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Analysis of Microplastics on Digestive and Respiration System of Horse-Eye Jack Fish (*Caranx latus*) in Coastal Bungus Bay, West Sumatra

Fitri Yuranda[1], Zulkarnaini Zulkarnaini[1*], Shinta Silvia[1], Calysta Deli Ad'hani[1]

[1] Department of Environmental Engineering, Faculty of Engineering, Andalas University, Padang, Indonesia

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Corresponding Author:
Zulkarnaini
zulkarnaini@eng.unand.ac.id

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Abstract: Microplastic pollution accumulates in the marine environment, thus impacting the health of marine organisms. The purpose of this study was to analyze and identify the abundance and characteristics of microplastics in Horse-Eye Jack fish (Caranx latus) in coastal Bungus Bay, West Sumatra. Fish samples were taken at three different locations, namely, Sako beach, Caroline Beach, and the east side of Bungus Bay. The average abundance of microplastics in horse-eye Jack fish samples in digestive is 21.63 ± 3.99 particles/fish, and in respiration, 9.23 ± 1.92 particles/fish. The shape identified forms of microplastics are fibers, films, fragments, granules, and foam, and the dominant color of microplastics is red. The most common size of microplastics found was <0.5 mm. FT-IR spectroscopy analysis showed that the polymer types of microplastics found were PE, PVC, PET, and PS. Statistical tests showed differences between the abundance of microplastics in two pathways of microplastic uptake (digestive and respiration), and the sampling location P<0.05. The results showed that microplastics were found in all Horse-Eye Jackfish samples in Bungus Bay, West Sumatra.These findings highlight microplastics' harm to the marine ecosystem and the necessity of managing plastic waste if we hope to avoid future plastic pollution catastrophes.

Keywords: Bungus Bay; *Caranx latus;* Digestive; Microplastics; Respiration

Introduction

Microplastics are small pieces of plastic with a diameter of less than 5 mm found in the environment (Saad et al., 2022). The interaction between microplastics and ecosystems is important for environmental risk assessment. Consumption and uptake of microplastics by aquatic biota are serious problems because they can threaten the survival of aquatic biota and food webs(Huang et al., 2021). These threats include the physical impact of digestive blockages and the toxicological effects of released chemicals (Wright & Kelly, 2017). Based on research from Cordova et al. 2019), microplastic concentrations in Indonesian seawater were estimated to vary from 30 to 960 particles/L, comparable to concentrations reported in the Pacific and Mediterranean seas.

Bungus Bay is one of the suitable places to study the abundance of microplastics in the marine environment. Many activities are carried out in the area, including a ferry port, Samudra Fishing Port (PPS) for fish landing, Pertamina ship activities, coal ships for PLTU and passenger transport ships, as well as aquaculture and quite busy tourism activities (Rahmawan & Wisha, 2020). Community household waste disposal carried by rivers also empties into Bungus Bay. 2 major rivers drain into Bungus Bay, namely the Sako Beach River and the Caroline Beach River (Al Tanto and Gunardi, 2016). In addition to the growing population in this area, various activities and waste disposal into the sea in the Bungus Bay area causes pollution and the production of

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microplastics, which is one of the components of marine debris. Based on this, it is necessary to conduct a study to determine the distribution of microplastics in the Bungus Bay area.

Pollution in waters can be dealt with in various ways, namely physically, chemically, and biologically. Biological components can also act as biomonitoring (Husamah et al., 2019). The study of monitoring the state of the environment using living organisms is known as biomonitoring. Biomonitoring monitors various environmental control measures by applying knowledge about ecosystems with different dynamics(Bhuyan, 2022). This makes it possible to estimate the effects of pollution on organisms more easily because the relationship between organisms and their environment united into a biological system can indicate environmental variables with the life (response) of organisms in a certain time and is relatively easier to measure (Schell et al., 2022).

Fish are one of the most commonly used species as bioindicators in water quality pollution monitoring because of their sensitivity to pollution, wide distribution, and ecological significance (Husamah et al., 2019). Recent research on microplastics was conducted by Nuamah et al. (2023) using two pelagic fish species (*S. maderensis* and *I. africana*) in the Gulf of Guinea, Ghana, in fish respiration; microplastic concentrations were found to range from 1 to 26 MP/individual for I. africana and 1-22 MP/individual for S. maderensis. Microplastic concentrations in fish gut ranged from 1 to 29 MP/individual for *I. africana* and 2-24 MP/individual for *S. maderensis*. Microplastics were found in fiber, fragment, film, pellet, and foam. (Zhang et al., 2020) found 8,895 particles/L of microplastics in the coastal areas of the South China Sea and (Edwin et al., 2023) found 26.67- 35 particles/L of microplastics in seawater off the coast of Padang, West Sumatra.

The characteristics of microplastics identified in this study include the shape, polymer type, color, and size of microplastics in the digestion and respiration of quail. The shape of microplastics will be grouped based on the type of classification (Kovac Virsek et al., 2016), including fragments, pellets, granules, films, fibers, and foam. The analysis is verified using a B-Optika microscope and then verified by testing the type of polymer using the Fourier Transform Infrared Spectroscopy (FT-IR) test method.

The analysis of microplastics in marine ecosystems is essential for studying the distribution and ecological impacts of microplastics (Bellasi et al., 2020). Fish, as one of the most diverse groups of aquatic organisms with great ecological and commercial value, are sensitive indicators for microplastic contamination in aquatic systems. The selection of horse-eye jack fish as a bioindicator is related to its economic and ecological value, as well as the high volume and value of production in the Bungus Bay area, West Sumatra. This study aims to analyze the abundance and characteristics of microplastics, including shape, polymer type, size, and color. Further research is also needed for commercially valuable fish species intended for human consumption. This aims to verify the potential transfer of contaminants to higher trophic levels in the environment, including humans (Miranda & de Carvalho-Souza, 2016).

Method

Research time and location

This research was conducted from June 2023 to November 2023, including the preparation stage, sampling, testing and analysis of laboratory samples, and data analysis. The research location was on the coast of Bungus Bay, West Sumatra. Sampling of horse-eye jackfish (*Caranx latus*) was carried out in the coastal area of Bungus Bay in 3 locations, namely, location 1 at Sako Beach, location 2 at Carolina Beach, and location 3 in the east of Bungus Bay. Determination of the sampling location is done by purposive sampling method by considering the conditions and circumstances of the research area based on the amount of potential plastic waste generated from human activities around the coast (Sherly Margaretha & Fauzi, 2022). The three areas are tourist beaches and harbor areas, located close to residential areas and have river mouths. Horse-eye jack fish samples were selected because they are fish that are often consumed by the community. Each location took ten fish samples to get more accurate concentration results.

Fish Sampling Process

Sampling is random sampling, and this study uses primary data (direct collection in the field). This study's horse-eye jack fish (*Caranx latus*) were taken directly from the local fishermen's catch at three sampling points. Each location was taken ten fish samples per sampling location. Fish were stored in a cool box filled with ice cubes to keep the samples fresh after arriving at the laboratory. After that, the fish were covered with aluminum foil and stored at -20 °C until analysis. Fish were measured in total length (TL) and width (WD) with a ruler, and total weight (W) was measured with digital scales. Fish were dissected to take digestion (digestif) and respiration (respiration). Fish were dissected by cutting from the anus dorsally to the lateral linea, then anteriorly to the back of the head and downward to the bottom of the abdomen until the contents of the fish's stomach were visible. Fish that have been dissected are then taken digestive and respiratory. Weighed and put into a container and labeled.

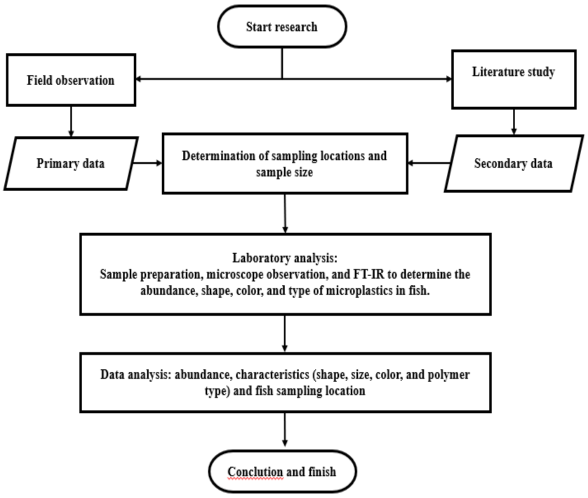

Figure 1. Research Flow

Sample analysis

The organic material was deconstructed to separate the microplastic sample from the fish tissue. This process aims to dissolve the sample and separate microplastics from other organic materials. Samples consisting of ten fish removed from the gills and digestive tract are then immersed in a NaOH solution of 50 ml/sample. Then heated at T = 60°C and t = 48 hours. After that, H2O2 (30%) was added, as much as 5 ml/sample, and allowed to stand overnight (t = 24 hours). The sample solution was then filtered using Whatman filter paper 42 under vacuum conditions.

Microplastic characteristics in fish samples were identified using a B-Optika microscope with 100 times magnification to analyze size, shape, and color. The type of microplastic polymer was identified using the PerkinElmer Frontier C90704 Spectrum IR Version 10.6.1 Fourier Transform Infra-Red (FTIR) Spectroscopy method with a 600-4000 cm-1 wavelength. Microplastic abundance was calculated using the following equation:

$$\text{Abundance} = \frac{\text{Number of microplastic particles}}{\text{Number of fish}} \qquad (1)$$

Statistical Analysis

Statistical analysis of data using SPSS IBM Statistic 29 software to see if there is a significant difference between the abundance and characteristics of microplastics in fish and seawater catchment pathways with all areas of the sampling point location. The inputted data is first seen for its normal distribution (normality of data) to determine whether the next test method is parametric or non-parametric. In this study, the hypothesis of a significant difference in the abundance of microplastics in the two sorption pathways (digestion and respiration) was tested using the T-test ($P < 0.05$). The second hypothesis, namely the existence of a significant difference in the abundance of microplastics in fish (digestive and respiration) and seawater samples for all sampling points, was tested using One-way ANOVA ($P < 0.05$).

Result and Discussion

Microplastic Abundance

Microplastics were identified in all fish and sampling points. The average abundance of microplastics in the digestive and respiration of horse-eye jack fish (*Caranx latus*) at each location is as follows: location 1 in the digestive of 16.2 ± 2.66 particles/fish and respiration of 8.1 ± 2.02 particles/fish, location 2 in the digestive of 27.00 ± 5.37 particles/fish and respiration of 11.20 ± 1.62 particles/fish, and location 3 in the digestive of 21.7 ± 3.95 particles/fish and respiration of 8.4 ± 2.12 particles/fish. The abundance of microplastics in digestif is higher than in respiration found in each location. The results can be seen in Figure 2.

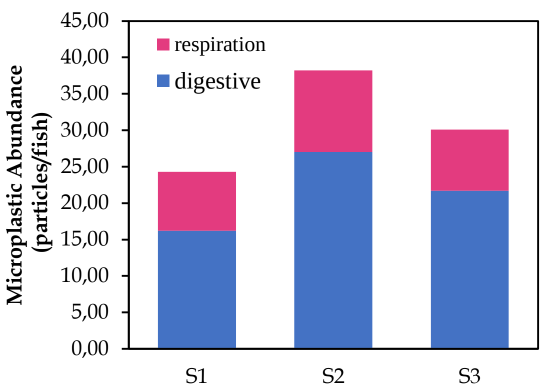

Figure 2. Microplastic Abundance in Horse-Eye Jack Fish

Based on the graph (Figure 2), the highest abundance of microplastics, both digestive and respiration, was found at location 2. The parametric t-test hypothesis was also carried out to see the significant difference between the abundance of microplastics through two pathways of uptake (digestion and respiration). The results of the parametric T-test obtained a data significance value of $P<0.05$, which states that there is a significant difference between the abundance of microplastics in the digestive and the respiration of horse-eye jackfish.

Microplastic pollution in fish tends to accumulate more in the digestive system than in the respiratory system. The results in the study are similar to those conducted by Nuamah et al. (2023) found in fish respiration, with concentrations ranging from 1 to 26 microplastic/individual for *I. africana* and 1-22 microplastic/individual for *S. maderensis.* Concentrations in fish digestion ranged from 1 to 29 microplastic /individual for *I. africana* and 2-24 microplastic /individual for *S. maderensis.* This is because microplastics in fish respiration are transient. Due to their particle size, most microplastics present in fish respiration are too large to move into the space between respiratory filaments, so they are only temporarily attached to respiratory surfaces (Grigorakis et al., 2017). Under natural conditions, these particles can be quickly removed through filtration processes, whereas in digestion, they can persist for a long time (Maina, 2002).

Bungus Bay is located in the city center and is visited by many people because it is one of the tourist areas in the city of Padang, West Sumatra. As a tourist area along the Bungus Bay coastal road, there are many street vendors and potential plastic waste generated from tourist activities. Moreover, seawater is the final disposal site for plastic waste in the environment that comes from rivers, marine debris, and other activities. Living organisms will eat these microplastics and cause microplastic pollution in aquatic organisms (Horton et al., 2017).

A one-way ANOVA parametric hypothesis test was conducted to see the significant difference between the abundance of microplastics in digestion and respiration to different sampling point areas. The research hypothesis is that there is a significant difference between the abundance of microplastics in the digestive and respiration of horse-eye jack fish at all sampling points. The results of the one-way ANOVA test obtained the significance value of the data for the abundance of microplastics in fish against the sampling location $P<0.05$, so it can be concluded that there is a significant difference between the abundance of microplastics in horse-eye jackfish (digestive and respiration) Bungus Bay against all sampling points.

Shape of Microplastics

The shape of microplastics is very diverse; in this study, the shape of microplastics was identified using a microscope (B - 350 Optika). In the study, five forms of microplastics were identified in the digestion and respiration of horse-eye jack fish: fiber, film, fragments, granules, and foam (figure 4). Based on the results of the analysis of the shape of microplastics carried out on horse-eye jack fish, fiber is the most common form found, which is highest in location 2 at 40% (digestive) and 39.68% (respiration) while in water the most fiber is found in location 1 at 48.01%. The percentage of microplastic forms found can be seen in the graph below (Figure 3).

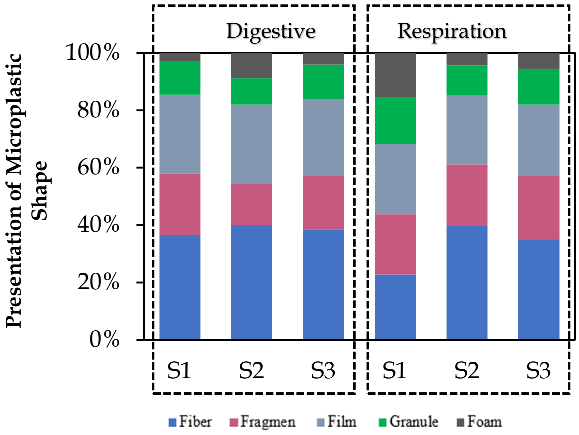

Figure 3. Abundance of microplastic forms in horse-eye jackfish digestion and respiration.

According to (GESAMP, 2015), fiber-shaped microplastics have a thin shape, so they are found floating in the water; besides that, according to (Ayuningtyas, 2019), fibers are thin and long like synthetic fibers. Many studies have reported fiber as sediment samples' most dominant form type (Abidli et al., 2021). Human activities such as textile production, laundering, fishing, and others contribute to fiber production (Zhu et al., 2019). According to (UNEP, 2016), microplastic in the form of fiber comes from clothing and fishing gear. Besides, according to Browne et al. (2011), waste from clothing is a source of microplastic fiber; washing one piece of clothing can release 1900 fibers. The washing of synthetic fibers from households flows along the river and empties into the sea (Fitri & Patria, 2019).

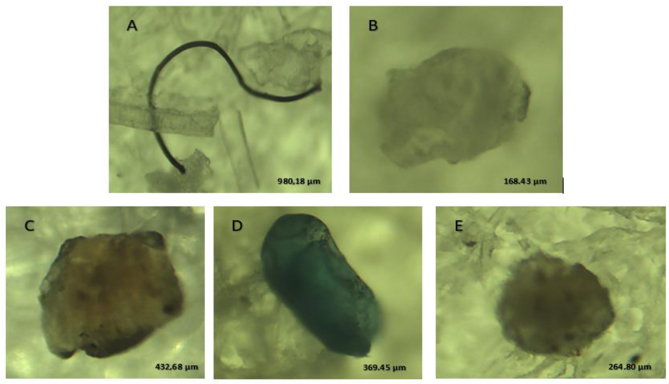

Figure 4. Forms of microplastics in horse-eye jack fish Fiber(A), Film (B), Fragment (C), Granules (D), and Foam (E)

Types of Microplastic Polymers

In this study, the types of microplastics identified in FT-IR were PE (polyethylene), PVC (polyvinyl chloride), PET (polyethylene terephthalate), PS (polystyrene), and PC (polycarbonate) (Figure 4). The most common type of polymer found in the digestion of horse-eye jack fish is PE with an average of 53.48%, followed by PET (25.98%), PVC (17.76%), and the least found is PS (2.78%). Whereas in breathing, the most commonly found polymer type was also PE, with an average of 39.52%, followed by PET (35.99%), PVC (19.09%), and the least found PS (5.40%). The highest type of PE was found at location 2 in digestion and respiration (figure 5).

Based on the characteristics analyzed, PE (polyethylene) and PET (polyethylene terephthalate) are the most commonly identified polymers in fiber, where fiber is the most common form found in the shape characteristics. Polyethylene has a lower density (0.91-0.97 g/cm-3), so it will float in water. The research of (Jiang et al., 2018) found that polyethylene is one type of microplastic commonly found in seawater, so many are consumed by fish or in fish respiration. PE is a type of polymer sourced from plastic bags and plastic packaging commonly found in food packaging and disposable products. Characterized by their lightweight nature, PE microplastics are easily carried by ocean currents. This may lead to their wider distribution in various waters and thus be consumed by marine ecosystems (Huang et al., 2021).

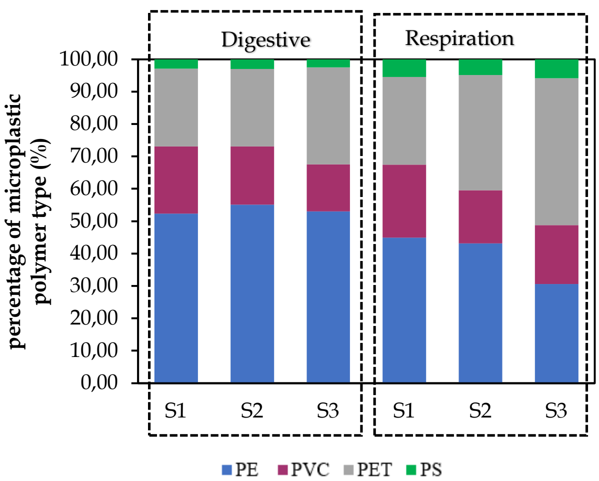

Figure 5. Percentage of Microplastic Polymer Types in horse-eye jack fish digestion and respiration

The PVC polymer type in the study was found in fragment form. PVC is often used for making water and sewer pipes due to its corrosion resistance, and in the construction industry, PS polymers are found in foam form (Bhuyan, 2022). Polystyrene is often used to make disposable plates and containers. Polystyrene is also found in the form of foam, which is used as a protective coating or wrapping for fragile items (Bellasi et al., 2020). Bungus Bay is a coastal area where the activities of the surrounding population are centered on the beach, the anchorage of fishing vessels, and tourist areas and has 2 rivers that drain into Bungus Bay. This allows much plastic waste to pollute the sea, causing microplastic pollution.

Some previous studies (Karbalaei et al., 2019) reported that the most common type of plastic polymer found in fish organisms in the sea was polyethylene (88.4%). This is also the case in the study Cordova et al. (2019), which stated that polyethylene is the main polymer often found in the analysis of microplastics in the gastrointestinal tract of fish. Polymers are the most widely produced by the plastics industry, often in different marine environments worldwide (Lopes et al., 2020). Polyethylene (PE) is the main type of plastic observed in the gastrointestinal tract of fish from fishing grounds, markets, beaches, and the sea. It has been identified as the most polluting in aquatic environments due to its durability and various applications in packaging (Erni-Cassola et al., 2019).

Microplastic Size

Microplastic size refers to the different shapes and types of microplastic particles present (Moto et al., 2024). Depending on their source and how the plastic degradation or fragmentation process occurs, they can have diverse shapes and structures (Su et al., 2019). The size of microplastics in this study was calculated based on images identified through a microscope with the help of the Moticplus application. The results were then classified into>0.5 mm, 0.5-1 mm, and 1-2 mm (Saad et al., 2022). The results of the percentage of microplastic size abundance in horse-eye jack fish at each sampling point can be seen in Figure 6.

The size of microplastics found in horse-eye jack fish samples (digestive and respiratory) ranged from less than 0.5 to 2 mm. The most dominant microplastic size found was less than 0.5 mm. More than 50% of the microplastics were less than 0.5 mm, with only 1-2 mm detected (figure 6). In horse-eye jack fish samples, the highest proportion of microplastics less than 0.5 mm was found in respiratory site three at 62.44%, the lowest at 1-2 mm, and the digestion at site two at 7.75% (Figure 6).

This study is based on previous research conducted by Zhang et al. (2020), where the size of microplastics found in water and fish samples ranged from 0.05 mm to 4 mm. More than 80% of microplastics were less than 0.5 mm in size, while only a small number of 2-4 mm microplastic engravings were detected. The study Markic et al. (2022) also found that most microplastic sizes were smaller than 0.5 by 40%. However, in another study, Edwin et al. (2023) found that the most dominant microplastic sizes in fish and water samples were 1-5 mm, and the least was less than 1 mm.

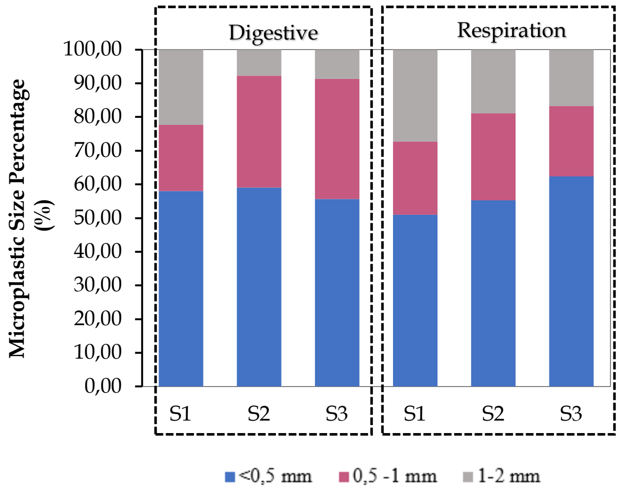

Figure 6. Percentage of Microplastic Size in hor se-eye jac k fish digestion and respiration.

These diverse sizes of microplastics can have varying impacts when they enter the environment, from absorbing pollutants to entering the food chain and potentially harming exposed organisms (Alberghini et al., 2023). For this reason, plastic waste management is critical to reducing the amount of microplastics entering the environment (Purayil et al., 2024). The further away the initial location of waste disposal or things that can be a source of microplastics into the waters, the further the transportation of microplastics will be to enter Bungus Bay, and the more chemical and physical degradation processes will be experienced (Avio et al., 2017). Initially, microplastics that emerge into water bodies can be larger particles and then break down into smaller particles over time and environmental exposure (Galloway et al., 2017).

Color Distribution of Microplastics

Microplastics can come in various colors, depending on the type of plastic, its source, and its degradation history and process (Markic et al., 2022). The color of microplastics can vary widely, ranging from transparent and white to various colors such as blue, red, green, yellow, or other colors (Andrady, 2011). Some plastics have a natural color from the dyes used in their manufacture, while others may acquire color from the dyeing process or the mixing of other substances in them (A. Lusher et al., n.d.). As plastics degrade into microplastics, their original color may fade or change depending on the environmental conditions and the degradation process (Hidalgo-Ruz et al., 2012).

The most frequently found color of microplastics in horse-eye jack fish digestion was red with an average in 3 locations of 21.10%, and the highest was in location 1 (Figure 7), followed by blue at 20.41%, brown at 15.16%, black 16.325, transparent 14.90%, yellow 8.52%, and the lowest was white 3.60%. In contrast, the most dominant color distribution of microplastics in the breath of horse-eye jack fish was found to be red with an average of 23.72% at location 1, followed by brown 17.19%, transparent 15.83%, black 14.46%, blue 12.64%, yellow 10.12%, and the laciest is white with an average of 6.03%. The color of microplastics present in horse-eye jack fish samples is shown in Figure 7.

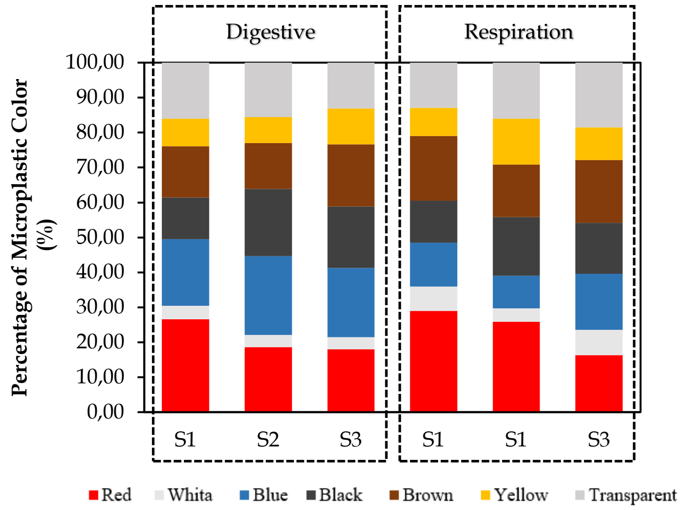

Figure 7. Percentage color distribution of microplastics in horse-eye jack fish digestion and respiration.

The results showed that colored particles were the most common colors found in the digestion and respiration of horse-eye jack fish. This finding aligns with previous research, which found similar results (Zhu et al., 2019). Colored particles can attract marine species and increase the chance of accidental ingestion, whereas white and transparent particles can easily be confused with small jellyfish and zooplankton (Nie et al., 2019).

The source of microplastic color can come from various plastic products. Plastic from drink bottles, shopping bags, or food containers has different colors (D'amelia et al., 2016). This plastic's original color can influence the microplastic's color after degradation. Microplastic environmental pollution can cause degradation and change color due to exposure to sunlight, oxidation, and chemical processes in the marine environment (Nie et al., 2019). This process can change the original color of the plastic into more fragmented and varied colors. The degradation process when plastic breaks down into microplastics due to long exposure to marine environmental conditions, such as friction, oxidation, and the actions of marine organisms, can result in greater color variations (A. L. Lusher et al., 2013). Microplastics can also digest or absorb other chemicals in the marine environment, such as pollutants or natural dyes. This causes discoloration of the microplastics (Grigorakis et al., 2017). Transport Process: Ocean currents can influence the movement of microplastics. Ocean currents carrying microplastics to various locations can be mixed with other materials, affecting their color (Hosseinpour et al., 2021).

Conclusion

The average abundance of microplastics in horse-eye jack fish (Caranx latus) samples in the digestive tract was 21.63 ± 3.99 particles/fish, and in respiration was 9.23 ± 1.92 particles/fish, while in seawater, it ranged from 55.89 ± 7.98 particles/L. The forms of microplastic identified are fiber, film, fragment, granule, and foam; the dominant color of microplastic is red. The most commonly found microplastic size is <0.5 mm. FT-IR spectroscopy analysis showed that the microplastic polymers found were PE, PVC, PET, and PS. The t-test showed that there was a significant difference between the amount of microplastics in the two pathways of microplastic absorption in fish (digestion and respiration) ($P<0.05$). The one-way ANOVA test showed that differences in sampling locations had a significant effect on the abundance of microplastics.

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Author Contributions
Conceptualization, Fitri Yuranda, Zulkarnaini, Shinta Silvia and Calysta Deli Ad'hani; methodology, descriptive qualitative; software, Microsoft Excel, SPSS; validation, Fitri Yuranda; formal analysis, Fitri Yuranda, Calysta Deli Ad'hani. ; preparation of initial draft, Fitri Yuranda; review and editing, Fitri Yuranda, Zulkarnaini; visualization, Fitri Yuranda; supervision, Shinta Silvia and Calysta Deli Ad'hani; project administration, Fitri Yuranda; acquisition of funds, Fitri Yuranda. All authors have read and approved the published version of the manuscript.

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Conflicts of Interest
The author declares no conflict of interest

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