Characteristics of microplastics in Dusky-tailed cardinalfish (*Taeniamia macroptera*) from natural reef and Fish Apartment in Pasir Putih Situbondo, East Java, Indonesia

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Abstract. In order to increase fish resources in Pasir Putih Situbondo area, hundreds of artificial reefs in the form of fish apartment (FA) units were laid on the seabottom in the area since 2008. The FA units made from plastic composed by Polypropylene (PP) dan High Density Polyethylene (HDPE) which claimed to be extremely durable for a long time. However, these types of plastic will still undergo weathering and fragmented along time and causes an increase in microplastic pollution in the seawater. The aims of the study are to determine the density as well as characteristics of microplastic in gills and gastrointestinal tract of Dusky-tailed cardinalfish (*Taeniamia macroptera*) inhabiting the FA. As comparison, fish specimens also collected from natural reef (NR) whereas microplastics in water column collected from both areas. The physical characteristics (colour, shape and size) observed using compound microscope and OptiLab while chemical characteristics analyzed with ATR-FTIR. In the water column, average density of microplastic was 44 particles/l; dominated by size of 0-20 μm in the surface and 20-40 μm at the dept of ±12 m. Results of independent sample t-test (for samples from the gills) and Mann-Whitney test (for samples from gastrointestinal track) show no difference in term of microplastic density from each organ. However, in the fish from FA, the average density was relatively higher, 8340 particles/gr in the gills and 14250 particles/gr in the gastrointestinal tract; compared to 6480 particles/gr in the gills and 11990 particles/gr in the specimens from NA. All microplastics particles dominated by black fragment with the size of 0-20 μm and type of the polymer is PP.

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1 Introduction

The coastal waters of Pasir Putih, Situbondo Regency, East Java, Indonesia have the potential for both economically fishery commodities and ornamental fish [1]. However, there have been numerous non-eco-friendly fishing practices that are damaging the marine ecosystem. The damage to the marine ecosystem, including coral reefs, can generate negative impacts on the diversity and abundance of fish. This loss occurs due to disrupted food sources, shelter, and optimal reproductive environments for fish. In an effort to increase the recovery of fish resources, hundreds of artificial reef units in the form of Fish Apartment (FA) have been placed in Pasir Putih Situbondo to restore the reef habitat as well as to enhance fish diversity and abundance. Fish species that are commonly found in around FA units and adjacent natural reefs is Dusky-tailed cardinalfish (*Taeniamia macroptera*), a planktivorous tropical marine fish belonging to the Apogonidae family. In their natural habitat, *T. macroptera* lives in small groups and is associated with coral reefs or reef-associated [2] and is considered as non-migratory species.

The FA is a hollow construction functioning as an artificial habitat that providing shelter, feeding, and spawning grounds for organisms, primarily fish [1]. The primary material used to make the FA partition unit is polypropylene (PP) plastic. On the other hand, high-density polyethylene (HDPE) plastic is the constituent material that serves as a supporting component for the FA partition structures. PP is a thermoplastic with good durability and resistance to chemicals and high temperatures [3]. HDPE, another thermoplastic, is characterized by high elasticity, low-temperature resistance, and lightweight properties [4]. However, PP and HDPE can be degraded by UV radiation, water movement, and abrasion, leading to increased microplastic (particles smaller than 5 mm) pollution in marine environments [5].

The presence of microplastic in aquatic environments will threaten the ecosystem and marine organisms, including coral fishes. Due to their small size, microplastics can easily enter the fish bodies, translocating into gills and the gastrointestinal tract [6]. Filtration mechanisms for respiration can cause microplastic accumulation in the gills [7]. Microplastic contamination in the gastrointestinal tract can occur actively through direct consumption or passively by contaminating prey. The impacts of consumed microplastics include false satiety, reducing the fish’s appetite [8, 9]. Microplastic entry into fish bodies can lead to digestive organ damage, oxidative stress, inhibited body growth, reproductive organ damage, and immune system impairment [10]. Accumulated microplastics in waters also have potential risks to humans. The FA made of PP and HDPE plastic that undergoes fragmentation into microplastics can transfer fine particles into the food web, distributing microplastics from lower to higher trophic level organisms. This process can lead to human exposure when consuming these fish, bringing microplastics into their bodies [11].

In this study, we accessed the density and characteristics of microplastics in the gill and gastrointestinal tract of *T. macroptera* from both natural reef and FA units. Density and characteristics of microplastics in the water column from both areas were also measured. The results are expected to provide data needed to evaluate the use of plastics in FA production and application that have potential to generate microplastics pollution.

2 Materials and method

2.1 Sampling sites and periods

The research conducted in March to August 2023. Fish specimens of Dusky-tailed cardinalfish (*T. macroptera*) collected from two sites, the Fish Apartment units (FA) and natural reef (NR) in Pasir Putih, Situbondo Regency, East Java. Both sites situated in Batu
Lawang reef and separated at a distance of about 500 m, as depicted in Fig. 1. Sample preparation and microplastic analysis were conducted at the Ecology Laboratory, Department of Biology, Faculty of Science and Data Analytics, Institut Teknologi Sepuluh Nopember (ITS) in Surabaya.

Fig. 1. Situational map of the sampling sites in Pasir Putih, Situbondo, East Java, Indonesia

2.2 Sampling procedures

2.2.1 Microplastics in fish organs

The fish samples collected using fish trap (Fig 2) by Scuba diving technique. The fish that caught from each location were then sorted and measured for total length and total wet weight. This procedure is to ensure that all individuals have relatively similar sizes, assuming that the fish specimens took up microplastics in a similar time and/or quantity [12]. For microplastic analysis, 10 individuals from each site were selected. Subsequently, the fish samples were stored in a freezer at -20°C to maintain sample quality until further examination [13].

In the laboratory, all selected specimens were dissected to collect gill (GI) and gastrointestinal tract (GT) organs which then measured for the wet weight (ww). All organs then separately placed in beaker glass containing 30% of H\textsubscript{2}O\textsubscript{2} to destruct organic substances or materials [14]. All beaker glass then covered by aluminium foil and put in an oven at 50°C for 24 hours to expedite the destruction of organic material [15]. After organic substances in the sample was destructed, microplastic filtration was carried out using vacuum filtration with a Buchner funnel filtering kit equipped with Whatman Grade 40 filter paper [16]. Vacuum filtration was used to accelerate the filtration of solid particles (microplastics) from the solid-liquid mixture [16]. After filtration, the filter paper was placed in a Petri dish and put in an oven at 40°C for drying [17].
**Fig. 2.** The Dusky-tailed cardinalfish (*Taeniamia macroptera*) (left picture) and sampling process in the Fish Apartments (right picture)

### 2.2.2 Microplastics in water column

Microplastics in water column around units of FA and NR collected from the surface (0 m) and near the sea-bottom (±8 m) by filtering the water using a plankton net with a mesh size of 75 µm [18]. After filtration, the outer side of the net was sprayed with 70% ethanol to preserve microplastic samples from microbes attaching to the microplastics [19]. Microplastics collected in the cod-end were then transferred to sample bottles and stored in a cool box for further analysis in the laboratory.

In the laboratory, a ±10 ml sample of water sample then transferred to test tubes. Subsequently, drying was conducted using an oven at 50°C for 24 hours. Organic material was destructed using 5 ml of 30% H₂O₂, and the samples were covered with aluminum foil and placed in an oven at 50°C for 24 hours [20]. The following procedures of filtration and drying were similar to the procedures for microplastics preparation form fish organs.

### 2.2.3 Analysis of physical characteristics

All microplastic samples from fish organs and water column were visually observed under stereo microscope for analysis of physical characteristics, including density, shape, colour and size. OptiLab Viewer software connected to the microscope was used to facilitate observation and documentation of microplastic particles. During observation, a melting test was conducted using a hot needle to confirm whether the observed particles were made of plastic or not. The classification of microplastic shape and color followed guidelines from literature sources such as [19, 21]. Raster images were used to measure the size of representative microplastic particles [22]. Size classification based on [23] which divided into 8 categories: 0-20 µm, 20-40 µm, 40-60 µm, 60-80 µm, 80-100 µm, 100-500 µm, 500-1000 µm, and 1000-5000 µm, respectively.

### 2.2.4 Analysis on type of polymer

Analysis of polymer type conducted in laboratory of Chemistry Department, Brawijaya University, Malang. The polymer composition of microplastics was analyzed using Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) [24]. Prior to analysis, the diamond crystal of ATR was rinsed with water or alcohol and a cloth to facilitate sample identification for high-quality results. The sample (microplastic particle) was then placed on the sample holder and pressed by the diamond crystal to allow infrared
radiation to be emitted or reflected on the sample. Polymers were identified based on the absorption bands.

2.2.5 Data analysis

This study is quantitative-descriptive research, which statistical tests used to compare the densities of microplastic particles between different organs (GI and GT) in different location. Prior to the tests, data normality was analyzed using the Shapiro-Wilk test. For the GI, the data were normal distributed, and a two independent sample T test was performed to compare the density of microplastic in GI from the NR and FA. Conversely, data of density from GT were not normal distributed, therefore the density comparison was performed using Mann-Whitney U test. This non-parametric test was also performed to compare density of microplastic from different organ in same location. All statistical tests were performed at p = 0.05; while data visualization was created using R Studio version 4.1.3.

3 Results and discussion

3.1 Microplastic density in the water column

In this study, we found that density of microplastics tends to be higher in deeper waters, and density of microplastics from the FA were higher compared to the NR. In the surface, the densities were 37 particles/l in FA and 20 particles/l in NR; while in the bottom were 84 and 31 particles/l, respectively (Fig. 3). These observations are in accordance with research in Svalbard, Norway by [25] and in Bohai Sea by [26]; in which deeper water have more microplastics particles compared to water surface.

The FA and NR are in the same area, indicating that microplastic contamination may originated from relatively similar sources. The microplastics in both locations possibly can be originated from three sources: inland-based, sea-based, and air-based. Inland-based waste includes household waste (food containers, water bottles, plastic bags, product packaging, clothing fibers, etc.) that are accidentally or intentionally disposed at the sea. Sea-based waste includes tools used for fishing (fishing nets, fishing lines), maritime transportation activities (anchor ropes), and floating cage nets (buoyant devices, fish aggregating devices). The air-based waste consists of microplastic particles carried by the air [27, 28]. These wastes enter the water body and gradually undergo degradation, leading to the accumulation of microplastics in the marine ecosystem [28].

Higher microplastics in FA is likely due to the partitions of polypropylene (PP) plastic and high-density polyethylene (HDPE) plastic ropes that undergo fragmentation through photodegradation when exposed to UV radiation; biodegradation by microorganisms and mechanical abrasion by sand and/or sediment [5]. According to the study by [29], polypropylene plastic exposed to UV radiation and mechanical degradation can form 6084±1061 microplastic particles within 12 months. Another study by [30] tested the degradation rate of plastics with different polymers; in which the degradation rate of PP is 7.5 µm/year, and HDPE is 4.3 µm/year.

The distribution of microplastics at different depths can be caused by several factors, including hydrodynamic effects in the water column, polymer type which affecting the density of microplastics, and fouling. Fouling of microorganisms on microplastic particles will form a biofilm on floating microplastic particles, gradually causing the microplastics to sink (negative buoyancy). The forms of the microplastics can also affect the buoyancy; in which cracks, fractures, brittleness, and surface roughness of microplastic will increase the
chances of foreign materials and/or particles attachment onto microplastic particles, thereby reducing the buoyancy of microplastics [26, 31].

![Fig. 3. Density of microplastics in the water column, in which density of microplastics tends to be higher in deeper waters, and density of microplastics from the Fish Apartment (FA) were higher compared to the Natural Reef (NR) ]

### 3.2 Microplastic density in the fish organs

Like in the water column; density of microplastics in the fish organs were also higher in the fish from the FA units. Result of Mann-Whitney U test showed significant difference (significance value was 0.049) in microplastic densities between the GI and GT organs at each location. However, the density of microplastic in the same organ from different location was not significantly differed, indicated by a significance value that higher than 0.05, that are 0.295 for GI and 0.226 for GT. In the GI from FA, average density was 834 particles/g wet weight (ww) while in the GT was 1425 particles/g ww. In the NR area, the density of microplastic was 648 particles/g ww from the GI and 1199 particles/g ww from the GT (Fig.4). These findings are in accordance with the research by [32] on coral fish in the South China Sea, where the abundance of microplastics in the GT was higher than in the GI, with a ratio of 3:1.

The ingestion of microplastics through the digestive system is considered as the main pathway for the accumulation of microplastics in fish bodies [6]. Smaller size of microplastics leading to misidentification by the fish and considering them as prey and resulting in accumulation of microplastic particles in the gastrointestinal tract [8, 9]. These particles will accumulate in the coiled structure of the intestines and intestinal villi [33, 34]. Moreover, according to [34], the narrow opening at the junction of stomach and intestine contributes to the retention of microplastics in the gastrointestinal tract, thus generate high accumulation of microplastics.
Microplastic accumulation also occurs in the gills, where microplastics enter passively through respiratory filtration processes. Water that carries microplastics enters the gills and is retained on the gill filament and gill rakers [35]. The lower accumulation of microplastics in gill organs compared to the gastrointestinal tract is due to the transient nature of microplastics, particularly within the gastrointestinal tract [6, 35]. When water enters the fish’s body, the fish can pump water to expel microplastic particles (or sediment captured) out of their body. This mechanisms causes larger microplastics to be trapped in the gills and resulting in lower microplastic abundance in the gills compared to the gastrointestinal tract [36].

![Fig. 4. Average density of microplastics in gill (GI) and gastrointestinal tract (GT) from the Fish Apartment (FA) and Natural Reef (NR).](image)

### 3.3 Physical characteristics of microplastic

#### 3.3.1 Size

In the water column, size of the microplastic particles ranged from 4-4612 µm. Most of small-sized particles are in the shape of fragment while larger particles are in the shape of fiber. From both area and depth (surface and sea-bottom), the particles dominated by size of 0-40 µm. Size of 0-20 µm dominating particles from the surface area in NR and in the sea-bottom of FA. In contrast, size of 20-40 mm dominating particles from the sea-bottom in NR and in the surface sample from FA area as depicted in Fig. 5. Based on their size, microplastics are classified into 2 categories: large microplastics (large MPs) with size ranging from 500 µm to 5 mm and small microplastics (small MPs) with sizes <500µm [24]. According to this classification, the dominant size of microplastics in all four water samples categorized into the small size category (<500 µm).
In the research by [26], larger particles (300-1000 µm) tend to be abundant at depth of 0-15 m while smaller particles (100-300 µm) will be more abundant in deeper water (20-30 m). This is because small-sized microplastics tend to sink compared to large-sized microplastics, as small-sized microplastics more easily lost their buoyancy due to a higher surface area-to-volume ratio. In the shallow water, especially in coral reef habitat, the occurrence of small-scale hydrodynamics may cause ‘stirring’ and allowing large-sized microplastics to move to greater depths. Furthermore, the variation in microplastic sizes found in the water column can be affected by the fragmentation and degradation process. Microplastic degradation over time is likely to produce smaller-sized microplastics. The dominance of small-sized microplastics can also be due to prolonged exposure in water bodies, in which various degradation processes such as biodegradation, photodegradation, thermal degradation, chemical degradation, and mechanical degradation may lead to the production of smaller-sized microplastics [37].

**Fig. 5.** Size classification of microplastics in the water column, dominated by small particles (0-40 µm)

In the fish organs (both GI and GT), dominant particle size was also in the range of 0-20 µm, followed by 20-40 µm (Fig. 6); where larger particles were more abundant in the GI while smaller particles were more abundant in the GT. These range of size in the organs related to the dominant size of microplastics in the water, where water carry essential substances for fish such as dissolved oxygen for respiration and food for consumption. Microplastics entry to the body through either filtration mechanisms in the gills or by ingesting microplastics in the digestive system, which leads to microplastics accumulation in the body. It is highly possible that when small-sized microplastics are dominant in the water, then the dominant size of microplastics in the fish body is also small [2, 8, 35]. Furthermore, the dominance of small-sized microplastics found in the organs of Dusky-tailed cardinalfish is related to their planktivorous nature [2]. Plankton generally consume prey ranging from 1-25 µm in size. Like fish, plankton can also misidentify microplastics as prey, leading to the accumulation of microplastics in their bodies.
3.3.2 Shape

In this study, shape of microplastics classified into film, beads, fiber, foam, and fragment forms [38]. The variety in microplastic shapes can be caused by the polymer type used, plastic degradation processes affected by the environment, and molds used in the production process [37]. From the water column in FA, five shapes were identified, namely fragment, fiber, film, foam and beads. Except of foam, all other shapes were found in NR area. In general, from both area and depths, the dominant shape was fragment with average density of 77% to 91%, followed by fiber. Microplastics in the shape of fragments may originate from various plastic products, including bottle packaging, plastic bags, pipe fragments, food packaging, disposable cutlery, and others [39]. Microplastics in the shape of fibers are suspected to come from fishing gear, fishing nets, floating cage nets, textile fibers, and household waste [40].

Fragment is also the most abundant microplastics particles in the fish organ, which also related to their presence in the water column. In the GI, the density of fragment was 616-766 particles/g ww, or 90.9-94.3% from the total density. Similarly, the density was 1148-1369 particles/g ww or 95.3-95.7% from the total density in the GT. Previous study by [41] stated that microplastics in the shape of fragments was the most dominant in the organs of planktivorous reef fishes. Microplastics in the shape of fragments have irregular forms and rough surfaces, making it easier for them to get trapped in the gills and gastrointestinal tract [42].

3.3.3 Colour

Microplastics have a variety of colors, which can be caused by the addition of pigments during the production or manufacturing process or by environmental factors on the plastic [21]. Our observations show that the most dominant colour is black with the relative density of 50-65% from the total density of microplastics in the water column (Fig. 7). In the organs of Dusky-tailed cardinalfish, we found 11 colour which dominated by black (Fig. 8). The density of black particles in the GI was 361-486 particles/g ww (56-58%) and 616-842...
particles/g ww (51-59%). Similar to the size and shape characteristics, there is a correlation between the color of microplastics in water and in fish organs. As a planktivorous fish, Dusky-tailed cardinalfish rely on plankton as food source. Although plankton comes in various colors, but generally, plankton bodies have black or dark patterns. This may confuse the fish in identifying black-colored microplastics as their prey, and the fish will consume black-colored microplastics that resulting in microplastics entering and accumulating in the fish bodies [8, 9].

![Fig. 7. Bubble grid of microplastics abundance based on shape and colour in the water column, dominated by black fragment](image)

Black colour was also dominant both at the surface and sub-surface in the study by [25], and in the coral reef habitat [43]. The high abundance of black-colored microplastics may also be due to the prevalence of black-colored plastic products. The extensive use of black color in plastic products is attributed to the minimal addition of pigments required to color unpigmented polymers, leading to enhanced colorfastness and strength, slowing down the degradation process of plastic [44]. It is known that black color has a higher UV-absorbing capacity than other colors, effectively preventing UV radiation from penetrating the polymer and inhibiting the aging and degradation of microplastics [21]. The black color may indicate a higher absorption capacity of contaminants in black-colored microplastics and other organic particles, as black-colored microplastics demonstrate high pollutant absorption ability.

Other colors frequently found in all four samples include gray, red, and blue. These colors are suspected to originate from human activities (anthropogenic) that degrade into microplastics and contaminate marine environments [40]. Gray-colored microplastics are likely derived from black-colored microplastics that undergo discoloration due to UV exposure [21]. Red-colored microplastics may come from household washing waste, fishing gear, and red-colored trash bags. Blue-colored microplastics may originate from fishing gear, boat paint, floating cage, buoyancy aids, and plastic bottle packaging [45].

In this study, we found that dominant microplastics either in water column or fish organs was black fragment followed by blue fiber. Fish Apartments units in Pasir Putih, Situbondo
consisted by partition unit made from PP plastic. Although highly durable, this type of plastic will be degraded or fragmented over time, by means of photodegradation (UV exposure), biodegradation (by microorganism) and/or mechanical abrasion by sand and sediment [5, 29]. Therefore, it is assumed that partition units of FA highly contribute to the abundance of fragment shape in the water column. Partition units of FA is black coloured; and it is assumed that dominance of black particles may originated from the degraded or fragmented units of FA, while the blue fiber may be come from degraded blue plastic rope used to tie the partition units of FA.

![Fig. 7. Bubble grid of microplastics abundance based on shape and colour in the fish organs, dominated by black fragment](image)

Exposure of plastics to UV radiation can initiate the cutting of C-C and C-H bonds, producing free radicals that ultimately form carbonyl (C=O) and hydroxyl (O-H) groups on the polymer chain. UV exposure to plastics can also initiate the integration of oxygen atoms into the polymer, forming ROO and leading to termination and a decrease in the molecular weight of the polymer chain. Shorter chains, with oxygen-containing functional groups at their ends, are more susceptible to degradation by microorganisms due to mineralization (increased hydrophilicity), enhancing microorganism adhesion. Microorganisms adhering to plastics can degrade them through their metabolic processes (biodegradation) [5, 30].

### 3.4 Chemical characteristics of microplastic

The results of ATR-FTIR indicate a ‘peak’ in the wave number ranged between 500-4500 cm⁻¹. Based on the translated results obtained from the dominant color and shape of the sample, polypropylene (PP) polymer was identified in the black fragment samples in both water column and fish organs in all locations (Fig. 8 and 9). The absorption bands (cm⁻¹) identifying the PP polymer type are 2950, 2915, 2838, 1455, 1377, 1166, 997, 972, 840, and 808 [46].
Fig. 8. Results of ATR-FTIR analysis for polymer type of black fragment in the water column from Natural Reef (NR, upper picture) and Fish Apartments (FA, lower picture). Black line: result of this study, red line: comparison with study by [45]
Fig. 9. Results of ATR-FTIR analysis for polymer type of black fragment in fish organs from Natural Reef (NR, upper picture) and Fish Apartments (FA, lower picture). Black line: result of this study, red line: comparison with study by [45].
PP is a petrochemical product derived from the propylene olefin monomer. The polymerization process involves connecting monomers with the addition of high-energy radiation and initiators/catalysts, resulting in the formation of propylene. Subsequently, PP molecules polymerize into long polymer chains [47]. Classified as a thermoplastic, PP has properties such as low density (0.85-0.94 g/cm$^3$), crystalline structure with high stiffness and melting point; and good durability against aldehydes, esters, aliphatic hydrocarbons, ketones, alcohols, acids, and bases [47]. This polymer is commonly found in marine water ecosystems; in a study by [48] in Bandon Bay of Thailand, 57% of tested microplastic samples showed the PP as polymer type. Due to its excellent resistance, especially to high temperatures, polypropylene plastic is widely used in various plastic products such as bottles, plates, food containers, straws, and ropes [47].

4 Conclusion

Based on the results of the research, in can be concluded that density of microplastics from water column and fish organs (gill and gastrointestinal tract) were higher in the Fish Apartments compared to natural reef. Particle density in the gills were higher than in gastrointestinal tract due to entry mechanism of microplastics into the fish body via respiration and feeding. Microplastic particles in both water column and fish organs share similar characteristics, dominated by small-sized (0-40 µm) black-coloured fragment; showing possibility that the microplastics pollution is originated from the same sources. Also, polymer type of the fragments is polypropylene (PP) which is used as materials for construction of the Fish Apartments. Therefore, it is strongly estimated that partition units of Fish Apartments highly contribute to the microplastics pollution in the study area.

This research only focuses on microplastics in planktivorous fish, therefore further research is needed to access the accumulation of microplastics in herbivorous and carnivorous fish in around Fish Apartments units. More complete and detailed data will become useful consideration for evaluation of the use of plastics as materials for construction of artificial reefs for coral reef rehabilitation and restoration.

References
