johnson, 2000). There is high possibility that MPs enter the body cavity through these two organs. The occurrence of MPs in the gonad may derive from coelomic fluid as it fills in the whole body cavity of sea urchins because it was found that all MPs were in the surface rather than inside of gonads with a microscopy analysis. In addition, there was a high correlation between the amount of the MPs in the coelomic fluid and gonad, which supports our speculation. However, this hypothesis still needs to be further proved in future studies.

4.4. The potential risk of MPs in sea urchins

A large number of studies have confirmed that MPs can harm marine organisms, such as reducing the appetite of marine invertebrates and hindering the passage of food through the intestinal tract (Derraik, 2002; Tourinho et al., 2010; Wright et al., 2013). It was reported that harmful chemicals, such as persistent organic pollutant, endocrine disrupting chemicals and heavy metal, carried on plastics, could cause liver stress, change the functional genes of endocrine system in fish and then transferred into the food chain (Rockström et al., 2009; Bakir et al., 2012; Rochman et al., 2013, 2015). Nobre et al. (2015) demonstrated that virgin microplastic particles had a huge impact on the development of anomalous embryonic of sea urchins although recent study showed that short-term exposure to MPs did not affect physiological performance of adult sea urchin (*Arbacia punctulata*) (Suckling and Richard, 2020). Sea urchins play important ecological function in the benthic marine environment. They are not only important feeders on algae, worms and molluscs, but also a prey for a variety of marine animals (Pearse, 2006). In addition, the creation of nutritious feces could add to the importance of sea urchins as a link to benthic communities that rely heavily on detritus for their development (Dethiera et al., 2019).

It is worth noting and worrying that the MPs were commonly found in the gonad of sea urchins in this study. Trifuoggi et al. (2019) reported

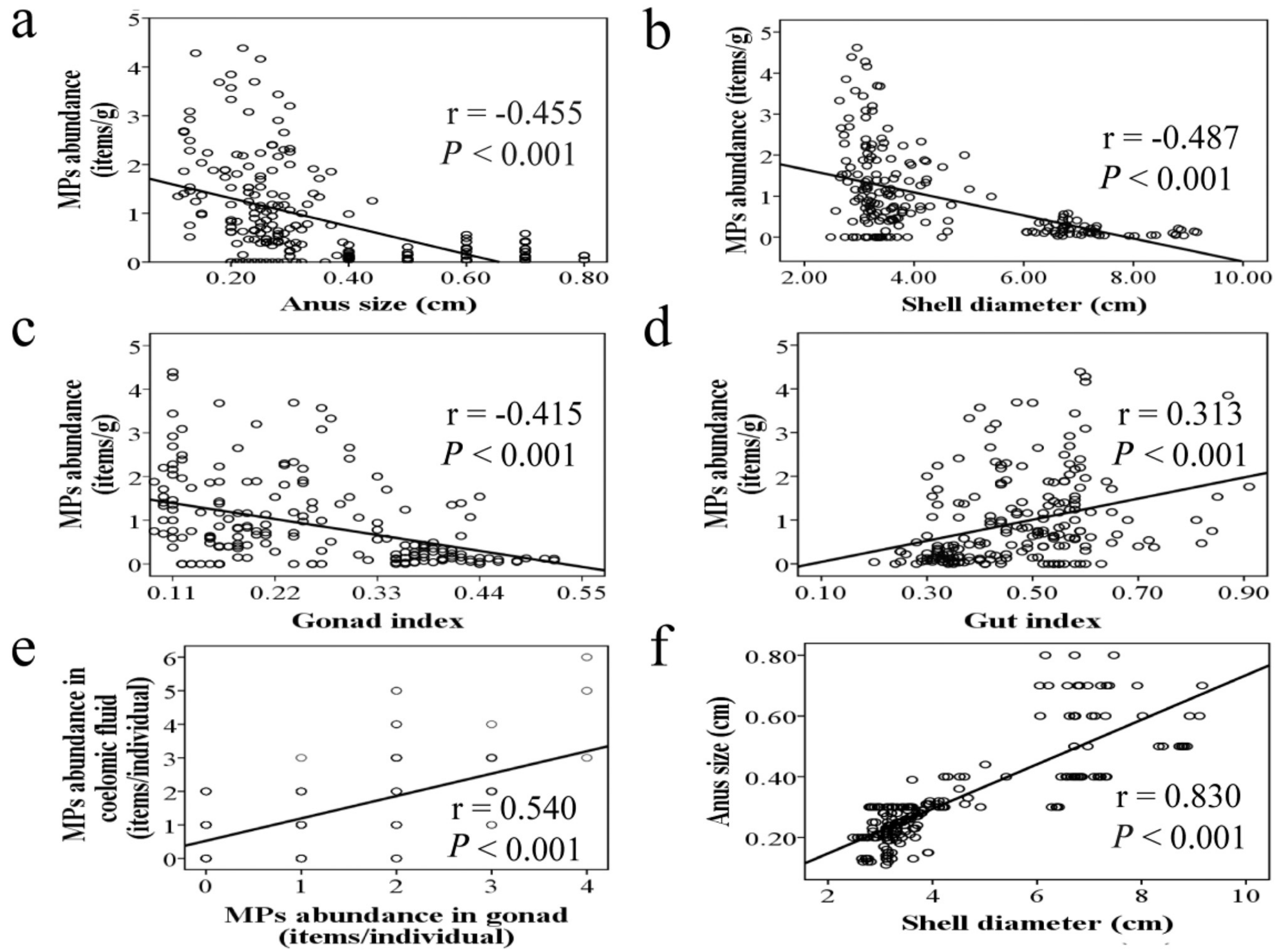

Fig. 10. Correlation analysis between MPs abundance and biological feature of sea urchins. Items/g represents items per gram wet weight of the total tissues. Gonad index and gut index represent the weight ratio of gonad or gut to total soft tissues, respectively.

that polystyrene nanoparticles can accumulate in the early development of sea urchin embryos and induce embryo toxicity. Moreover, the main components of gonad are macromolecular phospholipids and proteins with strong adhesion to MPs (Baião et al., 2019), which may lead to the persistent toxicity of MPs to sea urchin embryos. Therefore, MPs in gonad of sea urchins could induce noticeable harm to themselves and the related food web. What is worse, gonad is the edible part of sea urchins for humans, and its quality directly determines the market value of sea urchins (Suckling et al., 2011). It is difficult for humans to completely wash away the MPs on the gonad of sea urchins before cooking. Therefore, the fact that the MPs exist in the gonad of sea urchins may pose a potential risk to human health, which needs to raise concern.

5. Conclusions

In this study, the MPs contamination in wild sea urchins along the coastal areas in northern China was investigated for the first time. A very high detection rate was found. It seems that young sea urchins could ingest more MPs compared to older ones. Apart from gut, coelomic fluid and gonads can also accumulate MPs. The MPs in gonad may affect the embryonic development of sea urchin directly. Furthermore, the MPs in gonad may be transferred to human beings when being consumed since sea urchins are very popular seafood in Asian and European countries and gonad is the editable part. Therefore, more attentions need to be paid to remove MPs in gonad during the processing.

CRediT authorship contribution statement

Zhihua Feng: Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition. **Rui Wang:** Investigation, Methodology, Formal analysis, Visualization. **Tao Zhang:** Conceptualization, Methodology, Writing - review & editing. **Jiaxuan Wang:** Investigation. **Wei Huang:** Methodology. **Ji Li:** Writing - review & editing. **Juntian Xu:** Funding acquisition. **Guang Gao:** Conceptualization, Formal analysis, Writing - original draft, 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.

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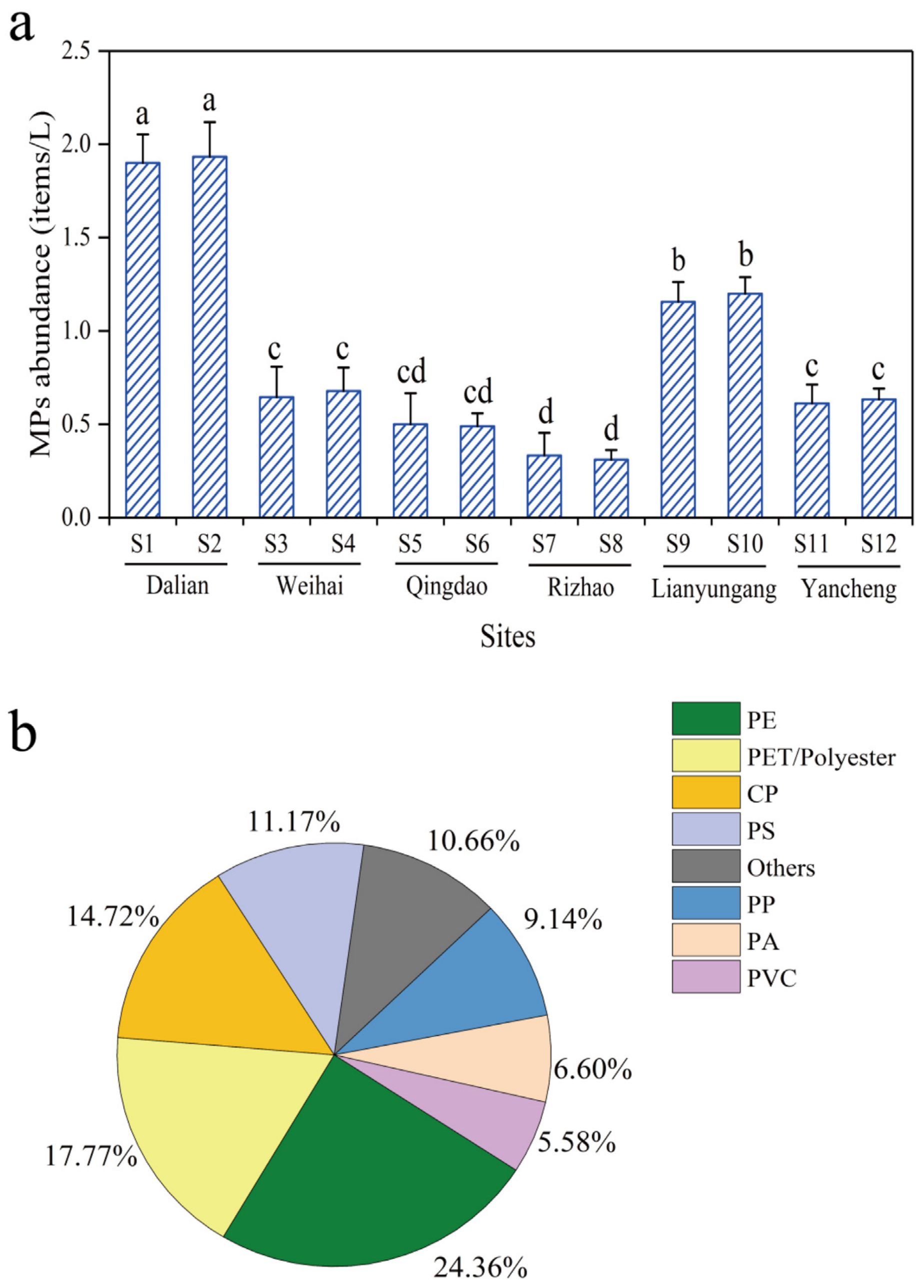

Fig. 11. Abundance (a) and type (b) of MPs in seawater at sampling sites in the Yellow Sea. Different letters above the bars indicate significant differences among areas (One-way ANOVA, $P < 0.05$). The proportions <5% were classified as others, including poly (propylene: ethylene) (PP-PE, 2.54%) > rayon (RY, 2.03%) > polyacrylonitrile (PAN, 1.52%) > polyether polyurethane (PEU, 1.52%) > polyethylene ethyl acrylate copolymer (PE-EA, 1.52%) > polyurethane (PU, 1.02%) > acrylonitrile butadiene styrene copolymer (ABS, 0.51%). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
A summary of MPs abundances (mean values) found in main benthic organisms in previous and present studies.

Area	Type of benthic organisms	Total species	Total individuals	Type of tissue	MPs abundance (items/ind.)	MPs abundance (items/g)	References
Oslofjord, Norway	Heart urchin	1	20	Soft tissue	1.1	/	Bour et al. (2018)
Coast of China	Mussel	1	/	Soft tissue	4.0	2.2	Li et al. (2016)
Coast of China	Oyster	6	/	Soft tissue	2.93	0.62	Teng et al. (2019)
Yellow Sea, China	Starfish	1	/	Soft tissue	/	4.3	Wang et al. (2019a)
Yellow Sea, China	Sand shrimp	1	10	Soft tissue	/	8.6	Wang et al. (2019a)
Yellow Sea, China	Decorator crab	1	30	Soft tissue	/	47.0	Wang et al. (2019a)
Bohai Sea and Yellow Sea, China	Sea cucumber	1	200	Gut	1.56–24.20	/	Mohsen et al. (2019)
				Coelomic fluid	0.40–9.88		
Yellow Sea, China	Sea urchins	4	210	Soft tissue	4.94	1.12	This study
				Gut	3.04	1.44	
				Coelomic fluid	1.08	0.99	
				Gonad	0.82	1.00	

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