



The occurrence of microplastics in the gastrointestinal tract of sea cucumbers on the conservation area of Mare Island, North Maluku, Indonesia

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Abstract. Sea cucumbers are benthic faunas that inhabit at seagrass beds as deposit feeders which are at risk of ingesting microplastics (MPs) in sediments. This study aims at determining the presence, abundance and characteristics of MPs in the gastrointestinal of sea cucumbers and the correlation between body weight and the abundance of MPs. Samples of sea cucumbers were collected at Mare Island Waters, namely Maregam Village (station 1) and Marekofo Village (station 2). Samples of sea cucumbers were dissected to take their digestive tract, then dried in an oven at 65°C for 24 hours. Subsequently, they were extracted using H₂O₂ solution for density separation. MPs were identified using microscope in the digestive tract of three species of sea cucumbers, namely *Holothuria atra*, *Holothuria scabra* and *Bohadschia vitlensis*. The average of MPs abundance in *H. scabra* was higher (128 particles ind⁻¹) than *H. atra* (70.67 particles ind⁻¹ at station 1; while at station 2 *H. atra* was recorded as 73.14 particles ind⁻¹ and no *H. scabra*). The types of MPs found were dominated by fragments followed by fibers, while the least amount ones were spheres. Generally, the MPs colors were dominated by black, brown, and gray, and the red ones were found very few. Statistically, there was no correlation between sea cucumbers body weight and MPs abundance with R² (coefficient of correlation) of 0.0198. The presence of MPs in the digestive tract of sea cucumbers allows their use as the bioindicators of the local marine environment pollution.

Key Words: Holothurian, marine protected area, micro-particles, Moluccas archipelago.

Introduction. Coastal waters of small islands are the important zones owing to their high biodiversity as a source of germplasm for the communities in coastal areas. Mangrove forest, seagrass meadows, coral reef ecosystems and their various associated faunas and floras are important components in supporting the sustainability of existing resources (Dueñas et al 2021; Huseyinoglu et al 2021). However, along with higher population growth accompanied by various anthropogenic activities such as urban, industry, tourism and domestics, it has produced various types of waste which eventually accumulates into the sea. One of the remaining wastes released into the sea is plastic waste (Seeruttun et al 2021; Binetti et al 2020). According to Jambeck et al (2015), plastic production is estimated entering into the marine environment in a range from 4.8 to 12.7 million metric tons in 2010. This number continues to increase from year to year and it is predicted that the total amount of plastic waste which accumulates in the sea will be ±155 million tonnes by 2025 (Iñiguez et al 2016). Small islands far from urban and industrial areas have also been contaminated by microplastics (MPs) such as the Orkney Islands – Scotland (Jones et al 2020) and Scilly Island – UK (Nel et al 2020).

These plastic waste materials will undergo weathering and photodegradation processes, fragmenting into small pieces to be MPs (< 5 mm) (Andrady 2011; Cole et al 2011), even into very small pieces which is so-called nanoplastics (Jaiswal et al 2022; Lim & Tian 2022). The issue of MPs has becoming a trending topic discussed by various society groups in recent years. This is because several research results have revealed that globally MPs have been found in both tropical and sub-tropical to polar regions,

especially areas adjacent to gyres (Jiang et al 2020). MPs have also been identified in various environmental matrices such as soil (Shi et al 2022), marine sediments (Coppock et al 2017; Loughlin et al 2021), fresh water (Jiang et al 2022; Wang et al 2022) and aerosols (Kernchen et al 2021; Shiu et al 2022). Meanwhile, at the coastal ecosystems, MPs have been found from coral reef ecosystems, seagrasses, and mangroves forest, where these ecosystems play the important roles as absorbers of MPs particles (Nor & Obbard 2014; Zhang et al 2019; Huang et al 2020; Jeyasanta et al 2020; Jones et al 2020). Moreover, MPs are also found in the digestive tract of several aquatic biota such as commercial fishes (Debbarma et al 2022), sea cucumbers (Mohsen et al 2018; Plee & Pomory 2020; Husin et al 2021), sand dollars (Plee & Pomory 2020), marine mussels (Heo et al 2022), marine mammals (Zantis et al 2022), and sea urchin gonads (Hennicke et al 2021; Sevillano-Gonzalez et al 2021), even in zooplankton (Taha et al 2021). On the other hand, MPs have the ability to bind other pollutant particles such as PAHs, PCBs, and pesticides (Fraser et al 2020; Fred-Ahmadu et al 2022) to harmful heavy metals (Wang et al 2017; Foshtomi et al 2019; Liu et al 2021). If this occurs, the higher the MPs absorbed in the body of an organism, the more dangerous it will be due to the possibility of biomagnification processes in the food chain in an ecosystem (Saley et al 2019; Krause et al 2020), and can be a threat to marine ecosystems as well such as seagrass beds and associated biotas (Huang et al 2020). One of the biota associated with seagrass beds that are potentially contaminated by MPs is sea cucumber (Plee & Pomory 2020). Sea cucumbers are a group of marine invertebrate faunas from the phylum Echinoderms that are ecologically important and have economic values. Through an ecological perspective, sea cucumbers, which are deposit feeders, act as 'bioturbators' or sediment processors in shallow marine ecosystems and as one of the keystone species for the shallow water ecosystems balance (Setyastuti et al 2019). While from an economic point of view, sea cucumbers have been widely used as tonic foods in Asian countries (Xu et al 2018), also sea cucumbers contain bioactive compounds that have the potential as antibacterial (Damanik et al 2019), antimicrobial (Dhinakaran & Lipton 2014), and antioxidants (Althunibat et al 2009; Esmat et al 2013).

Mare Island is one of the small islands that has been established as a conservation area in North Maluku through the Decree of the Minister of Maritime Affairs and Fisheries of the Republic of Indonesia No.66/Kepmen-KP/2020, as part of a group of small volcanic islands located in the western part of Halmahera Island. Various important coastal ecosystems can be found on Mare Island such as coral reef ecosystems, seagrass beds, and mangroves, as well as habitats for dolphins (Family Delphinidae) and blackfin sharks (*Carcharhinus melanopterus*) (DKP North Maluku Province 2019). The waters of Mare Island are directly connected to the waters of Ternate Island, Tidore and its surroundings which are islands with high urban, industrial, tourism and domestic activities in producing plastic wastes. According to Lessy & Najamuddin (2020), it was found a high abundance of plastic wastes in Ternate of 77% compared to other marine debris. It is feared that this will have a fairly high environmental impact and may become a source of MPs carried by local hydrodynamic conditions to other nearby islands such as Mare Island. Moreover, the lack of waste handling and management as well as low public awareness of the importance of environmental protection from the impact of plastic waste disposals are feared to worsen the health condition of this conservation area. The results of a previous study by Ramili & Umasangaji (2022) found that the MPs content in seagrass sediments on Mare Island ranged from $18,368 \pm 10,625$ to $27,090 \pm 13,908$ particles kg^{-1} dw. The accumulation and distribution of MPs in seagrass sediments can be a new concern since this ecosystem is the area to inhabit and a feeding ground for various marine biotas. Some of them are sea cucumbers which may be at risk of ingesting MPs depending on how they eat as deposit feeders. Several previous studies have revealed the presence of MPs in the digestive tract of sea cucumbers in Indonesian waters including Bintan Island and its surroundings (Idris et al 2022), on Tidung Besar Island and Bira Besar Island, Jakarta (Sayogo et al 2020), and Rambut Island (Wicaksono et al 2021). Due to the importance of the presence of sea cucumbers in seagrass ecosystems as sediment bioturbator, it is important to investigate the presence of MPs in the digestive tract of sea cucumbers in the waters of Mare Island as a conservation area. This study aimed to

reveal the presence, abundance, type and color characteristics of MPs in the digestive tract of three sea cucumber species namely *Holothuria scabra*, *Holothuria atra* and *Bohadschia vitiensis* and the relationship between body length and abundance of MPs. The results of this study are expected to provide the current data and information regarding the presence of MPs in the digestive tract of sea cucumbers, which are important biota associations in the seagrass ecosystem and become the basic data as a reference for further research and in making decisions related to conservation area management.

Material and Method

Study location. This work was conducted in Mare Island's waters, North Maluku, where station 1 was located in Maregam Village and station 2 in Marekofo Village in June 2022 (Figure 1).

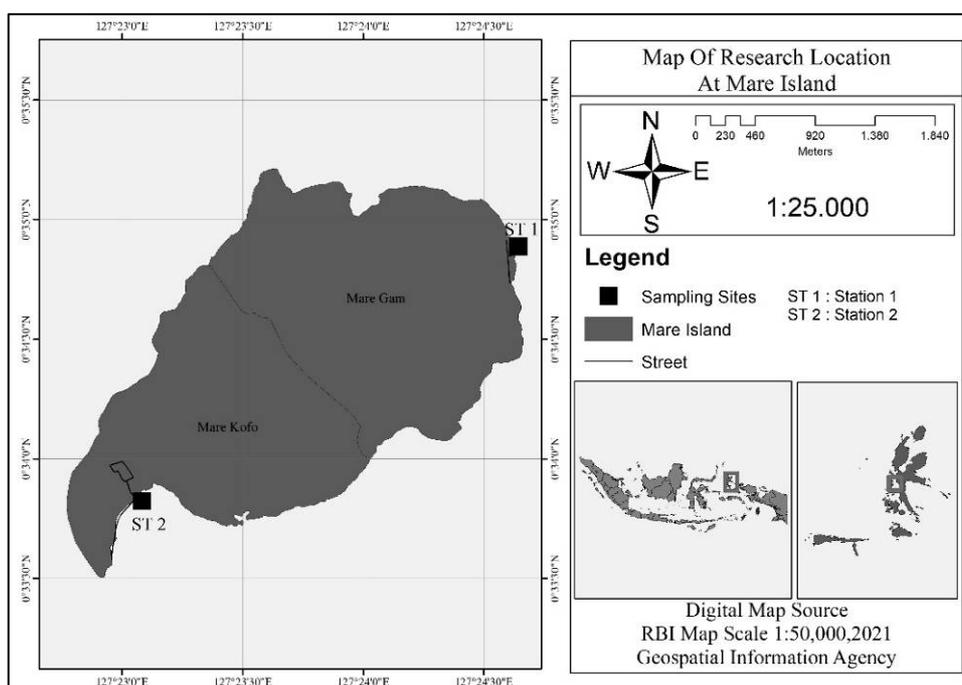


Figure 1. Sampling stations in Maregam (station 1) and Marekofo (station 2).

Samples collection. Sea cucumber samples were taken using the tracking survey method in the seagrass ecosystem with an area of 100 x 50 m at both research stations. The sea cucumbers found were measured for their total length, then photographed, and put in a plastic bag and stored in a coolbox filled with ice so that the samples remained durable for further identification in the laboratory. Subsequently, the sea cucumber samples were weighed prior to conserve them into the freezer. Furthermore, the digestive tracts of sea cucumbers were taken using a surgical instrument to identify the MPs.

Samples extraction. The digestive tracts of sea cucumbers that have been removed were placed in aluminum foil and then dried in an oven at 65°C for 24 hours. Then, the samples were put into a beaker which was added with ±15 mL of 3% hydrogen peroxide (H₂O₂) solution and left for ±1 hour. The supernatant from the samples that have been mixed with the H₂O₂ solution were then filtered using Whatman filter paper No. 40 (8 µm millipore). The filtered samples in filter papers were allowed to dry, then placed in a Petri dish to identify the presence of MPs in the digestive tract of sea cucumbers.

Identification of MPs. The filtered samples were then observed for the characteristics of the MPs using a digital microscope with a magnification of up to 1000x installed via cooling tech software (microscope USB drive/FCC/RoHS certified) on a computer. The

observed MPs particles were photographed for each sample, identified and recorded based on the types or shapes in the form of fibers, fragments or spheres as well as various colors of MPs according to Hidalgo-Ruz et al (2012).

Data analysis. The abundance of MPs in the digestive tracts of sea cucumbers was expressed in the number of MPs particles per individual (particles ind⁻¹). The correlation between the body weight of sea cucumbers and the number of MPs was analyzed using a simple linear regression equation. The results of MPs data analysis were displayed in the form of tables and graphs.

Result and Discussion

Distribution and composition of sea cucumbers. Overall, three sea cucumber species were found, namely *H. atra*, *H. scabra*, and *B. vitiensis* in the seagrass ecosystem of Mare Island (Figure 2). These three species of sea cucumbers are often found in seagrass beds as one of their habitats. *H. atra* is often found on sandy beaches, seagrass beds, coral reefs and exposed on sandy substrates and coral rubble (Setyastuti et al 2019). Furthermore, Lumbu et al (2020) stated that *H. atra* is often found in shallow waters and is active both during the day and at night. *H. scabra* is a species of sea cucumbers that can be found in seagrass beds and coral reefs, often exposed on the sand, and among seagrasses, sometimes burying itself in the sand (Setyastuti et al 2019). Meanwhile, *B. vitiensis* live at a depth of < 10 m in seagrass beds, hiding among seagrasses, behind rocks and dead coral (Setyastuti et al 2019).



Figure 2. The sea cucumber species found on Mare Island.

The three species of sea cucumbers found at the two research stations had different distributions and species compositions. The highest number of sea cucumber species was found at station 1 (Maregam Village) with three species, namely *H. atra*, *H. scabra*, and *B. vitiensis*, while at station 2 (Marekofo Village) only one species was found, namely *H. atra*. The total number of individual sea cucumbers found was 16 individuals (Table 1). It can be seen that *H. atra* was found in both research stations with more individual numbers than *H. scabra*, and *B. vitensis*. According to Setyastuti et al (2019) *H. atra* is a species of sea cucumbers that has a wide distribution and is most commonly found in Indonesian waters compared to the other two species. It is also one of the sea cucumber species which is able to adapt well in various environmental conditions.

Table 1

The composition and individual numbers of sea cucumbers found at Mare Island

Species	Stations		Number
	St. 1 (Maregam)	St. 2 (Marekofo)	
<i>Holothuria scabra</i>	4	-	4
<i>Holothuria atra</i>	6	5	11
<i>Bohadschia vitiensis</i>	1	-	1
	Total		16

The presence of MPs in the digestive tract of sea cucumbers. Identified MPs were found in all samples of the digestive tract (16 samples) of the three species of sea cucumbers, namely *H. scabra*, *H. atra* and *B. vitiensis* at both research stations. Overall, three types of MPs were found in the digestive tract of sea cucumbers, namely fibers, fragments and spheres (Figure 3). The results of this study were in accordance with several previous studies that also found fibers and fragments in the digestive tract of *H. atra* on Tidung Island (Sayogo et al 2020) and *H. scabra* on Bintan Island (Idris et al 2022). Various other types of MPs found in the digestive tract of several species of sea cucumbers are shown in Table 2. It can be seen that various types of MPs can be found in the digestive tract of various species of sea cucumbers both in natural habitats such as seagrass ecosystems (Plee & Pomory 2020; Idris et al 2022) and cultivated ones (Mohsen et al 2018). Additionally, it was seen that fibers and fragments were most commonly found in the digestive tract of sea cucumbers compared to other types such as films, foams, pellets and spheres. According to Pinheiro et al (2020), fibers and fragments are the most common forms of MPs found in various marine organisms.

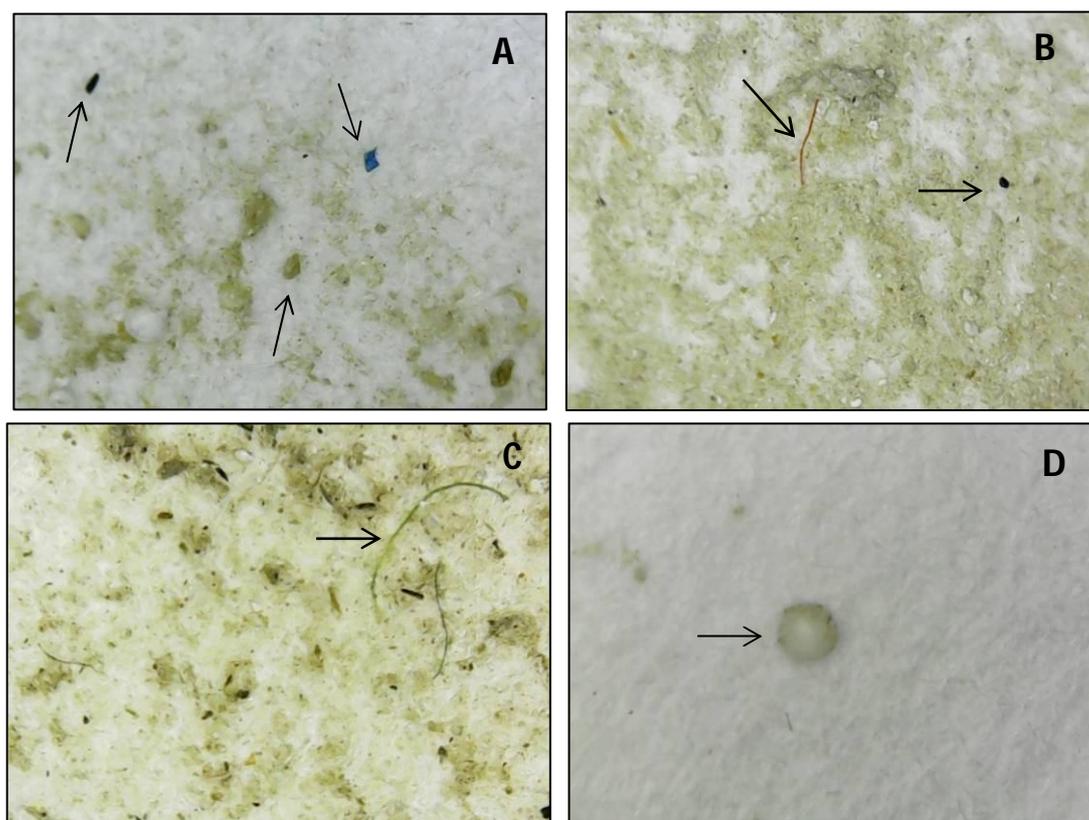


Figure 3. Various types and colors of MPs found in the digestive tract of sea cucumbers: (A) blue, black, and green fragments; (B), red fiber, and black fragment; (C) green fiber; and (D) gray sphere.

Table 2

Various types of MPs in the digestive tract of several species of sea cucumbers

Species	Sampling area	Microplastic types						References
		Fragment	Fiber	Film	Foam	Pellet	Sphere	
<i>H. atra</i> , <i>H. scabra</i> , <i>B. vitiensis</i>	Mare Island (seagrass meadow)	+	+	-	-	-	+	This study
<i>H. atra</i> , <i>H. scabra</i> , <i>Sticopus variegatus</i>	Bintan Island (seagrass meadow)	+	+	+	+	-	-	Idris et al (2022)
<i>H. atra</i>	Tidung Island, Bira Besar Island	+	+	+	-	+	-	Sayogo et al (2020)
<i>H. leucospilota</i>	Rambut Island	+	+	+	-	+	-	Wicaksono et al 2021
<i>H. mexicana</i> , <i>Actinopyga agassizi</i>	Florida Keys	+	+	-	-	-	-	Plee & Pomory (2020)
<i>Apostichopus japonicus</i>	China (farmed)	+	+	-	-	-	-	Mohsen et al (2018)
<i>Stichopus horrens</i>	Malaysia	+	+	+	-	-	-	Husin et al (2021)

Note: (+) = found; (-) = unfound.

The existence of these various types of MPs cannot be separated from the source of MPs in the local environment. The lack of handling and processing of plastic waste at the research site were indicated by the large amount of plastic waste on the coast of Mare Island, which were suspected to be a source of MPs. Plastic wastes in the form of plastic bottles, plastic packaging, and other plastic products that are thrown into the sea will undergo a process of fragmentation, degradation and weathering over time into small pieces in the form of plastic fragments (Andrady 2011; Graham & Thompson 2009). Moreover, the activities of local residents who use various fishing gear such as gill nets, tondas, and fishing rods (DKP Malut Province 2019) may be a source of MPs, especially the fiber types. As stated by Unsworth et al (2021) that a source of fiber is fishing nets and fishing line. Ramili & Umasangaji (2022) found MPs in seagrass sediments in high amount at both study sites. Jones et al (2020) indicated that seagrass beds are capable playing an important role as a reservoir for MPs contamination. The groups of benthic fauna such as sea cucumbers that inhabit in seagrass beds are very susceptible to uptake of MPs that accumulate in sediments (Plee & Pomory 2020).

Sea cucumbers are benthic fauna which are often found in the bottom substrates of seagrass beds as one of their habitats. Several previous studies showed the accumulation of MPs in seagrass beds, both in sediments or bottom substrates (Huang et al 2020; Dahl et al 2021; Kreitsberg et al 2021) and in the seagrass itself (Goss et al 2018; Jones et al 2020). Pinheiro et al (2020) revealed that groups of benthic faunas, one of which is sea cucumbers, which live in the bottom sediments of waters have a potential risk of interaction with MPs. According to Mohsen et al (2018), once MPs are present in the sediment, the sea cucumbers will swallow them. Furthermore, Pinheiro et al (2020) stated that one of the entry routes for MPs to the benthic fauna is through the ingestion process. Sea cucumbers have a feeding behavior as a deposit feeder so that the presence of MPs in their digestive tract cannot be separated from their feeding behavior which can swallow all materials contained in sediments, including MPs (Sun et al 2015; Viyakarn et al 2020). Ramili & Umasangaji (2022) reported the identification of MPs in seagrass sediments on Mare Island waters consisting of fibers, fragments, filaments, and pellets. This allows the transfer of these MPs into the digestive tract of the three species of sea cucumbers, *H. scabra*, *H. atra*, and *B. vitiensis* found in the seagrass beds of Mare Island through their feeding behavior as deposit feeders.

The abundance of MPs in the digestive tract of sea cucumbers. Identified MPs were found in all individuals of the three sea cucumber species found in both study sites. The abundance of MPs found in each individual sea cucumber was quite varied as shown in Figure 4. *H. scabra* was found in station 1 as many as four individuals with a range of MPs numbers between 78 and 154 particles ind⁻¹. The number of MPs found in six *H. atra* individuals ranged from 13 to 161 particles ind⁻¹. Meanwhile, *B. vitiensis*, which was only found in an individual, has a total number of MPs of 118 particles ind⁻¹. Five of *H. atra* individuals were found at station 2, with a range of MPs between 46 and 100 particles ind⁻¹. The total number of MPs particles found in the three species (*H. atra*, *H. scabra*, and *B. vitiensis*) found at station 1 was 1054 particles, while the total number of MPs at Station 2 was 367 particles from one sea cucumber species namely *H. atra* (Table 3).

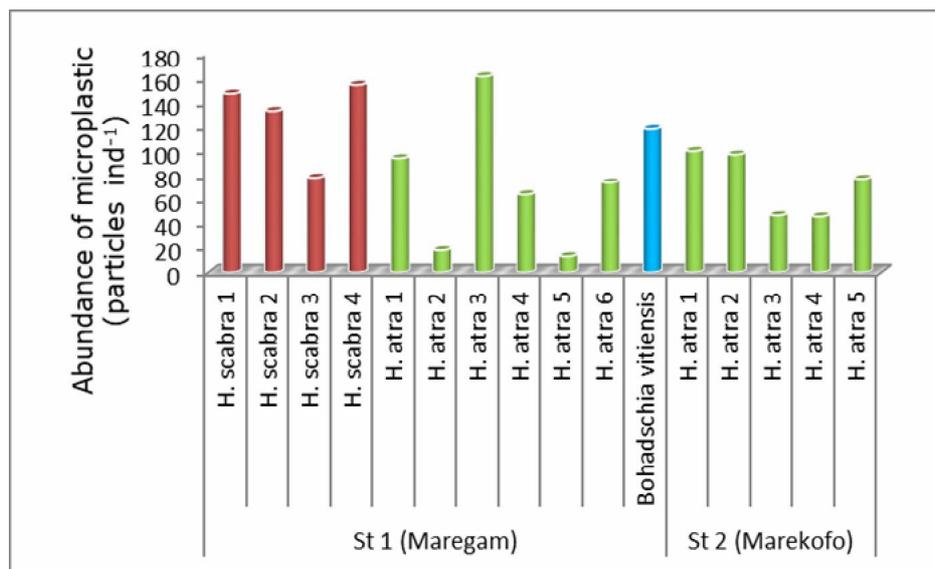


Figure 4. Distribution of the MPs numbers found in each individual sea cucumbers.

Table 3
Average body length, abundance of microplastics and total number of MPs

Stations	Species	Average of body length (cm)	Average abundance of MPs (particles ind ⁻¹)	Total number of MPs
St 1 (Maregam)	<i>Holothuria scabra</i>	15.63	128	512
	<i>Holothuria atra</i>	14.83	70.67	424
	<i>Bohadschia vitiensis</i>	20*	118*	118
St 2 (Marekofo)	<i>Holothuria atra</i>	19.30	73.4	367
Total				1421

Note: * the average value was uncounted due to only one individual found.

The results of this study also explained that the average abundance of MPs in the digestive tract of *H. scabra*, which was 128 particles ind⁻¹, was higher than the other two sea cucumber species, *H. atra*, which was 70.67 particles ind⁻¹ instation1, while in station 2 was 73.14 particles ind⁻¹, and *B. vitiensis* was 118 particles ind⁻¹. These values were higher than the abundance of MPs in sea cucumbers found on Bintan Island and its surroundings, which was 52±7.68 particles ind⁻¹ (Idris et al 2022), while Mohsen et al (2018) reported the highest average abundance on *Apostichopus japonicus* of 24.2±5.90 particles ind⁻¹. The high content of MPs in the digestive tract of *H. scabra* was probably related to their habitat, where according to Lumbu et al (2020), *H. scabra* is often found in waters which basically contain fine sand and overgrown with plants such as seagrass. Bakir et al (2020) stated that fine sediments have a higher density of MPs. Furthermore, Shi et al (2015) stated that this fine particle size can prolong the residence time of food in the digestive tract of sea cucumbers. Since the feeding behavior of these sea

cucumbers is as the deposit feeders, the possibility of ingesting these MPs is also related to how much sediment can be taken and processed by these sea cucumbers in their gastrointestinal tract. According to Setyastuti et al (2019), *H. atra* is able to process the sediments up to 19 kg dw year⁻¹ ind⁻¹. Meanwhile, sea cucumbers on coral reefs are collectively capable of consuming 8.3 x 10⁶ m³ of surface sediments (Roberts et al 2000).

The characteristics of MPs types in the digestive tract of sea cucumbers. The characteristics of MPs can be identified by the types or shapes. The identification of MPs characteristics according the types can describe the dominant number of MPs types in the gastrointestinal tract of sea cucumbers found at the research sites. MPs particles found in the three species of sea cucumbers, namely *H. atra*, *H. scabra* and *B. vitiensis* have various types with a total of 1421 particles observed. MPs types found in this study were grouped into three types, namely fragments, fibers, and spheres. The results of this study found that fragments followed by fibers were most commonly found in the gastrointestinal of these sea cucumbers. While the least ones were sphere found only in two of 6 individuals of *H. atra* and one individual of *B. vitiensis* at station 1.

The abundance of MPs by type in each species of sea cucumbers, namely *H. atra* and *H. scabra* at both study sites is shown in Figure 5. For *B. vitiensis* was not shown in the graph because only one individual was found. MPs types found in *B. vitiensis* were fragments of 107 particles, fiber of 10 particles and sphere of only one particle, with a total of 118 particles. The results shown in Figure 5 are the median and range values of each type of MPs from *H. scabra* and *H. atra*. Generally, it was seen that *H. scabra* have a median value with a higher range than *H. atra*. Moreover, fragments and fibers in *H. scabra* have median values with a range of 110.5 particles ind⁻¹ respectively with a range of 96 to 123.5 particles ind⁻¹, and 18.5 particles ind⁻¹ with a range of 8.75 to 28.75 particles ind⁻¹. Next, the median value and range of each type of MPs in *H. atra*, the median value for fragments was 66 particles ind⁻¹ with a range of 29 to 77.5 particles ind⁻¹, and fibers of 5 particles ind⁻¹ with a range of 2.5 to 9.75 particles ind⁻¹. Then, the median value for spheres was 0.5 with a range of 0 to 0.75 particles ind⁻¹. Eventually, the median value and range of each type of MPs in *H. atra* at station 2 has 76 particles ind⁻¹ of fragments with a range of 43 to 84 particles ind⁻¹ and the median value for fibers was 5 particles ind⁻¹ with a range of 4 to 6 particles ind⁻¹.

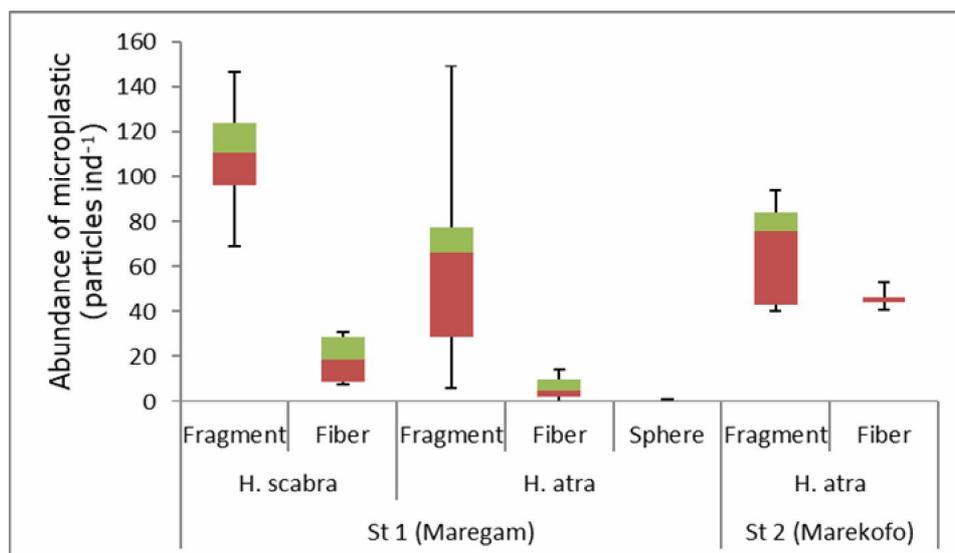


Figure 5. Characteristics of MPs types on the sea cucumbers found at the research site (here is not shown the type of sea cucumber *B. vitiensis*).

Overall, it was found that fragments were the most common in the gastrointestinal of those sea cucumbers, followed by fibers and spheres in both study sites. However, there were differences in composition where the composition of the fragments was higher at

station 2 which was 91.83% compared to station 1 which was 88.05%. This was followed by the fibers found at station 1 of 11.67% and station 2 of 8.17%. While sphere ones as the least composition of 0.28% at station 1. The distribution of MPs composition types in the sea cucumber digestive tract at the study site is shown in Figure 6.

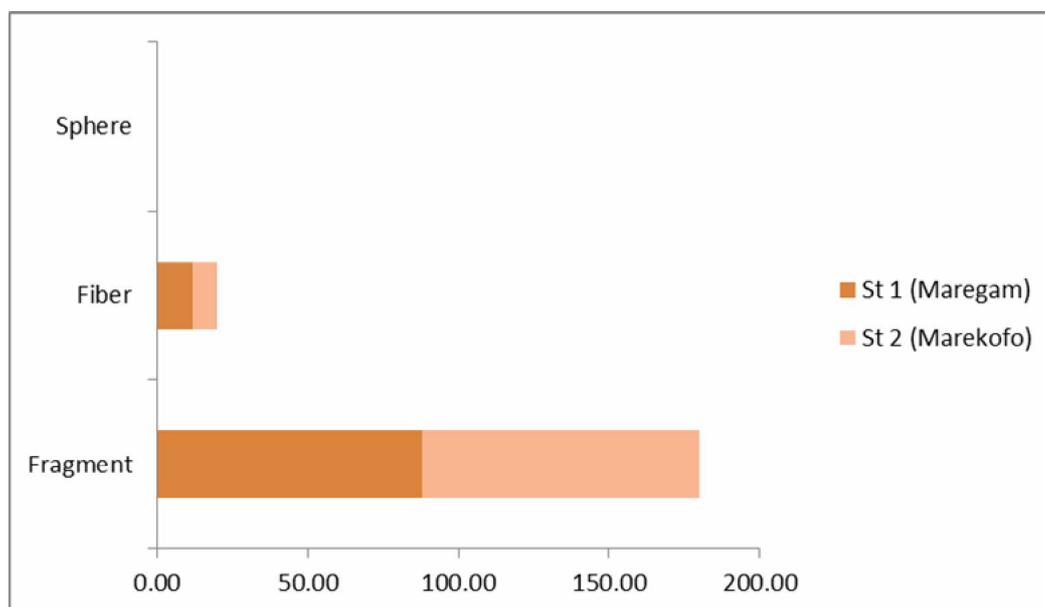


Figure 6. Composition of MPs types in the digestive tract of sea cucumbers in the waters of Mare Island.

Various types of MPs are often found in marine sedimentary, including fibers, fragments, pellets, films and spheres. The existence of various types of MPs highly depends on the sources. Generally, fibers and fragments were the most numerous particles which are found in the sediments compared to other types. The results of research conducted by Ramili & Umasangaji (2022) revealed that the percentage of fragments in the seagrass bed sediments of Mare Island was quite high, namely $\pm 31.37\%$, while the fibers were observed to be the highest at $\pm 76.36\%$. However, in this study, fragments were the most numerous particles discovered in the digestive tract of sea cucumbers at both stations. This is different compared to that reported by Idris et al (2022) who discovered a higher percentage of fibers in sea cucumbers on Bintan Island and its surroundings, as well as that was found on Tidung and Bira Besar islands (Sayogo et al 2020), also on Rambut Island (Wicaksono et al 2021). According to Mohsen et al (2018), the ingestion of MPs by sea cucumbers can occur as long as the size of the MPs is in accordance with the sea cucumber's mouth or as far as its tentacles can take sediment to be inserted into its mouth. Experiments in the laboratory found that sea cucumbers can ingest particles measuring ± 0.5 mm (Graham & Thompson 2009). According to James et al (2020), generally the marine sedimentary environment is dominated by plastic fragments measuring < 1 mm. Iwalaye et al (2020), stated that 90% of sea cucumbers *Holothuria cinerascens* ingested fragments through their tentacles. The high presence of fragments in the seagrass sediments of Mare Island allows the entry of these MPs into the digestive tract of sea cucumbers. Plee & Pomory (2020) revealed that the presence of fragment particles in the digestive tract of sea cucumbers is also related to their body size, where sea cucumbers with a larger body size allow them to ingest various types of MPs. While the sphere type is very rarely found because of its larger size to be caught by the sea cucumber's tentacles and enter its mouth. However, further investigation is still needed with a larger number of sea cucumber samples.

The color characteristics of MPs in the digestive tract of sea cucumbers. MPs with a total of 1421 particles found in the digestive tract of the three species of sea cucumbers were also identified based on color characteristics. Overall, eight MPs colors

were found, namely gray, black, brown, blue, red, white, green and yellow in the digestive tract of sea cucumbers. The various colors of MPs found in the three species of sea cucumbers indicated that *B. vitiensis* had six colors less than *H. atra* and *H. scabra*. Blue and white colors were not found in *B. vitiensis* while yellow colors were not found in *H. atra* at station 2 (Figure 7). Overall, the black color was found with a higher percentage followed by brown and gray in the three sea cucumber species. The color composition of *H. scabra* consists of black (58.2%), brown (17.0%), gray (16.2%) and yellow (0.2%). The percentage of MPs color in *H. atra* at station 1 and station 2 were black (40.8%; 49.9%), brown (23.8%; 21.3%), gray (17.1%; 16.1%), the lowest was red in both stations by 0.5%. Meanwhile, in *B. vitiensis*, the percentage of brown was higher (50%), black (24.6%) and gray (21.2%).

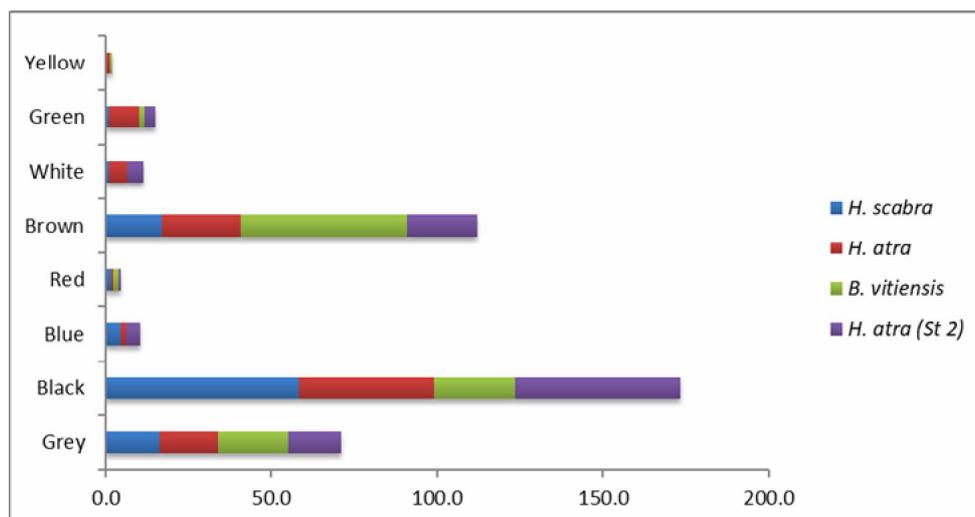


Figure 7. Percentage of MPs color composition on the different sea cucumber species.

The results of MPs colors identification in each species of sea cucumbers at the two research stations provided different results, where *H. scabra* had a higher distribution of median values and range of each color than *H. atra* (Figure 8). The color characteristics of MPs in *H. scabra* are gray with 21 particles ind^{-1} , range between 7.75 and 34, black of 72.5 particles ind^{-1} with a range of 61.5 to 85.5, blue of 6 particles ind^{-1} with a range of 1.75 to 0.25, red 1.5 particles ind^{-1} with a range of 0.75 to 3, brown of 19 particles ind^{-1} with a range of 17 to 23.75, white of 1 particle ind^{-1} with a range of 0.75 to 1.5, green of 1 particle ind^{-1} with a range of 0.75 to 1.5. Meanwhile, the median value and range of each color of MPs in *H. atra* were gray 12.5 particles ind^{-1} with a range of 3.25 to 18.75, black of 15.5 particles ind^{-1} with a range of 8.25 to 22, blue of 0.5 particles ind^{-1} with a range of 0 to 1.75, red of 0.5 particles ind^{-1} with a range of 0 to 0.75, brown of 13 particles ind^{-1} with a range of 5.75 to 27, white of 2 particles ind^{-1} with a range of 1.25 to 2.75, green of 0.5 particles ind^{-1} with a range of 0 to 4.75, yellow of 0.5 particles ind^{-1} with a range of 0 to 0.75.

While the median value and range of each types of MPs in *H. atra* at station 2 (Figure 9) are gray of 10 particles ind^{-1} with a range of 9 to 16, black of 33 particles ind^{-1} with a range of 26 to 50, blue of 1 particle ind^{-1} with a range of 0 to 3, red of 0 particle ind^{-1} , brown of 17 particles ind^{-1} with a range of 12 to 21, white of 1 particle ind^{-1} with a range of 0 to 7, and green of 0 particle ind^{-1} with a range of 0 to 2.

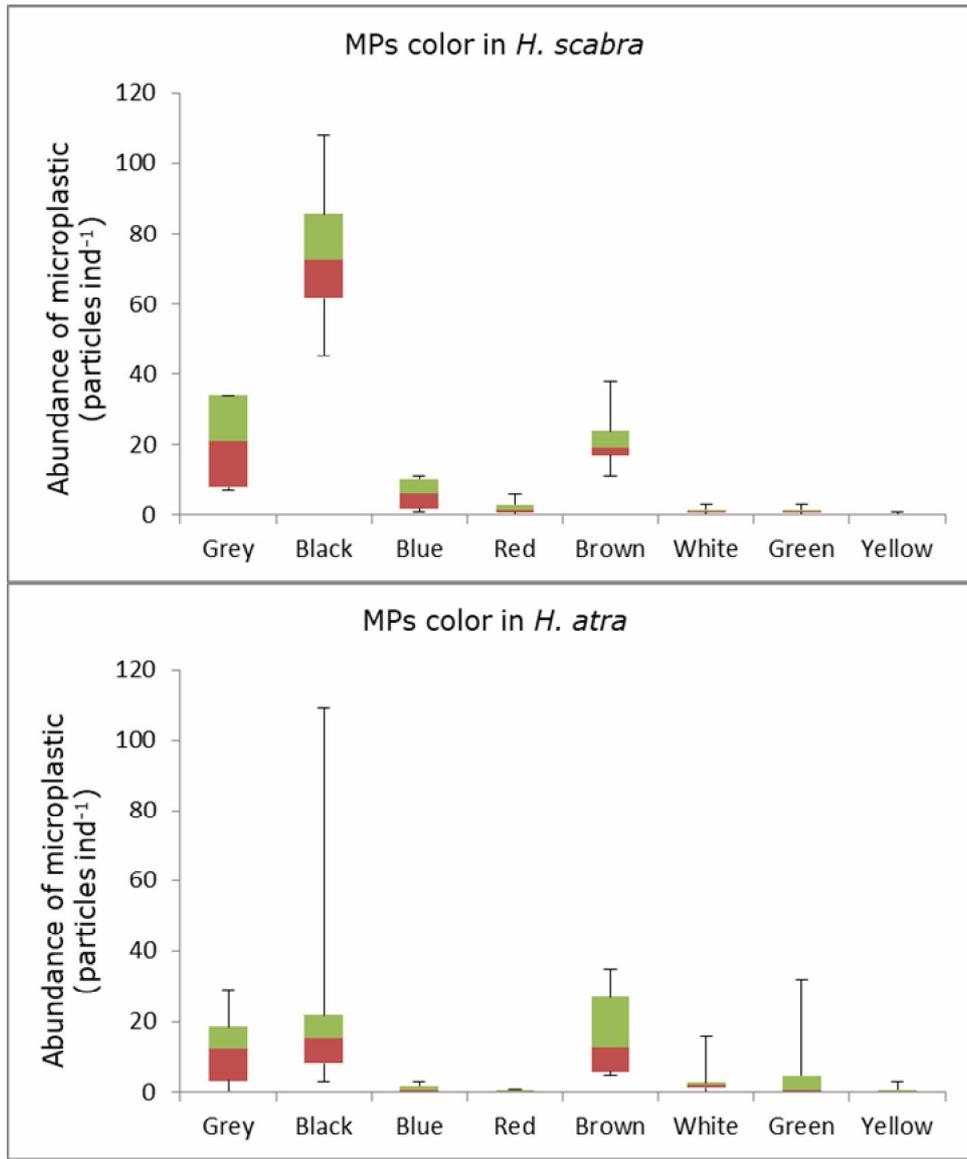


Figure 8. Characteristics of MPs colors in *H. scabra* and *H. atra* found at station 1 (*B. vitiensis* not shown).

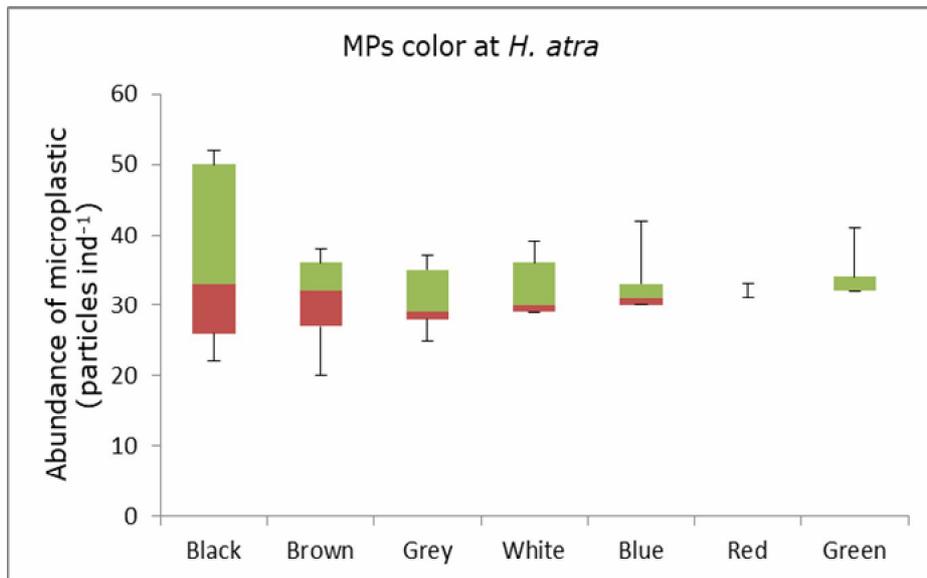


Figure 9. Characteristics of MPs colors in *H. atra* found at station 2.

The color composition of MPs in the gastrointestinal of sea cucumbers discovered in the two study sites was dominated by black, which was 47.44% at station 1 and 49.89% at station 2. Then followed by brown of 23.43% at station 1 and 21.25% at station 2, gray of 17.36% at station 1 and 16.03% at station 2. The least color found was yellow at station 1 and red in station 2. Yellow was not found at station 2 (Figure 10).

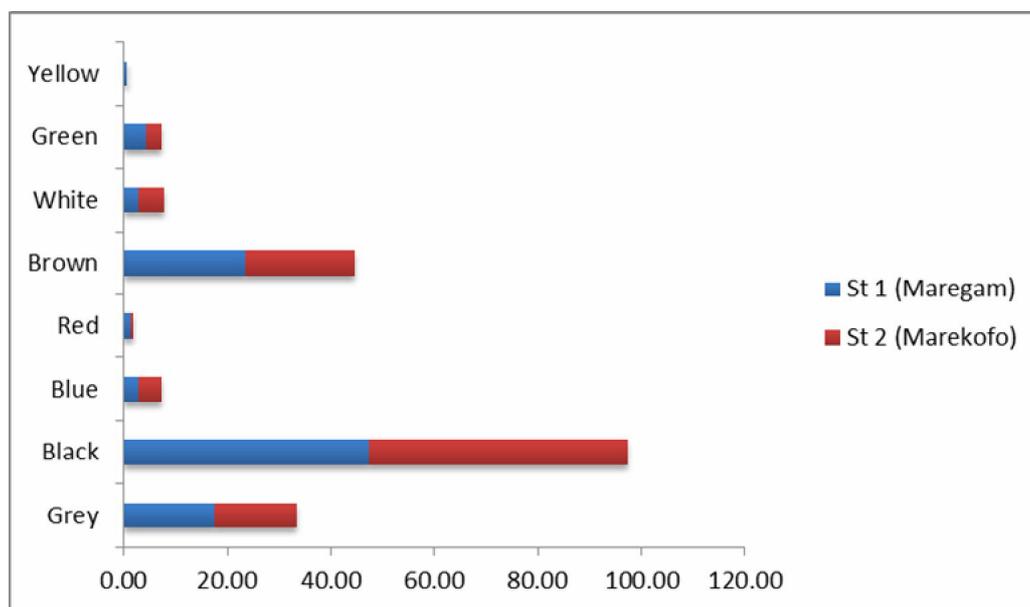


Figure 10. Percentage of microplastic color in the digestive tract of sea cucumbers in the waters of Mare Island.

The various colors of MPs (8 colors) found in the gastrointestinal tract of sea cucumbers represent the MPs colors found in the sediment matrix of seagrass beds on Mare Island as reported by Ramili & Umasangaji (2022) who found nine colors including orange which was unfound in the gastrointestinal tract of sea cucumbers. The dominance of black color found in the digestive tract of the three species of sea cucumbers in this study was similar to that of *Stichopus horrens* in Malaysia, which was 59% (Husin et al 2021). Several previous studies reported the dominance of different MPs colors, such as in sea cucumbers in the waters of Bintan Island, which were dominated by white followed by black (Idris et al 2022). Furthermore, in the gastrointestinal tract of *A. japonicus* cultivated in China was dominated by blue followed by transparent colors (Mohsen et al 2018). This difference in the colors dominance of MPs in the digestive tract of sea cucumbers in various places is probably related to the source of MPs deposited in local sediments. According to Idris et al (2020) the dominance of white color was presumably due to plastic particles deposited in the sediments that have occurred for a long time so that they had experienced the color changes. While the dominance of black or darker colors was probably induced by the plastic particles that have not been deposited in the sediment for a long time, so they had not experienced the colors degradation. Additionally, the black colors can indicate that in these waters there are many contaminants which had been absorbed in MPs and other organic particles (GESAMP 2015). This is in accordance with Wang et al (2018) who stated that black MPs particles are able to absorb chemicals more than white and other color particles. The dominance of black MPs is also found in fish groups such as croaker fish (*Johnius dussumieri*) in India (Debbarma et al 2022). This needs to be a new concern regarding the possible entry of MPs into the food chain system in seagrass beds through biotransfer between trophic levels.

The relationship between sea cucumbers body weight and MPs abundance. The relationship between body weight of sea cucumbers and the abundance of MPs has a very low coefficient of correlation performed by the equation of $y = 0.0744x + 77.331$ with R^2

= 0.0198 (Figure 11). According to the the graph, there is a positive trend between body weight of sea cucumbers and the abundance of MPs, but the coefficient of determination value of 1.98% indicates a very weak correlation between these two parameters. This indicates that the abundance of MPs is extremely low influenced by the sea cucumbers body weight and is more influenced by other factors. This is in accordance with that of reported by Mohsen et al (2018) on the sea cucumber species *A. japonicus* where there was no correlation between body weight and the amount of MPs ingested. The condition of the bottom substrate of the waters is indicated to be able to influence the growth of sea cucumbers, where the larger the grain size of the sediments will make the growth of sea cucumbers slower (Shi et al 2015). It definitely can affect the body weight of sea cucumbers.

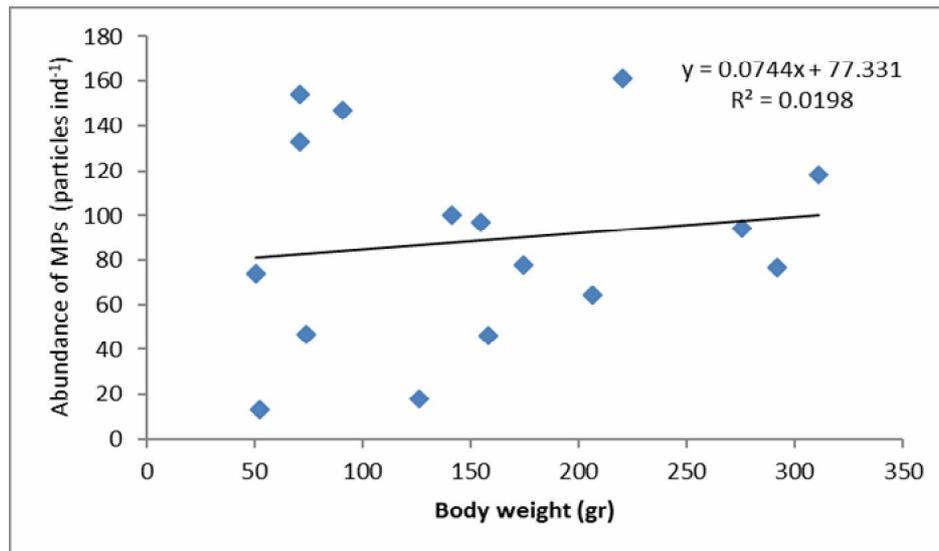


Figure 11. Graph of correlation between body weight of sea cucumber and microplastic abundance.

The occurrence of MPs in the three species of sea cucumbers found in the waters of Mare Island allows their use as bio-indicators for MPs entering benthic food webs, especially in seagrass ecosystems. One of the ecological roles of seagrass beds is as a place to forage for various organisms from various trophic levels. The accumulation of MPs in fauna that occupy lower trophic levels such as sea cucumbers, allows the bio-transfer to higher trophic levels. Nevertheless, it can also explain the tendency of sediments to be increasingly contaminated by MPs over time (Pinheiro et al 2020). Furthermore, Plee & Pomory (2020) revealed that the determination of MPs concentration levels ingested by sea cucumbers is not only related to the environmental conditions, but also relates to the possibility of contamination to humans. According to Teuten et al (2009), although the digestive tract is removed in the process of preparing sea cucumbers as food, there is a possibility that MPs contaminants are in the tissues that are consumed because they are absorbed during the digestive process. The three species of sea cucumbers obtained in this study have economic value in the categories of cheap or low value species (*H. atra*), medium value species (*B. vitiensis*) and expensive or high value species (*H. scabra*) (Setyastuti et al 2019). The high content of MPs found in *H. scabra* in this study needs attention considering that this species has a high commercial value. Although local people on Mare Island have not consumed sea cucumbers as a food source yet, the results of this study need special attention. This is based on the research data which MPs have been identified in one of the important ecosystems on Mare Island, namely in seagrass sediments (Ramili & Umasangaji 2022), and one of the associated biotas, namely sea cucumbers in this present study. This shows that stakeholders should pay special attention to the research in the environmental field by further work on the accumulation of MPs in other environmental matrices and the associated biota in determining policy steps for the management of coastal and marine resources in the future, especially in conservation areas as defense fortresses and maintenance of marine living resources.

Conclusions. MPs were identified in the gastrointestinal tract of three sea cucumber species, namely *H. atra*, *H. scabra* and *B. vitiensis* which consisted of three types, namely fragments, fibers, and spheres. The highest abundance of MPs was found in *H. scabra*. The characteristics of the MPs types were dominated by the fragments, while the colors were dominated by black followed by brown and gray. There was no correlation between body weight and the number of MPs found in the gastrointestinal of sea cucumbers at Mare Island.

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