INTRODUCTION

Plastic waste is one of the most serious threats to ocean pollution in the 21st century (Goldberg, 1995). Plastic waste is degraded in the environment into small pieces in the form of microplastics (MPs) measuring <5 mm to 1 mm in size and having a rounded, fibrous, and elongated shape (GESAMP, 2015). The MPs that enter marine waters come from the cosmetic industry and various kinds of plastic waste that have gone through the process of UV light exposure, biodegradation, and physical processes (Pettipas et al., 2016; Andrady, 2011). MPs are harmful for the exposed marine biota and cause problems with health, endocrine glands (Carbery et al., 2018), impair the digestive tract function, reduce growth rates, lower the levels of steroid hormones, cause disorders of the reproductive system; the exposure to plastic additives has toxic properties (Wright et al., 2013).

Microplastics contamination has occurred in various environments, such as seawater, freshwater, ice, and soil (Li et al., 2021). MPs move into aquatic biota from the immediate environment or their prey (Lusher et al., 2017a) so that they can increase the concentration in the body of the biota and cause bioaccumulation (Carbery et al., 2018). Bioaccumulation is an increase in the concentration of a dangerous chemical substance into an organism’s body, either through environment such as water, sediment, air, and through food or prey consumed (Arnot and Gobaz, 2006). The movement of MPs from an individual to individual can occur, and it is directly transferred to humans by consuming fish (Hantoro et al., 2019). Bioaccumulation of MPs has occurred in many marine species (Miller et al., 2020) such as fish and molluscs.

Grouper fish (Epinephelus) is a fishery product the consumption of which increases every year (KKP, 2018). High levels of consumption
can be comparable to the exposure by MPs to humans. Previous study results indicated that MPs were found in the digestive tract of fishery products in the world (Lusher et al., 2017a) i.e. *Epinephelus* spp., Indonesia (Hapitasari, 2016), *E. coioides*, Persia Bay (Akbarizadeh et al., 2017), *E. radiates* and *E. areolatus*, Saudi Arabia (Baalkhuyur et al., 2018) and *E. coioides*, Malaysia (Karbalaei et al., 2019). The contamination of MPs in grouper fish indicates that the aquatic environment has been polluted and bioaccumulation has occurred, which will then move to a higher trophic level (biomagnification).

Contamination and bioaccumulation information of MPs by the *Epinephelus* species in Indonesia is lacking. Limited information about MPs causes the seafood safety policy to be hampered and difficult to identify its risk profile (Lusher et al., 2017a). Therefore, the bioaccumulation of MPs in marine biota does not yet have a clear process and requires further testing (Miller et al., 2020). A study on number and types of MPs in digestive tract and sediment, the correlation between number of MPs with total body length and estimated uptake of MPs in fish from sediment using Bioaccumulation factor (BAF) are needed to observe.

### MATERIALS AND METHODS

#### Study site and sample collection

The samples of *Epinephelus* were taken on Pramuka Island, Seribu Islands, Jakarta, Indonesia at 3 points (East, Dermaga and Gosong) in October 2019 – January 2020 (Figure 1). Fish are collected using SCUBA diving and a speargun at a depth of 5–20 m, and the catch of fishermen or local people. The sediment was taken, at the same point with fish catch, with 2 depths (5 and 10 m) and was repeated 3 times. The sediment samples were taken in the amount of 1 kg and collected into plastic samples (Hildago-Ruz et al., 2012).

The samples of *Epinephelus* obtained were identified based on Allen et al. (2003) and the FishBase website (https://www.fishbase.de). Fish identification is based on body shape, body-color, line and spot specific characteristics, and total length (TL). Length measures are used to determine age (Abd-Allah et al., 2015). *Epinephelus* were measured from snout to tip of tail, to be analyzed using linear regression with the number of MPs in the digestive tract. All samples were stored in a cooling box filled with ice with a temperature range of 10–20°C to be brought to the laboratory for preparation and observation.
Sample preparation

Grouper fish

The digestive organs of fish were taken from the stomach to the intestines and separated from other parts that were not needed. The digestive organs that have been taken were then separated and weighed by digital scales. The sample preparation involved using the HNO₃ solvent to destroy the organic material contained in the sample (Lusher et al., 2017a). The sample preparation was divided into 3 stages: (a) smelting of organic matter (b) isolation and (c) visual observation of MPs. Organic matter was smelted using the HNO₃ solvent with a concentration of 68% and placed into a beaker glass along with the digestive contents of the grouper in a ratio of 1 gram of sample to 5 ml of HNO₃ solvent. The mixture of HNO₃ solvent with the digestive contents of grouper was heated at 60°C with a hotplate in a fume hood for 10 minutes. A saturated salt solvent was added to separate the MPs from the organic and liquid particles (1:1 with HNO₃). The mixed solvent was reheated for 10 minutes at 60°C.

Sediment

Sediment separation involved the following stages: (a) drying, (b) volume reduction, (c) density separation, (d) filtering, and (e) visual sorting (Hildago-Ruz et al., 2012). The sediment was mixed with 100 ml of H₂O₂ (6–10% concentration), and then stirred for 2 minutes to remove the organic matter content in the sediment. The sediment was allowed to stand until the reaction is complete with a sign of the loss of bubbles in the sediment (Frias et al., 2018). Sediment was dried in an oven at 70°C for 72 hours and then was separated by a 5 mm sieve; afterwards, 100 g was taken. Furthermore, saturated salt solvent (3:1) was added and stirred for 2 minutes.

The tools used in the laboratory, for observation and sample analysis, were cleaned with alcohol so that they are not contaminated with MPs materials. All samples were stored at room temperature for 24 hours, then the surface was separated to be observed under a microscope. Liquid samples (5 ml/sample) from the digestive tract of fish and sediment were observed using Olympus CH20 (400x magnification) and Olympus BX 51 microscopes. The numbers and types of MPs were calculated and measured and then categorized based on the shape of fragments, pellets, and fibers (Lusher et al., 2017b).

Data analysis

A descriptive statistical method was used to analyze the number and types of identified MPs. The data were analyzed by descriptive statistics that generally describe MPs in fish and sediments in tables and graphs. The correlation between the numbers of MPs with the body length of each individual Epinephelus was tested using linear regression. A linear regression test was performed using the Minitab 19 application program. One sample T-test was carried out on MPs in the sediment using the M.S. Excel application to determine the real difference in the number of MPs in sediment at each station.

The MPs data in Epinephelus digestive tract and sediment were analyzed using Bioaccumulation Factor (BAF) (Arnot and Gobaz, 2006). Estimation of MPs uptake in the sediment to digestive tract used BAF analysis with the formula:

\[
BAF = \frac{C_B}{C_{WD}}
\]

\(C_B\) is the number of MPs in the digestive tract of fish and \(C_{WD}\) is the number of MPs in sediment. The BAF value is obtained from the results of comparing the number of MPs particles that enter the digestive tract with the number of MPs in sediment. The MPs unit in Epinephelus was converted into particles/gr by dividing the number of MPs in digestive tract of each fish by digestive weight.

RESULTS AND DISCUSSION

MPs in the digestive tract

This study found 20 individuals and 4 types of *E. sexfasciatus*, *E. ongus*, *E. areolatus*, and *E. fuscoaguttatus* with average length of fish from 23 to 35 cm. The highest number of MPs was in *E. areolatus* with an average of 109.8 particles, and the lowest was found in *E. ongus* – 60 particles. In all samples of the digestive tract of fish, MPs with a total number of 1648 particles were found (Table 1).

On the basis of a total length of 25–35 cm, Epinephelus age in the juvenile category is 2 to 3 years, the same as the previous study of Abd-Allah et al. (2015) and Frias et al. (2018). Total length data also serves to map the distribution of MPs by age category (Jâms et al., 2020), which has been contaminated in different numbers and sources of samples (from habitats or experimental tests).
Table 1. The average total length of fish and MPs in the digestive tract

| Species                         | Average of total length (cm) | Average of MPs (particles) |
|---------------------------------|------------------------------|---------------------------|
| E. sexfasciatus (Valenciennes, 1828) | 23.84                        | 94                        |
| E. ongus (Bloch, 1970)           | 26.34                        | 60                        |
| E. areolatus (Forsskål, 1775)    | 26.68                        | 109.8                     |
| E. fuscoguttatus (Forsskål, 1775)| 35.26                        | 65.8                      |

The results of previous studies indicated that the number of MPs in Epinephelus was lower than in Pramuka Island (Table 2). Among them are E. areolatus with 1 ingested particle of MPs (Baalkhuyur et al., 2018), Epinephelus spp. 6 to 14 particles were found (Hapitasari, 2016), E. chlorostigma, E. radiatus, and E. epistictus was found with 1 particle (Baalkhuyur et al., 2018), E. coioides has a total of 7.75 items/10 gr (Akhbarizadeh et al., 2017) and 4 particles/species (Karbalaei et al., 2019), and E. merra were found 1.3 particle/individual (Garnier et al., 2019). On the basis of the experimental test, the results of E. moara found MPs in body tissue (Wang et al., 2020) and E. fuscoguttatus reported 10% ingestion of MPs (Xu and Li, 2021). The literature studies of species E. sexfasciatus, E. ongus, and E. fuscoguttatus came from nature (not laboratory-scale experiments), and ingestion of MPs has not been reported, so it is not easy to compare them.

Different habitat types, presence of MPs in the environment, consumption or prey behavior, and the preparation method used influence different numbers of MPs in Epinephelus. The results of previous studies showed differences in the number of MPs found, which based on the types and sizes of fish, density and color of MPs in environment, and sampling location (Wright et al., 2013; Akhbarizadeh et al., 2017; Baalkhuyur et al., 2018; Karbalaei et al., 2019; Xu and Li, 2021; Garnier et al., 2019; Liboiron et al., 2019; Tanaka and Takeda, 2016; Sbrana et al., 2020). Furthermore, the source of MPs in the digestive tract of Epinephelus is thought to have come from prey. Benthic invertebrates (Heemstra and Randall, 1993) such as crustacea, cephalopods (Salini et al., 1994), cnidarians (Freitas et al., 2017) and small fish (Freitas et al., 2017; Reñones et al., 2002; Erlangga 2021; Bessa et al., 2018) are the prey of Epinephelus, this allows contamination of MPs from the sediments that are directly ingested with their prey (Lusher et al., 2017a). Several species of fish and octopus (prey), which are food for Epinephelus (Freitas et al., 2017; Reñones et al., 2002, have been reported to have been contaminated with MPs (Bessa et al., 2018; Gündoğdu et al., 2020; Shabaka et al., 2020; Unpublished data for MPs in octopus).

The habitat of E. areolatus in seagrass beds or waters column have sediments close to coral reefs, dead coral, and soft corals with a depth of 2 to 100 m (Heemstra and Randall, 1993). The wide range and distribution of habitat are thought to make E. areolatus easily contaminated by surrounding waters. E. areolatus living in seagrass were found to have a higher MPs percentage than those living in mesopelagic (Baalkhuyur et al., 2018), in contrast to E. ongus and E. fuscoguttatus, where MPs contamination was relatively

Table 2. Number of MPs from Epinephelus species

| Species | Habitat/Sources | Number of MPs | Organs | Country/Location | Ref. |
|---------|-----------------|---------------|--------|-----------------|-----|
| E. areolatus | Seagrass | 1 particles/indv | Digestive tract | Saudi Arabian | a |
| Epinephelus spp. | Fish market | 6-14 particles/indv | Digestive tract | Indonesia | b |
| E. chlorostigma | Seagrass | 1 particles/indv | Digestive tract | Saudi Arabian | a |
| E. radiatus | Demersal | 1 particles/indv | Digestive tract | Saudi Arabian | a |
| E. epistictus | Demersal | 1 particles/indv | Digestive tract | Saudi Arabian | a |
| E. coioides | Fish market | 7.75 items/10 g | Muscle | Persian Gulf | c |
| E. coioides | Fish market | 4 particles | Viscera and gills | Malaysia | d |
| E. merra | Lagoon | 1.3 particles/fish | Digestive tract | French Polynesia | e |
| E. moara | Experiment | 0.375-9.60 μg kg⁻¹dw | Liver tissue | Laboratory | f |
| E. fuscoguttatus | Experiment | 10 % particles | Behaviour | Laboratory | g |

a: Baalkhuyur et al., (2018), b: Hapitasari (2016), c: Akhbarizadeh et al., (2017), d: Karbalaei et al., (2019), e: Garnier et al., (2019), f: Wang et al., (2020), and g: Xu and Li (2021).
smaller. It is due to its specific habitat, which is at a depth of 5–60 meters (Heemstra and Randall, 1993; Gibran, 2007) on the substrate of coral reefs, rock fragments, or rocks (Nanami et al., 2013), and its behavior which is slightly moving and stays in their habitat only.

Benthic invertebrates are the prey of predatory fish such as Epinephelus, which are directly exposed to MPs contamination on the waters column. Fishing equipment such as bottom nets, trawls, and various types of bottom ropes are a great potential source for MPs. Various types of fishing gear, mostly polyamide, polyethylene, and polypropylene, will be degraded to MPs and mostly settle on the seabed (Lusher et al., 2017a). Benthic invertebrates such as molluscs, crustacea, and echinoderms will easily be directly contaminated by MPs including bivalvia (Avio et al., 2015; Sussarellu et al., 2016; Tubagus et al., 2020), crabs (Watts et al., 2014), and sea cucumber (Graham and Thompson, 2009; Sayogo et al., 2020).

Human activities are a source of MPs in the estuary, coastal and deep-sea waters. Pramuka Island is a tourist destination island so that many human visit and increase population density. The result of previous studies indicated that macroplastics were found on Pramuka Island and its surroundings (Assuyuti et al., 2018) and most of the MPs were contaminated with sediments, seagrass, coral reefs, and other biotas in the Seribu Islands (Priscilla et al., 2019; Patria et al., 2020; Sayogo et al., 2020; Tubagus et al., 2020). The correlation between MPs abundance and population density that has human activity shows positive results and has been carried out in various locations (Browne et al., 2011).

Furthermore, the primarily source of MPs in marine ecosystems comes from domestic waste or abandoned, lost, or neglected fishing gear such as fishing gear, ropes, nets, and packaging materials (Lusher et al., 2017a).

**Types of MPs**

The types of MPs found are fiber, pellet, and fragment. The total number of MPs found was 1648 particles, with the most fiber 990 (60%), 570 fragments (35%), and 88 pellets (5%) particles (Figures 2 and 3) with a size range of 20–1000 μm (Figure 4). The highest average fragment values were in *E. areolatus* and lowest in *E. fuscoguttatus*. The highest pellets were in *E. areolatus* and lowest in *E. fuscoguttatus*. The highest fibers were in *E. areolatus* and lowest in *E. ongus* (Figure 2).

The size of MPs in four Epinephelus species varied and had similar with previous studies. MPs in *E. coioides* (from fishing ground) measuring < 100 to > 5000 μm (Akbarizadeh et al., 2017) and commercial fish from Pantai Indah Kapuk, Jakarta have < 20 to 100.000 μm (Browne et al., 2011). Different results from previous studies show the size of MPs in Epinephelus in the range of 1.8–2.71 mm from the Saudi Arabian Red Sea coast (Baalkhuyur et al., 2018) and a size range of 149–40.000 μm from Malaysia (Karbalaei et al., 2019). The results of plastic particle size were found in previous studies were not larger than 5 mm (Akbarizadeh et al., 2017; Baalkhuyur et al., 2018; Karbalaei et al., 2019), which indicates the MPs category. The plastics

![Figure 2. Average of number and types of MPs in the digestive tract.](image-url)
measuring 0.1 μm to <5 mm include the MPs category (Lusher et al., 2017a).

Fibers are the most abundant particles found on individual Epinephelus. The results of previous studies indicate that MPs fiber is dominant in Epinephelus (Hapitasari, 2016; Akhbarizadeh et al., 2017; Baalkhuyur et al., 2018). In addition, the commercial fish originating from Pantai Indah Kapuk, Jakarta, which is adjacent to Pramuka Island, dominate some predatory fish with an average number of 15.29 particles per individual (Hastuti et al., 2019). Different results are shown from the species E. coioides and E. merra, in which fragments are dominant (Garnier et al., 2019; Karbalaei et al., 2019).

Various types of fiber polymers that make up MPs, including polyamides and polyethylene, are widely used as raw materials for fishing rods, nets, and trawls (Lusher et al., 2017a), as well as polyester and nylon, which are used as raw materials for clothing (Browne et al., 2011; Al-Lihaibi et al., 2019). All of these types of polymers have been found to pollute water bodies. Various types of fiber polymers that make up MPs, including polyamides and polyethylene, are widely used as raw materials for fishing rods, nets, and trawls (Lusher et al., 2017a), as well as polyester and nylon, which are used as raw materials for clothing (Browne et al., 2011; Al-Lihaibi et al., 2019). All of these types of polymers have been found to pollute water bodies. Fiber has a high enough density so that it can be at the bottom of the water. Fiber is found mainly on the surface and bottom of waters (Bagaev et al., 2017), which is eventually ingested by bottom biota, directly or indirectly through their food or seawater (Galloway et al., 2017).

The type of MPs mostly found in Epinephelus that lives in island waters is a fragment type. These results are the same as previous studies that found fragment-type MPs in the digestive tract of E. coioides in the Persian Gulf (Akhbarizadeh et al., 2017) and commercial fish in Malaysia (Karbalaei et al., 2019). The fragments are
derived from polypropylene and polyethylene, which are degraded from plastic bottles, food wrappers, and various plastic-based utensils. The shape of the shards is sharp, tapered, rounded with a soft surface or with a rough surface (GESAMP, 2015). The fragments have varying densities, so that they can float or sink to the bottom of the water. This makes it easier for them to be eaten by biota that lives on the surface or bottom of the water. The results of previous studies indicated that planktivorous fish were contaminated with fragments because they seemed to look like food (Critchell and Hoogenboom, 2018; Gove et al., 2019). In addition, it is also suspected that the contamination of fragments on Epinephelus is its planktivore prey. The results of previous studies indicate that the fish larvae that fall prey to Epinephelus (Freitas et al., 2017) have been contaminated with MPs (Jatmiko et al., 2018).

Another form of MPs is pellets. In this study, the number of pellets was the least compared to other fibers and MPs fragments. These results are similar to study conducted by Akhbarizadeh et al., (2017). The small number of fish digestive tracts is thought to be influenced by the activity of the primary source. The source of the pellets comes from raw materials for the plastic industry, which involve the material molding process (Mugilarsan et al., 2015). Pellets come from the degradation of plastics during printing materials in the industry and have complex properties as well as high density to sink in sediment (GESAMP, 2015). In addition, pellets have usually been washed ashore and are found on almost all coasts of the world (Holmes et al., 2012; Zhang et al., 2015).

In this study, the types of MPs (fibers, fragments, and pellets) found in the digestive tract of Epinephelus were generally thought to have originated from the waste from human activities carried to the waters of Pramuka Island. Pramuka Island is an inhabited island used as a tourist and fishing destination, close to cities and adjacent to inhabited islands (BPS Statistik, 2020). High human activity causes a lot of plastic waste to be produced and disposed of directly into the water, which is then degraded into the form of microplastics (Karthik et al., 2018). This study found that the islands with the aim of tourism and aquaculture development have the second-highest MPs after the river estuary location. Tides (Lima et al., 2015; Karthik et al., 2018), distance from land and river flow also affect the MPs distribution to the coastal water column and its surroundings (Falahudin et al., 2020). In addition, the MPs color also affects the high number of MPs eaten by Epinephelus because they are considered prey (Xu and Li, 2021).

**MPs in sediment**

This study found the highest average number of MPs in the sediment was in the Dock, with 43 particles/gr, and the lowest in the Gosong was 28.5 particles/g. On the basis of the T-test, it is known that the average number of MPs in the sediment at the three sampling points is significantly different, with a significance value of 0.57 ($p < 0.05$) (Figure 5).

The number of sediment MPs on Pramuka Island has a range from previous studies (Lie et al., 2018; Septian et al., 2018; Asadi et al., 2019; Sayogo et al., 2020) which indicate that Indonesian coastal sediments have been contaminated (Manalu et al., 2017; Lie et al., 2018; Septian et al., 2018; Asadi et al., 2019; Cordova et al., 2019; Handyman et al., 2019; Falahudin et al., 2020; Sayogo et al., 2020; Tubagus et al., 2020; Sayogo et al., 2020; Yona et al., 2020). The distribution of plastic waste in Java has been carried out with varying intensities from 30% to 70% and found microplastics with various forms that are dominated by fragments (Dwiyitno et al., 2018). Microplastics distribution in fibers, fragments, and films types originating from household waste and anthropogenic activities (Septian et al., 2018; Maharani et al., 2018; Cordova et al., 2019).

The higher number of MPs in the Dock area is thought to be derived from high human activity on land which then enters the column and bottom of waters. The waste found at the bottom of the water is then continuously degraded by physical or biological processes, causing a lot of microplastics to accumulate in the sediment (Manalu et al., 2017). A sampling point of Gosong has a relatively small number of MPs members because this area is only used for diving and snorkeling and is relatively far from residential areas. According to Browne et al. (2011) and Dowarah et al. (2019), MPs abundance is positive correlated with population density and human activity. In addition, the interaction process between organic matter and microorganisms can increase the density and amount of sediment (Galloway et al., 2017).
MPs by body length

On the basis of the linear regression test, there was no relationship between Epinephelus body length and the number of MPs in the gastrointestinal tract ($p > 0.05$) (Figure 6). It shows that all sizes of fish can be contaminated with MPs. The results of previous studies showed were no significant effect between number of MPs with length or weight of fish (Possatto et al., 2011; Bessa et al., 2018; Garnier et al., 2019; Hastuti et al., 2019; Gündoğdu et al., 2020; Yona et al., 2020). Furthermore, fish body length did not correlate with consumption but the number of MPs correlates with sex (Sbrana et al., 2020). According to Jáms et al. (2020), the smallest to largest size of MPs can be digested by all animals.

The differences in habitat and fish behavior for each species are factors found in MPs in the body of Epinephelus. MPs are easily found in the environment due to the high disposal of plastic waste into the waters column. MPs < 5 mm in size can quickly enter the digestion of Epinephelus because they have wide mouth openings (Possatto et al., 2011). Therefore, there is no correlation between MPs numbers and fish size, but the habitat and behavior of Epinephelus do correlate.

![Figure 5](image1.png)

Figure 5. Average MPs of sediment

![Figure 6](image2.png)

Figure 6. Linear regression between the number of MPs in the digestive tract with total length of fish
The presence of MPs in the digestive tract of fish is a threat to fish and consumers. The MPs contamination in fish is of particular concern by the local government and the community not to consume the fish digestive tract.

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