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Research Article

Characteristics of Microplastic in Selected Marine Sponges from Pasir Putih Situbondo, East Java, Indonesia

Natasya Febriani Fauziah[1], Farid Kamal Muzaki[1,2*], Naurah Rizki Fajrinia[1], Aunurohim[1,2], Dian Saptarini[1,2]

[1]*Ecology Laboratory, Department of Biology, Faculty of Science and Data Analytics, Institut Teknologi Sepuluh Nopember, Surabaya 60111, Indonesia*
[2]*Research Center for Sustainable Infrastructure and Environment, Institut Teknologi Sepuluh Nopember, Surabaya 60111, Indonesia*

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ABSTRACT

Microplastics (MPs) are global concern due to their presence in various ecosystems and their tendency to have a negative impact on organisms. MPs are plastic fragments with a small size ranging from >1 µm to <5 mm, which are easily ingested by marine organisms, including marine sponges. As filter-feeding organisms, sponges can accumulate MPs in their bodies. This study aims to analyze the physical and chemical characteristics and the abundance of MPs in seawater and sponges. Three species of marine sponges (*Xestospongia testudinaria*/XT, *Aaptos suberitoides*/AS, and *Clathria* sp./CR) with ten replicate samples were collected from Batu Lawang reef in Pasir Putih, Situbondo, East Java, Indonesia. MPs samples were also collected from the water column (surface and sea bottom at a depth of ±6-7 m). Analysis of physical characteristics (shape, color, and size) performed using a stereo microscope and Optilab, whilst chemical characteristics (type of polymer) was analyzed using ATR-FTIR. A one-way ANOVA test followed by Tukey's HSD (both at p = 0.05) were performed to determine the difference in MPs density from each species. There was no significant difference in term of density in XT and AS, with average density of 60.6 and 66.9 particles/g wet weight (ww), respectively. The density in CR was significantly higher, with the value of 86.7 particles/g (ww). In the water column, the density was 20-27 particles/L (surface sample) and 37-84 particles/L (sea bottom). All particles dominated by black fragments with sizes ranging from 0 to 60 µm, and the polymer type is polypropylene (PP).

1. Introduction

Plastic pollution has become a global environmental issue (Ogunola and Palanisami 2016) for the health of ecosystems (John *et al.* 2022), with as much as 12.7 million metric tons of plastic waste entering marine ecosystems, and Indonesia is known as the second largest contributor to plastic pollution in the sea. In 2010, Indonesia generated 3.22 million tons of poorly managed plastic waste, contributing to an estimated 0.48 to 1.29 million metric tons of plastic pollution in the oceans, with 92.4% being microplastics (Jambeck 2015). In the marine environment, plastics will undergo degradation through several physical, chemical, and biological processes, including UV radiation, wave and current action, temperature, oxygen availability, and microorganism activities. This degradation results in smaller particles called microplastics (MPs) (Girard *et al.* 2021; Zhao *et al.* 2022; Zakaria *et al.* 2024). MPs can be defined as small plastic particles with a size less than 5 mm (John *et al.* 2022) and categorized into primary and secondary MPs. Primary MPs are microplastics that have been designed and produced for commercial uses, such as in cleaning and beauty products, pellets for animal feed, and resin powder (Ogunola and Palanisami 2016). Meanwhile, secondary MPs come from the degradation of large plastic fragments into microplastics; for example, plastic bags and fishing nets (Yona *et al.* 2023). MPs are synthetic materials that can be characterized by certain specifications such as density, type of polymer, shape,

* Corresponding Author
E-mail Address: rm_faridkm@bio.its.ac.id

color, and size of particle (Celis-Hernández *et al.* 2021; Li *et al.* 2021).

Because of their small size, MPs could be easily ingested by marine organisms (McEachern *et al.* 2019; Zhang *et al.* 2021). Accumulation of MPs in the tissues of marine organisms is reported widely in Indonesia, with the most concern being in fish and benthic organisms such as sea cucumber, shellfish, and sea urchin (Manullang *et al.* 2023). Consumption of MPs by organisms can cause bleeding or tissue inflammation, neurotoxicity, energy depletion, decreased skeletal growth rate, increased stress, decreased immune system, impaired digestive and reproductive function (Campanale *et al.* 2020; Fallon and Freeman 2021). Another issue concerning MPs accumulation in organisms is related to the potential of MPs to adsorb persistent organic pollutants (POPs), which then accumulate in the tissue and are translocated to marine food webs (Rodrigues *et al.* 2019). Several studies state that invertebrates may be more vulnerable to MPs accumulation than other organisms (Fallon and Freeman 2021).

Compared to other organisms, there are relatively few studies on the accumulation of MPs in sponges, despite their wide distribution across benthic ecosystems. Sponges are filter feeders, and their main diet is prokaryotes and plankton that are obtained through filtering from the water (Celis-Hernández *et al.* 2021). Sponges help supply nutrients primarily to coral reefs and other benthic organisms through their active role in the carbon, nitrogen, and phosphorus cycles, either independently or in association with other organisms. Sponges provide habitat for various benthic organisms and invertebrates, as well as food for predators, namely coral fish, crabs, starfish, and sea urchins (Pawlik and McMurray 2020). Sponges often exhibit high pumping rates of seawater; therefore, they process large volumes of seawater through their canals and aquiferous systems (Pawlik *et al.* 2018). Water enters the sponge's body through the ostia, is filtered by the choanocytes, and then is excreted through the osculum, where the microparticles carried along with the water tend to remain in the sponge's body cavity. Marine sponges have the potential to be indicators of microplastic contamination in marine waters, especially in waters around coral reefs because they have a wide distribution, their inherent nature of attaching to substrates, their ability to accumulate small particles through seawater filtration, and their high sensitivity to environmental changes (Celis-Hernandez *et al.* 2021; Fallon and Freeman 2021; Girard *et al.* 2021; Krikech *et al.* 2023). The effects of MPs accumulation on sponges include disruption of physiological functions, such as disrupted ostia opening, stunted growth, and disruption of respiration (Fallon and Freeman 2021).

In the mainland of East Java, marine sponges are widely distributed in almost all coastal areas, where higher diversity is estimated to occur in coral reef habitats of the north coast, which borders the Madura Strait, including Pasir Putih Bay in Situbondo Regency. Several species of sponges commonly found in Pasir Putih are *Aaptos suberitoides, Xestospongia testudinaria, Haliclona* spp., *Phyllospongia* spp., *Petrosia (Strongulopora) corticata, Neopetrosia* spp., *Gelliodes* sp., *Clathria* spp., and *Hyrtios* spp. (Subagio and Aunurohim 2013). Pasir Putih Bay is recognized as a marine tourism area, and the condition of its surrounding environment, including plastic pollution, could impact the diversity and composition of the sponge community. However, only a few studies have examined the MPs pollution in the area. A recent study by Yona *et al.* (2023) showed that the average density of MPs in the sediment of the west coast of Situbondo was 492.50±143.26 particles/kg. Moreover, information on MPs' pollution in the water column as well as benthic organisms is still limited. This study aimed to fill the gap by examining the density, physical characteristics, and types of plastic polymer in the water and tissue of sponges.

2. Materials and Methods

2.1. Sampling Area

The research was conducted from March to August 2023. Samples of MPs in sponges and seawater were collected from Batu Lawang reef in Pasir Putih Bay, Situbondo, East Java, Indonesia, as depicted in Figure 1. Sampling of seawater was conducted in two stations (BL and FA) while the sponges survey collection was conducted in the area between those stations with Scuba diving. The study area was situated in a local marine protected area (PerBup Situbondo 12/2022), in which permitted economic activities are beach tourism, limited traditional fishing (with cast net and fishing line), and underwater tourism (diving and snorkelling). Many tourism supporting facilities are available, including hotels, cottages, and restaurants. The settlement and fishing industry in the form of fishponds, floating net cages, and government-owned fisheries research facilities are also present in the nearby area. In addition, many units of fish aggregating devices in the form of fish apartments (made of black-colored polypropylene partition) are also present and deployed on the seafloor at a depth of 12-22 m near the FA station.

2.2. Collection of Sponge Samples

Samples of sponges were collected using a hand-collecting technique; a small section of sponges (±8 cm) was removed using a steel blade from 10 different individuals or colonies (replication = 10 samples) of three species: *Xestospongia testudinaria* (XT), *Aaptos suberitoides* (AS), and *Clathria* sp. (CR) (Figure 2). These species were chosen because they represent some of the most dominant sponge species in the sampling area (Subagio and Aunurohim 2013). Prior to sampling, all selected sponges were observed for the lifeform and life color, measured for their size, and documented using an underwater camera. Each sample was placed in a separate container and preserved in 10% formaldehyde.

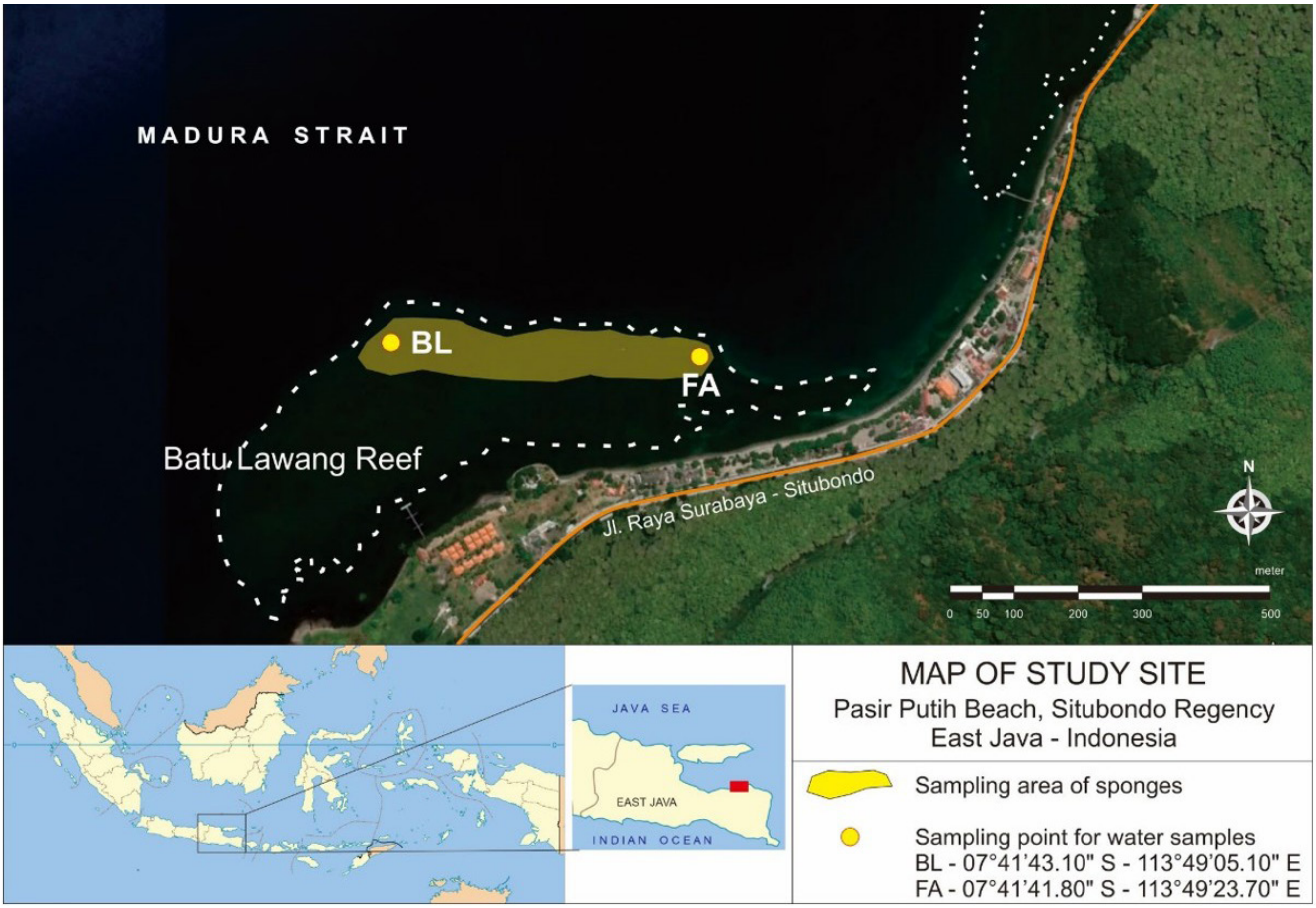

Figure 1. Location sampling area in Batu Lawang reef, Pasir Putih Beach, Situbondo Regency, East Java, Indonesia

Figure 2. Photographs of life specimens of marine sponges analyzed for microplastics in this study: *Xestospongia testudinaria* (left), *Aaptos suberitoides* (center), and *Clathria* sp. (right)

All samples were stored in a freezer with a temperature of -20°C in the laboratory (Fallon and Freeman 2021).

2.3. Microplastic Sampling from the Water Column

MPs in the water column were collected from the surface and near the sea bottom (at a depth of ±6-7 m) with two replications each. Samples were obtained by filtering water using a plankton net with a mesh size of 75 µm (Viršek *et al.* 2016). After filtration, the outer side of the net was sprayed with seawater, and the samples were collected in the cod end, rinsed with 70% alcohol to preserve the sample (Viršek *et al.* 2016). Each sample was placed in a separate glass jar and stored in a cool box until further analysis in the laboratory.

2.4. Sample Preparation

Each sponge sample was removed from the containers to a glass bottle containing 40 ml of NaOCl, wrapped with aluminum foil, and stored in an oven at 60°C for 2 hours to dissolve the organic materials in the samples (Fallon and Freeman 2021; Jaafar *et al.* 2021). The water samples were also removed from glass jars to glass tubes and added with 5 ml of 30% H_2O_2 (Li *et al.* 2021), wrapped with aluminum foil, and stored in an oven at 50°C for 24 hours (Fallon and Freeman 2021; Gurjar *et al.* 2022) to destroy any organic materials. After degradation of organic materials, all samples were filtered with Sartorius filter paper no. 389 (particle retention is 8-12 µm) and aided by a vacuum pump to speed up the filtration process. After filtration, the filter papers were then placed onto a Petri dish for drying in an oven at 40°C for 24 hours (Barboza *et al.* 2020).

2.5. Analysis of Physical Characteristics

Prior to observation, a melting test was conducted using a hot needle to confirm whether the observed particles were made of plastic or not. All samples were visually observed under an Olympus SZ61 stereo microscope (equipped with OptiLab) for analysis of physical characteristics, including density, shape, color, and size, following several guidelines such as Viršek *et al.* (2016) and Zhao *et al.* (2022). Measurement of particle size was facilitated by Image Raster software. Particle sizes were categorized into eight groups: 0-20 µm, 20-40 µm, 40-60 µm, 60-80 µm, 80-100 µm, 100-500 µm, 500-1,000 µm, and 1,000-5,000 µm (Nor and Obbard 2014). Analysis of physical characteristics was conducted in the Ecology Laboratory, Biology Department of ITS.

2.6. Analysis of Polymer Type

Following physical characteristics, the samples were analyzed for the type of polymer using Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) in the Integrated Laboratory of Sebelas Maret University, Surakarta. ATR-FTIR spectroscopy can produce a stable spectrum from the surface of irregular microplastics, and infrared rays can shoot certain parts of the particles from the sample being tested (Chen *et al.* 2020). The type of polymer was identified by comparing the spectrum of the absorbance band (Jung *et al.* 2018; Chen *et al.* 2020).

2.7. Data Analysis

The density of MPs was defined as the number of particles per liter (particles/l) for seawater samples and particles per gram wet weight (particles/g ww) for sponge samples. A one-way analysis of variance (ANOVA) followed by Tukey's HSD test was performed to determine the difference of MPs density between sponge samples (Fallon and Freeman 2021). All statistical tests were performed at $p = 0.05$; while data visualization was created using R Studio v4.1.3.

3. Results

3.1. Density of Microplastics in Seawater

The average density of MPs was 28.6 particles/L in the surface water and 57.58 particles/L in the bottom water. In this study, the density of MPs in water column is remarkably higher than in Bohai Sea (2.8-3.6 particles/L) and Sanya Bay of Hainan Island, China (15.5 particles/L), but lower than in Saigon Bay, Panama (107 particles/L).

3.2. Density of Microplastics in Marine Sponges

Based on the results of one-way ANOVA followed by Tukey's HSD test, there was no significant difference (significance values = 0.388) in terms of MP density in sponge XT and AS, with average densities of 60.6±7.49 and 66.9±12.64 particles/g wet weight (ww). However, the density of MPs in CR was significantly high (significance values = 0.000), with the value of 86.7±10.88 particles/g ww. Differences in the density of MPs across species may be related to differences in morphology, physiology, and availability of MPs in the surrounding seawater.

3.3. Physical Characteristics of Microplastics

3.3.1. Size

In the water column in BL, the surface sample was dominated by the MPs sized of 0-20 µm (50.12% from the total abundance of MPs) while the near-bottom sample was dominated by the MPs sized of 20-40 µm (30.88%). Contrarily, the surface sample from KP was dominated by MPs sized 20-40 µm (27.24%), and the near-bottom sample was dominated by MPs sized 0-20 µm (56.29%).

3.3.2. Shape

Five shapes of MPs particles were identified, including fragment, fiber, beads, foam, and film, in which fragment was the most dominant shape found in both water and sponge tissues, with relative abundance of >77% in water samples and >88% in sponge tissues (Figure 3). Fragments appear to be one of the dominant shapes of MPs particles in many marine waters around the world, such as in the Johor Strait, Baltic Sea, North Ionian Sea, Mediterranean, Wellington Harbour, and Korean waters.

3.3.3. Color

Various colors in MPs are influenced by the addition of pigments during the manufacturing process or by environmental factors during plastic degradation. Eleven colors were identified during the observation, including black, brown, red, orange, yellow, blue, green, purple, grey, white, and transparent. Based on our observations, the most dominant color from water samples is black, with a relative density of 50.12-63.18% of the total density of MPs in the water column. These results are consistent with the research in Johor Strait, Bohai Sea, China, Sanya Bay of Hainan Island, Bahía Blanca Estuary, and estuaries in the Gulf of Mexico. Dominant color of MPs in the sponge tissues was also black (Figures 4 and 5) with a percentage of 63.70% in XT, 71.95% in AS, and 67.62% in CR, respectively, showing strong similarity between the color of MPs in the water column and animal tissues.

3.4. Type of Polymer

Analysis on polymer type was performed only for the most dominant MPs found in both water and

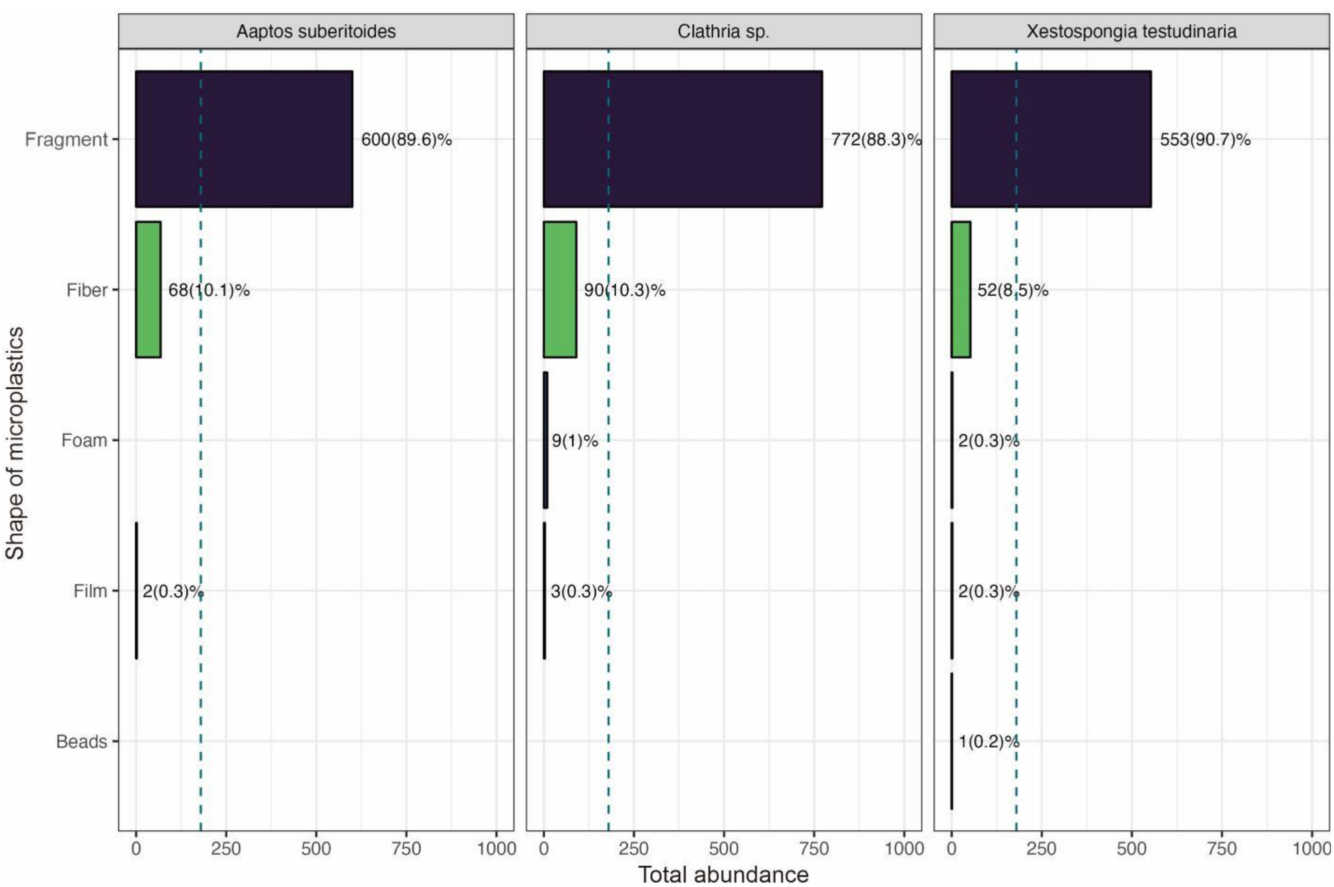

Figure 3. A diagram showing the shape distribution of microplastics in the tissue of the three sponges, which are highly dominated by fragment particles

sponge samples, which are the black fragments. Results of ATR-FTIR show a 'peak' in the wave number ranging from 500 to 4,500 cm^{-1}, which is then translated as polypropylene (PP) as shown in Figure 6. PP is a thermoplastic polymer produced via chain-growth polymerization from the monomer propylene. PP has low density (0.85-0.94 g/cm^3) and is resistant to high temperatures, making it suitable for items such as bottles, trays, carboys, pails, funnels, as well as instruments jars that have to be sterilized for use in clinical environments. PP was considered the second most abundant plastic polymer in seawater after polyethylene (PE), probably due to its wide use and lower density compared to water.

4. Discussion

4.1. Density of Microplastics in Seawater

Compared to several previous studies in Java, which use plankton net for sampling, this study resulted in relatively higher density (Manullang *et al.* 2023; Patria *et al.* 2023); possibly due to high anthropogenic activities in the area and the surroundings, which could be the main source of MPs pollution (Yona *et al.* 2023). Pasir Putih Bay is recognized as a tourist attraction area, surrounded by settlements and fisheries activities, which are presumed to significantly contribute to the high abundance of MPs in the seawater.

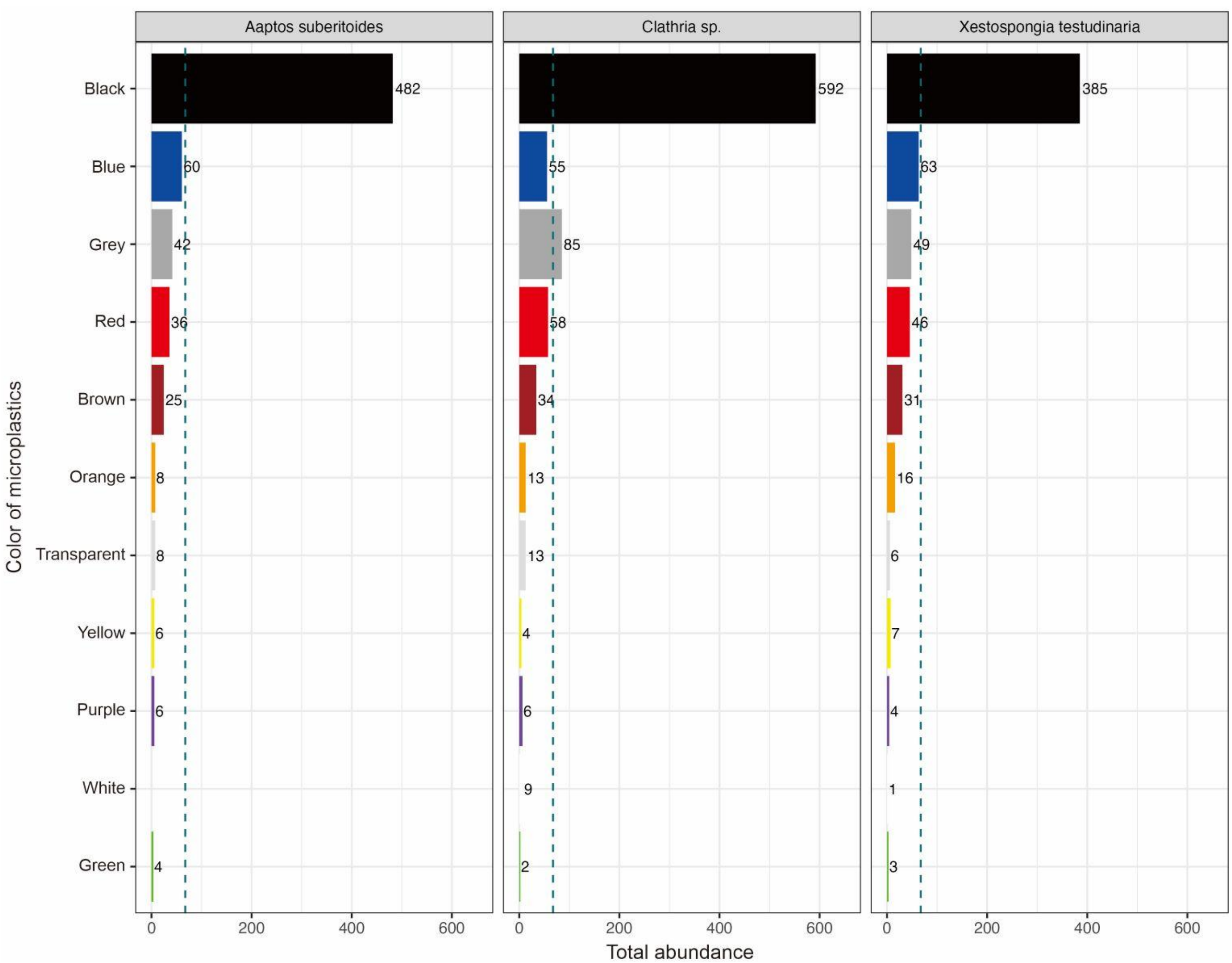

Figure 4. A diagram showing the color distribution of microplastics in the tissue of the three sponges, which is highly dominated by black color

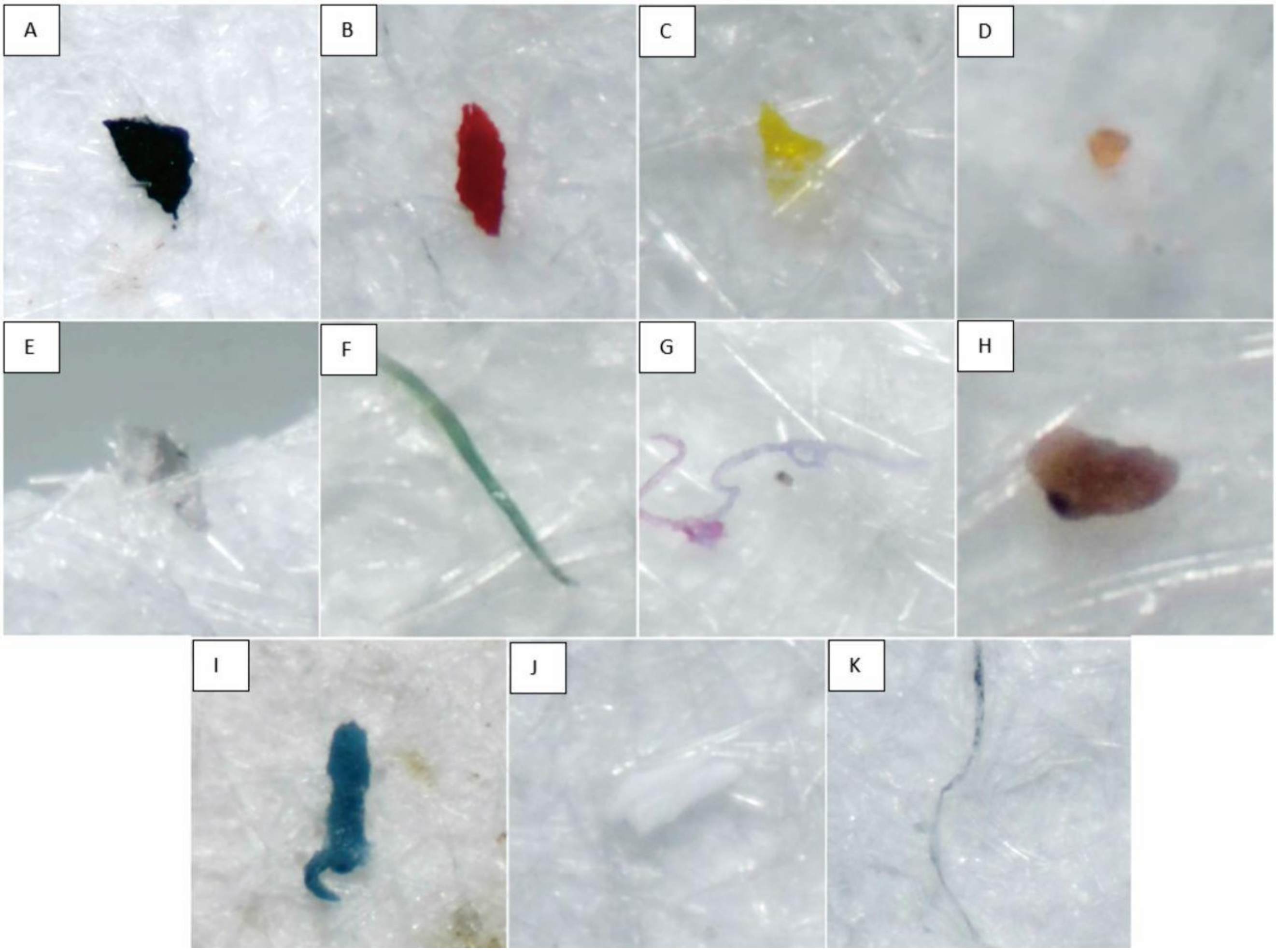

Figure 5. Photographs of several microplastic particles found in seawater and sponge samples: (A) black fragment, (B) red fragment, (C) yellow fragment, (D) orange beads, (E) transparent film, (F) green fiber, (G) purple fiber, (H) brown fragment, (I) blue fragment, (J) white foam, and (K) grey fiber

We found that the concentration of MPs in deeper water tends to be higher than on the surface. Aligning with this finding, Dai *et al.* (2018) reported that the density of MPs was higher at greater depth compared to the surface. The vertical distribution and sinking behavior of MPs are affected by the density of the particles, which is influenced by the type of polymer as well as the presence of fouling. Oxidative degradation by UV, temperature, and oxygen leads to plastics fragmentation, producing smaller fragments with rougher surfaces, which in turn increases the possibility of organisms to attach (usually diatoms) and other particles, thus lowering the buoyancy of MPs (Dai *et al.* 2018; Zhao *et al.* 2022).

4.2. Density of Microplastics in Marine Sponges

All sponge species in this study belong to subclass Heteroscleromorpha, class Demospongiae, which have a leuconoid type channel (Godefroy *et al.* 2019). Sponges obtain food by pumping water into their body through the ostia. The water is pushed by choanocytes (flagellated cells that form the internal layer), which create water movement in the body. Choanocytes take up food particles and oxygen in the water, while the remaining water comes out through the osculum (Flørenes 2013). MPs can enter the sponges via endocytosis and pinacocytes or absorption by choanocytes (Ogunola and Palanisami 2016; Girard

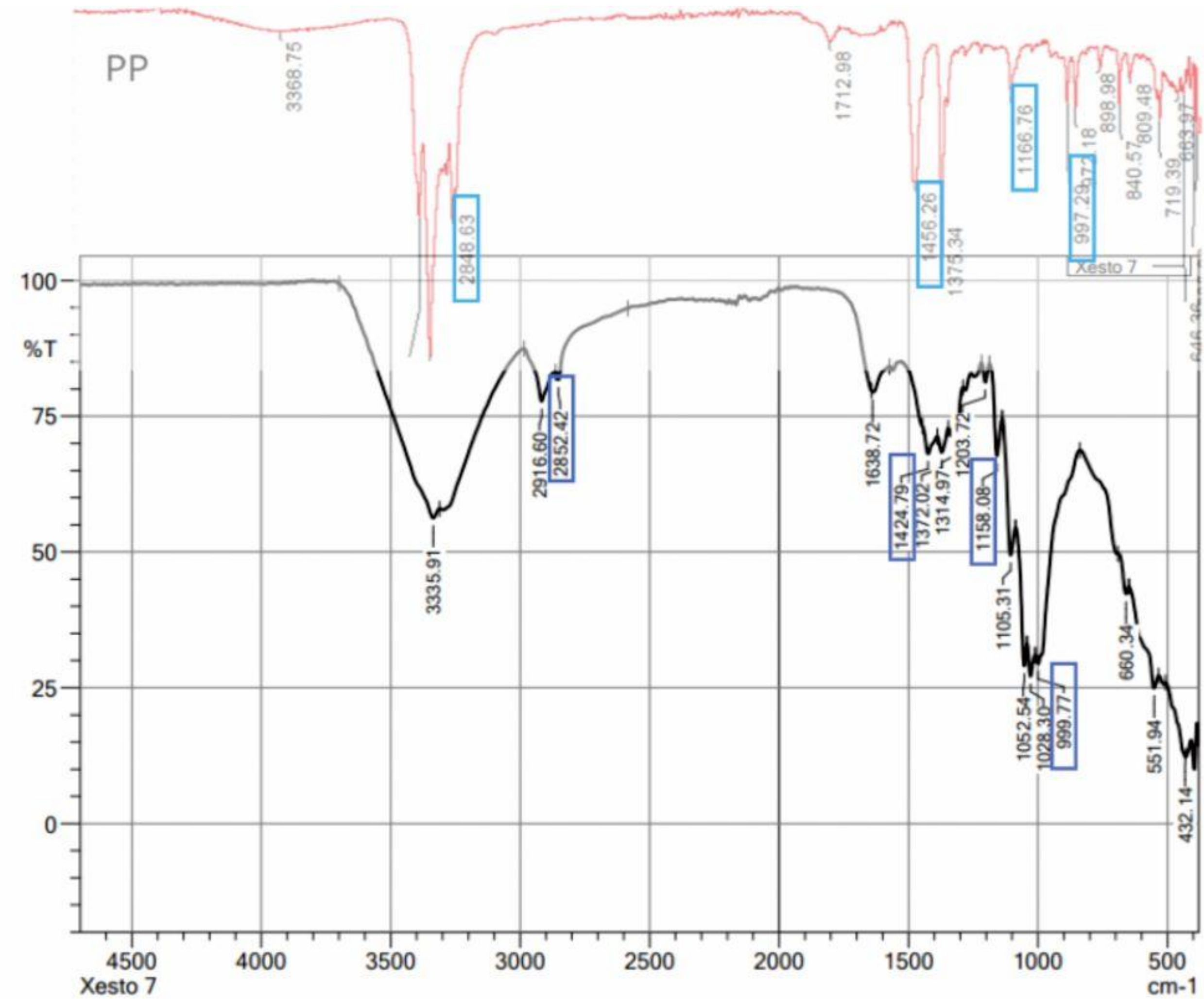

Figure 6. Results of ATR-FTIR analysis for the polymer type of black fragment from the tissue of *Xestospongia testudinaria*; polymer of black fragment type from other sponge species and seawater were similar. Black line: result of this study, red line: comparison with study by Sathish *et al.* (2020)

et al. 2021). Despite having the same type of channel, the three species of marine sponges showed different accumulations of microplastics, in which XT and AS display similar microplastic density in their tissues; this indicates that both species exhibit similar feeding behavior or have comparable abilities to filter and accumulate the microplastics (Krikech *et al.* 2023). Both species are also estimated to have high microbial abundance within their tissue, which plays a role in preventing or even increasing filtered MPs particles. This ability depends on the bacterial consortium on the surface of the sponge. However, further research on the relationship between microbial communities and the presence of microplastics in marine sponges is still needed (Krikech *et al.* 2023).

Higher density of MPs in CR was presumably caused by the differences in tissue density, which causes differences in the sponge's ability to accumulate microplastics (Fallon and Freeman 2021). Sponges with higher tissue density are known to have simpler aquifer systems with narrower channel cavities, fewer choanocyte chambers, and lower flow rates (Dahihande and Thakur 2021). Louden *et al.* (2007) showed that the density of several Demospongiae was found to be in the range of 3.6-8.2 kg/m^3, where the tissue density in the sponge is the quotient of the total weight by volume. Assuming that the density is a function of weight divided by volume, a higher weight will result in higher density. In this study, XT and AS have relatively greater weights of 63±6.4 g and 66±12.1 g, respectively, compared to CR with 27±9.56 g. Lower tissue density of CR was estimated to cause a higher flow rate, thereby absorbing more MP particles within the tissue.

Furthermore, morphological characters can also influence the accumulation of microplastics in marine sponges (Fallon and Freeman 2021). Vase branching sponges may accumulate a higher density of MPs, while encrusting sponges usually have a lower MP density. Three sponge species in this study have different lifeforms; XT with barrel-shaped lifeform, AS is massive to lobate, while CR is a branching sponge (Cleary *et al.* 2018).

4.3. Physical Characteristics of Microplastics
4.3.1. Size

The size of MPs can be influenced by the fragmentation time; the longer the fragmentation time, the smaller the size of the MPs and the greater the possibility of being ingested by organisms. Over time, various degradation processes, such as biodegradation, photodegradation, thermal degradation, chemical degradation, and mechanical degradation, may lead to the production of smaller-sized MPs particles (Zhang *et al.* 2021). Ingestion of MPs and their ecological impacts are dependent on the size, which is a crucial factor influencing the number of plastic particles ingested by small predators (Shamskhany *et al.* 2021). In the sponge tissues, the size dominance of MPs was in the range of 20-40 µm, with a percentage of 32.04% in AS, 35.93% in CR, and 38.39% in XT, respectively. Other small size ranges of 0-20 µm and 40-60 µm were also found, but in lower abundance (Figure 7). These findings indicate that marine sponges show selectivity in the accumulation of MPs by preferring small particles available in the environment (Krikech *et al.* 2023). The dominance of small-sized MPs in the sponges is highly correlated with the size of the food, which is usually less than 70 µm and comprises various organisms such as plankton, ciliates, flagellates, cyanobacteria, and others (Cole *et al.* 2011). The size of ostia is usually ±60 µm and more likely to retain particles larger than 50 µm from entering the sponge (Fallon and Freeman 2021). In the Heteroscleromorph sponges, as in this study, particles less than 50 µm are commonly found in the mesohyl tissue and accumulated in the aquiferous system and ectosome (Girard *et al.* 2021).

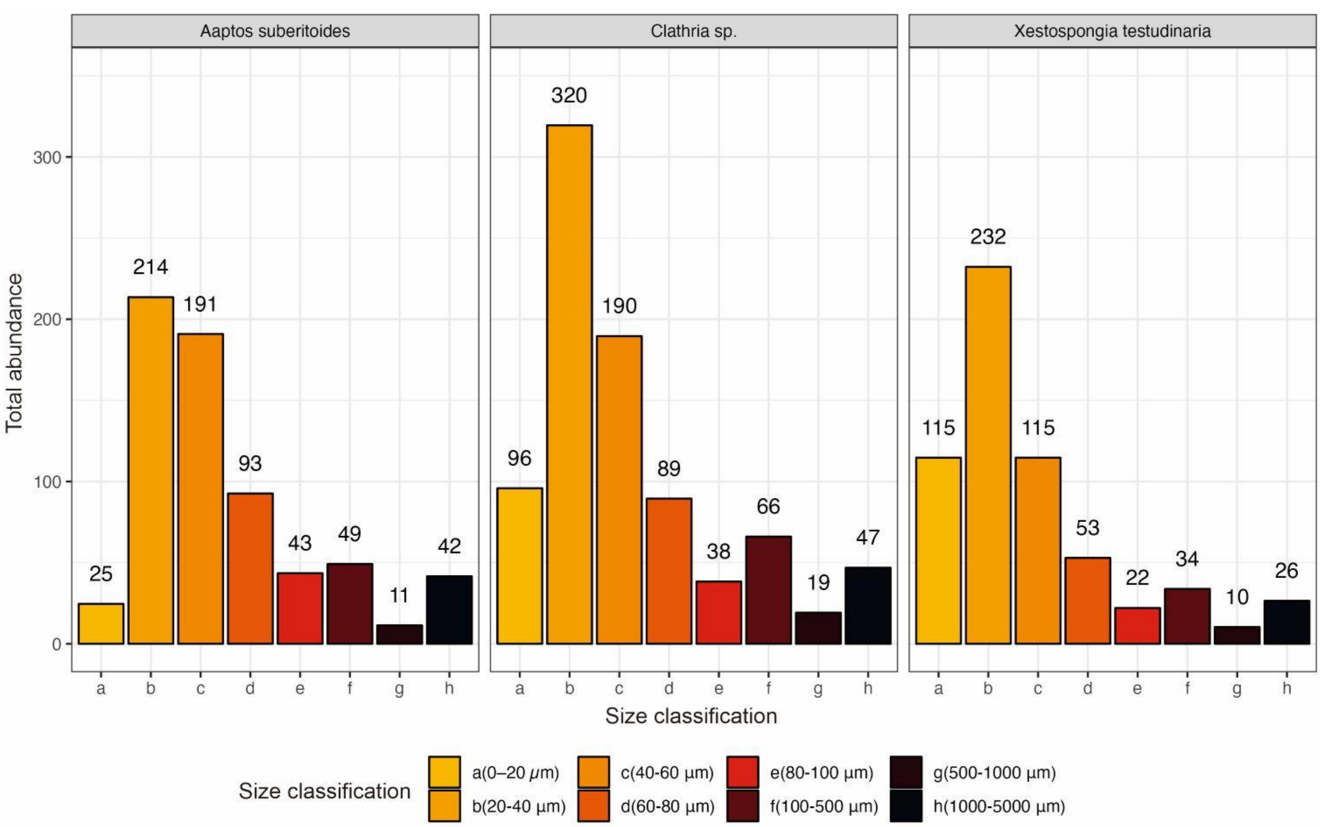

Figure 7. A diagram showing the size distribution of microplastics in the tissue of the three sponges, which are dominated by the size of 20-40 µm, followed by 40-60 µm and 0-20 µm

4.3.2. Shape

Fragments may originate from various plastic products, including bottles, plastic bags, pipes, food packaging, disposable cutlery, and others. The presence of plastic-made structures in the sea, e.g., drums and floats for aquaculture, is also a potential source for plastic fragments (Curren and Leong 2023). As in this study, the presence of a fish-aggregating device, known as a fish apartment, is also presumed to be the source of plastic fragments. Although highly durable, polypropylene will be degraded or fragmented over time, by means of photodegradation (UV exposure), biodegradation (by microorganisms), and/or mechanical abrasion by sand and sediment. The fragments were also found to be the most abundant MPs particles in the study by Parry *et al.* (2023), in which the observed microplastic characteristics show only a little variation or differences between species in the same location. This suggests that sponges have no preference for the particles they absorb, and their absorption is merely based on variations in particles surrounding the water (Krikech *et al.* 2023). Moreover, the fragments tend to have irregular forms, rough surfaces, and rigid edges, making them more likely to remain in the channels of the sponge compared to other shapes.

4.3.3. Color

As filter feeders, sponges obtain food by filtering water through an aquiferous system (Godefroy *et al.* 2019); consequently, it is plausible that the dominant black particles in the water column are also prevalent in their tissue. Black-colored plastics are the most extensively produced due to their minimum addition of pigments required to color unpigmented polymers, leading to enhanced colorfastness and strength as well as slowing down the degradation process of plastics (Huang and Xu 2022). Black color has a higher UV-absorbing capacity, which effectively prevents UV radiation from penetrating the polymer, thereby inhibiting the aging and degradation of plastics (Zhao *et al.* 2022). Black plastics are commonly used as plastic bags, disposable cutlery, toys, office stationery, and others, and can be abundant in coral reef habitats (John *et al.* 2022). Other identified colors that were less abundant than black were blue, grey, and red. Blue MPs may result from degradation of plastic bottles, boat paint, fishing gear (nets, ropes), floating cage, and buoyancy aids; while red MPs may be derived from household washing waste, fishing gear, and red-colored trash bags (Sathish *et al.* 2020). Gray-colored microplastics are likely to originate from black-colored microplastics that undergo discoloration due to UV exposure (Zhao *et al.* 2022).

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