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Microplastic contamination and risk assessment in blue shark (*Prionace glauca*) from the eastern tropical Pacific Ocean

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ABSTRACT

We quantified the abundance and characteristics of microplastics in the blue shark, *Prionace glauca*, found in the eastern tropical Pacific Ocean and investigated the potential microplastic pollution risks. Microplastics (MPs) were detected in 39.1 % of specimens, up to 0.15 ± 0.38 items/g wet weight of the posterior part of the pylorus, and were sized 45.87 to 3220.12 μm. The majority were fibrous in shape (83.3 %) and blue in color (72.2 %). Both sexes of sharks had similar MP abundance and characteristics, except for polymers, with polyethylene terephthalate and polypropylene representing the dominant type in males and females, respectively. Most individuals experienced low pollution, but one male *P. glauca* exhibited a high ecological risk level owing to the high MP abundance and detection of polyvinyl chloride. This study provides an important baseline for the ingestion of microplastics by pelagic shark species and is a preliminary quantitative measure that could be used in future studies of the risk of MPs.

1. Introduction

In recent decades, plastic pollution has become a global concern (MacLeod et al., 2021). Between 1950 and 2015, approximately 6300 million metric tons of plastic waste was generated (Uddin, 2021). Unfortunately, only 9 % of plastics are recycled while 12 % are incinerated. As a result, a large amount is emitted into the environment (Geyer et al., 2017). Plastics entering the ocean can be broken and degraded via UV radiation, wind, oceanic currents, and microbial degradation to form microplastics (MPs) with sizes <5 mm (Andrades et al., 2018; Barnes et al., 2009; Nauendorf et al., 2016). Several studies have confirmed that MPs can produce a series of toxicological effects such as oxidative stress, endocrine disruption, and developmental disorders in a wide range of marine taxa (Barboza et al., 2018; Uddin, 2021).

Studies investigating the ingestion and impact of MP on the lives of wild marine species are increasing (Avio et al., 2017; Barboza et al., 2020), but are still limited to sharks (Smith, 2018) and none of them have considered potential risk assessment. As a top oceanic predator, the blue shark (*Prionace glauca*) plays an important role in the open ocean food web, although it is also one of the most abundant bycatch shark species in tuna longline fisheries (Carvalho et al., 2010; da Silva et al., 2021). Recently, it was assessed as near threatened by the IUCN due to anthropogenic threats, such as overfishing and discard-induced mortality (Clarke et al., 2014; Sosa-Nishizaki et al., 2009; Zonn et al., 2021). *P. glauca* is a highly migratory species which can be found in tropical and temperate waters, and from the surface of the water to depths of >1000 m (Megalofonou et al., 2005; Stevens et al., 2010). It feeds on a variety of prey including cephalopods, fish, and crustaceans (Kubodera et al., 2007; Markaida and Sosa-Nishizaki, 2010). Such foraging behavior makes *P. glauca* vulnerable to MP pollution, either through direct capture or indirect ingestion of MPs via prey items. Moreover, *P. glauca* is entangled by accidental biting of plastics and hooked by ghost fishing gear (Colmenero et al., 2017; Parton et al., 2019). Previous studies have shown that *P. glauca* can eject its stomach contents by gastric eversion to get rid of indigestible items (Barreto et al., 2019; Brunnschweiler et al., 2005). However, as most studies have been conducted on *P. glauca* caught from nearshore areas and macroplastics (Table 1), knowledge of the presence of MPs in individuals from oceanic regions remains incomplete. Given the large-scale distribution of *P. glauca* in oceanic ecosystems, this lack of knowledge may threaten its conservation.

In the present study, we quantified and described MPs found in the posterior part of the pylorus of *P. glauca* in the eastern tropical Pacific

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Table 1

Studies on the occurrence of plastic in blue shark *Prionace glauca* globally.

Type of plastic	Study area	Maturity stage	N	Examined tissue	Size range	Main color	The main type of polymer	Frequency of occurrence (%)	Methodology	References
Microplastic	Eastern Tropical Pacific	Juvenile	23	The posterior part of the pylorus	45.87–3220.22 μm	Blue, transparent, brown	PET, PP, PVC	39.1 %	Dissection, 10 % KOH digestion, micro-FTIR	This study
Macroplastic and microplastic	Ligurian Sea (North-Western Mediterranean Sea)	Juvenile Adult	43 52	Stomach content	<5 mm:25.7 % 5-25 mm: 54.3 % >25 mm:20 %	Green, transparent, white	PE, PP PE, PP, PS	34.9 % 17.3 %	Visual sorting by a microscope, FT-IR	Bernardini et al. (2018)
Macroplastic	Southeastern and Southern off Brazil	nd	110	Stomach content	>25 mm	nd	nd	0.04 %	Dissection, Visual observation	Barreto et al. (2019)
Macroplastic	South Pacific Ocean (south of the Peruvian Sea)	nd	136	Stomach content	17.5–25.5 cm	Blue, white, yellow	PE	2.2 %	Dissection, Visual observation, FT-IR	Fernández and Anastasopoulou (2019)
Macroplastic	Off Nova Scotia, Canada	Juvenile Adult	270 279	Stomach	>25 mm	nd nd	nd nd	1.5 % 1.1 %	Dissection, Visual observation	McCord and Campana (2003)

N, number of samples; nd, not detected; PET, polyethylene terephthalate; PP, polypropylene; PVC, polyvinyl chloride; PE, polyethylene; PS, polystyrene.

Ocean. We also conducted an initial environmental risk assessment for MPs in *P. glauca*. The MP risk assessment process in wild marine organisms remains difficult because laboratory studies are hampered by the use of exposure conditions compared to environmentally relevant thresholds (Koelmans et al., 2017). Herein, based on the MP abundance in *P. glauca*, the pollution load index (*PLI*) approach was applied to assess the risk of MP with the aim of developing a preliminary risk assessment framework (Xu et al., 2018; Kabir et al., 2021; Wu et al., 2022).

2. Materials and methods

2.1. Sampling and sample preparation

Twenty-three *Prionace glauca* specimens were collected as bycatch in a tuna longline fishery operating in the eastern tropical Pacific Ocean (6°S–6.5°N, 98°–123°W) between September 2019 and January 2020 (Fig. 1). Biometric parameters such as fork length (range 150.0 to 184.0 cm, 172.8 ± 9.1 cm, mean ± standard deviation (SD)) and sex were recorded (Table 2). The posterior part of the pylorus was dissected and used for analysis. All subsamples were wrapped in aluminum foil bags, stored at −20 °C and transferred to the laboratory for further processing.

2.2. Isolation, observation, and identification of microplastics

Microplastic extraction was performed and modified according to the methods of previous studies (Kühn et al., 2017; Maes et al., 2020). Before digestion, the surface of each sample was carefully washed three times with ultrapure water (Milli-Q water) to remove any adhered impurities and placed in a clean petri dish to determine the wet weight. Each sample was then transferred into 300/500 mL conical flasks equipped with 20 mL of 10 % alkaline potassium hydroxide (KOH) solution per gram of tissue wet weight and covered with aluminum foil to avoid contamination. The conical flask was then placed in an oscillation incubator at 70 °C and 150 pm after 18 to 24 h. Then, the digestion solution was filtered through a glass fiber filter (2.7 μm pore size, 47 mm diameter, Whatman Inc.). The filters were observed under a stereomicroscope (SZX2-FOF; Olympus) coupled with a digital camera (U-TV0.63XC; Olympus). Each suspected microparticle was photographed, its shape and color were recorded, and the maximum length was measured using ImageJ version 1.50. A blank filter was left open to the air to control contamination and was checked immediately after the samples were observed. All suspected microparticles were further analyzed using a micro-Fourier transform infrared spectroscope (micro-FTIR, Spotlight 400, PerkinElmer) to validate the results and identify the polymers. Polymer identification was performed by comparing scanned spectra with a match of over 70 % or considered to have reliable spectral matches (after visual inspection).

2.3. Quality control

Procedural blanks were implemented in all experimental processes, that is, dissection of the shark, isolation, and observation of the MPs. Clean Petri dishes were placed next to the working zone and checked after each analysis. Reference materials such as vessel coatings and fishing gear were also collected from the ship. In the case of potential background contamination, the same characteristics of microparticles, according to shape, color, and size, were removed from the results. Researchers wore cotton lab coats and nitrile gloves, and all containers and tools were covered as much as possible to avoid the deposition of atmospheric fibers.

2.4. Risk assessment of microplastics

To the best of our knowledge, there is currently no standardized protocol for systematically evaluating the potential risks of MPs in

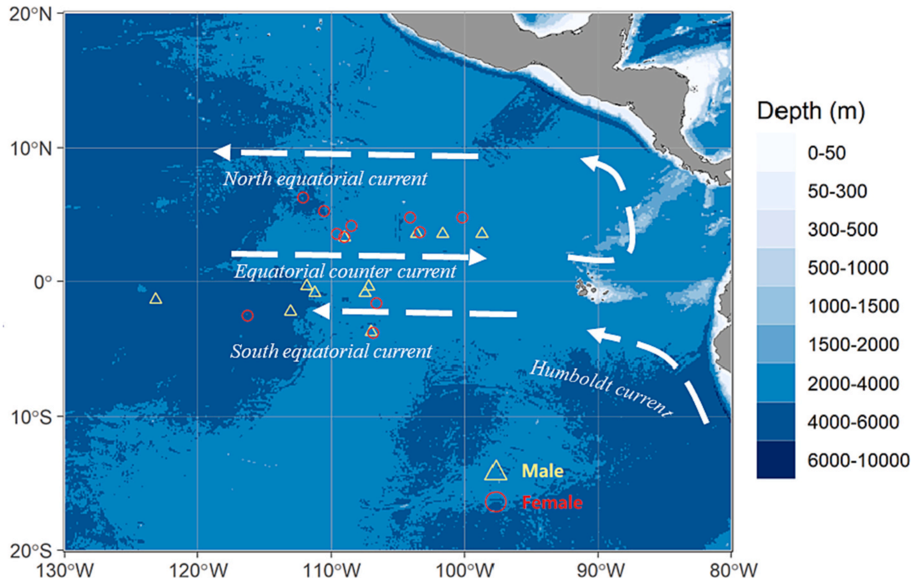

Fig. 1. Sampling locations of the blue shark *Prionace glauca* specimens in the eastern tropical Pacific Ocean between September 2019 and January 2020.

Table 2
Summary information of sampled *Prionace glauca*. M: male; F: female.

No.	Fork length (cm)	Sampling location	Date	Sex
1	173	6°16′N, 112°08′W	2019/10/01	F
2	183	5°16′N, 110°33′W	2019/10/03	F
3	178	3°49′S, 106°51′W	2019/10/24	F
4	177	4°48′N, 104°04′W	2019/10/28	F
5	150	4°48′N, 100°08′W	2019/10/28	F
6	162	4°09′S, 108°30′W	2019/11/04	F
7	168	3°40′N, 103°20′W	2019/11/05	F
8	184	3°19′N, 109°01′W	2019/11/06	F
9	182	1°38′S, 106°37′W	2019/12/23	F
10	164	3°34′N, 109°36′W	2019/12/29	F
11	181	3°34′N, 109°36′W	2019/12/29	F
12	168	2°33′S, 116°18′W	2020/01/05	F
13	180	1°20′S, 123°09′W	2019/09/18	M
14	165	0°21′S, 107°12′W	2019/10/14	M
15	180	3°45′S, 107°01′W	2019/10/23	M
16	179	3°34′N, 103°36′W	2019/10/27	M
17	184	3°34′N, 101°38′W	2019/10/27	M
18	164	3°34′N, 98°41′W	2019/10/27	M
19	159	3°19′N, 109°01′W	2019/11/06	M
20	171	0°51′S, 107°29′W	2019/11/09	M
21	171	0°50′S, 111°15′W	2019/11/21	M
22	178	0°19′S, 111°52′W	2019/11/22	M
23	173	2°14′S, 113°05′W	2019/11/25	M

pelagic predators. Consequently, we applied MP abundance to assess the MP pollution risk in the blue shark.

Risk assessment was expressed as pollution load index (*PLI*) which refers to the MP abundance (Tomlinson et al., 1980). The formulas employed for *PLI* were as follows:

$$\mathrm{CF_i} = \mathrm{C_i}/\mathrm{C_0}$$

$$\mathrm{PLI_i} = \sqrt{\mathrm{CF_i}}$$

where C_i is the MP abundance for individual i and C_0 is defined as the baseline MP abundance, which is theoretically a reference value of the minimum average MP abundance in shark species. Given the absence of a reference value for our study area and species, we followed the approach adopted in previous studies (Liu et al., 2022; Pan et al., 2021; Xu et al., 2018) and assigned C_0 to the published minimum average MP abundance (0.006 items/g) of shark species (blackmouth catshark *Galeus melastomus*, Alomar and Deudero, 2017). Variation in the selected constant does not affect the relative relationships of *PLI* among individuals or between sexes, only absolute values, and therefore does not influence the comparison results.

2.5. *Statistical analysis*

Since the data did not satisfy the supposition required to perform a parametric ANOVA, the non-parametric Mann–Whitney *U* test was used to test if there were any significant differences between the sexes. All statistical tests were carried out using SPSS version 19.0 (IBM Corp., Armonk, NY, USA) with a significance level of $p \leq 0.05$. All results are presented as mean ± standard deviation (SD).

3. Results

3.1. *Abundance of microplastics in shark*

A total of 93 suspected microparticles were identified by visual sorting. From these, 18 microparticles (19.4 %) were confirmed to be plastic polymers by micro-FTIR. For all *P. glauca* analyzed, 9/23 (39.1 %) specimens contained these MPs with an average abundance of 0.15 ± 0.38 items/g wet weight of the posterior part of the pylorus (Table 2). No difference in fork length was observed between male (176.18 ± 14.43 cm) and female (172.50 ± 10.38 cm) specimens (Mann–Whitney test, $U = 63$, $p = 0.85$). Both sexes of sharks also had a similar abundance of MPs (Mann–Whitney test, $U = 49$, $p = 0.24$). For male *P. glauca*, 5/11 (45.1 %) specimens contained 14 MPs and had an average abundance of 0.26 ± 0.54 items/g wet weight of the posterior part of the pylorus. In females, four MPs were detected in 4/12 (33.3 %) individuals, with an average abundance of 0.05 ± 0.08 items/g wet weight of the posterior part of the pylorus.

3.2. *Morphology, color, and chemical composition of microplastics*

These MPs were classified as fibers ($n = 15$, 83.3 %) or fragments ($n = 3$, 16.7 %), and no films or pellets were found (Fig. 2). The size range of all MPs was 45.87 to 3220.12 μm (794.88 ± 799.12 μm). Fibrous MPs ranged from 62.48 to 3220.12 μm (937.59 ± 802.61 μm), while fragments had a much narrower size range, from 45.87 to 134.36 μm (81.29 ± 46.81 μm). The size range of MPs in males was 63.65 to 3220.12 μm (908.83 ± 824.71 μm) and the average size of MPs in females was 396.02 ± 631.40 μm, and ranged from 45.87 to 1341.36 μm. The results

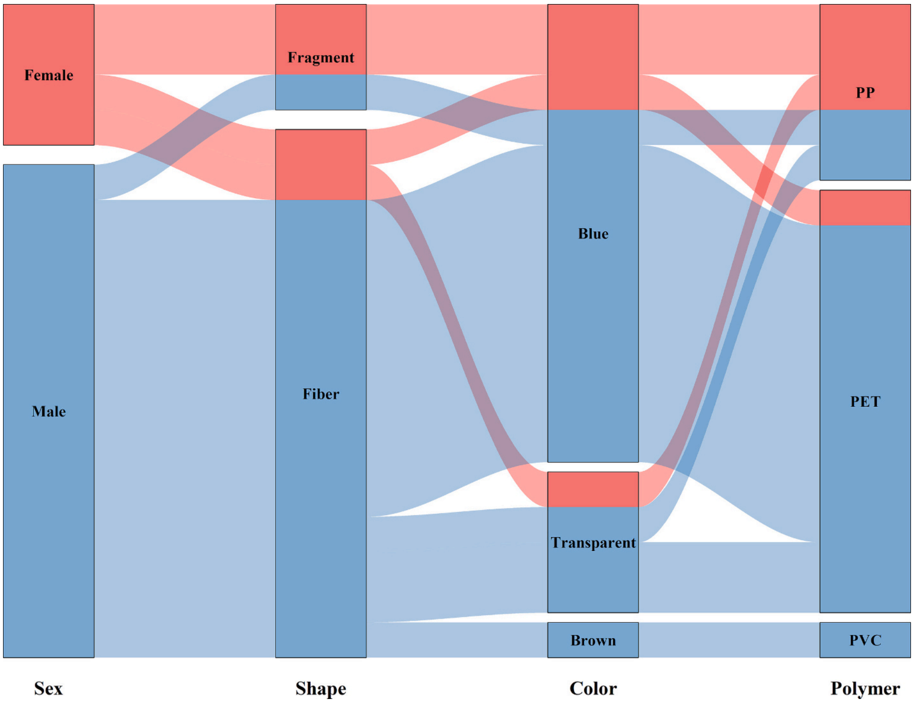

Fig. 2. Alluvial diagram summarizing the characteristics of microplastics in the posterior part of the pylorus of blue shark *Prionace glauca*. PET, polyethylene terephthalate; PP, polypropylene; PVC, polyvinyl chloride.

of the Mann–Whitney test showed that both sexes had a similar MP size (U = 13, $p = 0.11$), while the variations were considerable in contaminated individuals (Fig. 3). Three colors were observed in the detected MPs (Fig. 2; Fig. 4). The majority were blue (72.2 %), followed by transparent (22.2 %), and brown (5.6 %). Blue represents the dominant color of the fibers (66.7 %) and comprises 100 % of the fragments. It is also a common color of MPs in both sexes (male:71.4 %, female:75.0 %).

Three polymer types were identified: polyethylene terephthalate (PET), polypropylene (PP), and polyvinyl chloride (PVC) (Fig. 2; Fig. 4). The most frequently used polymers were PET (66.7 %) and PP (27.8 %). The fibers consisted of all polymers, mainly PET (80.0 %), followed by PP (13.3 %) and PVC (6.7 %), whereas the fragments only consisted of PP. Considerable variability in the polymer type of MPs was observed between sexes. MPs in male individuals had a higher proportion of PET (82.0 %), whereas PP (75 %) represented the dominant type of MPs in females.

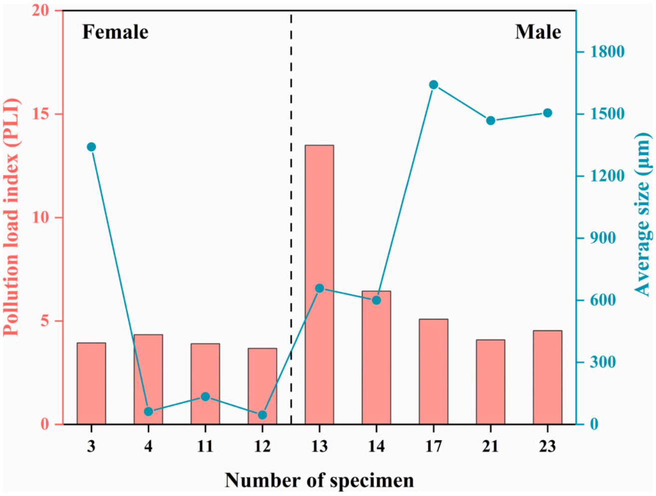

Fig. 3. Risk assessment and the average size of microplastics in each specimen of *Prionace glauca* (see Table 2 for detailed biometric parameters) by using the pollution load index (*PLI*) approach.

3.3. Risk assessment of microplastics

For *P. glauca* containing MP, 88.9 % (8/9) of the contaminated individuals had *PLI* values lower than 10, indicating that the pollution was relatively low, while specimen No. 13 had high pollution loads ($PLI = 17.41$), corresponding to the high MP abundance. Most of the samples had low pollution and specimen No. 13 was highly polluted.

4. Discussion

The blue shark *Prionace glauca* is a high-trophic-level predator that migrates over a wide range of horizontal and vertical scales, making it a potential bioindicator species of MP contamination in a vast three-dimensional oceanic region (Bernardini et al., 2018; Weigmann, 2016). In this study, the detection rate of MPs in *P. glauca* was much higher than in previous studies performed on this species from nearshore areas (0.04 to 2.2 %, Table 1) (Barreto et al., 2019; Fernández and Anastasopoulou, 2019). However, these studies only adopted a visual observation method; thus, the occurrence of MPs was not explicitly investigated. Our findings are similar to those reported by Bernardini et al. (2018), where 34.9 % of *P. glauca* specimens from the northwestern Mediterranean Sea contained MPs. This high detection rate may be explained by the large migratory movement of *P. glauca* (Kubodera et al., 2007; Nakano and Stevens, 2009). Such behavior can cause *P. glauca* to more likely be exposed to MP-contaminated water columns than other marine organisms. Indeed, several studies have quantitatively recorded up to 21,290 tons of MPs floating in the eastern Pacific Ocean (Law et al., 2014), and Alfaro-Núñez et al. (2021) detected the presence of MPs in 100 % of the collected seawater samples from the eastern tropical Pacific. These MPs accumulate around the Pacific subtropical gyres under the transportation of large-scale convergence in ocean currents, such as the Equatorial current and Humboldt current (Fig. 1) (Eriksen et al., 2013; Law et al., 2014) and greatly increase the possibility of randomly ingested MPs by highly migratory species. In addition, *P. glauca* is

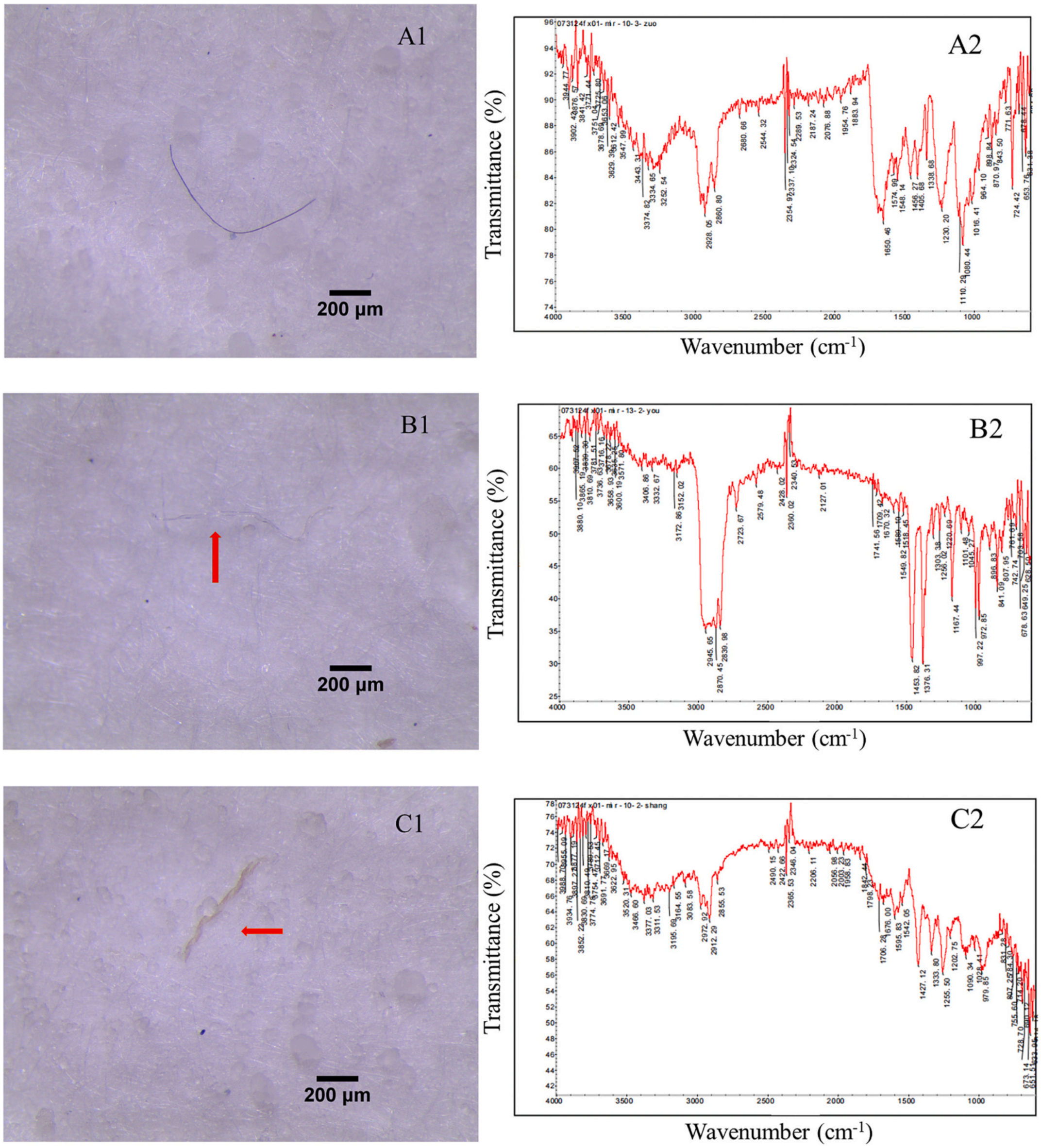

Fig. 4. Photographs of microplastic items identified using micro-FT-IR. A1–C1 were taken under a stereomicroscope, A2–C2 are the spectra for the items. A1, blue fiber; B1, transparent fiber; C1, brown fiber; A2, polyethylene terephthalate; B2, polypropylene; C2, polyvinyl chloride. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

characterized as an opportunistic predator (Cortés, 1999; Vaske Júnior et al., 2009) that can potentially consume prey containing MPs. It feeds on a variety of cephalopods (e.g. jumbo squid *Dosidicus gigas*), fish (e.g. chub mackerel *Scomber japonicus*) and crustaceans (Kubodera et al., 2007; Markaida and Sosa-Nishizaki, 2010). Several studies have demonstrated that *D. gigas* (Gong et al., 2021) and mesopelagic fish, including *S. japonicus* (Neves et al., 2015), had been contaminated by MPs. This high MP detection rate has also been reported in other pelagic sharks, such as the whale shark *Rhincodon typus* (Sampaio et al., 2018; Yong et al., 2021) and the porbeagle shark *Lamna dnasus* (Maes et al., 2020) with a range of 47.5 % to 100 %.

Compared to the observations of previous studies on the plastic ingestion of *P. glauca* (Table 1), no macroplastics were found in our specimens. The lack of macroplastics might be due to the tissue used in this study being the posterior part of the pylorus, which had a relatively small volume. Ingested food particles of *P. glauca* must pass through a relatively narrow junction between the pylorus and the stomach and may reduce the presence of large-sized plastic. In addition, sharks commonly regurgitate undigested remains; larger plastic particles may be expelled together with a relatively short residence time (Valente et al., 2019), and therefore may not enter the pylorus. Our results were similar to the MP size of other shark species, such as the small-spotted catshark *Scyliorhinus canicula* (range 380–3100 μm) from the Spanish Atlantic and Mediterranean coasts (Bellas et al., 2016) and the whale shark (1120 ± 700 μm) from the Philippines (Yong et al., 2021).

Evidence suggests that sexual foraging segregation exists in juvenile *P. glauca* (McCord and Campana, 2003), which might result in sex-specific MP ingestion. Unexpectedly, no significant difference was found in the abundance and size of MPs between male and female *P. glauca*, a result that has also been reported for other shark species (Parton et al., 2020). In contrast, Alomar and Deudero (2017) demonstrated a significant difference in MP abundance of juvenile blackmouth catsharks according to sex in some sampling locations in the western Mediterranean. This inconsistency among studies may be linked to the spatial foraging ecology of blackmouth catsharks. Thus, given the large-scale distribution of *P. glauca*, the potential difference in MP abundance between male and female individuals remains to be explored. A similar color composition of MPs was also found between male and female *P. glauca*, with blue being the predominant color recovered. This matches the high detection frequency and high amount of this MP color in seawater and other sharks (Kutralam-Muniasamy et al., 2020; Parton et al., 2020). Interestingly, different polymer types of MPs were observed between male and female individuals. The MPs in male *P. glauca* were dominated by polymers (e.g., PET, 1.34 g/cm^3 and PVC, 1.395 g/cm^3) with a density higher than that of seawater (1.025 g/cm^3). For females, however, low-density PP (0.95 g/cm^3) was the most abundant polymer.

There is currently no standardized method for monitoring the risk of MPs in wild marine organisms, although researchers have recently incorporated environmental influences (Liu et al., 2022; Pan et al., 2021; Xu et al., 2018). Moreover, the potential ecological risk posed by MPs has not been determined because laboratory studies are hampered by unrealistic exposure conditions (Koelmans et al., 2017). In this study, we conducted a preliminary risk assessment for MPs in *P. glauca*. According to the calculated pollution load index (Fig. 3), *P. glauca* could be evaluated as having a low pollution load; however, this was not true for specimen No. 13, which was assessed as having a high pollution load owing to the high MP abundance. The specimen also contained PVC polymer. PVC is considered one of the most harmful polymers in the aquatic environment (Lithner et al., 2011), and can release carcinogenic monomers and intrinsic plasticizers, resulting in lethal effects for marine animals (Green et al., 2016). The strongly uneven *PLI* values and the associated high polymeric risk indicate that *P. glauca* is exposed to highly variable MP risk levels in the study area. The MP size-frequency distributions indicated that small-sized MPs were the most abundant in the posterior part of the pylorus of *P. glauca*. And given the size-dependent toxicity of MPs (Rebelein et al., 2021), the MPs detected might have adverse effects on the tissues of *P. glauca.* However, there is no evidence that MPs have toxic effects on sharks, and further research is necessary. In addition, fibers constituted the largest proportion (83.3 %) of MPs. Fibrous MPs have larger aspect ratios with a rough surface, are more easily embedded in tissues, and reside longer, which can cause mechanical damage and more severe toxicological effects in organisms compared to other types of MPs (Kutralam-Muniasamy et al., 2020; Rebelein et al., 2021).

This is the first study to investigate the abundance and characteristics of MPs in *P. glauca* from the eastern tropical Pacific Ocean and further provide a risk assessment based on the abundance of MPs for a better understanding of the MP pollution in *P. glauca*. Future research efforts should focus on the distribution patterns of MPs in different tissues, to better assess the potential impact of oceanic sharks. Future studies with different geographic populations of *P. glauca* are also needed to more precisely and comprehensively explore the individual specialization of MP contamination levels and potential risks.

CRediT authorship contribution statement

Xuemin Huang: Conceptualization, Methodology, Software, Formal analysis, Writing – original draft. **Huachen Gao:** Methodology, Validation, Formal analysis, Investigation. **Zezheng Li:** Methodology, Validation, Formal analysis, Investigation. **Feng Wu:** Methodology, Validation, Formal analysis, Investigation. **Yi Gong:** Resources, Writing – review & editing, Supervision, Funding acquisition. **Yunkai Li:** Resources, Writing – review & editing, Supervision, Funding acquisition.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2022.114138.

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