Microplastic pollution at Qilianyu, the largest green turtle nesting grounds in the northern South China Sea

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As new persistent pollutants, microplastics have recently attracted considerable attention. When they are present in beach sediments, microplastics may adversely affect the nesting and hatching of sea turtles, which rely on beaches to reproduce. In this study, microplastic pollution at Qilianyu was investigated. Qilianyu is located in the northeastern Xisha Islands and has the largest known nesting grounds for green turtles in China. The present results indicate that the average abundance of microplastics in the beach surface sediments was 338.44 ± 315.69 thousand pieces·m^{-3} or 1353.78 ± 853.68 pieces·m^{-2}, with foam and plastic blocks being the main microplastics identified. The microplastic particles were categorized as small and were predominantly within the 0.05–1 mm size category. Most microplastic particles were white. Polystyrene and polyethylene were found to be the most common forms of plastic present. Microplastic pollution was observed not only on the surface of the nesting grounds but also at the bottom of the nests at approximately 60 cm depth, which may be harmful to the incubation of sea turtle eggs. Based on the present findings, removing plastic litter on beaches is suggested to reduce the threat of microplastic pollution to marine life, including sea turtles. This is particularly the case for small pieces of plastic. Furthermore, the foam used in aquaculture should be recovered and replaced before it becomes fragmented owing to age. In addition, regional cooperation between stakeholders in the South China Sea should be strengthened to collectively promote the reduction and cleanup of marine litter.

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Abstract:

As new persistent pollutants, microplastics have recently attracted considerable attention. When they are present in beach sediments, microplastics may adversely affect the nesting and hatching of sea turtles, which rely on beaches to reproduce. In this study, microplastic pollution at Qilianyu was investigated. Qilianyu is located in the northeastern Xisha Islands and has the largest known nesting grounds for green turtles in China. The present results indicate that the average abundance of microplastics in the beach surface sediments was 338.44 ± 315.69 thousand pieces·m^{-3} or 1353.78 ± 853.68 pieces·m^{-2}, with foam and plastic blocks being the main microplastics identified. The microplastic particles were categorized as small and were predominantly within the 0.05–1 mm size category. Most microplastic particles were white. Polystyrene and polyethylene were found to be the most common forms of plastic present. Microplastic pollution was observed not only on the surface of the nesting grounds but also at the bottom of the nests at approximately 60 cm depth, which may be harmful to the incubation of sea turtle eggs. Based on the present findings, removing plastic litter on beaches is suggested to reduce the threat of microplastic pollution to marine life, including sea turtles. This is particularly the case for small pieces of plastic. Furthermore, the foam used in aquaculture should be recovered and replaced before it becomes fragmented owing to age. In addition, regional cooperation between stakeholders in the South China Sea should be strengthened to collectively promote the reduction and cleanup of marine litter.

Keywords: Beach sediment; *Chelonia mydas*; Fourier transform infrared spectrometer; Qilianyu; Microplastics; Nesting grounds.

1. Introduction

Marine plastic pollution is a common worldwide environmental problem (Martin et al., 2019). Plastic fragments in the ocean are easily broken down into many miniature fragments or particles as a result of long-term physical and chemical action (Andrady, 2011; Hopewell et al., 2009). Microplastics are defined as plastic fragments or particles that have a diameter of less than 5mm (Arthur et al., 2009). It is estimated that approximately 51 trillion plastic particles are present in the world's oceans (Tirkey et al., 2021; Wessel et al., 2016). Microplastics in the environment can migrate over long distances through external forces such as wind, rivers, and ocean currents. They can pollute some of the most remote corners of the earth, from mountain lakes to deep-sea sediments (Gago et al., 2018; Moreira et al., 2016; Nelms et al., 2017). Microplastics are a new pollutant in the current marine environment and are gaining increasing global attention (Andrady, 2011). However, most studies focus on offshore waters, with research focused on remote areas such as deep seas, polar regions, islands and reefs still being extremely limited (Auta et al., 2017; Imhof et al., 2017).

Sea turtles are highly loyal to their nesting grounds, with most adult female sea turtles returning to the beach where they were born to lay their eggs (Triessnig et al., 2012). The presence of microplastics on the beach can have a negative effect on the reproductive cycle of sea turtles (Beckwith et al., 2018; Duncan et al., 2018). Sea turtles have temperature-dependent sex determination, meaning that the sex determination for this species depends on the incubation temperature. This is determined by the nest temperature during the mid-stage of the incubation period (Mrosovsky & Yntema, 1980). The specific heat capacity of plastics is higher than that of sand. Microplastics that have become incorporated into the beach sand will increase the overall temperature of the beach. This will then affect the nest temperature, which will cause a gender imbalance in sea turtles (Andrady, 2011; Beckwith & Fuentes, 2018). Furthermore, microplastics can absorb harmful chemical pollutants. This can affect the embryonic development of sea turtles through osmosis, which can decrease hatching success (Bergeron, 1994; Yang et al., 2011). Duncan et al. (2018) first discovered microplastics at a depth of 60 cm (nest depth) in nesting areas for sea turtles in Mediterranean Cyprus, which considered that the presence of microplastics may affect the hatching success rate and sex ratio of sea turtles, threatening their population sustainability.

The populations of sea turtles in China have dropped sharply owing to massive illegal trade and habitat loss, resulting in the loss of almost all nesting beaches (Lin et al., 2021). The Xisha Islands in the South China Sea are currently the largest nesting grounds for green turtles in China. A total of 100 green turtle nests were recorded per year from 2016 to 2019 (Jia et al., 2019; Wang et al., 2019). These islands are the last surviving relatively intact land area in China where sea turtle reproduction still occurs. This is due to the remoteness of the islands from the mainland and low human population owing to low levels of development. The green turtle population in the Xisha Islands has a unique genetic makeup, representing a newly defined population (Gaillard et al., 2019). It is therefore vital to protect this distinct turtle population and their nesting grounds to ensure the survival of these turtles into the future.

In this study, the abundance of microplastics in the nesting grounds of green turtles at Qilianyu, Northeastern Xisha Islands is evaluated. The characteristics and possible sources of microplastic pollutants are also described. Revised management practices are also proposed, in line with survey results. These data will help fill gaps in knowledge about microplastic pollution in green turtle habitats in China. Furthermore, it will provide basic information and references for the protection, management, and ecological restoration of beaches as sea turtle nesting grounds in the South China Sea.

2. Materials and methods

2.1 Study area

The Qilianyu cluster (16°55′N–17°00′ N, 112°12′E–112°21′ E) is located in the northeastern Xisha Islands in the South China Sea, approximately 330 km from Hainan Island. The eight small islands are connected by reefs, with a total area of approximately 1.32 km^2. The current permanent population is approximately 200, primarily living on Zhaoshu Island. With the exception of Zhaoshu Island and North Island, all other islands in the archipelago are uninhabited. In this study, sampling points were set up on six islands, including North Island (NI), Middle Island (MI), South Island (SI), North Sand (NS), Middle Sand (MS), and South Sand (SS). These islands have very good quality nesting sites for green turtles and have records of green turtles laying eggs there in recent years (Figure 1).

2.2 Sample collection and separation

The geographic coordinates of the sample points were recorded using a global positioning system. Sediment samples within an area of 25 cm × 25 cm and a depth of 0–2 cm were collected from both the strand line and the turtle nesting line (TNL) for six nesting grounds. This sampling process was repeated three times at each nesting ground. Additional samples were collected from the TNL using a custom-made cylindrical galvanised steel core with a diameter of 20 cm. Samples were collected at depths of 0–60 cm (0–2 cm, <2–20 cm, <20–40 cm, and <40–60 cm). This was to further explore the presence and extent of microplastic pollution at the depth where sea turtles nest at approximately 60 cm (Duncan et al., 2018).

The saturated sodium chloride density method described by Zhang et al. (2021) was used to separate the microplastics from the sediment. For each sample, 250 cm^3 was weighed and placed in a beaker. A total of 500 mL of saturated sodium chloride solution was added. The mixture was then stirred for 2 minutes, after which it was left to settle for 10 minutes. The supernatant was then passed through a 300-mesh sieve. The remaining compositions from the beaker were added to sodium iodide solution and stirred for 2 minutes, after which the mixture was left to settle for 10 minutes. After density separation had taken place the sample was transferred to a 100 mL beaker. A solution of 10% potassium hydroxide was added, and the mixture was left to digest for two days. Finally, the supernatant solution was decanted and filtered through a 0.45 μm glass fiber membrane (GF/F, 47 mm Ø, Whatman, Shanghai, China). This was done using a vacuum filtration device (GM-0.33A, Zhengzhou, China), while waiting for the one-step analysis.

2.3 Observation and identification of microplastics

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. The microplastics were classified and counted according to their morphological characteristics, color and size (Zhang et al., 2021).

Samples suspected to be microplastics that were representative of each group were randomly selected, and their surface structures were tested for polymer types using a Fourier transform infrared spectrophotometer (IRTracer-100, SHIMADZU, Japan). The detector spectral range was 600–4,000 cm^{-1}, co-adding 16 scans at a resolution of 8 cm^{-1} (Zhang et al., 2021). The resulting atlas was compared to the IR polymer spectral library, with only readings at a confidence level of 70% or higher being considered reliable and accepted.

2.4 Experiment quality control

All containers were rinsed at least three times with Milli-Q water and then dried before the start of the experiments. All plastic equipment was replaced with non-plastic if possible. If this was not possible, they were rinsed three times with Milli-Q water and then inspected to ensure that no plastic fragments were generated during sample processing. In addition, all containers were always covered with aluminum foil to avoid contamination. Nitrile gloves and cotton lab coats were constantly worn throughout the experiment, with lab windows also remaining closed. Three procedural blanks were set to minimize contamination from the environment.

2.5 Statistical analysis

Statistical analysis was performed using Excel and SPSS 19.0 statistical software. All data were tested for normal distribution and variance homogeneity before the statistical analysis. One-way analysis of variance was used to analyze the difference in microplastic abundance between the six nesting grounds. The relevant data are shown as mean ± standard deviation. $P < 0.05$ was considered a significant difference, and $P < 0.01$ was considered a highly significant difference according to the two-tailed test.

3. Results

3.1 Distribution and abundance of microplastics pollution at Qilianyu

The quantity of microplastics found in the nesting grounds at Qilianyu ranged from 92—782 thousand pieces·m^{-3} or 368—3128 pieces·m^{-2}, with an average abundance of 338.44 ± 315.69 thousand pieces·m^{-3} or 1353.78 ± 853.68 pieces·m^{-2}. The distribution of microplastics across the six islands had a degree of spatial variation (Figure 2). MS was the nesting ground that was most severely polluted with microplastics, followed by NS, SI, and NI, respectively ($df = 17$; $F = 7.202$; $P = 0.002$). In contrast, MI and SS were found to be less polluted in comparison to the other sampling sites. The abundance of microplastics in the sediment samples exhibited a gradual increase from northwest to southeast, with the exception of MI and SS.

Although many investigations and studies into beach microplastic pollution exist, the particle size survey range is not uniform. This makes it difficult to compare the variation in abundance of microplastics at the regional level. Therefore, only broad comparisons were made for the same particle sizes across different studies. When comparing the abundance of beach microplastics with other areas (Table S1), results showed that the abundance of microplastics (0.05–5 mm in size) in nesting grounds of green turtles at Qilianyu was lower than in Hainan Island, Hong Kong, and Guangdong Province but similar to Ganquan and Quanfu Island in the Xisha Islands. Moreover, microplastics with a particle size range of 0.05–0.33 mm accounted for 27.79 % of all particles in this study. The actual abundance of microplastics at Qilianyu is therefore considerably lower than that found in Guangdong and Hong Kong. It is likely that the lower abundance of microplastics at Qilianyu in the Xisha Islands is associated with increasing distance from the mainland.

3.2 Morphological characteristics of microplastics

When they were separated, the microplastics were shown to have different morphological characteristics. Figure S1 shows the shape category of the microplastics found in the samples. Among the microparticles observed, plastic blocks formed the largest proportion at 58.74 %, followed by foams at 36.01 % and fibers at 4.76 %. Meanwhile, microbeads and films were found to be relatively rare, accounting for only 0.75 % of the total microplastic particles (Figure 3).

The most common color of the sampled microplastics was white. This included both transparent and white microplastics (68.71 %). Among these, white foam was the most common type. The second most common color was black (23.87 %), Multicolored microplastics such as yellow, green, gray, and blue were relatively rare, as shown in Figure 4. The average size of the microplastics at Qilianyu is indicated in Figure 5. Small microplastic particles (<1 mm) comprised the majority of the microplastics (90.22 %).

3.3 Polymer compositions of microplastics

The polymer compositions of the microplastics included polyethylene (PE), polypropylene (PP), and polystyrene (PS) (Figure S2). The most common polymer compositions were PS (40.74 %) and PE (40.74 %). Foams comprised PS, fibers and microbeads comprised PE, and plastic blocks primarily included PE or PP (Figure 6).

3.4 Changes in microplastic density with increasing sampling depth

Results showed that as the sampling depth increased, the average density of the microplastics decreased. When the depths were 0–2, <2–20, <20–40, and <40–60 cm, the average microplastic densities were 418.89 ± 270.41, 415.11 ± 301.35, 277.85 ± 140.14 and 264.67 ± 200.40 thousand pieces·m^{-3}. However, there was no significant difference between microplastic densities at each depth ($df = 66$; $F = 2.043$; $P = 0.117 > 0.05$) (Figure 7). This indicated that microplastic pollution was not limited to the surface of green turtle nests but can also be found deeper underground. This means that microplastics can come into close contact with the turtle eggs, which usually lie at a depth of 60 cm.

4. Discussion

4.1 Current status of microplastic pollution at Qilianyu

Beaches are gathering points for ocean microplastics and key areas of environmental pollution (Nelms et al., 2016; Poeta et al., 2014). Although the island is relatively remote from the mainland, the nesting grounds of the green turtles at Qilianyu have still been exposed to microplastic pollution. Microplastic pollution is closely related to regional population activities and economic development (Fang et al., 2021). The overall abundance of microplastics (size range 0.05–5 mm) at Qilianyu was lower than that at other places such as Hainan and Guangdong, China. This is likely the small population of the islands, implying that it is less severely affected by land-sourced plastic litter. However, microplastics are stable and can exist in the environment for a long time. Therefore, their abundance may increase with time. Measures need to be taken to prevent the microplastic pollution increase in Qilianyu.

4.2 Sources of microplastic pollution for Qilianyu

The types of microplastics found at the nesting grounds at Qilianyu were primarily plastic blocks and foams, with the main compositions being PS and PE. However, Huang et al. (2020)

showed that the types of microplastics found in the sea water of the Xisha Islands are primarily fibers and films, with the composition predominantly being polyethylene terephthalate (56.2%) and PP (20.3%). The different types and compositions found indicate that microplastics in beach sediment at Qilianyu may not be directly derived from local sea water but instead come primarily from beach debris.

Previous investigations have found that the greatest proportion of beach debris at Qilianyu is plastic and foam (Zhang et al., 2020) (Figure S3). Plastic blocks and foam can easily break in a beach environment. This is more common under higher temperatures as a result of weathering and degradation (Fok & Cheung, 2015; Fok et al., 2017). Owing to its tropical climate, Qilianyu experiences strong direct solar radiation, accounting for 60–70% of the world's solar radiation. The average annual average temperature is approximately 27.4 ℃ (Xu et al., 2018), which is conducive to the breakdown of plastic. Furthermore, the highest percentage of the microplastics at Qilianyu were white in color, followed by black. In line with the results of 24 investigations analyzed by Hidalgo-Ruz et al. (2012), this is likely to be because of the weathering and fading of plastics in beach or ocean environments. Therefore, it is likely that most of the microplastics found as part the current study were from broken plastic debris on the beach. Items such as plastic bottle caps being broken into small plastic particles on the beach were commonly seen during field work (Figure S4).

Furthermore, Zhang et al. (2020) confirmed that the geographic source of beach litter at Qilianyu was primarily from abroad, primarily from Southeast Asian countries, such as Vietnam and Malaysia. Therefore, it is likely that the microplastics in the nesting grounds for the green turtles at Qilianyu were produced from plastic litter from abroad that drifted there with the ocean currents, washed ashore, and then was broken into smaller fragments on the beach.

4.3 Potential threats of microplastic pollution to sea turtles at Qilianyu

Consistent with previous research results (Mohamed Nor & Obbard, 2014; Peng et al., 2017; Vianello et al., 2013), the microplastics found in the nesting grounds on Qilianyu were predominantly small particles (0.05–1 mm). However, the smaller the microplastic particle size, the larger their specific surface area. Implying that they can absorb more pollutants, which may cause greater harm in the hatching of green turtles (Duncan et al., 2018).

The presence of microplastics is extensive in the sea turtle nesting grounds at Qilianyu, with them even coming into close contact with their eggs. Owing to the effects of climate change and the presence of microplastics, the beach temperature at Qilianyu has been increasing annually. Temperatures have increased by 1 to 2 °C in 2021 in comparison with data collected in 2018 (T. Zhang and L. Lin, unpublished data.). An increased incubation temperature may change the sex ratio of any sea turtles which are born locally. In addition, the microplastic surfaces can accumulate heavy metals and organic pollutants (Bergeron, 1994; Yang et al., 2011). Jian et al. (2020) indicated that heavy metals can enter the embryo through penetration of the shell membrane. Therefore, we opined that microplastics near the green turtle nests may adversely affect the development of turtle embryos. However, the degree of harm to the hatching of green turtle eggs due to microplastics at Qilianyu is not yet clear. Therefore, research and monitoring in this field needs to be strengthened going forward. Important areas for future research include the impact of microplastic enrichment on turtle hatching temperature, and the impact of attached microplastic surface pollutants on sea turtle hatching.

4.4 Management Suggestions

Foketal et al. (2017) opined that cleaning up plastic litter on beaches may reduce the generation of microplastics there. However, the current beach litter cleaning at Qilianyu primarily removes large plastic litter of a size > 10 cm. The overall amount of large litter has been reduced to a low level by regular cleaning efforts. However, a large amount of small litter (1–10 cm) still remains after cleaning has taken place. The proportion of smaller pieces of litter being removed requires improvement (Zhang et al., 2020). In addition, during the peak period for sea turtle nesting, the accumulation rate of plastic litter on the green turtle nesting ground beach at North Island of Qilianyu was 1.01 pieces$\cdot$m$^{-2}\cdot$month^{-1}. This was higher than that found by other studies (D. Q. Li and L. Lin, unpublished data.). This plastic litter should be cleaned up in time to prevent it from breaking down to form microplastics. Therefore, it is suggested that the strength and frequency of beach litter cleaning should increase. This should particularly focus on the removal of small plastic particles and foam, with cleaning frequency being increased from once a week to once every 2–3 days. In view of the increasing number of foam plastics, it is suggested that the foam used for local commercial activities such as aquaculture and seafood transportation should be recovered and replaced before aging and fragmentation into smaller pieces of plastic litter occurs.

Considering the litter at Qilianyu being primarily from abroad, regional cooperation between stakeholders in the South China Sea should be strengthened, with joint promotion taking place about the appropriate treatment of marine litter. This will help to reduce the generation of large plastic litter and prevent large plastics from breaking down to form more microplastics, to the benefit of the conservation of the green turtle populations of the South China sea.

Credit author statement

Ting Zhang: Conceptualization; Methodology; Investigation; Formal analysis; Writing-original draft. **Deqin Li:** Conceptualization; Methodology; Investigation; Formal analysis; Writing-original draft. **Jichao Wang:** Validation; Visualization; Writing-review & editing. **Yunteng Liu:** Methodology; Writing-review & editing. **Rui Li:** Visualization; Investigation, Methodology. **Shannan Wu:** Methodology; Writing-review & editing. **Liu Lin:** Conceptualization; Writing-review & editing. **Haitao Shi:** Conceptualization; Writing-review & editing.

Conflicts of interest

The authors declare no conflicts of interests.

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Figure 1

Figure 1. Map of study area and sampling points.

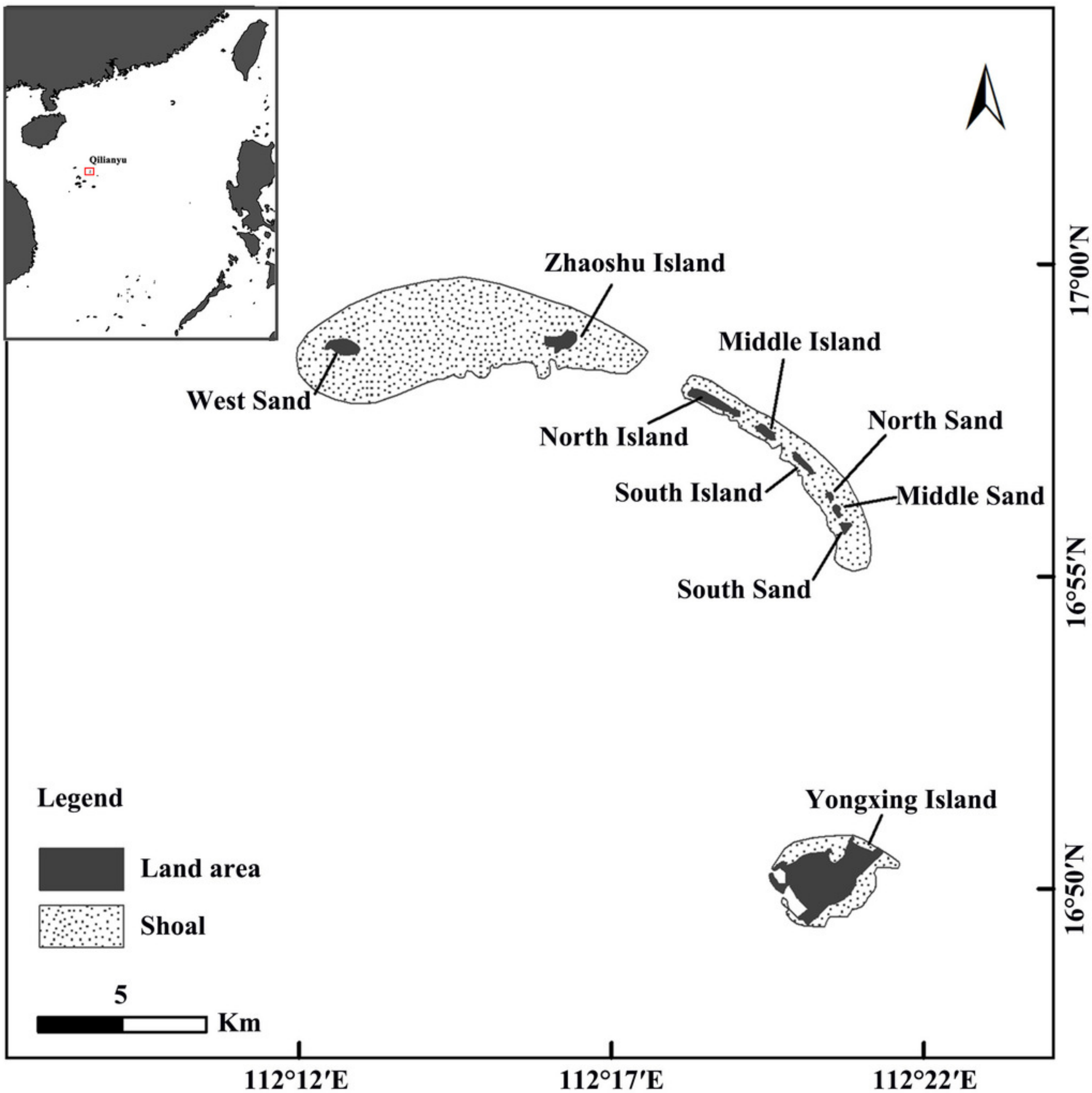
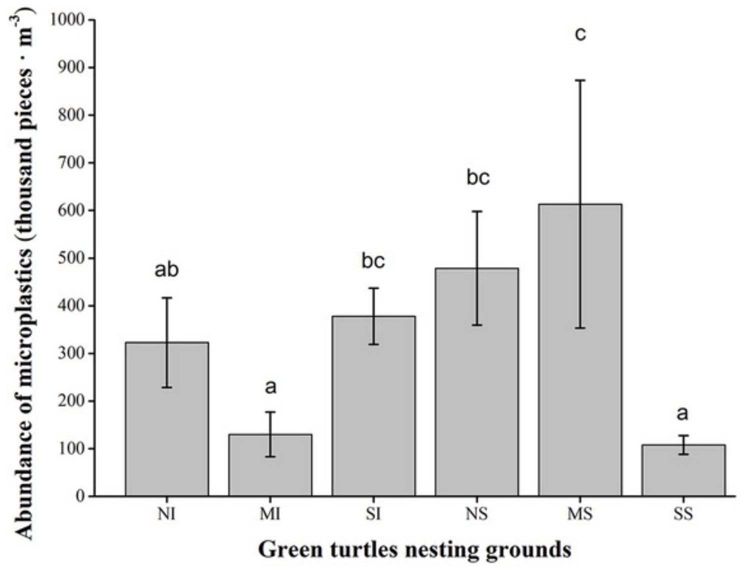


Figure 2

Figure 2. Microplastic abundance in surface sediments of six nesting grounds at Qilianyu. Different lowercase letters in the figure indicate significant differences at the $P<0.05$ level.

Different lowercase letters in the figure indicate significant differences at the $P<0.05$ level.


Abundance of microplastics (thousand pieces · m-3)
1000
900
800
700
600
500
400
300
200
100
0
ab
a
bc
bc
c
a
NI
MI
SI
NS
MS
SS
Green turtles nesting grounds

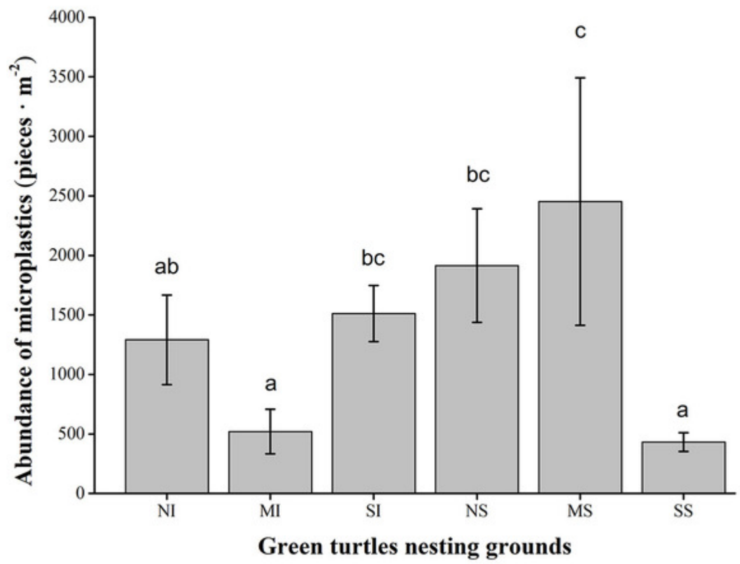

Abundance of microplastics (pieces · m-2)
4000
3500
3000
2500
2000
1500
1000
500
0
ab
a
bc
bc
c
a
NI
MI
SI
NS
MS
SS
Green turtles nesting grounds

Figure 3

Figure 3. Composition (%) of microplastics with different shapes (n = 18).

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 are outliers.

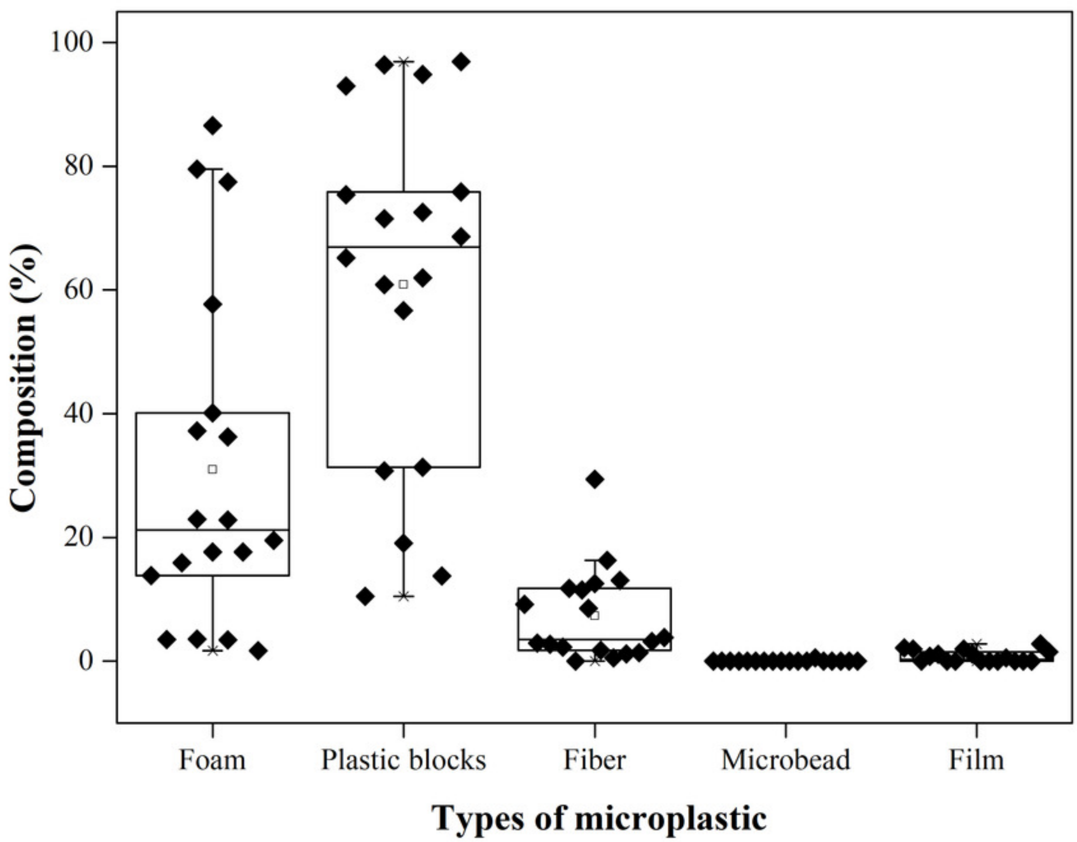


Figure 4

Figure 4. Composition (%) of microplastics with different colors (n = 18).

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 are outliers.

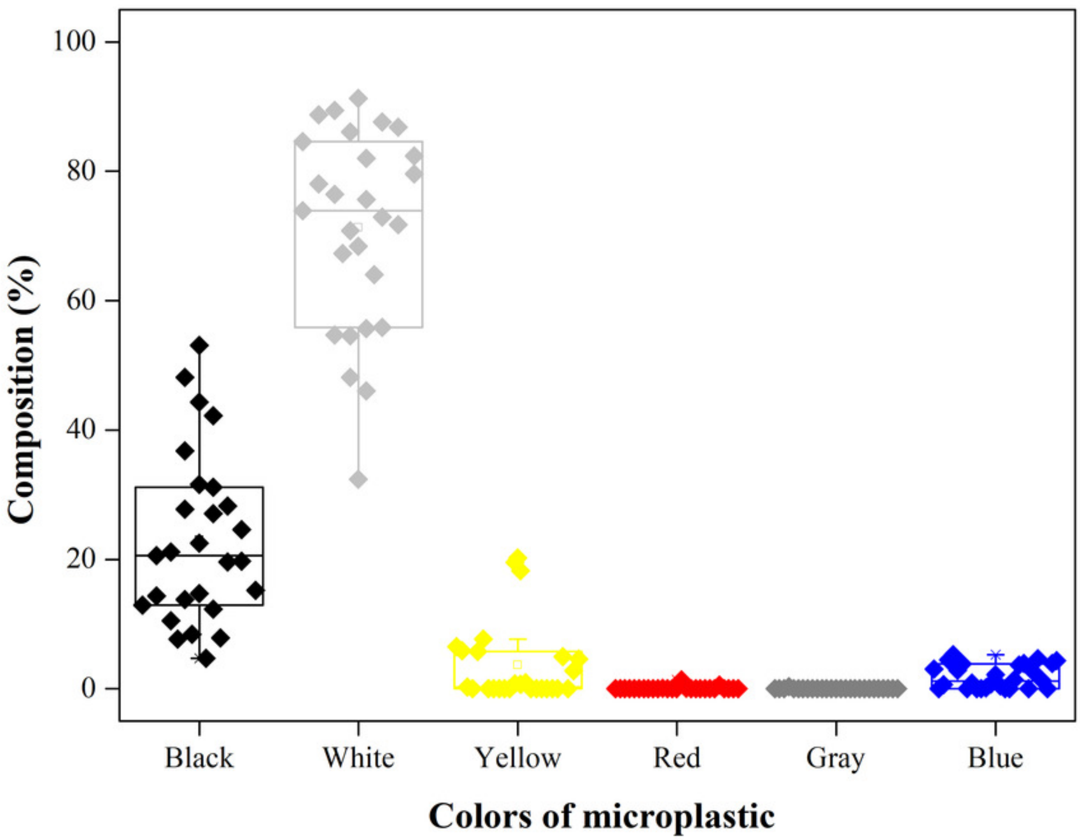


Figure 5

Figure 5. Composition (%) of microplastics with different grain sizes (n = 18).

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 are outliers.

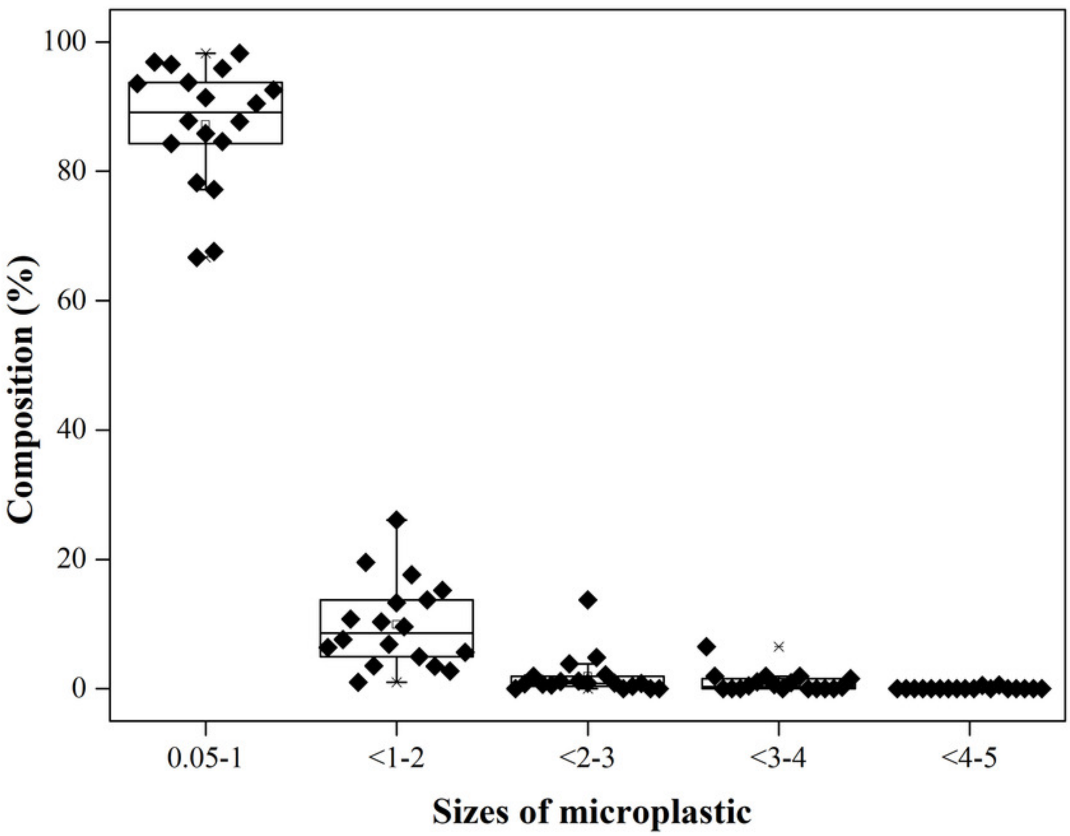


Figure 6

Figure 6. Composition of the selected items from six nesting grounds at Qilianyu.

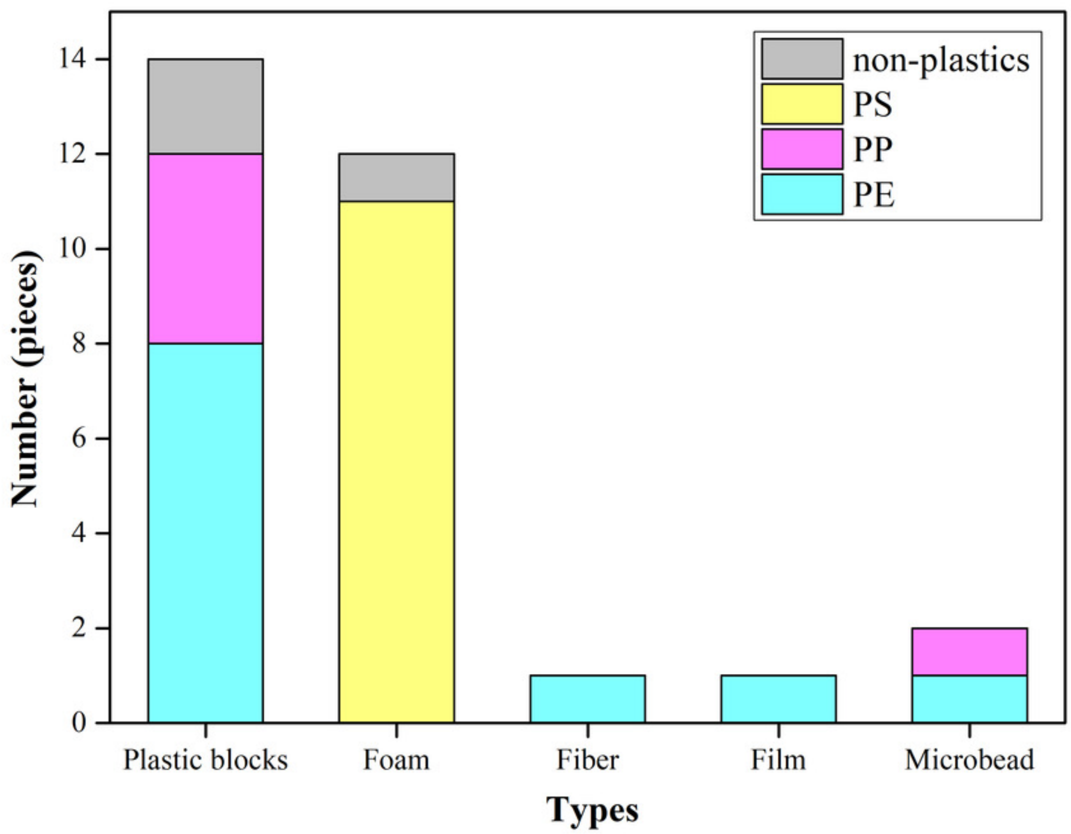


Figure 7

Figure 7. Values of average microplastic abundance at different depths in the nesting grounds of green turtles.