

Microplastics in the digestive tract of sunu grouper (*Plectropomus leopardus* Lacepede, 1802) from coastal waters of North Maluku

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Abstract. Microplastic pollution threatens marine fish resources as it contaminates various types of fish. This study aimed to analyze the characteristics and abundance of microplastics in the digestive tract of sunu groupers. Data were collected at the Ternate Archipelago Fisheries Port and Ternate Hygienic Fish Market. The data collected included the fish's length and weight, stomach, and microplastics from 30 sunu grouper samples. The results of this study showed that all samples of sunu grouper tested contained microplastics in their digestive tract. Microplastics consist of fibers, films, and fragments, consisting of black, grey, red, white, green, brown, blue, purple, and yellow. The abundance of microplastics in the grouper's digestive tract ranges from to 6-32 particles/individual. The presence of microplastics in marine biota has a high potential to pose a threat to marine food sources. Therefore, it is very important to conduct a massive awareness campaign about the threat of microplastics to the environment and marine food sources so that mitigation efforts are urgent and carried out simultaneously and jointly by all parties.

1 Introduction

Sunu grouper (*Plectropomus leopardus*) is one of the fish that has high economic value and is a leading commodity in the fisheries sector is the sunu grouper (*Plectropomus leopardus*). Several studies have found the benefits of grouper fish, such as being able to reduce the risk of coronary heart disease, containing omega 3 with a content of around 200 mg, containing vitamin A, lowering high cholesterol levels, anti-inflammatory effect, preventing Alzheimer's disease, containing amino acids, vitamin B complex, taurine and selenium, increasing body immunity, caring for the digestive system and being an antioxidant [1]. However, marine fish resources, including grouper fish are currently threatened because they are contaminated by dangerous chemicals such as heavy metals and microplastics.

Microplastics are the result of fragmentation of macro-sized plastic waste through various processes, such as photolysis by ultra violet light, fragmentation by hydro-

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oceanographic activities such as waves and currents, or other activities. Fragmentation of macroplastics which are classified as microplastics, are plastic particles with a size of <5 mm and greater than 330 µm [2,3].

Microplastics consist of primary and secondary microplastics. Primary microplastics are microplastics that are directly produced or produced for certain purposes by processing raw and completely unprocessed polymer materials that are generally found in soap, detergent, cosmetics and clothing. Secondary microplastics originate from the fragmentation or decomposition of large plastics. Microplastics are very dangerous because they persist in the environment for a long period of time. The presence of microplastics in the environment is increasing because they enter the food chain and bioaccumulate in top predators, including humans.

The very small size of microplastic particles means that fish can ingest microplastics either directly or indirectly by eating zooplankton that have accumulated microplastics [4,5]. Microplastics ingested by fish translocate into the digestive tract and gills and enter the circulatory system. Microplastics that enter the blood circulation have the potential to enter fish organs [6,7]. When microplastic particles accumulate in large quantities in the fish body, they can block the fish's digestive tract [8].

Several research results in Indonesian waters have also proven the presence of microplastics in several types of marine fish such as [9-12]. The above study was carried out on the digestive tract of several types of marine fish such as mackerel, flying fish, herring, Carangidae rabbitfish, lemuru, snapper and grouper. Research conducted in various locations has proven the presence of microplastics in fish which are widely consumed by the community. What about the fish in the waters of Ternate Island that have also been contaminated with microplastics? To complete and strengthen the existing research result, it is necessary to carry out research regarding the microplastic content in the digestive tract of sunu groupers in Ternate City, which is one of the coastal cities where people are highly dependent on marine fish. The aim of this study was to analyze the content and abundance of microplastics in the digestive tract of the sunu grouper (*Plectropomus leopardus*).

2 Materials and methods

Samples of sunu grouper (*Plectropomus leopardus*) were collected from fishermen's catches at the Ternate Nusantara Fisheries Harbor (Station 1) and the Ternate Hygienic Fish Market (Station 2) each with 15 fish from each location so that the total samples collected were 30 fishes. The data collected included the length and weight of the fish, the length and weight of the fish digestive tract, and the characteristics of the microplastics. To avoid contamination from the surrounding environment, all equipment used was protected by cleansing with distilled water. Fish samples were dissected to determine the contents of their digestive tracts. Fish sample dissection was based on previously published guidelines [13]. Fish samples were dissected along the gastrointestinal tract from the anterior part (esophagus) to the posterior part (anus).

The microplastic analysis procedures were based on [14]. The weight and length of the digestive tract were determined, after the tract was removed from the body of the fish, placed in a test tube and oven-dried at 80 °C for 24 h. The samples were dried placed in a solution of 2 ml 30% H₂O₂ and 6 g of NaCl/20 ml left to stand for 24 h and then incubated in a water bath at 80 °C. This is the process of organic material destruction. The use of a 30% H₂O₂ solution eliminates a large amount of biogenic organic materials on the surface of microplastics (biofilms and macrofouling organisms), and the use of NaCl is recommended to separate microplastics from other materials. The sample was then filtered using a vacuum pump and Whatman filter paper number 42 with a size 2.5 µm. The type of microplastic was determined using a digital microscope connected to laptop integrated

cooling tech software with 100-fold magnification.

Types of microplastics are plastic fragments (compact color), pellets (compact color), filaments/fibers (compact color), and plastic films (transparent color). The process of observing microplastics has been standardized to avoid selection errors. The characteristics of the microplastics used as standards are 1) no visible cellular or organic structure; 2) the fibers are of the same thickness throughout their entire length and must not taper at the ends; 3) colored particles have a homogeneous color; 4) the fibers are not segmented, or appear to be bent on the ribbon; and 5) the particles are not shiny [14]. Most plastic pieces are flexible and do not break when produced. Plastic pieces often bounce or spring when produced. If a piece broke when touched, it was not counted as plastic. The concentration of microplastics in the fish samples was measured as particles per individual.

The distribution of microplastics in the digestive tract of grouper fish at the two stations was analyzed using one-way ANOVA and the relationship between fish biometrics consisting of fish length and weight and fish digestive tract length-weight with the abundance of microplastics in the digestive tract of sunu grouper fish at the two stations used correlation analysis.

3 Results and discussion

3.1 Biometrics of sunu grouper (*Plectropomus leopardus*)

The results of biometric measurements of 30 samples of sunu grouper (*Plectropomus leopardus*) consisting of the length and weight of the fish, and the length-weight of the fish's digestive tract are presented in Fig 1 and 2. The results of biometric measurements of the sunu grouper at the first location of the Nusantara Fisheries Port have a length ranging from 29.5 cm – 50.3 cm with an average of 41.9 cm, a weight ranges between 1.12 kg – 2.14 kg with an average of 1.4 kg, a length of the digestive tract ranging between 4 cm – 10.5 cm with an average of 7.0 cm and a weight of the digestive tract ranges from 2.93 g – 47.01 g with an average of 12.8 g.

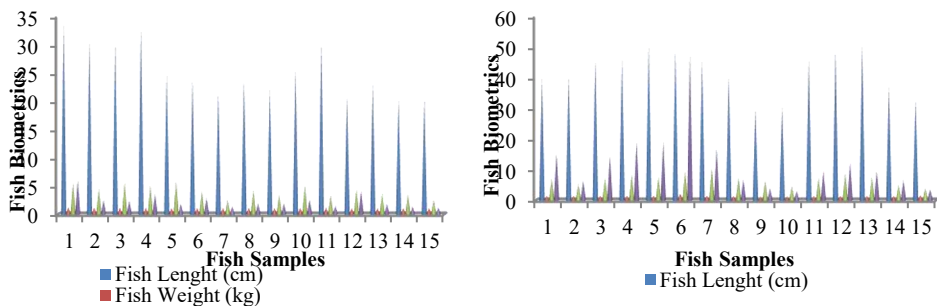


Fig 1. Biometric samples of sunu grouper (*Plectropomus leopardus*) at Station 1 (left side) and Station 2 (right side)

The results of this study are similar to [15], who found that the length range of sunu grouper (*Plectropomus leopardus*) caught in the waters around the Spermonde Islands, South Sulawesi ranged from 15.0-83.0 cm with a weight ranging from 100-4,610 g. Likewise [16] stated that the average length of sunu grouper is around 40 cm. Several studies have found that the length of the fish's digestive tract ranges from <1 cm to 20 times its body length. The highest length of the fish digestive tract generally occurs in herbivorous and detritivorous species, whereas the lowest is found in carnivorous and predatory species [17].

The dimensions of the size of the fish digestive tract are important for analysis as a morphological indicator of trophic levels in the ecological system. Several factors influence the dimensions of the fish's digestive tract, including food factors, fish size (mass and length), fish body shape, feeding history, ontogeny and phylogeny [18].

3.2 Characteristics and abundance of microplastics in the digestive tract of sunu grouper (*Plectropomus leopardus*)

There are three types of microplastics found in the digestive tract of sunu grouper consist of 3 types, namely fiber, film and fragments (Fig 2). The microplastics found in the digestive tract of sunu grouper consist of nine colors, namely black, grey, red, white, green, brown, blue, purple and yellow. The diverse colors of microplastics reflect the diverse of sources of plastic pollution in the environment [19]. The percentage of microplastic types and microplastic color found in the digestive tract of sunu grouper fish at the two sampling locations are presented in Fig 3 and 4.



Fig 2. Samples of microplastics found in the digestive tract of sunu grouper fish (a) fiber, (b) film, (c) fragment

The types of microplastics include plastic fragments (compact color), plastic fibers (compact color), and plastic films (transparent color). The fragments are microplastic shaped like fragments which generally come from the fragmentation of hard plastic such as plastic food boxes, plastic bottles and other hard plastic products. The fibers are microplastic-shaped like threads and generally come from the fragmentation of fabric, clothing, nets, ropes, sacks and textile waste. The films are microplastic shaped like a thin sheet, which generally comes from the fragmentation of thin plastic materials, such as plastic packaging and plastic bags.

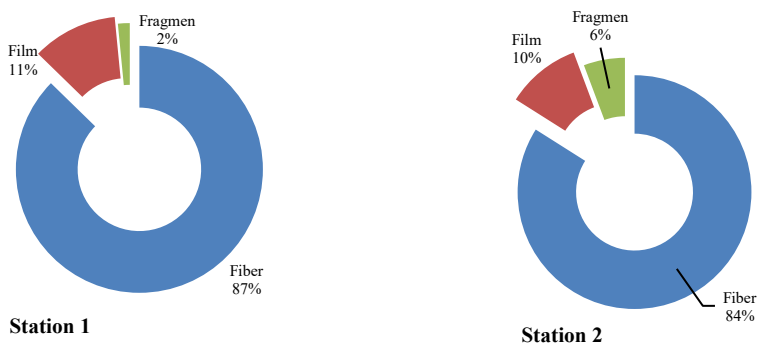


Fig 3. Percentage of microplastics in the digestive tract of sunu grouper

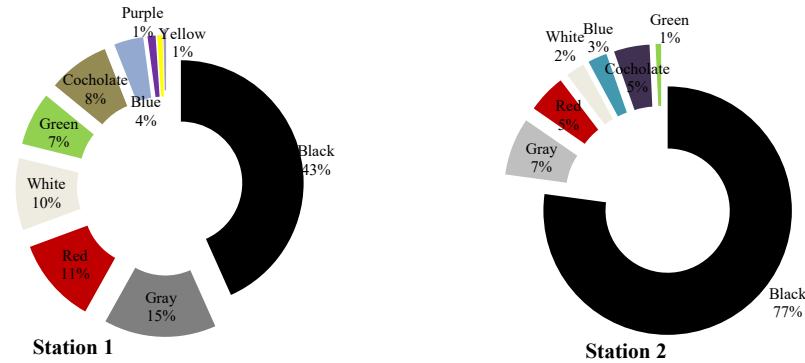


Fig 4. Percentage of microplastic colors in the digestive tract of sunu grouper

The dominant color of the microplastics was black that is 43% at sampling location one and 77% at sampling location 2. The results of this study are similar to [16] in research conducted in Tuban Sea Waters, East Java which found black microplastics as the dominant color in pelagic fish at (82%) and in demersal fish at (69%). Similarly, [20,21] reported that the color variations of microplastic particles found in marine biota were dominated by black (45%). Research by [22] also stated that the accumulation of microplastics in seafood was dominated by black (70%).

The dominant black color was found because it is similar to the color of fish food and some common prey [23]. The ingestion of colorful microplastics by fish is considered food by marine organisms because of their limited ability to recognize food selectivity [24]. Likewise [6,25,26] suggested that microplastics with various attractive colors might be ingested by biota because of their attractiveness and similarity to real prey or food. Meanwhile, the very small size of microplastics and their high buoyancy mean that microplastics are always available in the water column so that the potential for ingestion by demersal and pelagic fish is very high.

The color characteristics of microplastics have also been used as an index of photodegradation and the length of time the plastic remains on the sea [27]. The black color found in microplastics can change because of weathering and transportation in water. When microplastics are in the environment, they undergo degradation simultaneously. This mechanism does not only depend on environmental conditions, but is also influenced by the physical and chemical characteristics of the polymer materials that make up microplastics [28]. Black microplastics are predominantly found in the gastrointestinal tract of fish and in the environment, indicating that these microplastics have been in the tissues and environment for a long time, thereby absorbing a number of pollutants and causing the color to change to black [29,30].

The presence of other colors such as yellow, blue, and red indicates that the original color of the microplastic source and the change in the presence of these colors is due to polyethylene polymer compounds that change color because they contain or have absorbed PCB compounds. The color change process is an indication of long exposure times in seawater, resulting in the polymer oxidation [27].

The results of the microplastics abundance analysis in the digestive tract of sunu grouper at the two sampling locations are presented in Figure 5. The abundance of microplastics at Station 1 of the Nusantara Fisheries Harbor ranged between 6-32 particles/individual and at Station 2 of the Ternate Hygienic Fish Market ranged from 11-29 to individual. The abundance of microplastics obtained in this study was similar to that of [31] of 14-20 particles/ individual in the digestive tract of pelagic fish in the southwest

Plymouth Beach.

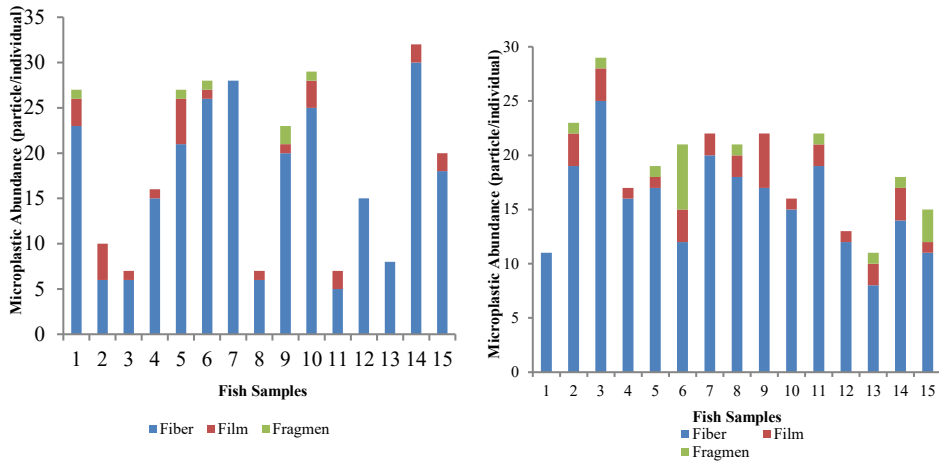


Fig 5. Abundance of microplastics in the digestive tract of sunu grouper at Station 1(left side) and Station 2 (right side).

The types of microplastics found in this study also strengthen the results of previous research such as [16], which found three types of microplastics, namely fibers, fragments, and pellets in the gastrointestinal tract of pelagic and demersal fish in the waters of the Tuban Sea. The dominant types of microplastics were fiber (74%) in pelagic fish and fiber (84%) in demersal fish. Several other studies such as [22, 31-36] also reported the fiber form as the most dominant microplastic in the gastrointestinal tract of fish samples.

The types of microplastic particles found in this study also strengthen the results of previous research such as [16], which found three types of microplastics, namely fiber, fragment and pellet, in the gastrointestinal tract of pelagic and demersal fish in the waters of the Tuban Sea with the dominant type of microplastic being fine fiber in the pelagic and demersal fish. Several other studies have also reported fiber-type microplastics to be the most dominant microplastics found in the gastrointestinal tract.

The main source of fiber microplastics is household waste as a result of washing clothes where these products are made from polyethylene and polyamide polymers. The fabric washing process can release 1900-700,000 fibers per 6 kg into the environment [37-41]. In addition, microplastic fibers can also come from nets or ropes containing polyamide polymers which are often used in fishing gear by the fishing industry [42-44].

Fiber type microplastics are often found in the gastrointestinal tract of pelagic fish because of their thin shape and size and many float on the surface of water [3,4]. In addition, fibers released from wastewater treatment plant drainage pipes can enter the water surface and eventually accumulate in sediment

[26] and fiber microplastics have also been found in demersal fish species. Fiber type microplastics are generally made from acrylic, polyester or polyamide polymers.

In addition, the presence of microplastic fragments in the gastrointestinal tract of fish is secondary microplastic originating from the degradation of larger plastic debris through natural weathering processes in accordance with [45,46]. Fragment types are secondary microplastics whose presence in the environment and biota is closely related to the population size of an area [47,48]. The city of Ternate is a coastal city where most people carry out activities in coastal areas so that it has the potential to produce macro and microplastic waste.

Fragments type microplastic generally consist of polyethylene, which is commonly used in consumer products (e.g., plastic bags, bottles, hats, and containers [6]. In addition, they

can originate from daily use of food packaging materials, containers, and other items is also the main source of fragments [49].

Pellet microplastics are primary microplastics whose presence in the environment and biota is direct waste from microplastic products and not the result of fragmentation of macroplastics. However, if the shape is round and the edges are sharp and irregular, macroplastic fragmentation is indicated. In demersal species, microplastics exist in the form of pellets, which are generally considered primary microplastics [50]. Pellets as a primary source are usually directly produced by raw material factories for making plastic products [51], such as industrial applications including medicine and the medical device plastic industry which are produced on a micro scale including those used to form larger plastic products (resin pellets pre-production) [27].

The type of microplastics in the marine environment and biota depends on the source and fragmentation process of the microplastics as well as the length of time the microplastics remain in the environment. Variations in the type of microplastics are the result of visualization of the source and fragmentation process of macroplastics which then enter the digestive tract because they are preyed upon by fish owing to their shape resembling prey [52,53].

The microplastic fragmentation process can be the result of degradation or erosion on the surface of the particles resulting from biological processes, photodegradation, chemical weathering, or physical influences (wave action, wind, weather and ultraviolet radiation). This result in various forms of microplastic particles [40].

[26,27] reported that the type of plastic polymer most often found in marine environments and fish organisms in the sea is polyethylene, which can reach 88.4%. Meanwhile [3,4,20,21], polyethylene polymer is most widely produced by the plastics industry because it is used on a large scale such as daily household needs, in cosmetics, detergents, cleaning and maintenance products, packaging, shopping bags, food containers and drinking water bottles [54]. Polyethylene is the most dangerous plastic polymer in aquatic environment because of its durability and is mostly used in packaging [55]. Polyethylene is a low density polymer often found in the digestive tract of pelagic fish. Polyethylene production is high because it has a density below one, so there is a high probability that this type of polymer will be abundant [56].

The distribution of microplastics in the digestive tract of fish is strongly influenced by suitable habitat conditions [7]. In addition, there are other factors such as fish eating patterns and habits, sources of microplastics, and oceanographic dynamics. For distribution of microplastics in the digestive tract of grouper fish in both locations, the results of one-way ANOVA showed an F count of 2.079, F table of 4.20, and a significant value of 0.101. Because the significant value obtained was greater than 0.05 and the F count < F table, it can be concluded that the abundance of microplastics in the digestive tract of sunu grouper fish at the two locations was not significantly different.

3.3 Relationship between fish biometrics and the abundance of microplastics in the digestive tract of sunu grouper (*Plectropomus leopardus*)

The results of the analysis of the relationship between fish biometrics consisting of fish length and weight and fish digestive tract length weight with the abundance of microplastics in the digestive tract of sunu grouper fish at two sampling locations are presented in Fig. 6 and 7.

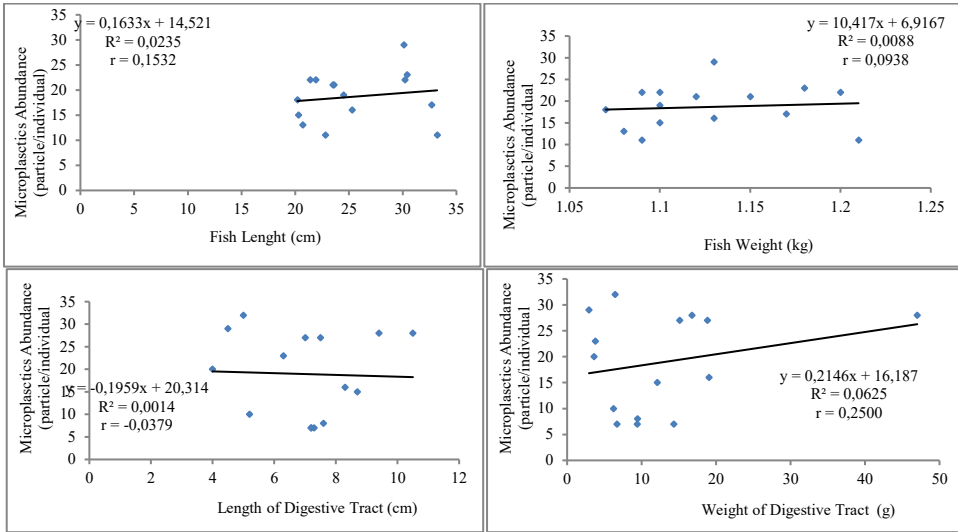


Fig 6. Correlation between microplastic abundance and fish biometrics at Station 1.

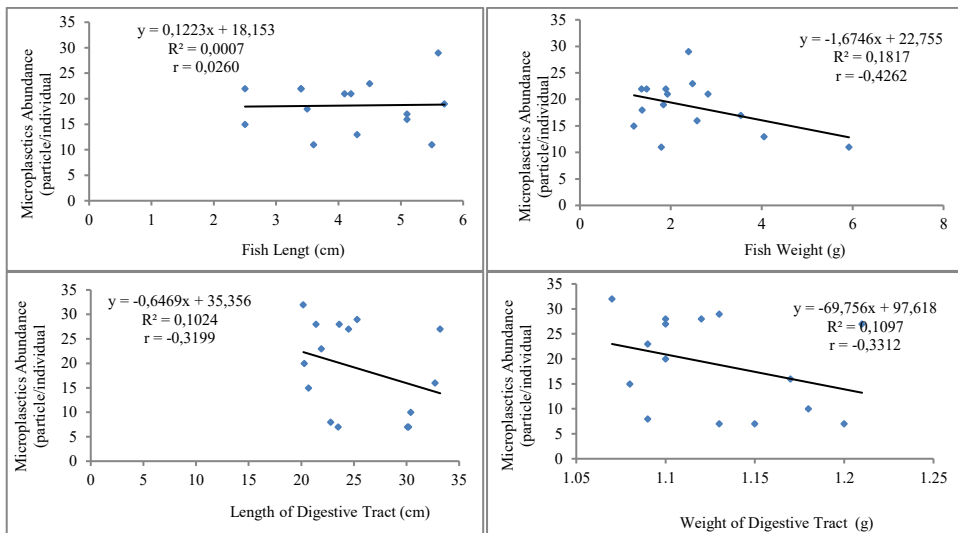


Fig 7. Correlation between microplastic abundance and fish biometrics at Station 2

The results of the analysis of the relationship between fish biometrics and the abundance of microplastics in the digestive tract of sunu grouper fish in general at sampling location one showed a positive relationship, meaning that if there was an increase in the biometric size of the fish, the abundance of microplastics also increased. However, based on the regression coefficient value which was <0.25 , there was a very weak correlation. At the second sampling location, a negative correlation was obtained, which means that if there is an increase in the biometric size of the fish, there will be a decrease in the abundance of microplastics in the digestive tract of the sunu grouper. The correlation coefficient was <0.4 indicates a weak correlation. This shows that other factors that influence the abundance of microplastics in the fish digestive tract have a greater influence than fish biometrics. The influencing factors were feeding patterns, habits, habitat conditions, and oceanographic dynamics.

4 Conclusion

The presence of microplastics in marine biota has a high potential to pose a threat to marine food sources. All sunu grouper samples tested contained microplastics found in their digestive tracts. The microplastics found consisted of three types, namely fiber, film and fragments and consisted of nine colors, namely black, gray, red, white, green, brown, blue, purple and yellow. The abundance of microplastics in the grouper's digestive tract ranges from to 6-32 particles/individual. Analysis of the relationship between fish biometrics and abundance of microplastics in the fish digestive tract showed a very weak relationship. The recommendation suggested from this research is the importance of a massive awareness campaign about the threat of microplastics to the environment and marine food sources so that mitigation efforts are urgent and carried out simultaneously and jointly by all parties.

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