

Contents lists available at ScienceDirect

Marine Pollution Bulletin



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

Microplastics in different tissues of a pelagic squid (*Dosidicus gigas*) in the northern Humboldt Current ecosystem

Yi Gong ^{a,b,c,d,*}, Yaxin Wang ^a, Ling Chen ^e, Yunkai Li ^{a,b,c,d,*}, Xinjun Chen ^{a,b,c,d}, Bilin Liu ^{a,b,}

^a College of Marine Sciences, Shanghai Ocean University, Shanghai, China

^b Key Laboratory of Sustainable Exploitation of Oceanic Fisheries Resources, Ministry of Education, Shanghai, China

^c National Engineering Research Centre for Oceanic Fisheries, Shanghai Ocean University, Shanghai, China

^d Key Laboratory of Oceanic Fisheries Exploration, Ministry of Agriculture and Rural Affairs, Shanghai, China

^e Library of Shanghai Ocean University, Shanghai, China

ARTICLE INFO

Keywords: Dosidicus gigas Pelagic squid Microplastic Gill Intestine Stomach

ABSTRACT

Microplastics (MPs) found in marine invertebrates have aroused great concern, but MP ingestion by cephalopods is rare. To evaluate MP contamination in commercially important pelagic squids, we examined the abundance and characteristics of MPs in the gill, intestine, and stomach of jumbo squid *Dosidicus gigas* (30.9 to 65.0 cm mantle length), collected from the northern Humboldt Current. The average abundance ranged from 4.0 to 7.4 items/individual and 0.2 to 0.7 items/g wet weight for the three tissues. The MPs were sized 80.75 to 4632.27 µm, with larger MPs generally found in the stomach. The majority of MPs were fibrous in shape, blue or black-gray in color, and cellophane in composition. These results revealed the MP distribution in *D. gigas* and could be driven by its movement pattern and habitat use. Furthermore, this study provides evidence that adherence to gills is probably an alternative means by which pelagic squid accumulate MPs.

1. Introduction

Plastics, particularly in the form of microplastics (MPs), are ubiquitous in marine environments (Wright et al., 2013; Provencher et al., 2017; Galloway et al., 2017). MPs float and sink through the water column. They are highly bioavailable and readily ingested by marine organisms, either through direct capture or through feeding on contaminated prey (Nelms et al., 2018). There is a considerable amount of literature documenting the ingestion of MPs by marine organisms; however, as most studies have been conducted on crustaceans and fish, MPs' potential presence in cephalopods has not yet been studied extensively. This is especially true for pelagic squids, which play crucial roles in marine ecosystems due to their voracious prey consumption, and which have economic and provisioning roles for oceanic fisheries around the world (Hunsicker et al., 2010; de la Chesnais et al., 2019).

In the present study, we focused on an iconic species of pelagic squids, jumbo squid *Dosidicus gigas*, which is highly abundant in and endemic to the eastern Pacific Ocean (Nigmatullin et al., 2001). This species supports the world's largest cephalopod fishery, with the commercial annual catch ranging between 0.8 and 1.2 million tons from

2014 to 2018 (FAO yearbook. Fishery and aquaculture statistics). D. gigas is recognized as a voracious and adaptable predator of a broad range of prey, such as crustaceans, fish, and cephalopods (including cannibalism). The estimated trophic level of D. gigas ranges from almost 4.0 to 4.4 across different geographic stocks, indicated by stable isotope and stomach content analyses (Markaida and Sosa-Nishizaki, 2003; Field et al., 2007; Espinoza et al., 2015). D. gigas is also considered highly migratory, undertaking diel vertical migration to the hypoxic oxygen minimum layer and ontogenetic migration between the continental shelf and open ocean (Gilly et al., 2006; Stewart et al., 2013a). Thus, D. gigas could potentially represent a significant bioindicator of MP contamination in a vast three-dimensional space, given the high frequency of MP ingestion documented in crustaceans (Watts et al., 2014; Zhang et al., 2021) and fish (Boerger et al., 2010; Ory et al., 2018; Pereira et al., 2020). In addition, plastics have been identified in cephalopods, although only four pieces of peer-reviewed literature have been published to date. Using stomach content analysis, plastic pellets and fishing tools were found in D. gigas from the California Current (Braid et al., 2012) and Ecuador (Rosas-Luis, 2016), respectively; however, neither study investigated MPs. For coastal cephalopods, recent studies

https://doi.org/10.1016/j.marpolbul.2021.112509

Received 2 April 2021; Received in revised form 12 May 2021; Accepted 13 May 2021 0025-326X/ $\$ 2021 Elsevier Ltd. All rights reserved.

^{*} Corresponding authors at: College of Marine Sciences, Shanghai Ocean University, Shanghai, China. *E-mail addresses:* ygong@shou.edu.cn (Y. Gong), ykli@shou.edu.cn (Y. Li).

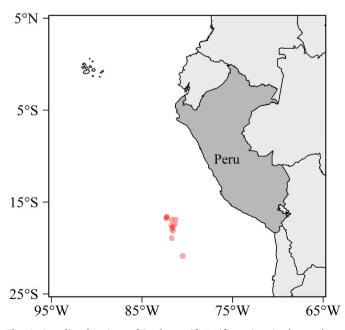
of common cuttlefish *Sepia officinalis* (Oliveira et al., 2020) and Indian squid *Uroteuthis duvaucelii* (Daniel et al., 2021) have reported a predominance of fibrous MPs in their digestive systems and edible tissues, respectively. Several studies have shown that MPs have toxic effects on tissues because of additives in their composition and adsorbed chemicals (Bakir et al., 2014; Jeong et al., 2016; Oliveira et al., 2020), both of which may hinder the stability and survival of marine organisms (Fossi et al., 2018; Rebelein et al., 2021). It is therefore vital to determine the occurrence and characteristics of MPs in pelagic squid such as *D. gigas* in order to lay the foundations for future monitoring and toxicology testing, especially given the rapid rise in squid landings.

The present study arose from the lack of MP quantification in pelagic squid and from the need to compare results for other cephalopods and high-trophic-level marine predators. We investigated the presence of MPs in the gill, intestine, and stomach tissues of *D. gigas* specimens taken from the northern Humboldt Current ecosystem, which is one of the most productive ecosystems in terms of fishery production (Chavez et al., 2008). The aims of this study were (i) to quantify the abundance and characteristics of MPs in *D. gigas*, and (ii) to assess the distribution patterns of MPs in squid tissues.

2. Materials and methods

2.1. Sample collection and preparation

Dosidicus gigas specimens were collected between October and November of 2019 by scientific observers on commercial jigging cruises operating in the waters off the Peruvian Exclusive Economic Zone (Fig. 1), which is the main fishing ground of this commercially important species off the coast of western South America (Liu et al., 2013; Csirke et al., 2015). All squid were frozen on board and transported to the laboratory, then frozen at -20 °C until further analysis. Before dissection, *D. gigas* specimens were thawed at room temperature. Basic measurements were recorded for each squid, including dorsal mantle length (ML) and body weight (BW). The outer regions of the squid specimens were rinsed with ultrapure water (Milli-Q-Water) to remove any adhered particles. Each specimen was carefully dissected in a metal tray, using tweezers and scissors to prevent damage to internal tissues. Sex was determined according to Lipiński and Underhill (1995). The gill, stomach, and intestine of each squid were extracted in sequence, their



respective wet weights were recorded, and they were wrapped in aluminum foil bags and stored in a freezer for further processing.

2.2. Isolation, observation, and identification of microplastics

Microplastic extraction was performed according to the modified alkaline method described by Dehaut et al. (2016). Before digestion, all tissue samples were dried at 60 °C for 24 h, then divided into three to five subsamples depending on size (Oliveira et al., 2020). The dry weight of each tissue sample was measured and used to estimate the required volume of 10% KOH solution. Tissue samples were individually transferred into conical flasks, followed by the addition of 20 mL per gram of dried tissue of KOH solution, and covered with aluminum foil to avoid contamination. The conical flasks were then set at 60 °C for 24 h, with continuous agitation (130 rpm) in an oscillation incubator. The digestion solution was sequentially cooled and vacuum filtered through a glass fiber filter (2.7 μ m pore size, 47 mm diameter, Whatman Inc.), and the filters were then placed in clean Petri dishes.

All filters were observed under a stereomicroscope (SZX2-FOF) coupled with a U-TV0.63XC digital camera (both from Olympus, Tokyo, Japan). Every potential microplastic (>50 μ m) was photographed, and the maximum length was measured using ImageJ Version 1.50 software and used to classify items as small microplastics (50 μ m to <1 mm) or large microplastics (1 to 5 mm). Shapes were identified based on visual evaluation of the morphometric characteristics. In terms of color, suspected microplastics were clustered into six color groups: black-gray, blue, green, red, multicolored, and yellow-brown.

Attenuated total reflection Fourier transform infrared spectroscopy (ATR FT-IR) (Nicolet iN10, Thermo Fisher Scientific, USA) was carried out for polymer identification. A total of 216 common items (~53%) were randomly selected across all individuals and tissue types and identified via ATR FT-IR. The spectral range was set at 455 to 4000 cm⁻¹, at a resolution of 8 cm⁻¹. Identifications with a level of certainty of at least a 70% match, or considered to have reliable spectral matches (after visual inspection), were accepted. To prevent potential airborne, container, and tool contamination, stringent preventative measures were applied.

2.3. Statistical analysis

Analysis of variance (ANOVA) with a post hoc Tukey's honestly significant difference (HSD) test was used to explore differences in ML between sexes, as well as the wet weight and average abundance and size of MPs among tissue types. All statistical analyses and graphics were conducted using R Version 3.5.3 (R Core Team, 2019) or OriginPro software Version 2020 (OriginLab Corp., Northampton, MA, United States). The significance level was set at 0.05. All results are presented as the mean value \pm standard deviation (SD).

3. Results

3.1. Biometric parameters

A total of 72 tissue samples were taken from 10 female and 14 male individuals (ML: 53.5 \pm 9.7 cm, BW: 4870.9 \pm 1828.0 g), and all were within the common size range of *D. gigas* from the northern Humboldt Current ecosystem (Liu et al., 2013; Csirke et al., 2015). No difference in ML (ANOVA, $F_{1,22} = 0.75$, p = 0.40) and BW ($F_{1,22} = 1.99$, p = 0.17) was observed between sexes (female: 55.6 \pm 8.7 cm, 5480.2 \pm 1746.4 g; male: 52.1 \pm 10.1 cm, 4435.7 \pm 1819.7 g). Therefore, MPs identified in ferences ($F_{2,69} = 33.93$, p < 0.01) were found in the wet weights of the three tissue types using ANOVA with post hoc Tukey's HSD comparisons, indicating that the intestine weight (6.5 \pm 3.7 g) was significantly lower than the weights of the gill (52.6 \pm 26.3 g) and stomach (43.5 \pm 23.6 g), with no significant difference between the latter two tissues (p > 0.23).

3.2. Abundance of microplastics in squid tissues

From the 72 tissue samples analyzed, 55 (76.39%) were found to contain MPs. Nonetheless, MPs were found in all individuals examined, i.e., at least one tissue sample from each squid contained MPs. Overall, a total of 403 items were identified and were distributed unevenly among the tissue types. The number of items was highest for stomach samples (n = 178), with 20/24 (83.33%) samples containing MPs, which represented an average abundance of 7.42 \pm 4.88 items/individual. A total of 130 items were detected in 16/24 (66.67%) gill samples, with an average abundance of 5.42 \pm 5.96 items/individual. For the intestine tissues, 79.17% of samples contained 95 items and had an average abundance of 3.96 \pm 3.62 items/individual. The ANOVA results showed that the stomach had a higher number of MP items per squid than the intestine (p = 0.04), and the difference between the gill and stomach was not significant (p = 0.34). MP abundance was also expressed as the number of items per gram of tissue (items/g, wet weight). Significant differences were found in the MP abundance of items/g among tissue types ($F_{2.69} = 8.93$, p < 0.01, ANOVA). The values for the intestine samples (0.74 \pm 0.77 items/g) were significantly higher than those for the gill (0.20 \pm 0.24 items/g) and stomach (0.30 \pm 0.24 items/g) samples, with no significant differences between the last two tissue types (p = 0.76).

3.3. Morphology, color, and chemical composition of microplastics

The MPs identified in the squid consisted of either fibers or fragments, while no films or pellets were found. Fibers represented the dominant shape of MPs in all tissues sampled. The percentage of fibers reached 97.69% in gill samples, followed by 93.82% and 92.63% in stomach and intestine samples, respectively. Fragments made up the remaining 2.31% to 7.37% of the total counts of MPs identified. When pooling all tissue samples together, the size range of MPs was 80.75 to 4632.27 μ m (1072.17 \pm 845.40 μ m), with the smallest MPs found in the intestine and the largest found in the stomach (Fig. 2A). Considerable variability in the size of MPs was observed among tissue types (ANOVA, $F_{2,400} = 6.70$, p < 0.01). Post hoc Tukey's HSD tests indicated that the average size of MPs was significantly larger in the stomach tissues (1238.43 \pm 1003.67 μm) than in the gill and intestine tissues (980.60 \pm 733.23 μm and 885.93 \pm 568.21 $\mu m,$ respectively), with no significant differences between the last two tissue types (p = 0.68). The most common size group of MPs in all tissue types was smaller than 1 mm, accounting for 52.81% to 64.62%, indicating that the majority of MPs identified in this study were small MPs (Fig. 2B). Fibers had a much wider size range (100.03 to 4632.27 μ m) and comprised 100% of the large MPs, while fragments were all small MPs (range 80.75 to 451.89

µm). Six color groups of MPs were found in the studied D. gigas specimens. Black-gray and blue were the dominant forms, accounting for more than 33.85% and 31.58% of MPs in squid tissues, respectively (Fig. 3A). The other colors of items recovered were green, red, and yellow-brown. Interestingly, we also found one and four multicolored fragments in the intestine and stomach samples, respectively (Figs. 3A, 4). Out of 403 MPs initially recovered, a total of 216 common items (consisting of 209 fibers and 7 fragments to reflect the overall pattern of potential MPs) were randomly selected for chemical composition verification by ATR FT-IR. Of these, 163 items were confirmed to be polymers. Five polymer types were determined: cellophane, polyacrylic acid (acrylic), alkyd, polyethylene terephthalate, and polypropylene. The most frequent polymers were cellophane (79.33%) and acrylic (10.06%). The predominant colors of cellophane MPs were black-gray and blue, while all acrylic MPs were black-gray (Fig. 3B). Each remaining polymer represented less than 5% of the items detected.

4. Discussion

MPs are frequently found in high-trophic-level marine taxa, such as dolphins (Novillo et al., 2020), sharks (Maes et al., 2020; Parton et al., 2020), and tunas (Markic et al., 2018). It is well accepted that MPs may be ingested through a pathway of trophic transfer from contaminated prey (Hipfner et al., 2018). As a highly migratory pelagic squid, D. gigas undertakes ontogenetic migration between the continental shelf and open ocean (Stewart et al., 2013b; Alegre et al., 2014). During this migration, D. gigas consumes a wide range of prey from both neritic and oceanic food webs, including pelagic and demersal forage fish, cephalopods, and crustaceans (Argüelles et al., 2012; Alegre et al., 2014; Gong et al., 2020). Cephalopods (including cannibalism) and myctophids (or lanternfish) were reported to be the main prey of D. gigas from the central Gulf of California (Markaida et al., 2008) and waters off Ecuador and Peru (Alegre et al., 2014; Rosas-Luis, 2016; Rosas-Luis and Chompoy-Salazar, 2016). Several studies have demonstrated that mesopelagic fish, including myctophids (Boerger et al., 2010; Gassel and Rochman, 2019) and cephalopods (Oliveira et al., 2020; Daniel et al., 2021; this study), had been contaminated by MPs. Moreover, a small fraction of planktivorous fish species from the South American Pacific coast reportedly contain MPs in their digestive tracts (Ory et al., 2018). Although direct trophic transfer has not been seen in pelagic squid, laboratory feeding studies have demonstrated the trophic transfer of MPs in invertebrates (Farrell and Nelson, 2013), as well as in a top marine predator, the gray seal Halichoerus grypus (Nelms et al., 2018). There is no doubt that the stomach and intestine tissues of D. gigas specimens contain MPs (as discussed below). Moreover, MPs identified in tissues independent of the digestive system (i.e., gills) indicate that

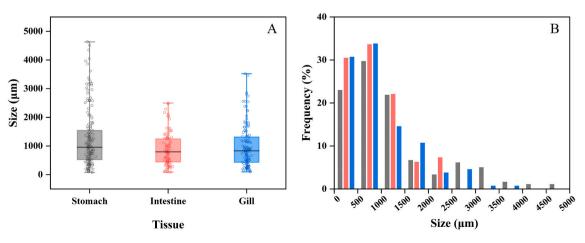


Fig. 2. Size of microplastics in three tissue types of jumbo squid *Dosidicus gigas*. (A) The boxes represent the interquartile ranges and the horizontal line within each box represents the median value. Whiskers indicate minimum and maximum values. (B) Frequency distribution of microplastics by size, grouped into 500 µm ranges.

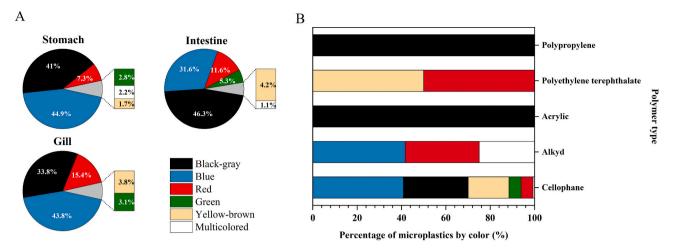


Fig. 3. Color distribution of microplastics (A) in different tissues, and (B) across polymer types.

other mechanisms may be significant for the uptake and, potentially, the translocation of MPs in pelagic squid. It has been suggested that adherence is probably a novel pathway for the accumulation of fibrous MPs in the gills of marine animals, since gills have permanent contact with the aquatic environment (Watts et al., 2014; Kolandhasamy et al., 2018; Zhang et al., 2019). Alternatively, several studies have reported mechanisms for the translocation of MPs from the intestine to other tissues (Von Moos et al., 2012; Collard et al., 2017; Barboza et al., 2020). For example, the detection of MPs in the liver of European anchovy (*Engraulis encrasicolus*) was explained by the agglomeration of smaller particles which pass through the intestinal barrier by intracellular or paracellular endocytosis (Collard et al., 2017). However, the MPs found in our gill samples were of a larger size (70% larger than 500 µm) than those found in previous studies (438 µm or smaller); thus, the exact mechanism of transfer of larger MPs in this case remains to be explored.

Our results show that MPs were found in all sampled squid from the waters of the northern Humboldt Current ecosystem. This high detection rate indicates that the studied region is commonly contaminated by MPs. At the tissue level, however, the percentage of individuals containing MPs was reduced to 76.39% (ranging from 66.67% in the gill samples to 83.33% in the stomach samples). The tissue-specific detection rate presented here suggests that one tissue alone cannot be used to evaluate the true incidence of MP accumulation in a species (Zhang et al., 2019). Moreover, these findings somewhat contradict the results of studies of D. gigas specimens found exclusively in the California Current (Braid et al., 2012) and Ecuador (Rosas-Luis, 2016). These authors reported that a much lower percentage of D. gigas specimens contained plastic items (<27% using the visual analysis of stomach content); however, the occurrence of MPs was not explicitly evaluated. To the best of our knowledge, there are no published reports identifying MPs in pelagic squid available for direct comparison with our results. However, recent studies investigating MPs in coastal cephalopods can be used to put our data into context. The MP detection rate of our study is similar to that reported by Oliveira et al. (2020) for common cuttlefish Sepia officinalis, with 100% of the digestive system samples containing MPs, and higher than the results reported for Indian squid Uroteuthis duvaucelii, with 18% edible tissue (soft tissue without gut and viscera) samples containing MPs (Daniel et al., 2021). This may be explained by the hypothesis that only small MPs can translocate or be absorbed into the edible tissues of squid (Von Moos et al., 2012; Barboza et al., 2020). Indeed, the size of MPs in the edible tissues of U. duvaucelii was estimated to be less than 300 µm (Daniel et al., 2021), while 39.46% of the MPs identified in our study were larger than 1000 µm. Moreover, a high MP detection rate has been reported in other high-trophic-level marine predators, such as the Western Mediterranean striped dolphin Stenella coeruleoalba (90.5%, Novillo et al., 2020) and demersal sharks from the

waters of the United Kingdom (67%, Parton et al., 2020). However, given the site-specific dietary sources and environment-driven switch in the prey selectivity of *D. gigas* (Portner et al., 2019; Gong et al., 2020), it is unknown whether the high detection rate in our study would also occur in other geographical locations and environmental conditions.

Of the MP items detected in D. gigas tissue samples, fiber was the predominant shape observed, which corresponds with previous studies on cephalopods (Rosas-Luis, 2016; Oliveira et al., 2020; Daniel et al., 2021) and high-trophic-level marine predators (Alomar and Deudero, 2017: Parton et al., 2020). Several other studies have also identified fiber as the most abundant shape of MPs in the marine environment (Taylor et al., 2016; Murphy et al., 2017; Li et al., 2019). The source of the fibrous MPs found in *D. gigas* specimens may be plastic fishing tools, such as fishing nets and ropes, which would be consistent with previous results reported for marine animals from fishing grounds (Rosas-Luis, 2016; Zhu et al., 2019; Zhang et al., 2019, 2021). Black-gray and blue were the most frequent colors of MPs recovered, a result that has also been reported in several other studies of seawater and organisms (Zhang et al., 2019; Parton et al., 2020). The MP size-frequency distributions indicated that small-sized MPs were the most abundant in D. gigas tissues, which is also in agreement with several previous studies performed on cephalopods (Daniel et al., 2021) and fish (Markic et al., 2018; Zhu et al., 2019; Zhang et al., 2019). This is likely due to the peculiar feeding behavior of cephalopods: ingested food particles must pass through a relatively narrow esophagus, which is located in the middle of the brain. Prey of any size is bitten and sliced by the beak, while the radula shreds them further (Hanlon and Messenger, 2018). This feeding behavior may also break up large plastic items into small pieces, especially large fragments. For example, small-sized plastic fragments with similar characteristics were found in one of the D. gigas stomach samples (Fig. 4). Considering the size-dependent toxicity of fibrous MPs (Jeong et al., 2016; Rebelein et al., 2021), we concluded that the MPs detected may represent adverse effects on the tissues of D. gigas. However, there is no evidence at present that MPs have toxic effects on cephalopods, and further research is necessary. Material analysis through ATR FT-IR determined that cellophane (CP) was the most abundant polymer found in D. gigas tissues. CP is employed primarily as a packaging material, with applications in food and cigarette wrappers, and can be manufactured in coatings that are combined with synthetic polymers. It is known that polymers (e.g., CP, 1.42 g/cm^3) with a density higher than seawater (1.025 g/cm³) tend to sink (Woodall et al., 2014). Thus, once CP packages degrade into MPs in the marine environment, they can sink into deeper water layers or sediments, making themselves available to organisms living in these areas. CP has been highlighted as a commonly identified polymer in benthic fish species (Alomar and Deudero, 2017; Zhu et al., 2019; Zhang et al., 2020) and invertebrates (Li et al., 2019;

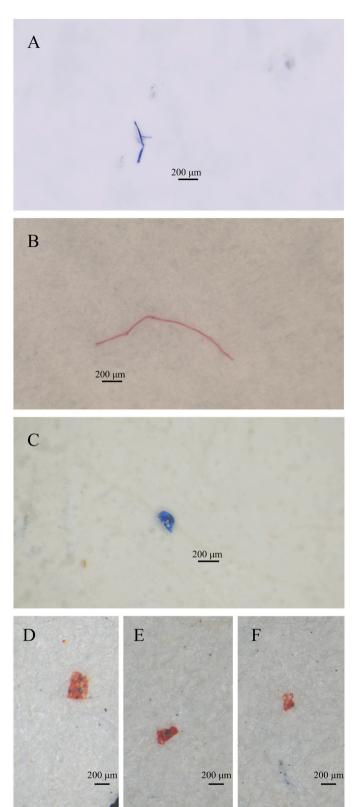


Fig. 4. Photographs showing examples of the types of microplastics found in jumbo squid *Dosidicus gigas* from the northern Humboldt Current ecosystem. (A) Blue fiber, (B) red fiber, (C) blue fragment, and (D-F) multicolored fragments found in the stomach of a 54.2 cm mantle length *D. gigas* specimen. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Feng et al., 2020; Zhang et al., 2019, 2021). This pattern could also have resulted in the high amount of CP items in our study, since *D. gigas* spends the vast majority of its time in the deep-sea hypoxic environment (Gilly et al., 2006; Stewart et al., 2013b), which provides a refuge from predators and is favorable for *D. gigas* when foraging for prey (Davis et al., 2007; Stramma et al., 2010). This also explains the absence of polymers with a low density (e.g., polyethylene, 0.96 g/cm³); however, several items of polypropylene (0.95 g/cm³) were found in our *D. gigas* samples, possibly because of biofouling and subsequent vertical transport (Kooi et al., 2017; Hipfner et al., 2018).

The average MP abundance in D. gigas tissues was 5.60 \pm 5.05 items/ individual and 0.42 \pm 0.54 items/g. However, these results should be treated with caution, given that similar fragments were found in tissues within the same individual, especially in the intestine and stomach samples. This could lead to an overestimation of the amounts of MPs in *D. gigas*, as they probably came from the fragmentation of a larger item. Comparisons with results reported for other cephalopods and hightrophic-level marine predators suggest that our findings are similar to the results reported for demersal sharks (2 to 7 items/individual, Parton et al., 2020), but lower than those reported for wild common cuttlefish (39 items/individual and 1.85 items/g digestive gland, Oliveira et al., 2020), striped dolphin (14.9 items/individual, Novillo et al., 2020), and porbeagle shark Lamna nasus caught in the Celtic Sea (9.5 items/g, Maes et al., 2020), while they are higher than those reported for Indian squid (0.18 items/individual and 0.008 items/g, Daniel et al., 2021). These differences are probably due to interspecific variations in biometric and ecological characteristics (e.g., movement pattern, trophic ecology, and habitat use). Compared with offshore waters, higher MP abundance is associated with a high level of anthropogenic activity in nearshore waters or urbanized areas (Murphy et al., 2017; Chan et al., 2019). As mentioned above, D. gigas uptakes food resources from both neritic and oceanic food webs (Gong et al., 2020); these foraging strategies may result in a lower MP abundance than that found in predators that hunt for prey mainly in nearshore waters or close to urbanized areas (e.g., common cuttlefish; Oliveira et al., 2020). In addition, D. gigas exhibits diel vertical movements between surface waters and a low dissolved oxygen environment, i.e., the upper regions of oxygen minimum zones (Gilly et al., 2006; Stewart et al., 2013a). It has been shown that large pelagic fish (e.g., sharks) cannot enter the oxygen minimum zones, since they are not adapted to anoxia (Davis et al., 2007; Stramma et al., 2010); thus, D. gigas may ingest prey containing MPs from deeper water layers than other predators are able to reach (Bazzino et al., 2010). Another possible explanation of such heterogeneity and variability in MP abundance relates to differences in the methodology used to quantify MP abundance (Covernton et al., 2021), including the different chemicals used to digest organism tissues and the tissue types investigated (Provencher et al., 2017). For example, the extraction of MPs from common cuttlefish (Oliveira et al., 2020) and D. gigas specimens in this study were performed using acid digestion and alkaline digestion techniques, respectively. Moreover, the digestive gland and edible tissues were used to evaluate the MP abundance in common cuttlefish and Indian squid, respectively (Oliveira et al., 2020; Daniel et al., 2021), while three tissue types (i.e., gill, intestine, and stomach) were used in this work. For sharks, spiral valve tissue samples were used to estimate MP abundance in the porbeagle shark (Maes et al., 2020), while Parton et al. (2020) examined MPs in the stomachs and digestive tracts of demersal sharks. Indeed, differences in MP abundance between tissues have been recognized in crustaceans, echinoderms, and fish (Abbasi et al., 2018; Zhang et al., 2019; Feng et al., 2020). This phenomenon is also supported by our results, and higher abundances of MPs were found in D. gigas gill and stomach tissues by individual, and in intestine tissues by tissue weight. Therefore, further efforts are required to finalize and adopt standardized methods that can be used to quantify MP contamination in different marine organisms.

CRediT authorship contribution statement

Yi Gong: Conceptualization, Methodology, Software, Formal analysis, Writing – original draft, Funding acquisition. Yaxin Wang: Conceptualization, Methodology, Software, Formal analysis, Writing – original draft, Funding acquisition. Ling Chen: Methodology, Validation, Formal analysis, Investigation. Yunkai Li: Resources, Writing – review & editing, Supervision. Xinjun Chen: Resources, Writing – review & editing, Supervision. Bilin Liu: Resources, Writing – review & editing, Supervision.

Declaration of competing interest

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

Acknowledgements

This work was supported by the National Key R&D Program of China (2019YFD0901404), National Natural Science Foundation of China (31900333, 31872573), and the Program for Professor of Special Appointment (Eastern Scholar) at Shanghai Institutions of Higher Learning (0810000243). We thank all our colleagues at SHOU for their assistance with sample collection and preparation.

References

- Abbasi, S., Soltani, N., Keshavarzi, B., Moore, F., Turner, A., Hassanaghaei, M., 2018. Microplastics in different tissues of fish and prawn from the Musa Estuary, Persian Gulf. Chemosphere 205, 80–87. https://doi.org/10.1016/j. chemosphere.2018.04.076.
- Alegre, A., Ménard, F., Tafur, R., Espinoza, P., Argüelles, J., Maehara, V., Flores, O., Simier, M., Bertrand, A., 2014. Comprehensive model of jumbo squid *Dosidicus gigas* trophic ecology in the northern humboldt current system. PLoS One 9. https://doi. org/10.1371/journal.pone.0085919.
- Alomar, C., Deudero, S., 2017. Evidence of microplastic ingestion in the shark Galeus melastomus Rafinesque, 1810 in the continental shelf off the western Mediterranean Sea. Environ. Pollut. 223, 223–229. https://doi.org/10.1016/j.envpol.2017.01.015.
- Argüelles, J., Lorrain, A., Cherel, Y., Graco, M., Tafur, R., Alegre, A., Espinoza, P., Taipe, A., Ayón, P., Bertrand, A., 2012. Tracking habitat and resource use for the jumbo squid *Dosidicus gigas*: a stable isotope analysis in the Northern Humboldt Current System. Mar. Biol. 159, 2105–2116. https://doi.org/10.1007/s00227-012-1998-2.
- Bakir, A., Rowland, S.J., Thompson, R.C., 2014. Enhanced desorption of persistent organic pollutants from microplastics under simulated physiological conditions. Environ. Pollut. 185, 16–23. https://doi.org/10.1016/j.envpol.2013.10.007.
- Barboza, L.G.A., Lopes, C., Oliveira, P., Bessa, F., Otero, V., Henriques, B., Raimundo, J., Caetano, M., Vale, C., Guilhermino, L., 2020. Microplastics in wild fish from North East Atlantic Ocean and its potential for causing neurotoxic effects, lipid oxidative damage, and human health risks associated with ingestion exposure. Sci. Total Environ. 717, 134625 https://doi.org/10.1016/j.scitotenv.2019.134625.
- Bazzino, G., Gilly, W.F., Markaida, U., Salinas-Zavala, C.A., Ramos-Castillejos, J., 2010. Horizontal movements, vertical-habitat utilization and diet of the jumbo squid (*Dosidicus gigas*) in the Pacific Ocean off Baja California Sur, Mexico. Prog. Oceanogr. 86, 59–71. https://doi.org/10.1016/j.pocean.2010.04.017.
- Boerger, C.M., Lattin, G.L., Moore, S.L., Moore, C.J., 2010. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. Mar. Pollut. Bull. 60, 2275–2278. https://doi.org/10.1016/j.marpolbul.2010.08.007.
- Braid, H.E., Deeds, J., DeGrasse, S.L., Wilson, J.J., Osborne, J., Hanner, R.H., 2012. Preying on commercial fisheries and accumulating paralytic shellfish toxins: a dietary analysis of invasive *Dosidicus gigas* (Cephalopoda Ommastrephidae) stranded in Pacific Canada. Mar. Biol. 159, 25–31. https://doi.org/10.1007/s00227-011-1786-4.
- Chan, H.S.H., Dingle, C., Not, C., 2019. Evidence for non-selective ingestion of microplastic in demersal fish. Mar. Pollut. Bull. 149, 110523 https://doi.org/ 10.1016/j.marpolbul.2019.110523.
- Chavez, F.P., Bertrand, A., Guevara-Carrasco, R., Soler, P., Csirke, J., 2008. The northern Humboldt Current System: brief history, present status and a view towards the future. Prog. Oceanogr. 79, 95–105. https://doi.org/10.1016/j.pocean.2008.10.012.
- Collard, F., Gilbert, B., Compere, P., Eppe, G., Das, K., Jauniaux, T., Parmentier, E., 2017. Microplastics in livers of European anchovies (*Engraulis encrasicolus*, L.). Environ. Pollut. 229, 1000–1005. https://doi.org/10.1016/j.envpol.2017.07.089.
- Covernton, G.A., Davies, H.L., Cox, K.D., El-Sabaawi, R., Juanes, F., Dudas, S.E., Dower, J.F., 2021. A Bayesian analysis of the factors determining microplastics ingestion in fishes. J. Hazard. Mater. 413, 125405 https://doi.org/10.1016/j. jhazmat.2021.125405.

- Csirke, J., Alegre, A., Argüelles, J., Guevara-Carrasco, R., Mariátegui, L., Segura, M., Tafúr, R., Yamashiro, C., 2015. Main biological and fishery aspects of the jumbo squid (*Dosidicus gigas*) in the Peruvian Humboldt Current System. S. Pac. Reg. Fish. Manag. Organ. 33. doi:10.13140/RG.2.1.4924.7443.
- Daniel, D.B., Ashraf, P.M., Thomas, S.N., Thomson, K.T., 2021. Microplastics in the edible tissues of shellfishes sold for human consumption. Chemosphere 264, 128554. https://doi.org/10.1016/j.chemosphere.2020.128554.
- Davis, R.W., Jaquet, N., Gendron, D., Markaida, U., Bazzino, G., Gilly, W., 2007. Diving behavior of sperm whales in relation to behavior of a major prey species, the jumbo squid, in the Gulf of California, Mexico. Mar. Ecol. Prog. Ser. 333, 291–302.
- de la Chesnais, T., Fulton, E.A., Tracey, S.R., Pecl, G.T., 2019. The ecological role of cephalopods and their representation in ecosystem models. Rev. Fish Biol. Fish. 29, 313–334. https://doi.org/10.1007/s11160-019-09554-2.
- Dehaut, A., Cassone, A.L., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivière, G., Lambert, C., Soudant, P., Huvet, A., Duflos, G., Paul-Pont, I., 2016. Microplastics in seafood: benchmark protocol for their extraction and characterization. Environ. Pollut. 215, 223–233. https://doi.org/10.1016/j. envpol.2016.05.018.
- Espinoza, M., Munroe, S.E.M., Clarke, T.M., Fisk, A.T., Wehrtmann, I.S., 2015. Feeding ecology of common demersal elasmobranch species in the Pacific coast of Costa Rica inferred from stable isotope and stomach content analyses. J. Exp. Mar. Biol. Ecol. 470, 12–25. https://doi.org/10.1016/j.jembe.2015.04.021.
- http://www.fao.org/fishery/static/Yearbook/YB2018_USBcard/booklet/web_CB1213T. pdf, FAO 2020-. (Accessed 1 March 2020).
- Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: Mytilus edulis (L.) to Carcinus maenas (L.). Environ. Pollut. 177, 1–3. https://doi.org/10.1016/j. envpol.2013.01.046.
- Feng, Z., Wang, R., Zhang, T., Wang, J., Huang, W., Li, J., Xu, J., Gao, G., 2020. Microplastics in specific tissues of wild sea urchins along the coastal areas of northern China. Sci. Total Environ. 728 https://doi.org/10.1016/j. scitotenv.2020.138660.
- Field, J.C., Baltz, K., Phillips, A.J., Walker, W.A., 2007. Range expansion and trophic interactions of the jumbo squid, *Dosidicus gigas*, in the California current. Calif. Coop. Ocean. Fish. Investig. Rep. 48, 131–146.
- Fossi, M.C., Pedà, C., Compa, M., Tsangaris, C., Alomar, C., Claro, F., Ioakeimidis, C., Galgani, F., Hema, T., Deudero, S., Romeo, T., Battaglia, P., Andaloro, F., Caliani, I., Casini, S., Panti, C., Baini, M., 2018. Bioindicators for monitoring marine litter ingestion and its impacts on Mediterranean biodiversity. Environ. Pollut. 237, 1023–1040. https://doi.org/10.1016/j.envpol.2017.11.019.
- Galloway, T.S., Cole, M., Lewis, C., 2017. Interactions of microplastic debris throughout the marine ecosystem. Nat. Ecol. Evol. 1, 1–8. https://doi.org/10.1038/s41559-017-0116.
- Gassel, M., Rochman, C.M., 2019. The complex issue of chemicals and microplastic pollution: a case study in North Pacific lanternfish. Environ. Pollut. 248, 1000–1009. https://doi.org/10.1016/j.envpol.2019.03.002.
- Gilly, W.F., Markaida, U., Baxter, C.H., Block, B.A., Boustany, A., Zeidberg, L., Reisenbichler, K., Robison, B., Bazzino, G., Salinas, C., 2006. Vertical and horizontal migrations by the jumbo squid *Dosidicus gigas* revealed by electronic tagging. Mar. Ecol. Prog. Ser. 324, 1–17. https://doi.org/10.3354/meps324001.
 Gong, Y., Li, Y., Chen, X., Yu, W., 2020. Trophic niche and diversity of a pelagic squid
- Gong, Y., Li, Y., Chen, X., Yu, W., 2020. Trophic niche and diversity of a pelagic squid (*Dosidicus gigas*): a comparative study using stable isotope, fatty acid, and feeding apparatuses morphology. Front. Mar. Sci. 7, 642. https://doi.org/10.3389/ fmars/2020.00642
- Hanlon, R.T., Messenger, J.B., 2018. Cephalopod Behaviour. Cambridge University Press, Cambridge.
- Hipfner, J.M., Galbraith, M., Tucker, S., Studholme, K.R., Domalik, A.D., Pearson, S.F., Good, T.P., Ross, P.S., Hodum, P., 2018. Two forage fishes as potential conduits for the vertical transfer of microfibres in Northeastern Pacific Ocean food webs. Environ. Pollut. 239, 215–222. https://doi.org/10.1016/j.envpol.2018.04.009.
- Hunsicker, M.E., Essington, T.E., Watson, R., Sumaila, U.R., 2010. The contribution of cephalopods to global marine fisheries: can we have our squid and eat them too? Fish Fish. 11, 421–438. https://doi.org/10.1111/j.1467-2979.2010.00369.x.
- Jeong, C.B., Won, E.J., Kang, H.M., Lee, M.C., Hwang, D.S., Hwang, U.K., Zhou, B., Souissi, S., Lee, S.J., Lee, J.S., 2016. Microplastic size-dependent toxicity, oxidative stress induction, and p-JNK and p-p38 activation in the monogonont rotifer (*Brachionus koreanus*). Environ. Sci. Technol. 50, 8849–8857. https://doi.org/ 10.1021/acs.est.6b01441.
- Kolandhasamy, P., Su, L., Li, J., Qu, X., Jabeen, K., Shi, H., 2018. Adherence of microplastics to soft tissue of mussels: a novel way to uptake microplastics beyond ingestion. Sci. Total Environ. 610–611, 635–640. https://doi.org/10.1016/j. scitotenv.2017.08.053.
- Kooi, M., Van Nes, E.H., Scheffer, M., Koelmans, A.A., 2017. Ups and downs in the ocean: effects of biofouling on vertical transport of microplastics. Environ. Sci. Technol. 51, 7963–7971. https://doi.org/10.1021/acs.est.6b04702.
- Li, J., Lusher, A.L., Rotchell, J.M., Deudero, S., Turra, A., Bråte, I.L.N., Sun, C., Shahadat Hossain, M., Li, Q., Kolandhasamy, P., Shi, H., 2019. Using mussel as a global bioindicator of coastal microplastic pollution. Environ. Pollut. 244, 522–533. https://doi.org/10.1016/j.envpol.2018.10.032.
- Lipiński, M.R., Underhill, L.G., 1995. Sexual maturation in squid: quantum or continuum? S. Afr. J. Mar. Sci. 15, 207–223. https://doi.org/10.2989/ 02577619509504844.
- Liu, B., Chen, X., Chen, Y., Tian, S., Li, J., Fang, Z., Yang, M., 2013. Age, maturation, and population structure of the Humboldt squid *Dosidicus gigas* off the Peruvian Exclusive Economic Zones. Chinese J. Oceanol. Limnol. 31, 81–91. https://doi.org/10.100 7/s00343-013-2036-z.

Maes, T., van Diemen de Jel, J., Vethaak, A.D., Desender, M., Bendall, V.A., van Velzen, M., Leslie, H.A., 2020. You are what you eat, microplastics in porbeagle sharks from the North East Atlantic: method development and analysis in spiral valve content and tissue. Front. Mar. Sci. 7, 1–17. https://doi.org/10.3389/ fmars.2020.00273.

- Markaida, U., Sosa-Nishizaki, O., 2003. Food and feeding habits of jumbo squid *Dosidicus gigas* (Cephalopoda: Ommastrephidae) from the Gulf of California, Mexico. J. Mar. Biol. Assoc. UK 83, 507–522. https://doi.org/10.1017/S0025315403007434h.
- Markaida, U., Gilly, W.F., Salinas-Zavala, C.A., Rosas-Luis, R., Booth, J.A.T., 2008. Food and feeding of jumbo squid *Dosidicus gigas* in the Gulf of California during 2005–2007. CalCOFI. Rep. 49, 90–103.
- Markic, A., Niemand, C., Bridson, J.H., Mazouni-Gaertner, N., Gaertner, J.C., Eriksen, M., Bowen, M., 2018. Double trouble in the South Pacific subtropical gyre: increased plastic ingestion by fish in the oceanic accumulation zone. Mar. Pollut. Bull. 136, 547–564. https://doi.org/10.1016/j.marpolbul.2018.09.031.
- Murphy, F., Russell, M., Ewins, C., Quinn, B., 2017. The uptake of macroplastic & microplastic by demersal & pelagic fish in the Northeast Atlantic around Scotland. Mar. Pollut. Bull. 122, 353–359. https://doi.org/10.1016/j.marpolbul.2017.06.073.
- Nelms, S.E., Galloway, T.S., Godley, B.J., Jarvis, D.S., Lindeque, P.K., 2018. Investigating microplastic trophic transfer in marine top predators. Environ. Pollut. 238, 999–1007. https://doi.org/10.1016/j.envpol.2018.02.016.
- Nigmatullin, C.M., Nesis, K.N., Arkhipkin, A.I., 2001. A review of the biology of the jumbo squid *Dosidicus gigas* (Cephalopoda: Ommastrephidae). Fish. Res. 54, 9–19. https://doi.org/10.1016/S0165-7836(01)00371-X.
- Novillo, O., Raga, J.A., Tomás, J., 2020. Evaluating the presence of microplastics in striped dolphins (*Stenella coeruleoalba*) stranded in the Western Mediterranean Sea. Mar. Pollut. Bull. 160, 111557. https://doi.org/10.1016/j.marpolbul.2020.111557.
- Oliveira, A.R., Sardinha-silva, A., Andrews, P.L.R., Green, D., Cooke, G.M., Hall, S., Blackburn, K., Sykes, A.V., 2020. Microplastics presence in cultured and wild-caught cuttlefish, Sepia officinalis. Mar. Pollut. Bull. 160 https://doi.org/10.1016/j. marpolbul.2020.111553.
- Ory, N., Chagnon, C., Felix, F., Fernández, C., Ferreira, J.L., Gallardo, C., Garcés Ordóñez, O., Henostroza, A., Laaz, E., Mizraji, R., Mojica, H., Murillo Haro, V., Ossa Medina, L., Preciado, M., Sobral, P., Urbina, M.A., Thiel, M., 2018. Low prevalence of microplastic contamination in planktivorous fish species from the southeast Pacific Ocean. Mar. Pollut. Bull. 127, 211–216. https://doi.org/10.1016/j. marpolbul.2017.12.016.
- Parton, K.J., Godley, B.J., Santillo, D., Tausif, M., Omeyer, L.C.M., Galloway, T.S., 2020. Investigating the presence of microplastics in demersal sharks of the North-East Atlantic. Sci. Rep. 10, 1–11. https://doi.org/10.1038/s41598-020-68680-1.
- Pereira, J.M., Rodríguez, Y., Blasco-Monleon, S., Porter, A., Lewis, C., Pham, C.K., 2020. Microplastic in the stomachs of open-ocean and deep-sea fishes of the North-East Atlantic. Environ. Pollut. 265 https://doi.org/10.1016/j.envpol.2020.115060.
- Portner, E.J., Markaida, U., Robinson, C.J., Gilly, W.F., 2019. Trophic ecology of Humboldt squid, *Dosidicus gigas*, in conjunction with body size and climatic variability in the Gulf of California, Mexico. Limnol. Oceanogr. 1–17 https://doi. org/10.1002/lno.11343.
- Provencher, J.F., Bond, A.L., Avery-Gomm, S., Borrelle, S.B., Bravo Rebolledo, E.L., Hammer, S., Kühn, S., Lavers, J.L., Mallory, M.L., Trevail, A., Van Franeker, J.A., 2017. Quantifying ingested debris in marine megafauna: a review and recommendations for standardization. Anal. Methods 9, 1454–1469. https://doi. org/10.1039/c6ay02419j.

- R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. Available at. https://www.R-project. org/.
- Rebelein, A., Int-veen, I., Kammann, U., Scharsack, J.P., 2021. Microplastic fibersunderestimated threat to aquatic organisms? Sci. Total Environ. 777, 146045 https://doi.org/10.1016/j.scitotenv.2021.146045.
- Rosas-Luis, R., 2016. Description of plastic remains found in the stomach contents of the jumbo squid *Dosidicus gigas* landed in Ecuador during 2014. Mar. Pollut. Bull. 113, 302–305. https://doi.org/10.1016/j.marpolbul.2016.09.060.
- Rosas-Luis, R., Chompoy-Salazar, L., 2016. Description of food sources used by jumbo squid Dosidicus gigas (D'Orbigny, 1835) in Ecuadorian waters during 2014. Fish. Res. 173, 139–144. https://doi.org/10.1016/j.fishres.2015.08.006.
- Stewart, J.S., Field, J.C., Markaida, U., Gilly, W.F., 2013a. Behavioral ecology of jumbo squid (*Dosidicus gigas*) in relation to oxygen minimum zones. Deep. Res. Part II Top. Stud. Oceanogr. 95, 197–208. https://doi.org/10.1016/j.dsr2.2012.06.005.
- Stewart, J.S., Gilly, W.F., Field, J.C., Payne, J.C., 2013b. Onshore-offshore movement of jumbo squid (*Dosidicus gigas*) on the continental shelf. Deep. Res. Part II Top. Stud. Oceanogr. 95, 193–196. https://doi.org/10.1016/j.dsr2.2012.08.019.
- Stramma, L., Schmidtko, S., Levin, L.A., Johnson, G.C., 2010. Ocean oxygen minima expansions and their biological impacts. Deep. Res. Part I Oceanogr. Res. Pap. 57, 587–595. https://doi.org/10.1016/j.dsr.2010.01.005.
- Taylor, M.L., Gwinnett, C., Robinson, L.F., Woodall, L.C., 2016. Plastic microfibre ingestion by deep-sea organisms. Sci. Rep. 6, 1–9. https://doi.org/10.1038/ srep33997.
- Von Moos, 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, 11327–11335. https://doi.org/10.1021/ es302332w.
- Watts, A.J.R., Lewis, C., Goodhead, R.M., Beckett, S.J., Moger, J., Tyler, C.R., Galloway, T.S., 2014. Uptake and retention of microplastics by the shore crab Carcinus maenas. Environ. Sci. Technol. 48, 8823–8830. https://doi.org/10.1021/ es501090e.
- Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., Thompson, R.C., 2014. The deep sea is a major sink for microplastic debris. R. Soc. Open Sci. 1 https://doi.org/10.1098/ rsos.140317.
- Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492. https:// doi.org/10.1016/j.envpol.2013.02.031.
- Zhang, F., Wang, X., Xu, J., Zhu, L., Peng, G., Xu, P., Li, D., 2019. Food-web transfer of microplastics between wild caught fish and crustaceans in East China Sea. Mar. Pollut. Bull. 146, 173–182. https://doi.org/10.1016/j.marpolbul.2019.05.061.
- Zhang, D., Cui, Y., Zhou, H., Jin, C., Yu, X., Xu, Y., Li, Y., 2020. Microplastic pollution in water, sediment, and fish from artificial reefs around the Ma'an Archipelago, Shengsi, China. Sci. Total Environ. 703, 134768 https://doi.org/10.1016/j. scitotenv.2019.134768.
- Zhang, T., Sun, Y., Song, K., Du, W., Huang, W., Gu, Z., Feng, Z., 2021. Microplastics in different tissues of wild crabs at three important fishing grounds in China. Chemosphere 271, 129479. https://doi.org/10.1016/j.chemosphere.2020.129479.
- Zhu, L., Wang, H., Chen, B., Sun, X., Qu, K., Xia, B., 2019. Microplastic ingestion in deepsea fish from the South China Sea. Sci. Total Environ. 677, 493–501. https://doi.org/ 10.1016/j.scitotenv.2019.04.380.