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Baseline

Microplastic sink that cannot be ignored in chemosynthetic organisms

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ABSTRACT

Marine microplastic (MP) pollution is a widespread concern; however, to date, MP pollution in chemoautotrophic ecosystems remains largely unknown. This study focuses on the cold seep in the South China Sea. Two dominant species, namely mussel (*Gigantidas platifrons*) and squat lobster (*Shinkaia crosnieri*), were collected for examining the MP pollution. MPs were present in both mussels and squat lobsters with abundances of 0.13 ± 0.04 and 0.17 ± 0.06 items/ind., respectively. MPs were mainly fibrous (62.5 %) and transparent (45.8 %). The main polymer type was polyester (54.2 %). About 86.5 MPs/m^2 were found inhabiting mussels and squat lobsters—a value comparable to those reported in benthos. This pilot report on MP pollution in cold-seep species provides key information for studies on MP pollution in chemoautotrophic ecosystems and evidence regarding a potential biological MP sink. The role of cold-seep organisms in MP retention and transport in the regional sea merits further attention.

1. Introduction

Marine plastic pollution has been listed as a major global environmental problem (Galloway and Lewis, 2016) and has attracted widespread attention. Plastic particles have invaded the most remote areas of the planet, including the Antarctic (Cincinelli et al., 2017), the Arctic (Lusher et al., 2015), abyssal regions (Fischer et al., 2015), hadal regions (Jamieson et al., 2019), and even the Mariana Trench (Peng et al., 2018). Microplastics (MP) are plastic particles ranging in size from 0.1 μm to 5 mm (Galloway et al., 2017) that can be easily ingested by organisms. Once ingested, MPs are involved in biogeochemical cycles in ecosystems. MP contamination has been identified not only in coastal organisms, but also in deep-sea organisms, including echinoderms, molluscs, and crustacean species (Courtene-Jones et al., 2017; Taylor et al., 2016; Zhang et al., 2020). In addition to the physical damage caused by MPs, chemical hazards arising from additives, heavy metals, microorganisms, and persistent organic pollutants absorbed by MPs can also have toxic effects on organisms through ingestion. Some studies have shown that MPs reduce the clearance rate and fecundity (Rist et al., 2016; Sussarellu et al., 2016), influence the allocation of energy (Detree and Gallardo-Escarate, 2018), and cause oxidative stress responses in bivalves (Revel et al., 2019).

The significant discrepancy between the amount of plastic entering the ocean and the abundance of MPs in surface waters has resulted in public efforts to trace the "missing plastic" (Cozar et al., 2014; Reichert et al., 2022). Existing studies on MP ingestion and transfer have been conducted mainly in photoautotrophic ecosystems, with chemoautotrophic ecosystems being largely ignored. Unlike in photoautotrophic ecosystems, the energy source in chemoautotrophic ecosystems is not the sun, but rather the energy released from chemosynthetic processes. The "dark food chain" of chemoautotrophic systems has recently received attention (Cheng et al., 2021). The deep-sea cold seep is the most widespread chemosynthetic environment formed by hydrocarbon infiltration from the seafloor, and it is also known as deep-sea oasis as it can sustain high biomass (Feng et al., 2018). To obtain nutrients and energy, most invertebrates in deep-sea cold-seep ecosystems form

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symbiotic relationships with chemosynthetic bacteria. For example, *Gigantidas platifrons*, which is extensively distributed in hydrothermal vents and cold seeps in the Pacific Northwest, obtains 95 % of its energy needs from chemosynthetic symbionts (Wang, 2018), with filter feeding accounting for a much smaller proportion. With such a small proportion of filter-feeding effects, questions arise with regard to MPs in chemosynthetic organisms; nevertheless, research in this field is sparse. With extremely high biomass, whether cold seep ecosystems could be potential MP reservoirs is also unclear.

Site F cold seep was chosen as the study area for detecting MP pollution in chemoautotrophic ecosystems. It represents the most active cold-seep area in the South China Sea region, where many biogeochemical investigations have been conducted. This study aims to address the following questions: 1) What are the abundances and characteristics of MPs in chemosynthetic organisms? 2) Are there differences in MPs among different chemosynthetic organisms? 3) As a unique part, what role do cold-seep species play in the fate of MPs?

2. Materials and methods

2.1. Sample collection

Site F cold seep (119°17′ E, 22°06′ N) (Fig. 1) is located on the Formosa Ridge, with a water depth of 1120 m. The temperature near the seafloor is around 3.4 °C, the pH is approximately 7.7, the salinity is close to 34.5 ppt, and the current velocity is up to 0.35 m/s (Cao et al., 2021). The local ecosystem is characterised by low biodiversity, with the "mussel–squat lobster" community being the structure-forming part. *Gigantidas platifrons* and *Shinkaia crosnieri* are the dominant species in this ecosystem, with densities of up to 273 and 300 ind./m^3, respectively (Zhao et al., 2020).

Biological samples were collected by the remotely operated underwater vehicle (ROV) "*Faxian*" during the *Kexue* cruise in July and September 2017. Mussels (*Gigantidas platifrons*) and squat lobsters (*Shinkaia crosnieri*) were collected using shovels and suction samplers controlled by the ROV robotic arm, stored in a closed bio-box, and transferred to the deck. The collected samples were stored at −20 °C in the laboratory.

2.2. MP extraction and characterisation

After thawing, samples were rinsed three times with pure water, and the body length and weight of the organisms were measured. The soft tissues of the mussels were dissected; for small individuals, tissues from two to four of them were pooled as one batch, while large individuals were treated singly. The entire body of each squat lobster was kept and treated singly. Due to the large mass of the samples, the acidic digestion method was adapted from Sun et al. (2019) for digestion efficiency. Samples were placed in a conical flask with a lid and 65 % HNO_3 solution was added at a ratio of 1:6 (w/v). Subsequently, the samples were heated at 80 °C for 4 h. The digested solution was filtered through a membrane (0.7 μm; GF/F Whatman) after cooling to room temperature. MPs were then identified under a microscope (Stemi SV11, Zeiss, Shanghai, China), and the sizes of the particles were measured using a software connected to the camera (Carl Zeiss AxioVision 4.9.1SP2). All suspected MP particles were identified using a micro-Fourier transform infrared spectroscope (μ-FTIR, PerkinElmer Spectrum Spotlight 400, Perkin Elmer Inc., USA) equipped with an attenuated total reflection accessory. For identification, the spectral resolution was set to 16 cm^{-1}, and the spectrum was scanned over the 4000–750 cm^{-1} range. The

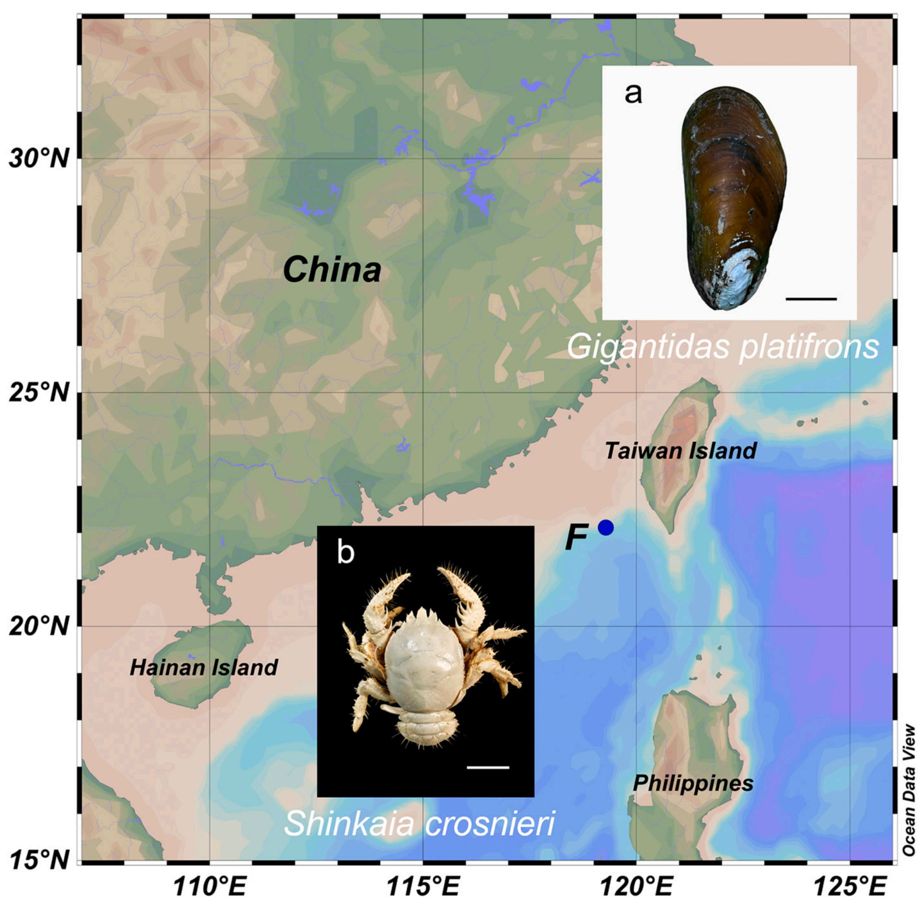

Fig. 1. Sample collection. The blue dot indicates the sampling location (Site F cold seep). Insets a and b are photographs of representative biological samples, with the scale bars representing 2 cm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

obtained spectra were compared to a library; results were deemed acceptable when the match score was >70 %. The MP surface morphologies were observed using scanning electron microscopy (SEM, TM4000 Plus, Hitachi, Japan).

2.3. Quality assurance and controls

A cotton lab coat and nitrile gloves were worn throughout the experiments. To prevent air contamination, the experiment was conducted in a fume hood. All devices were rinsed three times with pure water before use, while the pure water used during the experiments was filtered using a GF/F membrane. Three groups of blanks were used as controls, i.e., with HNO_3 solution only and no samples.

2.4. Data analysis

The particles identified as MPs were counted and, for each batch, MP abundance was calculated. The average MP abundance was based on different batches of samples and presented as mean ± standard error (SE). The Mann–Whitney test was used to analyse differences in MP abundance between different species using the SPSS software (version 26.0), with $P < 0.05$ considered as statistically significant. A map of the sample site was created using the Ocean Data View software; other figures were created using the Origin 2022 software.

3. Results and discussion

3.1. MP abundance

MPs were detected in both mussels and squat lobsters from the cold seep. MP occurrence in both mussels and crustaceans in coastal and farmed areas is well known; however, the case of the cold seep reveals MP occurrence in organisms from a chemoautotrophic ecosystem as well. In this study, 97 mussels (*Gigantidas platifrons*) (Fig. 1a) were collected, with 82 and 15 being collected in July and September 2017, respectively. The average length of the mussels was 8.72 ± 1.54 cm, with a range of 4.04–12.30 cm. In total, 89 squat lobsters (*Shinkaia crosnieri*) (Fig. 1b) were collected, with 58 and 31 being collected in July and September 2017, respectively. The average length of squat lobsters was 6.63 ± 2.38 cm, with a range of 2.12–9.74 cm. No MPs were found in the blank controls. A total of 10 and 14 MPs were detected in all mussels and squat lobsters, respectively. MP abundances in mussels and squat lobsters were 0.13 ± 0.04 and 0.17 ± 0.06 items/ind. (Fig. 2a), with MP occurrence rates of 10.31 and 12.36 %, respectively. Microscopic observations of MPs in the cold-seep species by SEM revealed severe breakages (Fig. 2b and c).

In coastal waters, mussels have been proposed as bioindicator species for monitoring MP pollution (Cho et al., 2021; Li et al., 2019; Santana et al., 2016). Gedik and Eryasar (2020) found 0.69 MPs per individual in mussels collected in Turkey. Nam Ngoc et al. (2018a) found an average of 0.61 MPs per mussel on the French Atlantic coast. Li et al. (2016) reported that MP abundance in mussels in Chinese coastal waters ranged from 1.5 to 7.6 items/ind. Compared with the reported abundance of MP pollution in coastal mussels, MP abundance in mussels from Site F cold seep is much lower. In addition, the MP occurrence rate for mussels in chemosynthetic ecosystems is lower than that for coastal mussels. The MP occurrence rate in mussels was 46.25 % in Greece (Digka et al., 2018), 48 % in Turkey (Gedik and Eryasar, 2020), and 75 % in the Santos estuary in Brazil (Santana et al., 2016). Differences among these values may depend on the distance from the continent, with regions further from the coast being less affected by human activities, and may also be related to a rate of filter feeding on algae and particulate organic matter of deep-sea mussels lower than that of coastal mussels (Page et al., 1990). In addition, *Gigantidas platifrons* covers most of its material and energy needs from chemosynthetic symbiotic bacteria, with only a small fraction of the energy being obtained by filtering particulate matter from seawater; this may also be a factor regarding the aforementioned differences.

Squat lobster (*Shinkaia crosnieri*) belongs to subphylum Crustacea, order Decapoda, infraorder Anomura, family Munidopsidae, and genus *Shinkaia*. It usually obtains its nutrients through symbiotic bacteria on its setae or by feeding on deep-sea mussels or other organisms through its own chelipeds. There are few reports of MP contamination in squat lobsters. Horn et al. (2019) reported an MP abundance of 0.65 items/ind. in another anomuran, *Emerita analog*, i.e., the Pacific mole crab, which is higher than that in *Shinkaia crosnieri* in this study. MP abundance here is lower than that in other decapods (D'Costa, 2022), and the occurrence rate here is also lower than that in other decapods. The MP occurrence rate in shrimp (*Aristeus antennatus*) from the Mediterranean Sea living at 630–1870 m depth was 39.2 % (Carreras-Colom et al.,

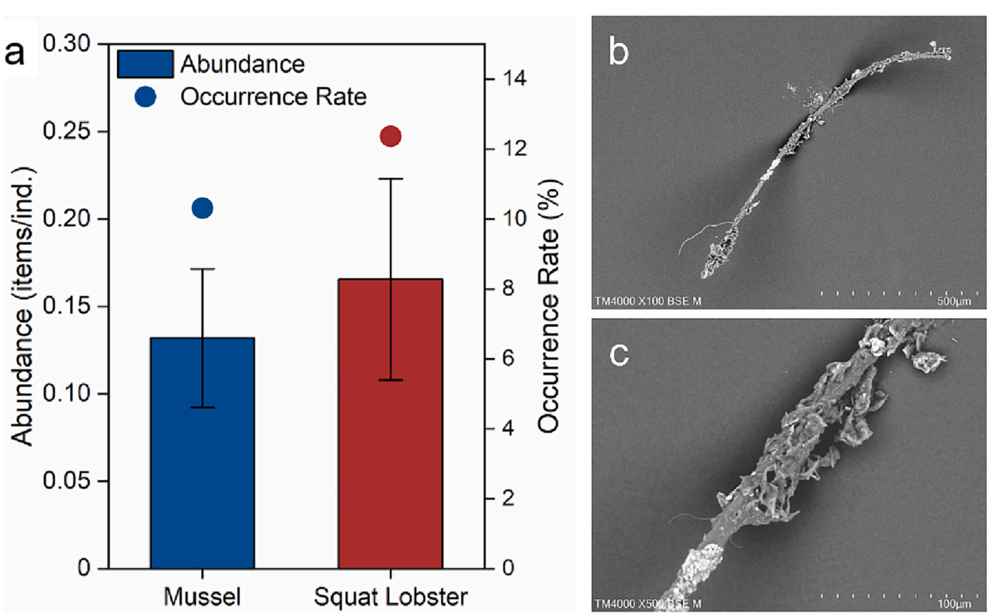

Fig. 2. a) MP abundance and occurrence rate (items/ind.) in mussel (*Gigantidas platifrons*) (n = 97) and squat lobster (*Shinkaia crosnieri*) (n = 89). SEM micrographs of MPs found in the cold-seep species at b) 100× and c) 500× magnifications.

2018). The MP occurrence rate in Pacific mole crabs was 35 % (Horn et al., 2019), and that in brown shrimp was up to 63 % (Devriese et al., 2015). It can be speculated that the low occurrence rate and low abundance of MPs in squat lobsters may be related to the low MP occurrence in the ambient environment.

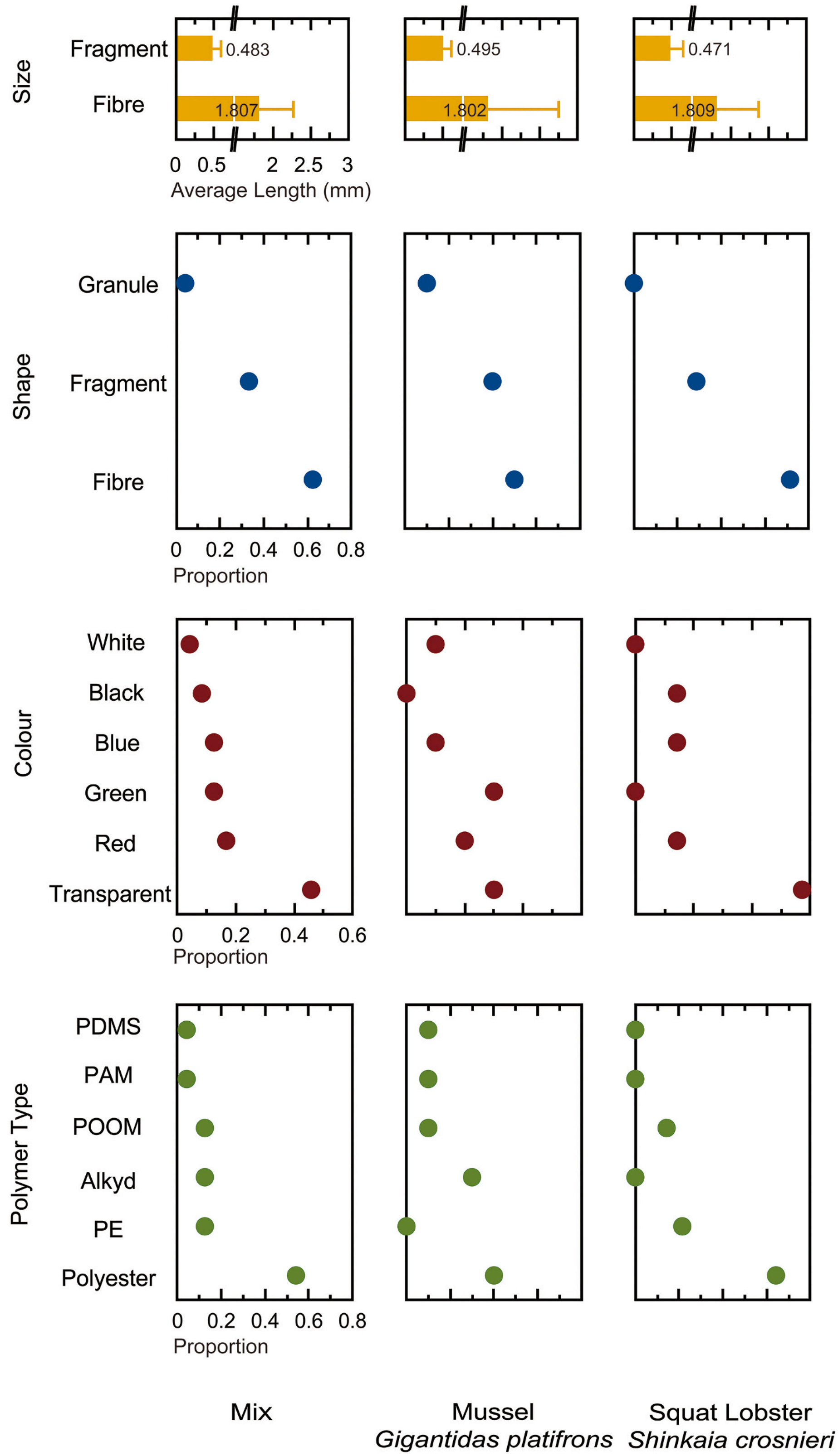

Fig. 3. MP characteristics (size, shape, colour, and polymer type) in the cold-seep species. PDMS: polydimethylsiloxane; PAM: polyacrylamide; POOM: polymerised, oxidised organic material; and PE: polythene.

3.2. MP characteristics

MPs found in the cold-seep species were mainly fibres (62.5 %) and fragments (33.3 %), with fibres accounting for 71.4 and 50.0 % of MPs in squat lobsters and mussels, respectively (Fig. 3). The average length of MPs in squat lobsters was 1809 ± 559 and 471 ± 181 μm for fibres and fragments, respectively; the average length of MPs in mussels was 1802 ± 954 and 495 ± 124 μm for fibres and fragments, respectively (Fig. 3). The similar lengths of MPs in the two species probably relate to the fact that the MPs were from the same environment. Large MPs (i.e., >0.5 mm) were more frequent than small ones, which may be due to the tendency of small particles to be egested and large ones to be retained. Large particles have been shown to be retained by the stomachs of crustaceans (Cau et al., 2020), and the Antarctic krill and deep-sea Norwegian lobster can cause MP fragmentation (Cau et al., 2020; Dawson et al., 2018).

MPs in the cold-seep species exhibited a range of colours, mainly transparent (45.8 %), red (16.7 %), blue (12.5 %), and green (12.5 %) (Fig. 3). A total of five colours of MPs were found in mussels, with their proportions being uniformly distributed and no colour being dominant. Conversely, a total of four colours of MPs were found in squat lobsters, with the proportion of transparent MPs being much higher than those of other colours. The difference in MP colour distribution between the two species may be related to the ingestion mode of each organism. Mussels are less selective for MPs through filter feeding; therefore, the resulting colours of the MPs are more uniform and exhibit a wider range. Squat lobsters ingest MPs through predation and may be relatively selective.

MPs in the deep-sea chemosynthetic organisms were dominated by polyester (54.2 %), polythene (PE) (12.5 %), alkyd (12.5 %), polymerised, oxidised organic material (12.5 %), and others (Fig. 3). Polyester was the dominant polymer type in both organisms (Fig. 3); in general, polyester-dominated MPs are common in deep-sea organisms (Carreras-Colom et al., 2018; Taylor et al., 2016) and sediments (Woodall et al., 2014). PET, which is the raw material for polyester, was the main polymer type found by Teng et al. (2022) in cold seep species. Polyesters and PE are widely used plastics. The higher density of polyester than that of PE may result in a greater proportion of polyester in deep water. Alkyd is a coating widely used in ships and vehicles and it is also found in large quantities in the seawater of the South China Sea (Cai et al., 2018).

3.3. Comparison between the cold-seep species

There was no significant difference (Mann–Whitney test; $P > 0.05$) in MP abundance between mussels and squat lobsters. This result was also obtained by Teng et al. (2022) after comparing the MP abundance of these two species collected in 2018 and 2020. Although the trophic level of the squat lobster is higher than that of the mussel, part of its food source comes from the mussel, and the lack of a significant difference indicates that there is no biomagnification of MPs in the mussel–squat lobster food chain. MPs do not accumulate in the body after being ingested by mussels and they are excreted by organisms, as has been observed for mussels in coastal waters (Goncalves et al., 2019; Woods et al., 2018).

Site F cold seep has received attention from other scholars. Teng et al. (2022) sampled both cold-seep species in 2018 and 2020 and found MP abundances of 2.80 and 2.30 items/ind., respectively. The reason for our results being lower than these values could be temporal variations. In addition, Teng et al. (2022) reported high levels of cellophane, with a proportion of 36 %; however, this study did not corroborate their finding, which may also be a reason for the different MP abundances.

3.4. Implications

Our results indicate that chemoautotrophic ecosystems have been contaminated by MPs, thereby demonstrating the ubiquity of MPs. Similar to the ecosystems of Antarctica and deep waters elsewhere, the ecosystems of cold seeps are relatively fragile because of their higher biomass and lower biodiversity. MPs have the potential to act as vectors for pathogenic bacteria and harmful algae (Lyons et al., 2010; Maso et al., 2016; Zettler et al., 2013); thus, MP ingestion may pose a risk to cold-seep organisms. Bacteria carried on MPs may disrupt the inherent symbiotic bacterial community structure in mussels; therefore, the trophic relationship of mussels with symbiotic bacteria may be affected by MPs. For example, it has been demonstrated that α-proteobacteria are more strongly associated with some MPs (Bryant et al., 2016; Dussud et al., 2018). Methanogenic symbiotic bacteria from γ-proteobacteria are predominant in mussels (*Gigantidas platifrons*) (Duperron et al., 2006), and introduction of other bacteria may lead to a change in the symbiotic community. Such changes could eventually threaten the stability of the entire cold-seep ecosystem. Teng et al. (2022) found higher abundance of MPs in gills hosted by symbiotic bacteria than in other parts of the mussel, which is noteworthy. Further experiments are needed to verify the harmful effects of MPs on cold-seep organisms, including but not limited to the distribution and hazards of MPs in various organs of cold-seep organisms.

Given their extremely high biomass, cold-seep organisms may be potential sinks for MPs. Based on the biological density (Zhao et al., 2020) and the MP abundance in each individual (mussels and squat lobsters only) obtained in this study, 86.5 MPs were estimated to be in 1 m^2 of organisms in the Site F cold seep. It is worth noting that the MP abundance should be higher in reality because the acid digestion method has been shown to decrease the recovery of MPs (Nam Ngoc et al., 2018b). This value can be as high as 1454.4 items/m^2, as extrapolated from the MP abundance in cold-seep organisms by Teng et al. (2022). Liu et al. (2021) conducted a survey of MP pollution in the South China Sea using a manta trawl (with a mesh size of 330 μm, a height of 0.5 m, and a width of 1 m) and found that the abundance of MPs in the surface seawater was 0.05–0.26 items/m^3. In that survey, the MP abundance at site H1 (119°21′ E, 21° N), located near the Site F cold-seep area, was 0.145 items/m^3. Considering either the entire South China Sea area or the waters around the cold-seep area, the abundance of MPs in organisms living in the cold seep is much higher than that in the surface seawater. It is likely that by ingesting MPs, cold-seep organisms enrich the MPs in the environment and temporarily store them in their bodies. The abundance of MPs in benthic organisms is ~45 items/m^2 in a bay (Zhang et al., 2023) and about 35.7–987.0 items/m^2 in the South Yellow Sea, China (Wang et al., 2019; Xu et al., 2016). After comparison, the MPs in cold seep organisms per unit area are comparable to the values of MPs in familiar organisms. The cold seep ecosystem is presumably a repository for missing MPs that has been ignored previously. In photoautotrophic systems, biological sinks could shape the MP distribution (Kvale et al., 2020). Therefore, when studying the transport and fate of MPs in regional seas with cold seeps, the role of cold-seep organisms as MP vectors or sinks cannot be ignored.

4. Conclusions

MPs were detected in two dominant species, mussel and squat lobster, in a cold seep in the South China Sea. The MP abundances in these two species were 0.13 and 0.17 items/ind. The main characteristics of MPs in these chemosynthetic organisms were that they were in the shape of fibre, composed of polyester, and transparent in appearance. Due to their high biological density, cold-seep organisms may play an important role as sinks in the MP transport in regional seas. Therefore, more effort should be devoted to the study of MP pollution in cold seeps to reveal the impact of MPs on chemosynthetic ecosystems and quantify the role of cold seeps in the retention of MPs in regional marine ecosystems.

CRediT authorship contribution statement

Kangning Zhang: Methodology, Data curation, Formal analysis, Visualization, Writing – original draft. **Junhua Liang:** Methodology, Data curation, Visualization. **Zhongli Sha:** Funding acquisition, Resources. **Li Zhou:** Resources. **Shan Zheng:** Conceptualization, Funding acquisition. **Xiaoxia Sun:** Conceptualization, Funding acquisition, 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.

Data availability

Data will be made available on request.

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