Spread of microplastics in the digestive systems of grouper fish (Genus epinephelus) from the Pasar Bengkulu coastal zone in Indonesia

Y. Johan[1], *Y. P.* Sari[1,2*], and *R. H.* Wibowo[2]

[1]University of Bengkulu, Study Program of Marine Science, Faculty of Agriculture, Kandang Limun, Bengkulu, Indonesia
[2]University of Bengkulu, Department of Biology, Faculty of Mathematics and Natural Sciences, Kandang Limun, Bengkulu, Indonesia

Abstract. Microplastics (MPs) have infected fish, crustaceans, and mollusks, among other marine fisheries products. This investigation was to determine the microplastic content of the gills, musscle, and intestines of Grouper (*Genus epinephelus*) in Pasar Bengkulu, Indonesia. This study was conducted in July-August 2022 with a random sample of 25 participants. At the Laboratory of Fisheries Marine Science Univerisity of Bengkulu, microplastics were analyzed. In the gills, musscle, and intestinal of Grouper Fish (*Genus epinephelus*), researchers discovered three types of microplastics, including fiber, film, and fragments, with a total abundance of 3.60 particles per individual. Transparent, black, and yellow were the hues discovered. Black predominated the color palette of the microplastic. 40.32µm - 675.31µm was the range of microplastic size. The FTIR analysis identifies the polymer as Polyethylenimine, Epichlorohydrin-Modified, 17 Wt% Solution In Water.

1 Introduction

In recent years, plastic has been identified as the most significant marine trash component worldwide [1]. Indonesia is the second-largest ocean polluter in the world [2]. Approximately 80% of the world's oceans are thought to be contaminated by plastic trash [3]. In the maritime environment, plastics decompose extremely slowly. Plastic presents a threat to the marine ecosystem if its quantity continues to rise, as this could cause pollution [4]. According to Hanif et al. [5], plastic garbage in the ocean that has been exposed to ultraviolet radiation undergoes a degradation process that can result in a change in size. The four phases of size change consist of nanoplastics (1 µm), microplastics (5-1 µm), mesoplastics (5-25 mm), and macroplastics (> 25 mm) [6]. According to Purnama et al. [7] plastic trash in the ocean will disintegrate into microplastics by the year 2021.

Microplastics are difficult to remove from the marine environment because plastic is a particularly tenacious material [2]. Multiple forms of plastic were discovered in the intestinal of a dead sperm whale (Pyhseter macrocephalus), illustrating the harm posed by

* Corresponding author: yenniputrisari@unib.ac.id

microplastics in the ocean [8]. A'yun [9] has conducted research on identifying microplastics in fish (2019). On fifteen samples of mullet (*Mugil cephalus*), 78 microplastics were discovered, with an average of five per sample. Yona et al. [10] identified microplastic fiber types in the gills and digestive tracts of reef fish, with the predominant size being >1000 µm. Snapper (*Lutjanus sp.*) and grouper (*Epinephelus sp.*) contain an average of eight and sixteen microplastic particles per fish, respectively, in their digestive tracts [11]. The accumulation of microplastics in aquatic environments will disturb fish feeding chains [12]. Microplastic contamination has a negative effect on fish, hence it is essential to identify and assess the kind and quantity of microplastics.

Bengkulu's waters have the potential to become contaminated by microplastics. The waters of Bengkulu will amass microplastics carried by currents. In addition to getting input from other regions, it is believed that the source of microplastics is internal to Bengkulu. Due to the diversity of Bengkulu's internal operations, the possibility for an abundance of microplastics to be created will likewise vary. Consequently, it is required to perform study to determine the quantity and types of microplastics in the seas of Pasar Bengkulu, at various sites.

2 Materials and methods

2.1 Study site and sample collection

This research was conducted from July to August 2022 in the waters surrounding Pasar Bengkulu, Indonesia. Laboratory of Fisheries Marine Science University of Bengkulu analyzed microplastics found in the gastrointestinal tract. Grouper (*Genus epinephelus*) samples were collected in up to 25 fish of each species were collected as samples. The acquired sample is then placed within the Styrofoam container. In addition, the sample is transported to a laboratory for the identification and analysis of the microplastic content. In addition, interviews with local fisherman were undertaken to determine the location of the catch.

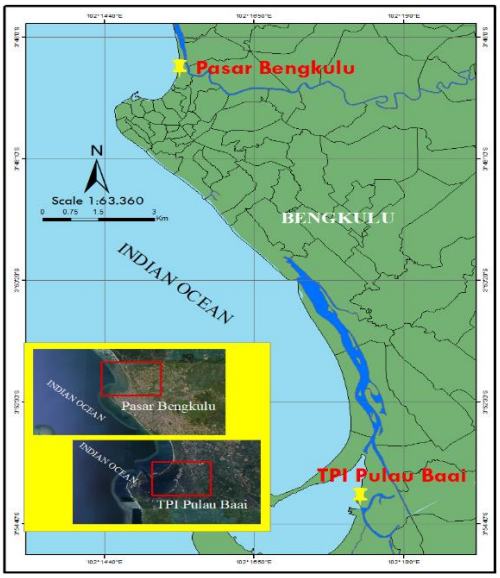

Fig. 1. Site sampling of this study.

2.2 Sample preparation

2.2.1 Grouper fish

The common research equipment was sanitized using both wet and dry processes. To prevent contamination of research equipment, the wet method of sterilization involves washing all instruments with antibacterial soap, while the dry method use an oven heated to 115 ºC for 90 minutes [13]. Researchers employed gloves, masks, and sterile laboratory conditions to prevent contamination during the investigation.

The digestive organs of fish were taken from the intestinal 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 KOH 10% solvent to destroy the organic material contained in the sample. 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 KOH solvent with a concentration of 10% and placed into a beaker glass along with the digestive contents of the grouper in a ratio of 1 gram of sample to 3 ml of KOH solvent. The mixture of KOH solvent with the digestive contents of the grouper was heated at 40°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 (1:3 with KOH).

Then a second incubation was carried out by adding a solution H2O2 30% as much 5 ml, sample to which the solution has been added H2O2 30% then allowed to stand again for 24 hours at room temperature, After the fish sample has been crushed, it is then filtered with filter paper whatman (1,2 µm) using a vacuum filter to facilitate sample filtering, then closed and coated with aluminum foil, After that, it was dried using an oven at a temperature of 60°C to simplify the identification process [14].

2.3 Data analysis

The quantity and types of detected MPs were examined using a descriptive statistical approach. The data was evaluated using descriptive statistics that, in general, characterize MPs in fish. The following formula can be used to determine the amount of microplastics present :

$$Microplastic\ abundance = \frac{\text{the quantity of microplastic particles (particles)}}{\text{the number of fish (individual)}} \quad (1)$$

According to the findings of Yudhantari et al. [14], the formula for determining the abundance of microplastics is the number of microplastic particles obtained divided by the number of available samples. Analysis of data using Fourier Transform Infra-Red (FT-IR) to determine the presence of polymers in microplastics and the degree of similarity between microplastic polymers. Using Microsoft Excel, data analysis is performed.

3 Results and discussion

3.1 MPs in the digestive tract, Gill, and Et., al.

The amount of microplastics discovered in the gills, intestinal, and musscle of grouper (*Genus epinephelus*). There were 18 particles of microplastics per fish. Microplastics are absent in the intestinal and musscle of grouper (*Genus epinephelus*). According to Yona et al. [10] that groupers in the wild do not move as frequently as other fish, the habitat of other

fish tends to be larger than the habitat of groupers. Figure 1 depicts grouper (*Genus epinephelus*) found in the waters of Pasar Bengkulu, Indonesia.

In the study, grouper (*Genus epinephelus*) measuring between 32.000 and 21.00 cm in length were detected. The presence of microplastics in grouper gills (*Genus epinephelus*) can be attributed to a number of causes. In the waters of Pasar Bengkulu the presence of discarded fishing gear, such as fishing rods and nets, used by fisherman for fishing is the primary factor. In addition, synthetic fabrics from domestic garbage (the result of the washing process) are an additional source of microplastics, with up to 1900 fibers per garment being released [15].

3.2 Type of microplastic

Microplastics detected in the intestinal of grouper (*Genus epinephelus*) were categorized by kind, color, and size based on the findings of an analysis. The features of the microplastics detected in the samples are shown in Table 1. The research of Grouper (*Genus epinephelus*) showed the presence of microplastic fibers, films, and fragments. In this investigation, pellet like microplastics were not discovered. Figure 1 depicts a graph showing the number of microplastic types detected in grouper (*Genus epinephelus*).

Table 1. Classification of Shape, Color and Size of Microplastics in fish.

No	Microplastic Characteristics	Sample		
		Grouper (*Genus epinephelus*)		
		Gill	Intestinal	Musscle
Type				
1	Fiber	+	-	-
2	Film	+	-	-
3	Fragmen	+	-	-
Color				
1	Black	+	-	-
2	Transparent	+	-	
3	Yellow	-	-	-
Size				
1	Class 1	-	-	-
2	Class 2	+	-	-
3	Class 3	+	-	
4	Class 4	+	-	-
5	Class 5	+	-	-
6	Class 6	+	-	-
7	Class 7	-	-	-
8	Class 8	-	-	-

Information:
+ (Microplastics Found)
- (No Microplastics Found)

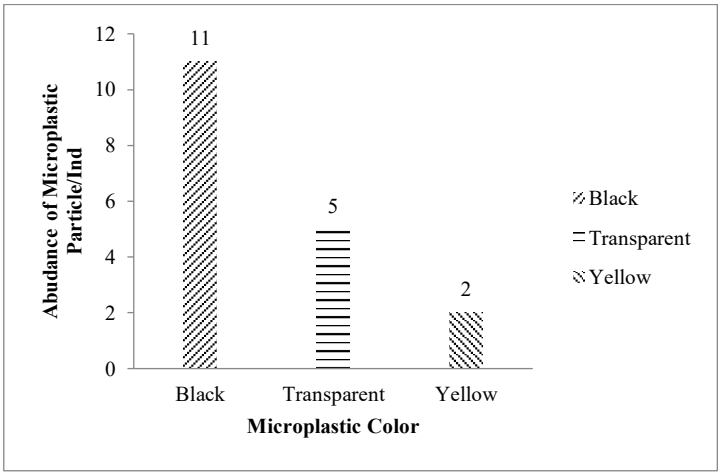

Fig. 2. Abundance of microplastic color.

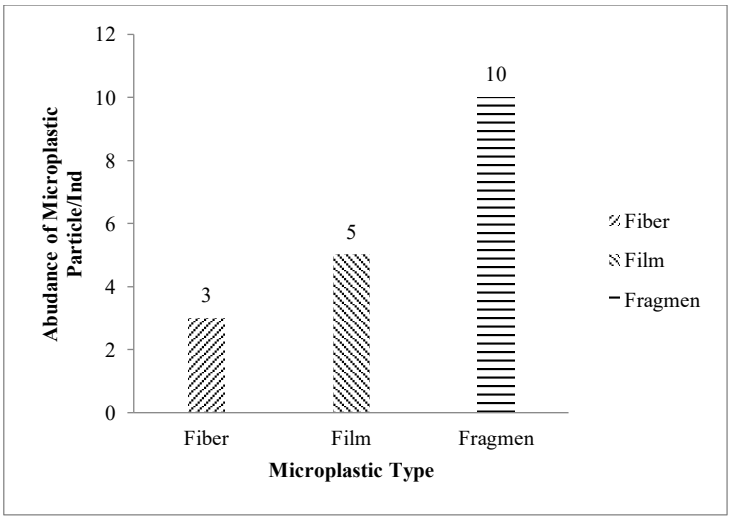

Fig. 3. Abundance of microplastic type.

Microplastics are abundant in grouper (*Genus epinephelus*) Based on observations done with a microscope on as many as 18 particles, the overall abundance of microplastics per individual was 3.60 particles. The fragment type with a total of 10 particles is the most prevalent form of microplastic.

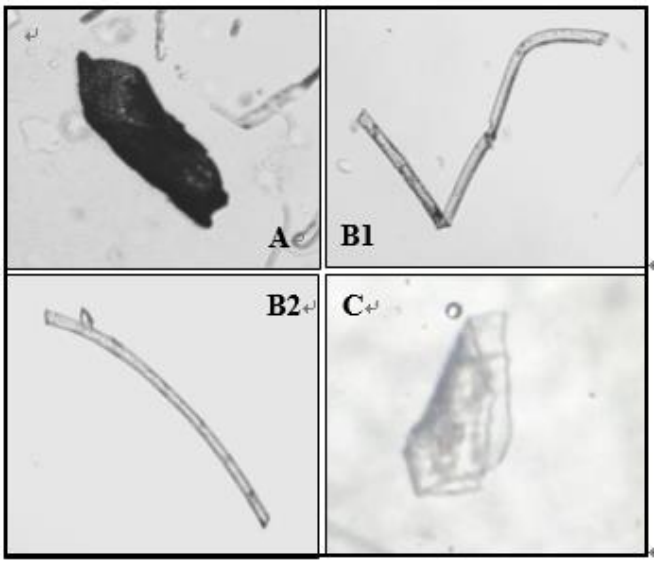

Fig. 4. Types of MPs in the gills, muscle and intestinal of Grouper (*Genus epinephelus*). A.Fragment; B1. Fiber; B2. Fiber; C. Film.

Seven particles make up the film type, which is the second most prevalent form. The third category consists of five particles of fiber. Based on grouper sampling location (*Genus epinephelus*), it is assumed that the microplastics found came from the activities of coastal communities, specifically from the waters of Pasar Bengkulu, where there is a high concentration of tourism near residential areas. Microplastic contamination is higher the closer a sampling location is to an area of human activity [16]. The number of microplastics identified in the form of fragments as a result of activities in the research area is consistent. The causes that cause microplastic fragments to predominate in the studied samples because they originate from areas where there are littering community activities, such as small pipe fragments, mica folders, used beverage bottles, and discarded jars [17].

The results of several studies have shown that the dominant type of microplastic is found in grouper fish (*Genus epinephelus*), which is a particle of microplastic that is prevalent at the water's bottom. According to Yudhantari et al. [14] that fragment-type microplastics have a high density and buoyancy (heavy buoyancy) in comparison to other types of microplastics, therefore they will sink quickly. This establishes that the particles of microplastic most typically detected in grouper fish (*Genus epinephelus*) living at the bottom of the ocean are fragments of microplastic. The type of fragment with the color on the microplastic that was still concentrated indicates that the microplastic has largely lost its color. Figure 4 displays the color of the microplastic.

The color abundance data were obtained for microplastics that were transparent, black, and yellow. Based on the observations and computations, the most prevalent microplastic color is black with 11 particles, followed by clear with 5 particles and yellow with up to 2 particles. The most prevalent color is black According to research conducted by Abbasi et al. [18], the hues of microplastics that were most prevalent were black, green, and blue. Sembiring et al. [19] showed that the microplastics most commonly found in the intestines of milkfish are typically black. According to Tata et al. [20], fish consume black microplastics, oysters and shellfish consume white or transparent microplastics, and species consume blue microplastics copepoda. According to the results of this investigation, this is the case. The dominant color in the sample is black. This remark is supported by Kartini [21],

who states that species living in the water column and at the bottom of the sea will find it impossible to distinguish between microplastics and natural prey, result in a harmful impact.

Microplastic size refers to Cole et al. [22], grouped into 8 size classes: Class 1 (20-40 μm), Class 2 (40-60 μm), Class 3 (60-80 μm), Class 4 (80-100 μm), Class 5 (100-500 μm), Class 6 (500-1000 μm), Class 7 (1000-2000 μm), and Class 8 (2000-<5000 μm). Figure 4 shows the sizes of the microplastics detected in this research.

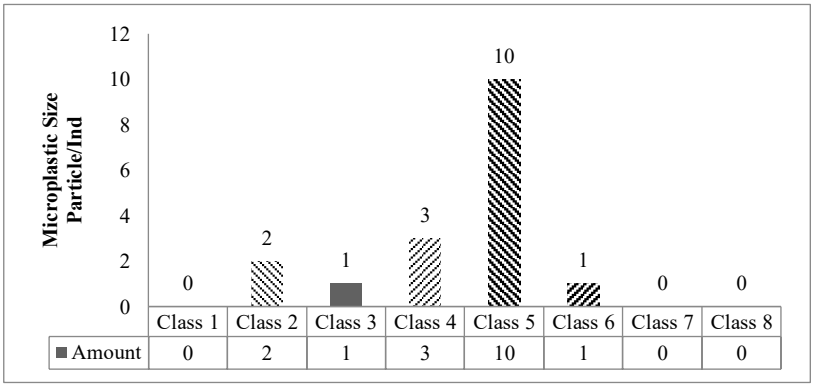

Fig. 5. Microplastic size abundance chart.

The highest abundance of microplastic sizes obtained was in class 5 with a size of (100-500 μm), obtaining as many as 10 particles/individual. Then in grade 4 with size (80-100 μm), obtained as many as 3 particles/individual, in class 2 with a size of (40-60 μm) obtained as many as 2 particles / individual, and in class 3 with a size of (60-80 μm) and in class 6 with a size of (500-1000 μm), obtained as much as 1 particle/individual. For class 1, class 7 and class 8 microplastic sizes were not identified. The different sizes of microplastics found in this study are due to the fact that the plastics produced have different types, shapes, sizes, and components of the constituent materials, as well as physical and chemical factors that occur in the water, which also influence the transformation of large plastics into smaller plastics. Microplastics become smaller over time as a result of solar radiation and strong ocean waves [23]. According to Claessens et al. [24], ocean waves and strong ultraviolet radiation influence the rate of change in the size of microplastics throughout time.

3.3 Microplastic Polymer

Polymer Polyethylenimine, Epichlorohydrin Modified, 17 Wt% Solution In Water, was discovered in the gills, musscle, and intestines of grouper fish (*Genus epinephelus*) in the seas of Pasar Bengkulu. This is a *polyethlenimine* based polymer (PEI). It is believed that what was discovered originated from heavily populated residential areas and communal activities. Polyethylenimine (PEI) is composed of the chemical composition H3C-CH2-NH-[CH2CH2-NH]n-CH2-CH3+. Polymer polyethylenimine (PEI) comprises secondary amines or, in the case of the sample, molecules including methylene and nihonium. Figure 4 represents the results of the FTIR test for microplastic polymer types as a wave graph. Microplastic polymer was detected in as many as one sample of grouper fish (*Genus epinephelus*), according to the findings of tests. Table 2 displays the proportion of polymer.

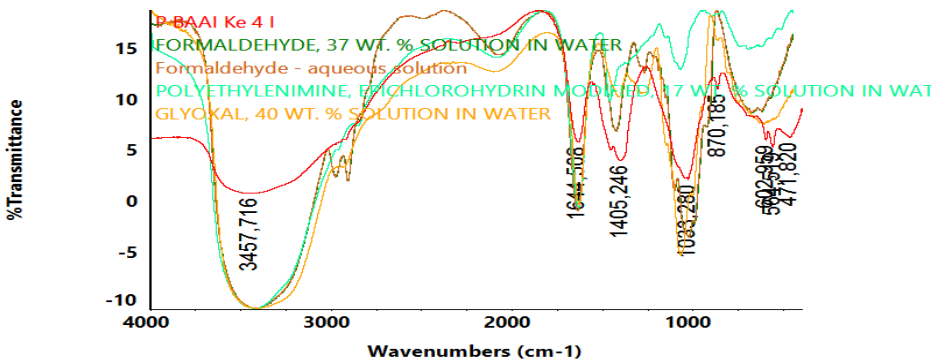

Fig. 6. Polymer spectrum graph polyethylenimine, epichlorohydrin modified, 17 Wt % solution in water.

Table 2. Microplastic polymer similarity percentage.

Polymer type	Percentage (%)	Source
Polyethylenimine, Epichlorohydrin Modified, 17 Wt % Solution In Water	61,05	This polymer material is often used in the medical industry, aircraft, automobiles, general tableware/catering, telecommunications, moulded interconnect device (MID), electricity and lighting.

4 Conclusions and recommendations

4.1 Conclusions

Microplastics are abundant in grouper (*Genus epinephelus*) in the waters surrounding Pasar Bengkulu. It is available in numerous varieties, hues, and sizes. The type of fiber, film, and particles of microplastic found in the intestinal, gills, and musscle of groupers (*Genus epinephelus*). The ratio of microplastic kinds is dominated by 10-particle-per-individual pieces. Grouper (*Genus epinephelus*) has the hues of transparent, black, and yellow. Microplastics are primarily black and contain 11 particles per individual. Twenty five groupers (*Genus epinephelus*) had a total of 18 microplastic particles, with an abundance of 3.60 particles per individual. The microplastic ranges in size from 40.32 µm to 675.31 µm. Polyethylenimine, Epichlorohydrin Modified, 17 Weight Percent Solution In Water is the type of polymer.

4.2 Recommendations

The risk of microplastics to the health of the impacted community as a result of fish consumption should be further investigated in future studies.

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