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The microplastic pollution in beaches that served as historical nesting grounds for green turtles on Hainan Island, China

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ABSTRACT

This study evaluated microplastic pollution in beaches that have served as historical nesting grounds for green turtles in Hainan Island, China and explored the sources of microplastic pollutants to conduct habitat restoration for sea turtles. The average abundance of the microplastics in the beach surface sediments was 2567.38 ± 2937.37 pieces·m^{-2} or 641.85 ± 734.34 thousand pieces·m^{-3}, foam and plastic block were the main microplastics identified. Microplastic size was predominantly within the 0.05–1 mm category (small microplastic particles), and most microplastic particles were white. Polystyrene and polyethylene were the dominant plastic compositions. The type and compositions of microplastics indicate that most microplastics in this study were broken from large plastic blocks and foam. To reduce the threat of microplastic pollution to marine life, including sea turtles, we suggested removing plastic litter, especially small plastic on beaches, and replacing and recovering the foam used in aquaculture before it ages and fragments.

Increasing amounts of ocean plastic litter is a common problem worldwide (Martin et al., 2019). Plastic litter floating on the sea surface is rapidly moved to the beach under the action of wind, waves and ocean currents, and continuously stranded and accumulated (Fang et al., 2021). Beach environments are conducive to the fragmentation of plastic litter (Fok et al., 2017). The chemical and mechanical breakdown of plastic debris is promoted during saltation in a beach environment (Corcoran et al., 2009; Hopewell et al., 2009) and plastic litter on the beach is also prone to embrittlement and degradation under the influence of ultraviolet light, tidal erosion and biological effects which form plastic fragments of different sizes and even microplastics (Andrady, 2011; Munari et al., 2017). According to the National Oceanic and Atmospheric Administration (NOAA) "microplastics are not specific types of plastics, but any type of plastic fragments with a diameter of less than 5 mm" (Andrady, 2011). Compared with large-size plastics, microplastics are more widely distributed in the environment, more abundant and easier to be ingested by marine organisms (Moreira et al., 2016; Nelms et al., 2016).

Microplastics are a new type of pollutant in the marine environment today and global attention on microplastic pollution continues to increase (Andrady, 2011). A number of studies have recently investigated microplastics in beach sediments and have indicated their prevalence in South Korea (Lee et al., 2015), New Zealand (Bridsona et al., 2020), the Canary Islands (Herrera et al., 2018), Hong Kong, Guangdong and Taiwan of China (Fok and Cheung, 2015; Fok et al., 2017; Kunz et al., 2016) and other coastal ares.

Sea turtles rely on beaches to reproduce, and most adult females return to their birthplace to lay eggs (Triessnig et al., 2012). Microplastics can increase overall beach temperature which affects nest temperature and leads to gender imbalance of sea turtles (Andrady, 2011; Beckwith and Fuentes, 2018). In addition, microplastics often contain harmful chemical pollutants such as heavy metals, organic pollutants, or plasticizers, which can move into sea turtle eggs *via* osmosis, affecting embryonic development, decreasing hatching success and ultimately threatening population sustainability (Bergeron and Mclachlan, 1994; Yang et al., 2011). Duncan et al. (2018) suggested that the presence of microplastics in nesting grounds may affect the hatching success rate and sex ratio of sea turtles.

In 2018, the Chinese Government issued the "Sea Turtle Conservation Action Plan (2019–2033)" which encourages the restoration of historical nesting grounds (Chinese Ministry of Agriculture and Rural Affairs, 2019). As an important nesting ground for green turtles

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historically, the beginning of sea turtle habitat restoration is imminent in Hainan. Therefore, it is necessary to evaluate microplastic pollution of these historical nesting grounds and propose management practices to prepare for the return of the sea turtles to the local areas to lay eggs. In the present study, we surveyed microplastic pollution at 13 historical nesting grounds of green turtles on Hainan Island to: 1) evaluate the status of microplastic pollution, 2) describe the morphological characteristics of microplastics, and 3) identify the sources of microplastic pollutants. We also proposed management practices based on the survey results. This study will provide guidance for future beach monitoring and the formulation of measures to prevent plastic pollution in sea turtle habitats.

Geographic coordinates of 13 historical nesting grounds were recorded using a global positioning system (Fig. 1). Sediment samples with an area of 25 × 25 cm and a depth of 0–2 cm were collected on the "strand line" (SL) and the "turtle nesting line" (TNL) of 13 historical nesting grounds, and sampling was repeated three times at each nesting ground (Beckwith and Fuentes, 2018). The samples were stored in sampling bags and transported back to the laboratory for separation and detection of microplastic.

Following the saturated sodium chloride density method described in Thompson et al. (2004), microplastics in the sediment samples were separated, and the experimental procedures were optimized. Details are as follows. For each sample, 250 cm^3 of sand was placed in a beaker; 500 mL of saturated sodium chloride solution (1.2 $g \cdot cm^{-3}$), the mixture was stirred for 2 min and left to settle for 10 min. Then, the supernatant was passed through a 300-mesh sieve, and the sodium chloride solution was recovered and reused. These steps were repeated three times. The remaining components from the beaker were added to a 500 mL 60% sodium iodide solution (1.8 $g \cdot mL^{-1}$) and stirred for 2 min, after which the mixture was left to settle for 10 min. Then, the supernatant was passed through a 300-mesh sieve, and the sodium iodide solution was recovered and reused. These steps were repeated twice. The residue on the screen was rinsed with distilled water into a 100 mL Erlenmeyer flask, 10% potassium hydroxide solution was added and the mixture was left to digest for two days. After two days, the supernatant solution was decanted and filtered through a 0.45 μm glass fiber membrane (GF/F, 47 mm Ø, Whatman, Shanghai, China) using a vacuum filtration device (GM-0.33A, Zhengzhou, China). Finally, the filter membrane was stored in a clean glass petri dish for one-step analysis (Wang et al., 2018; Thompson et al., 2004).

All samples on the filter membrane were observed under a stereo microscope (SMZ-168 SERIES, MOTIC, Xiamen, China), and images were obtained with a SONY DSC-RX10M2 digital camera. According to their morphological characteristics, microplastics were divided into five categories based on type (foams, plastic blocks, fibers, microbeads, and films) and seven colors (black, white, yellow, green, gray, blue, and other). Nano Measurer 1.2 software was used to count the number of microplastic particles, and particle size was determined by measuring the length of the longest side of the microplastic particle (Harrison et al., 2012) and categorized based on size (0.05–1.00, 1.01–2.00, 2.01–3.00, 3.01–4.00 and 4.01–5.00 mm) (Fok et al., 2017). For simplicity, integers will be used when the size classes are mentioned below. Among them, microplastics with the particle size of 1–5 mm were considered large, and those with the particle size of <1 mm were considered small (Van Cauwenberghe et al., 2015).

Beaches are gathering areas of ocean microplastics and are considered key areas of environmental pollution (Barnes et al., 2009; Poeta et al., 2014; Nelms et al., 2016). In the present study, we found that the 13 investigated historical nesting grounds of green turtles on Hainan Island were all polluted by microplastics. The abundance of microplastics ranged from 456 to 13,056 $pieces \cdot m^{-2}$ or 114 to 3264 thousand

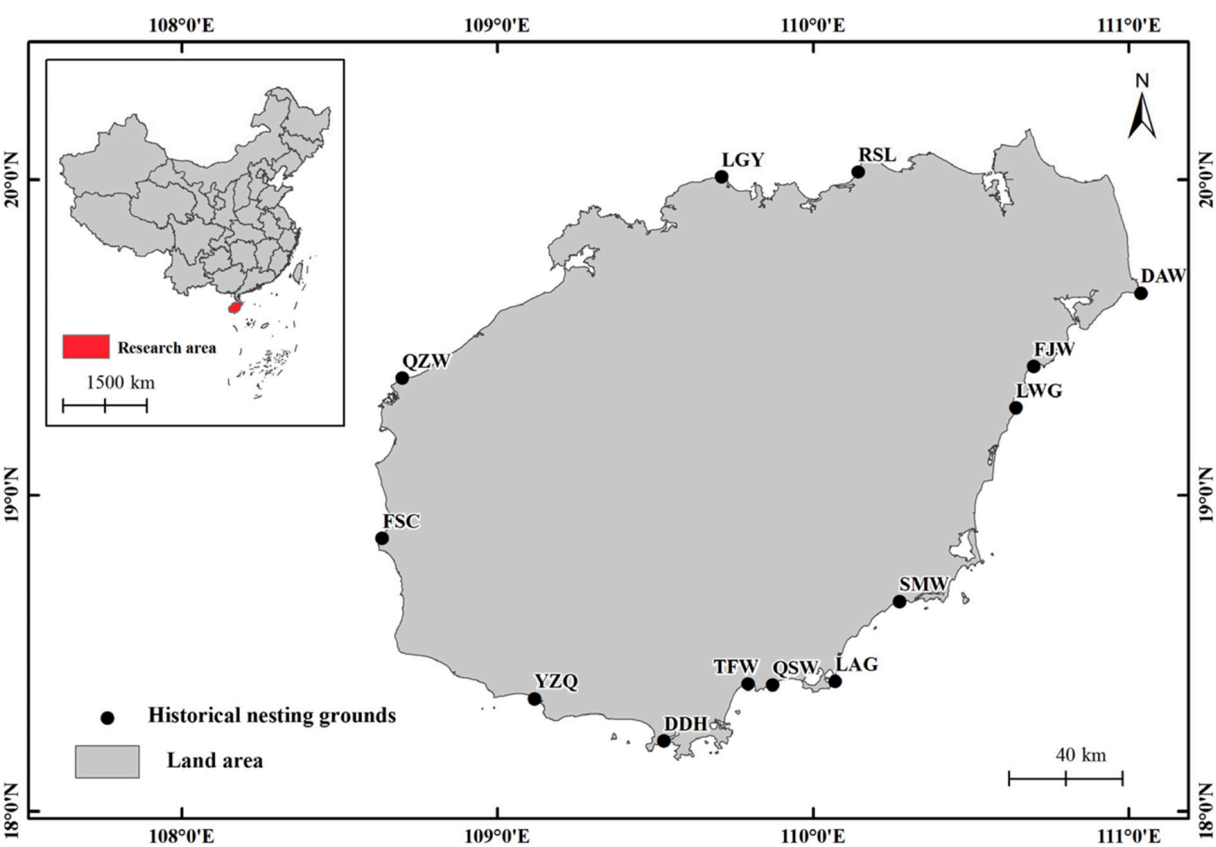

Fig. 1. Map of Hainan Island and 13 historical nesting grounds of green turtles. The historical nesting grounds were namely Da'aowan (DAW), Fengjiawan (FJW), Longwan'gang (LWG), Shimeiwan (SMW), Li'an'gang (LAG), Qingshuiwan (QSW), Tufuwan (TFW), Dadonghai (DDH), Yazhou Qu (YZQ), Fushicun (FSC), Qiziwan (QZW), Lingaojiao Fishermen Village (LGY), and Rongshanliao (RSL).

pieces·m^{-3}, and the average abundance was 2567.38 ± 2937.37 pieces·m^{-2} or 641.85 ± 734.34 thousand pieces·m^{-3}. The distribution of microplastics in the 13 historical nesting grounds had a high degree of spatial difference (Fig. 2). The abundance of microplastics in FJW was significantly higher than in FSC, and both were significantly higher than in the other 11 nesting grounds ($r = 11.324$, $P < 0.001$). Based on our field investigation, there are several shrimp ponds and fish farms near FJW and FSC which are the sites with the highest abundance of microplastics. Several studies have demonstrated that coastal shrimp ponds and fish farms can contribute to microplastic pollution in the local offshore marine environment (Sun et al., 2016; Xu et al., 2020). Thus, we confer that the microplastic pollution in these two nesting sites is a serious issue and is caused by nearby aquaculture farms.

Microplastic pollution is closely related to regional population activities and economic development, however, it is hard to directly compare the concentrations of microplastics in different studies because of the variations in experimental methods (Qiu et al., 2015; Fang et al., 2021). Thus, we only conducted some broad comparisons on the same particle size in different studies. When comparing the abundance of beach microplastics with other areas (Table S1), we found that the abundance of microplastics (0.05–5 mm in size) in the nesting grounds of green turtles on Hainan was lower than in Hong Kong and Guangdong Province. Moreover, the microplastics with a particle size range of 0.05–0.33 mm accounted for 27.79% of all sizes in this study, and the actual abundance is much lower than that of Guangdong and Hong Kong, but higher than Quanfu Island and Ganquan Island of Xisha. We suspect that the lower abundance of microplastics on Hainan and Xisha are associated with distance from the mainland (*i.e.*, the further from the mainland results in less plastic litter).

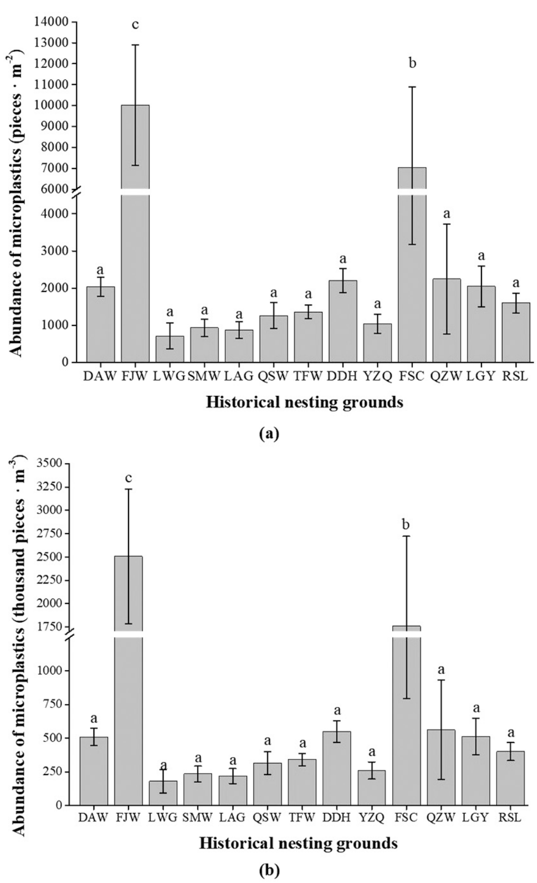

Fig. 2. Distribution and abundance of microplastics in the surface sediments collected from 13 historical nesting grounds on Hainan Island. The lowercase letters above the bars indicate significant differences ($P < 0.05$).

Microplastics can be directly derived from microscale polymers (Zitko and Hanlon, 1991), and large-scale plastic litter through physical, chemical, and biological processes that cause division and volume reduction (Jambeck et al., 2015; Horton et al., 2017). However, secondary microplastics occupy most of the source, compared with primary microplastics (Zhou et al., 2020). In our study, the proportion of the five different microplastic types varied. Plastic blocks accounted for the largest proportion at 67.36%, followed by foams at 24.38% and fibers at 7.56%, whereas microbeads and films were 0.70% (Fig. 3). Previous studies determined that plastic blocks were mainly derived from plastic products commonly used in daily life, such as plastic bottles (Wu et al., 2017), and foam plastic is widely used in coastal marine aquaculture facilities, fishing equipment, seafood storage, and transportation (Fang et al., 2021). In our study site, fishermen are often observed using foam containers for seafood and as alternatives to fishing rafts. Furthermore, earlier investigations have determined that the greatest proportion of beach litter on Hainan is plastic and foam (Zhang, 2020), and Fok et al. (2017) demonstrated that plastic blocks and foam can easily break in a beach environment. Thus, we speculated that most of the microplastics in our study were split from plastic litter, indicating that they were likely of secondary origin (Hazimah and Obbard, 2014).

Plastic degradation mainly occurs through the oxidation process induced by solar ultraviolet radiation (Andrady, 2011). Due to the high temperatures with sufficient strength of solar ultraviolet radiation and weathering, beaches are more suitable than other natural environments for the breakdown of plastic debris (Zhao et al., 2015). Small microplastic particles (0.05–1 mm) comprised the majority of microplastics (68.25%) in this study (Fig. 4), which correlated with the findings of many studies on microplastic distribution in sediments (Vianello et al., 2013; Hazimah and Obbard, 2014; Peng et al., 2017). The average size of microplastics was 0.69 ± 0.89 mm, ranging from 0.05 to 5 mm (Table S2), and the average microplastic size in each nesting ground is indicated in Fig. 5a. There was a significant negative correlation between the mean size and abundance of microplastics in the 13 historical nesting grounds ($R^2 = 0.32$, $F = 5.183$, $P = 0.044 < 0.05$; Fig. 5b). We found that as particle size declined, the abundance of microplastics increased, which was consistent with the apparent decrease in the mean size of plastic debris on Earth along with the increase in abundance of such particles due to continuous degradation (Barnes et al., 2009). However, the smaller the microplastics particle size, the larger their specific surface area, indicating that microplastics are absorbing more

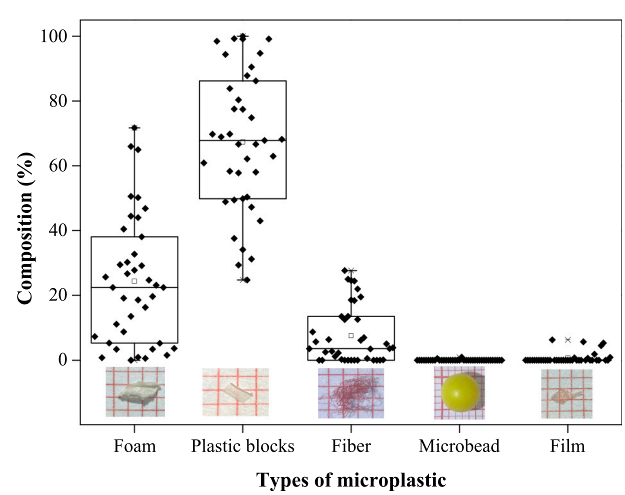

Fig. 3. Composition (%) of microplastics with different types in the surface sediments collected from 13 historical nesting grounds on Hainan ($n = 39$). The solid horizontal lines from the top to the bottom of each box plot indicate the maximum value, 75% quartile, median, 25% quartile, and minimum value. Empty boxes indicate average values, and solid circles indicate the outliers. The small grid represents 1 mm × 1 mm.

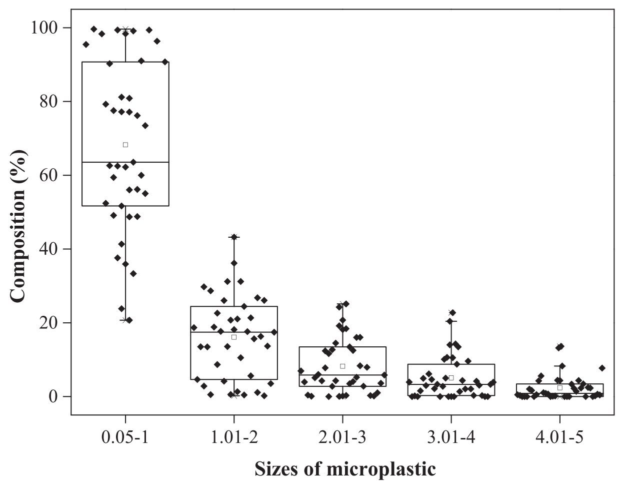

Fig. 4. Composition (%) of microplastics with different sizes in the surface sediments collected from 13 historical nesting grounds on Hainan ($n = 39$). The solid horizontal lines from top to bottom of each box plot indicate the maximum value, 75% quartile, median, 25% quartile, and minimum value. Empty boxes indicate average values, and solid circles indicate the outliers.

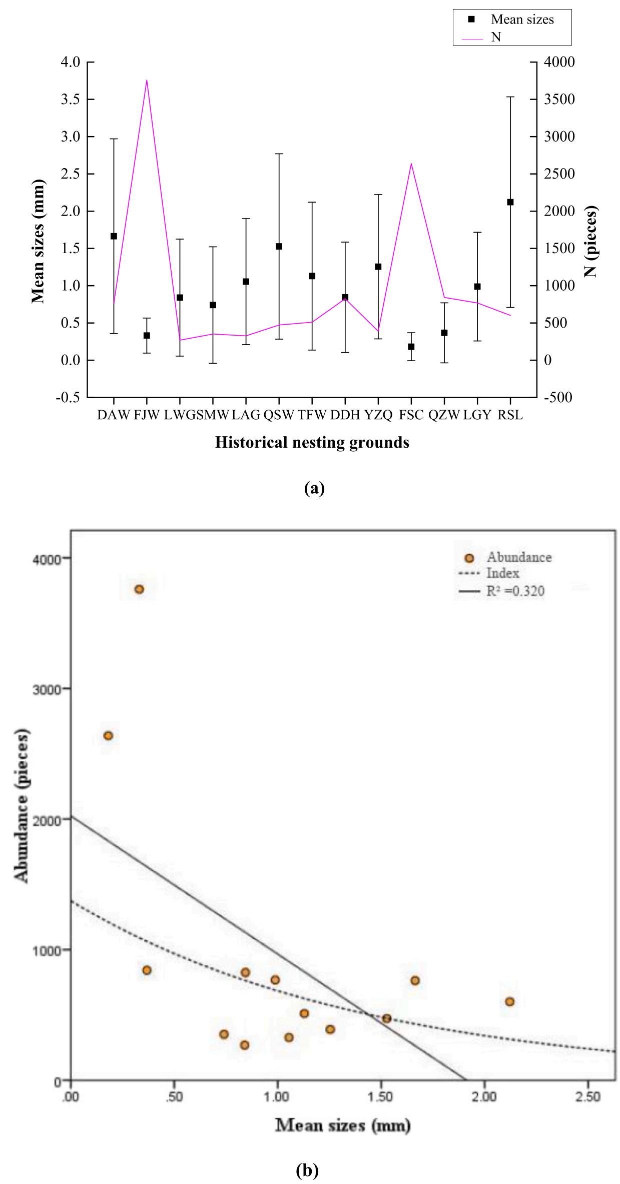

Fig. 5. (a) Mean microplastic size in each nesting ground; (b) correlation between mean microplastic size and abundance in each nesting ground.

pollutants, which may cause greater harm to the hatching of green turtle eggs (Duncan et al., 2018).

The majority of microplastics found in our study were white color (including transparent and white; 70.02%); among them, white foam was the most common type. The second most common color was black (25.60%), whereas multicolored microplastics such as yellow, green, gray, and blue were relatively rare (Fig. 6). This is likely to be the result of weathering and fading of plastics in beach or ocean environments (Hidalgo-Ruz et al., 2012).

We selected a representative subset of microplastics from each group, and their surface structure was tested for polymer types using a Fourier transform infrared spectrophotometer (IRTracer-100, SHIMADZU, Japan). Microplastic particles were placed onto the sample area, and during data acquisition the ATR imaging attachment was in direct contact with microplastics on the filter membrane. The detector spectral range was 600–4000 cm^{-1}, co-adding 16 scans at a resolution of 8 cm^{-1}. The spectra were processed by the Lab Solutions IR software and compared with the IR polymer spectra library. When interpreting the Fourier-transform infrared spectroscopy (FTIR) output, only the readings with confidence levels of 70% or higher were considered reliable and accepted (after visual inspection). All confirmed polymer types were included in our results.

The compositions of microplastic particles among the nesting grounds were heterogeneous; the most common polymer components were Polystyrene (PS) (40.35%) and Polyethylene (PE) (33.33%) (Figs. 7 and 8), which was consistent with the results of previous surveys conducted in Haikou and Wanning of Hainan (Qiu et al., 2015). This is because the sampling sites were located on beaches, which are usually tourism areas and fisherman's wharves where plastic blocks and foam are often discarded. When the plastic blocks and foam degrade into microplastics, PE and PS are usually abundant in the sediments. The result of microplastic compositions once again confirmed that most of the microplastics may have originated from the fragmentation of plastic litter in this study. The most common type of plastic in beach sediments is polystyrene foam (EPS) (Fang et al., 2021). Polystyrene foam has a wide range of uses and a huge output, and it is a typical plastic pollutant in the marine environment (Fok and Cheung, 2015; Xu et al., 2020). The density of EPS is small (0.05 $g \cdot cm^{-3}$), so it easily floats on the sea surface and becomes stranded on beaches. The heat distortion temperature range of EPS is 60–85 °C; temperatures that frequently are reached on beaches. Due to weathering and degradation, coastal beaches have become gathering places for lightweight plastics represented by EPS (Fok and Cheung, 2015).

Beach cleaning activities that remove large plastic debris may be highly effective in preventing the generation of microplastics on beaches (Fok et al., 2017). However, several studies have demonstrated that large amounts of small plastic debris remained at the studied beaches despite beach clean-ups (Ivar do Sul et al., 2011; Zhao et al., 2015; Zhang et al., 2020). These small plastic particles are likely to split into microplastics, thus causing greater harm to the marine environment and organisms. In order to create a good nesting environment for sea turtles, measures should be taken to reduce beach microplastic pollution when restoring these historical nesting grounds. Firstly, increased attention should be paid to nesting grounds with high microplastic abundance. For example, FJW and FSC should be prioritized for restoration due to their high plastic content and it would take longer to repair. Next we should also increase the frequency of beach litter cleaning, especially removing small plastic particles. In addition, we suggest that the foam used in activities such as aquaculture and seafood transportation should be replaced and recovered before aging and fragmentation in order to reduce the pollution with microplastics.

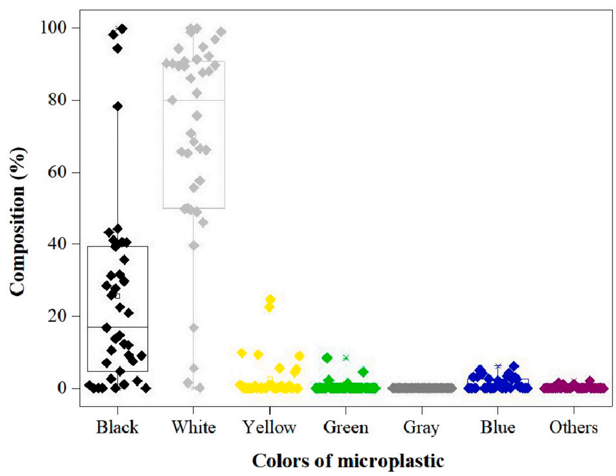

Fig. 6. Composition (%) of microplastics with different colors in the surface sediments collected from 13 historical nesting grounds on Hainan ($n = 39$). The solid horizontal lines from the top to the bottom of each box plot indicate the maximum value, 75% quartile, median, 25% quartile, and minimum value. Empty boxes indicate average values, and solid circles indicate the outliers.

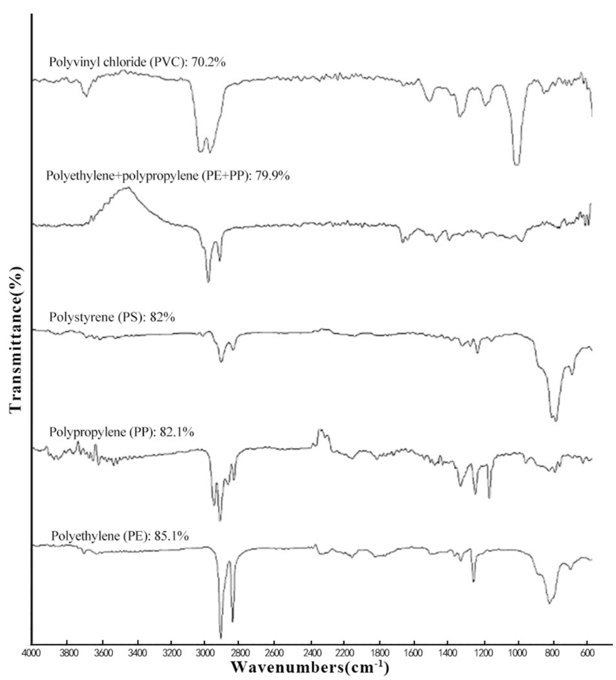

Fig. 7. Raman spectroscopy spectra of the microplastics collected in the present study.

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CRediT authorship contribution statement

Ting Zhang: Conceptualization, Methodology, Investigation, Validation, Formal analysis, Writing – original draft, Writing – review & editing. **Liu Lin:** Conceptualization, Methodology, Investigation, Validation, Formal analysis, Writing – original draft, Writing – review & editing. **Deqin Li:** Visualization, Investigation, Methodology. **Shannan Wu:** Methodology, Writing – review & editing. **Li Kong:** Methodology, Investigation. **Jichao Wang:** Validation, Visualization, Writing – review & editing. **Haitao Shi:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

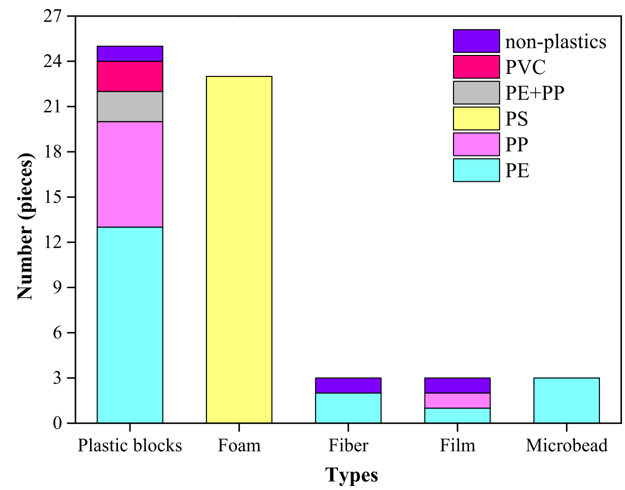

Fig. 8. Components of the selected items from 13 historical nesting grounds on Hainan.

Declaration of competing interest

The authors declare no conflicts of interests.

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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.2021.113069.

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