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# Occurrence of Microplastic Ingestion by Commercial Fish Species from the Pangempang Estuary in Indonesia

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**Abstract**

The aim of this study was to assess the occurrence of microplastic in digestive organs (esophagus to intestine) of popular marine commercial fish species: Jack (*Caranx* SP.), blackspot snapper (*Lutjanus fulviflamma*), coral grouper (*Epinephelus carallicala*), golden snapper (*Lutjanus johnii*) and pickhandle barracuda (*Sphyraena jello*) from Pangempang Estuary in Indonesia. To separate the microplastic particles, each sample was digested using alkaline digestion solution, filtered on Whatman paper and then dried. Microplastic particles in the processed digestive organ contents were identified and enumerated using a dissecting stereo microscope. Microplastics were observed in 94 % of fish digestive organs sampled: 100 % jacks (n = 10), 80 % blackspot snapper (n = 10), 100 % coral grouper (n = 10), 90 % golden snapper (n = 10) and 100 % pickhandle barracuda (n = 10). In these fish samples we found in average of 41 ± 6.0, 26 ± 11.0, 15 ± 2.0, 11 ± 1.0 and 14 ± 2.0 SE microplastic particles per individual and an average of 499.5 ± 94.4, 501.5 ± 198.8, 83.6 ± 18.4, 79.8 ± 11.6 and 40.2 ± 25.0 SE microplastic particles per kg fish weight, in jacks, blackspot snapper, coral grouper, golden snapper and pickhandle barracuda, respectively. Fiber was the most dominant type accounting for 78.6 % of all observed microplastics. Microplastic beads were not detected. The correlation of the average consumed microplastic and the average fish weight per species showed a negative relationship ($R^2$ = 0.36), but was not significant (*p*-value = 0.28). Our finding suggests that demersal fish like jacks, blackspot snapper and coral grouper may have ingested a higher quantity of microplastic particles compared to pelagic species like pickhandle barracuda.

**Keywords:** Aquatic pollution, Borneo, Indonesia, Ingestion, Plastic

## Introduction

Following its invention over 110 years ago, plastic materials and their debris have become a ubiquitous pollutant across the globe. Plastic material is made from polymers that can be shaped easily for many varieties of uses. The desire to use plastic materials due to their affordability, bio-inertia, high strength to weight ratio and resistance to degradation has made plastic an important material for human use. By the year 2017, the total global production of plastic was estimated to reach 8,300 million metric tons [1], with the rate of production reaching over 300 million metric tons per year [2]. Approximately 10 % of the annual plastic production is projected to end up in the ocean [3]. Countries in Asia, including Indonesia are responsible for 88 - 95 % of global marine debris [4] of which 70 % is estimated to be plastic [5].

Initially regarded as a safe material, several decades of plastic production have brought a wide range of environmental problems, particularly in marine ecosystems. Continuous exposure to weathering factors such as sunlight, wind and waves, along with the process of oxidation, causes plastic items to break down into smaller fragments. On the shore, it may be fragmented into smaller pieces due to grinding from rocks and sand [6]. The breakdown process leads to smaller particle known as microplastics (< 5 mm in diameter) [7]. These smaller plastic fragments can be easily mistaken for prey and subsequently ingested by aquatic organisms. There is growing concern regarding microplastic issues because they have the potential to physically affect animals and pose toxicological risks due to potentially harmful compounds added during plastic production. These concerns highlight the possibility of these contaminants entering the food chain [8,9]. These added compounds encompass fillers and plasticizers used for texture adjustment, coloring agents, antimicrobials, flame retardants and other substances that modify plastic properties to meet specific preferences [10].

Microplastic particles can also act as transport vector, meaning that they can accumulate and spread harmful chemicals and pathogenic microorganisms [11]. Cole *et al.* [12] reported that ingestion of microplastic particles by zooplanktons shows negative impacts on feeding, hatching and overall health. Similarly, the ingestion of microplastics by oysters also showed negative impact on their reproduction and feeding habits [13]. To fish, exposure to microplastics or in combination with other contaminants that attached on them can induce various health problems [14].

The marine region situated in the eastern part of Kalimantan Island is an integral component of the tropical waters within the Indo-Pacific Coral Triangle. This area is renowned for its abundant marine biodiversity [15]. Estuarine waters are often utilized for fisheries [16,17] and are a sociocultural and economically important fishing ground used by both traditional and commercial fishermen [18]. Estuary ecosystems face increasing rates of microplastic pollution and accumulation, which may have negative impacts on biota. A large portion of microplastic pollution originates from urban areas, including inland urban sources, and is carried by rivers and streams to oceans, a portion of which will become trapped and accumulate in estuaries [19]. Pollution in estuaries is problematic as they provide crucial habitat for a variety of commercially and recreationally important species such as fish and shellfish [20]. Numerous studies have investigated microplastics in both marine and inland environments [21-23]. In fact, Meicahayanti *et al.* [24] reported the presence of microplastics in waters of Mahakam River located in the East Kalimantan Island. However, there have been limited evaluation on microplastic contamination in transitional waters between the 2 ecosystems, such as estuarine waters, allowing analysis of microplastic concentrations ingested by some commercial fish. To address this knowledge gap, we evaluated the types and abundance of microplastic particles in available commercial fish samples collected from Pangempang Estuary in East Kalimantan (Borneo), Indonesia.

## Materials and methods

### Site description and Sampling

The annual mean air temperature in East Kalimantan is 28.44 °C (83.19 °F). This region experiences the effects of an equatorial climate, resulting in 2 periods of intense rainfall and 2 periods of reduced rainfall, with a total annual precipitation of approximately 97.22 mm (3.83 inches) [25]. The Pangempang Estuary (0°12'52.52"S, 117°25'30.65"E) is situated on the eastern side of Kalimantan Island, Indonesia. This estuary serves as the confluence point for waters from the Pangempang River and seawater from the Makassar Strait, situated between Kalimantan and Sulawesi Island (**Figure 1**). The Pangempang River spans approximately 26 km, originating from the Marangkayu reservoir. Its width ranges from 8 m upstream to 140 m downstream. The river passes through the Marangkayu Town settlement, situated roughly 20 km upstream from the Pangempang Estuary. Additionally, about 7 km upstream from the estuary, there is a coal loading port in operation. The estuary plays a vital role as an intercoastal waterway for the nearby villages. In addition to residential areas in the surrounding estuary, several recreational beaches are privately owned and operated and are economically important to the region’s tourism industry. Furthermore, local fishermen actively engage in fishing activities within this area or use it as a passage to access the open waters of the Makassar Strait.

To evaluate the ingestion of microplastics by fish, we collected fish specimens from local fishermen operating in the waters of the Pangempang Estuary on October 15$^{th}$, 2022, which falls within the period commonly designated as the “wet season”. However, Kalimantan/Borneo Island lacks a distinct wet/dry season, unlike the rest of the Indonesian archipelago [26]. We were able to collect ten replicates of each 5 different species from these fishermen. The species we analyzed were jack (*Caranx* SP.), blackspot snapper (*Lutjanus fulviflamma*), coral grouper (*Epinephelus carallicala*), golden snapper (*Lutjanus johnii*) and pickhandle barracuda (*Sphyraena jello*). We identified the fish species using keys in “Marine Fishes and Crustaceans of the Southeast Asian Region” [27]. Each individual fish was placed in a plastic bag, and then stored on ice during transport to the Water Quality Laboratory at Mulawarman University. The samples were preserved in freezer before processing and analysis (**Figure 2**).

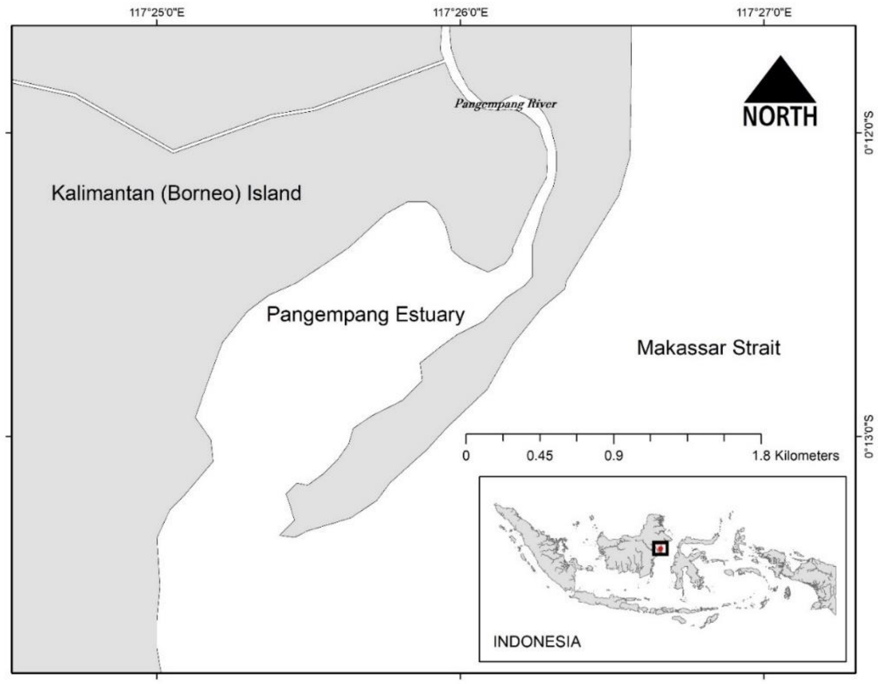


**Figure 1** The Pangempang Estuary (East Kalimantan, Indonesia).

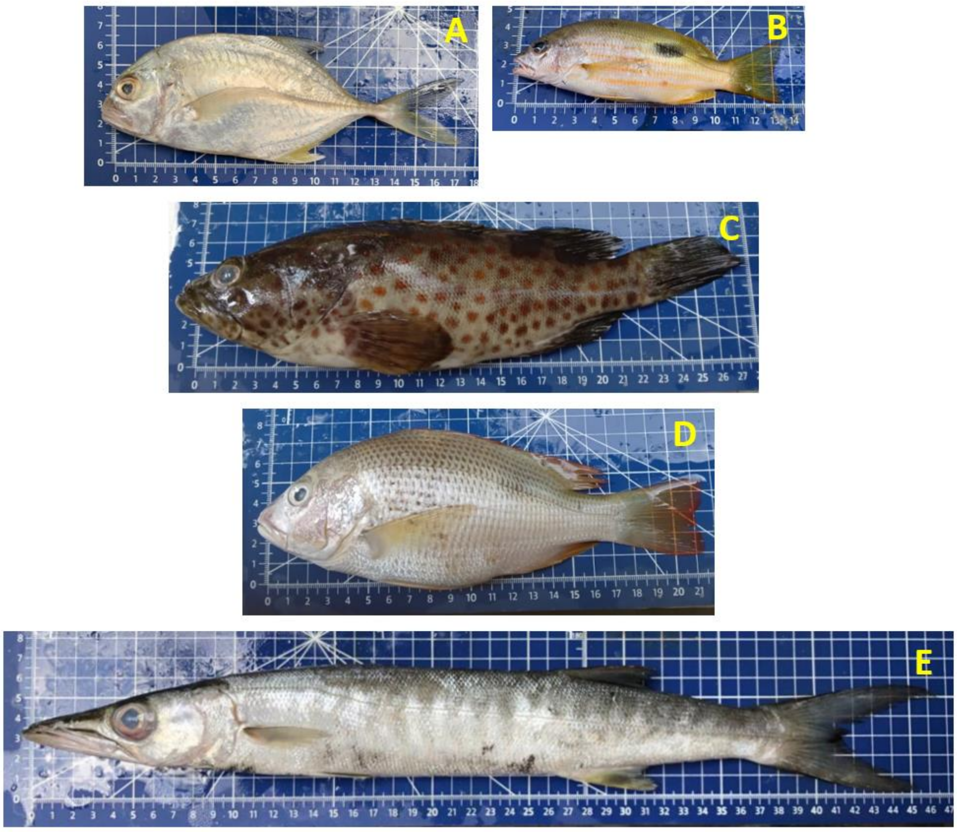


**Figure 2** Photographs illustrating the observed fish species: (a) jack (*Caranx* SP.), (b) blackspot snapper (*Lutjanus fulviflamma*), (c) coral grouper (*Epinephelus carallicala*), (d) golden snapper (*Lutjanus johnii*) and (e) pickhandle barracuda (*Sphyraena jello*).

**Table 1** Taxonomy, common name and foraging ecology.

| No. | Taxonomy and common name | Foraging ecology |
|---|---|---|
| 1. | Family: Carangidae<br>Genus: Caranx<br>Common names: Jacks or trevallies | Most species are **demersal**, or bottom dwelling, while others are **pelagic**, moving long distances in the upper water column [28]. All species are predatory fish, taking smaller fish, cephalopods as prey [29]. |
| 2. | Family: Lutjanidae<br>Species: *Lutjanus fulviflamma* (Forsskal, 1775)<br>Common names: Blackspot snapper | Many species under this family are **demersal** and associated with coral reefs [30]. Some of the larger red snapper species descend into deeper waters, at least to 200 m in depth [31]. Lutjanus is predatory fish usually found in tropical and subtropical reefs and mangrove forests. |
| 3. | Family: Serranidae<br>*Epinephelus carallicala* (Kuhl & Hasselt, 1828)<br>Common names: Coral grouper | Predominantly sedentary species that resides near the ocean floor (**demersal**), and it goes through a **pelagic larval** stage of approximately 40 days before eventually settling in shallow coastal hardbottom habitats during its juvenile phase [32]. They usually eat fish, octopuses and crustaceans. Some species prefer to ambush their prey, while others are active predators. They swallow prey rather than biting pieces off of them [33]. |
| 4. | Family: Lutjanidae<br>*Lutjanus johnii* (Bloch, 1792)<br>Common names: Golden snapper or John's snapper | Lutjanus exhibits a 2-part life cycle: an initial stage with **pelagic eggs and larvae**, succeeded by a later stage as juveniles and adults that are oriented towards the seafloor (**demersal**) [34]. Feed on fishes and benthic invertebrates including shrimps, crabs and cephalopods. Adults probably frequent coral reef areas; juveniles found in mangrove estuaries [35]. |
| 5. | Family: Sphyraenidae<br>*Sphyraena jello* Cuvier, 1829<br>Common names: Pickhandle barracuda or Banded barracuda | They are migratory **pelagic** predators and usually be found in schools swimming in the tropical waters, predominantly nearby coral reefs [36]. Prey on fish, cephalopods and shrimps [37]. |

**Laboratory processing**

Each specimen was lengthened to the nearest mm and weighed using an analytical balance to the nearest 0.001 kg. Weight represents fish wet weight; excess water was removed prior to weighing. We conducted a dissection of the fish's stomach and gathered its contents, spanning from the esophagus to the intestine. To separate the microplastic particles, digestive tract contents were digested using alkaline digestion solution of 10 % potassium hydroxide (KOH) [38,39] in a 150 mL glass beaker. Each beaker was covered with aluminum foil. We stirred the samples in the beaker using magnetic spinner at 350 rpm for 2 h. Then we collected the particles after the digestion using a sieve of 125 µm and transferred to a previously cleaned beaker using distilled water. Into the beaker 30 % $H_2O_2$ was added and then stirred. After 1 h stirring, samples were vacuum pump filtered on 1.6 µm pore size Whatman paper and then dried in an oven at 60 °C for 24 h.

Using a 10× - 45× magnification dissecting microscope, microplastic particles in the processed digestive tract contents were identified and enumerated. The microplastic particles were observed and categorized into 4 major types (fiber, fragment, film and bead; [40]) following protocols from Hidalgo-Ruz *et al.* [41]; Eppehimer *et al.* [42]. To minimize contamination by airborne synthetic fibers during sampling processing, the researchers wore 100 % cotton lab cloth. We consistently cleaned all material used in this sample processing with distilled water. During the preparation and observation, the beakers and aluminum cups were always covered when not in use to limit potential airborne microplastic deposition. All these processes were conducted in the laboratory behind closed doors and windows.

**Data analysis**

We measured the fish body weight and examine the relationship with observed ingested microplastics in each specimen. We used linear regression analysis using statistical program R (version 3.5.1) [43] to determine the trend and significance of fish body weight (independent variable) in affecting the abundance of ingested microplastics (dependent variable).

## Results and discussion

**Results**

In this study, the majority of observed fish species are demersal (**Table 1**), or at least in part of their life phase, demersally as adults and pelagically in the juvenile phase. Only the Pickhandle barracuda species live as pelagic fish. All observed fish are predator species that prey on marine biota including fish, shrimps, crabs and cephalopods. When considering average length and weight, the Pickhandle barracuda stands out as the longest and heaviest, while the blackspot snapper is the shortest and lightest among the species studied (**Table 2**).

**Table 2** Observed fish length and weight.

| No. | Fish | Length (cm) | | | | Weight (kg) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Average | SE | Min. | Max. | Average | SE | Min. | Max. |
| 1. | Jack | 18.24 | 0.85 | 16.20 | 25.20 | 0.10 | 0.02 | 0.06 | 0.25 |
| 2. | Blackspot snapper | 14.65 | 0.35 | 13.50 | 16.90 | 0.05 | 0.004 | 0.04 | 0.08 |
| 3. | Coral grouper | 25.47 | 0.88 | 21.00 | 30.50 | 0.21 | 0.02 | 0.12 | 0.36 |
| 4. | Golden snapper | 19.99 | 0.87 | 16.80 | 24.10 | 0.16 | 0.02 | 0.09 | 0.23 |
| 5. | Pickhandle barracuda | 43.61 | 1.02 | 38.40 | 47.70 | 0.36 | 0.02 | 0.26 | 0.46 |

We observed microplastic fibers, film, fragments in fish digestive systems (**Figure 3**). Microplastic beads were not detected in any of our samples. Microplastic particles were identified in 100 % of jacks, coral grouper, golden snapper and pickhandle barracuda samples. The 80 % of blackspot snapper contained observable microplastics.

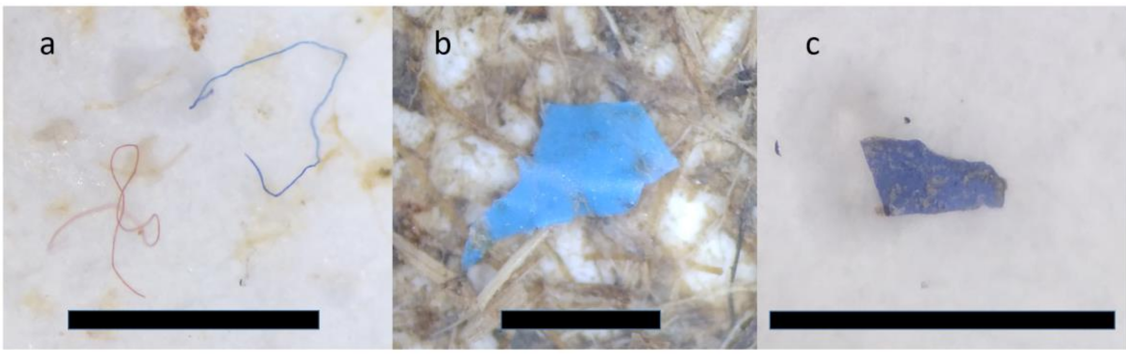


**Figure 3** Photographs illustrating the observed microplastic types found in digestive organs from sample fishes collected in the Pangempang Estuary: (a) fiber, (b) film and (c) fragment. Horizontal scale bar ≈ 0.5 mm.

In fish samples of jacks, blackspot snapper, coral grouper, golden snapper and pickhandle barracuda, we found in average of 41 ± 6.0, 26 ± 11.0, 15 ± 2.0, 11 ± 1.0 and 14 ± 2.0 SE microplastic particles per individual, respectively (**Figure 4(a)**), and we found in average of 499.5 ± 94.4, 501.5 ± 198.8, 83.6 ± 18.4, 79.8 ± 11.6 and 40.2 ± 25.0 SE microplastic particles per kg fish weight, respectively (**Figure 4(b)**). The blackspot snapper had the highest number of microplastic particles per kg of fish weight: 501.5 ± 198.8 SE microplastic particles. The lowest number of particles per kg of fish was found in the pickhandle barracuda: 40.2 ± 25.0 SE microplastic particles.

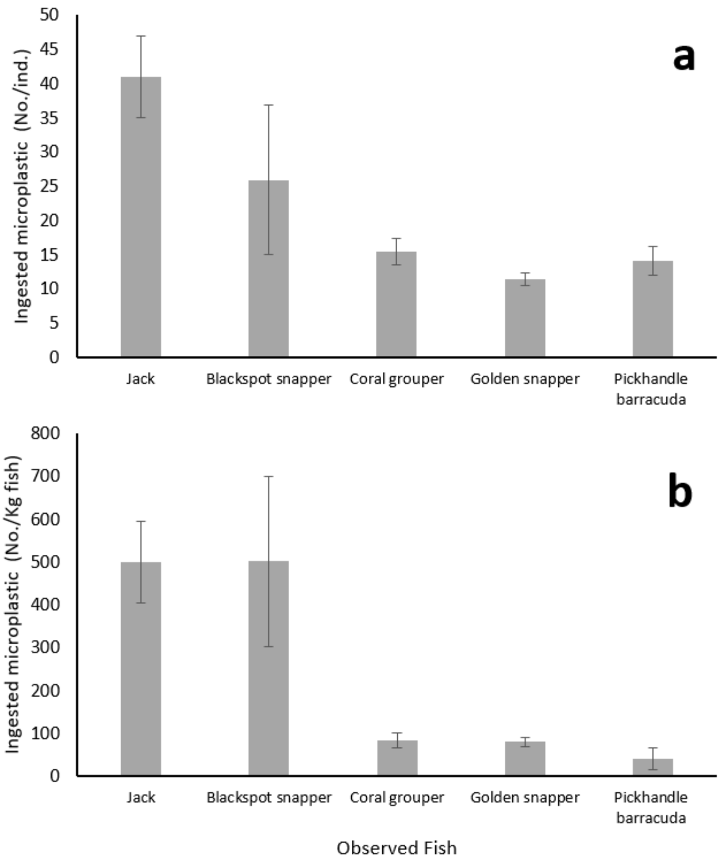


**Figure 4** Average microplastic particles per individual (a) and per kg fish wet weight (b).

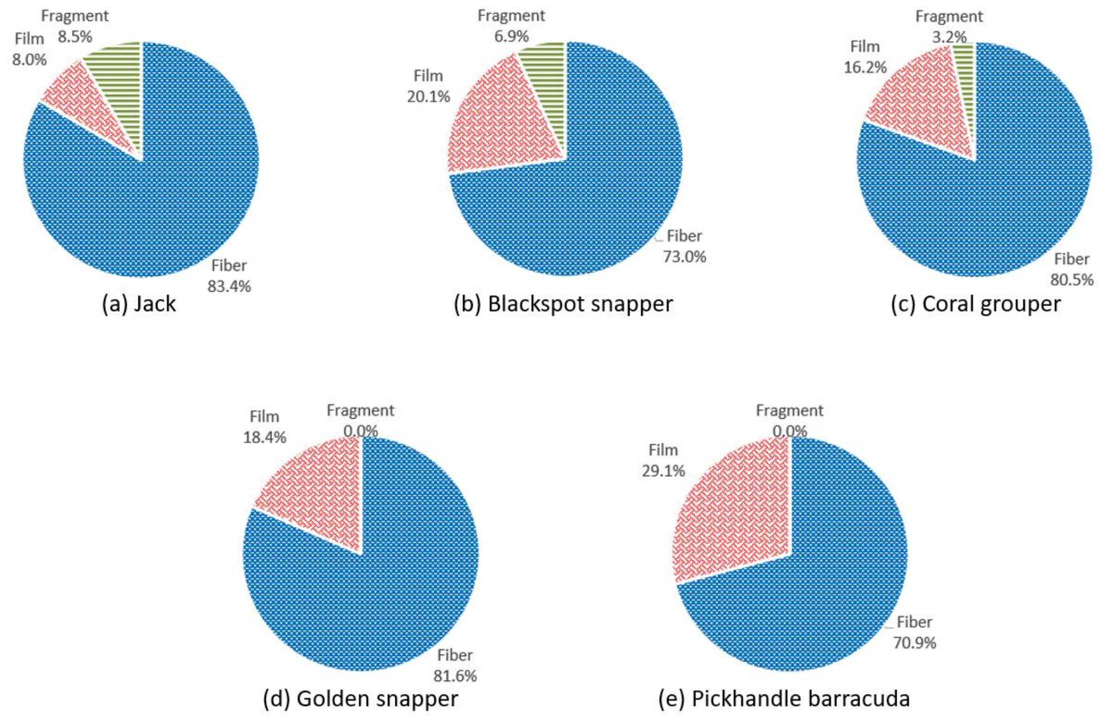


**Figure 5** Percentages of the observed microplastic types found in digestive organs from sample fishes collected in the Pangempang Estuary.

In jack samples, fibers were most prevalent representing 83.4 % of all microplastic pieces, film and fragment were much lower at 8.0 and 8.5 %, respectively. Fibers were observed in all samples, film was found in 80 % of samples, and fragments were found in 90 % of our jack fish samples (**Figure 5(a)**). Our blackspot snapper samples exhibited that fiber was the most observed microplastic type 73 %, and the followed by film (20 %) and fragments (7 %). The fibers, film and fragments were detected in 80, 70 and 50 % of all samples, respectively (**Figure 5(b)**). The coral grouper samples showed that fiber particles were the most abundant with 80.5 %, as compared to percentages found for film (16.2 %) and fragments (3.2 %). In this species, fibers, film and fragments were detected in 100, 90 and 40 % of our samples, respectively (**Figure 5(c)**). From our golden snapper samples, we observed that fibers were the most detected type (81.5 %) and followed by film (18.5 %). No fragments were found in this species samples. The fibers shape particles were found in all this species samples. The film shape particles were found in only 77.8 % of the samples (**Figure 5(d)**). Finally, our pickhandle barracuda samples indicated that fiber was the most dominant as well (71.0 %) microplastic particles and then followed by film (29.0 %). There was no fragment shape particle detected in this species samples. Either, the fibers and film were detected in all this species samples (**Figure 5(e)**).

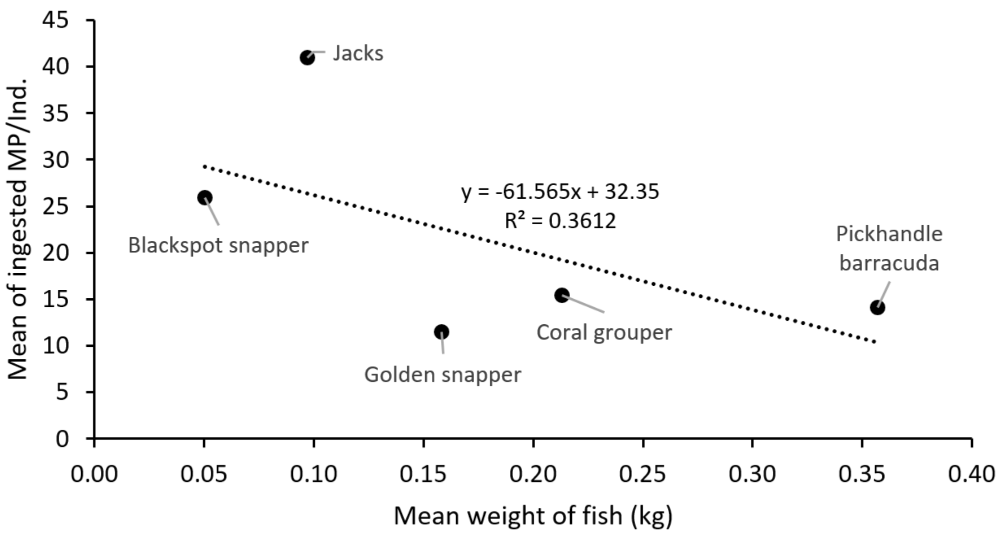


**Figure 6** Relationship of mean weight of observed fish and mean number of ingested microplastic particles per species individual.

Among the 5 fish species we observed (n = 5), the average weight per individual blackspot snapper in our sample was the smallest, at 0.05 kg, while the pickhandle barracuda was the largest, at 0.36 kg (**Table 2**). The relationship of the average consumed microplastic particles and the average fish weight per species (**Figure 6**) show a negative relationship ($R^2 = 0.36$), but the relationship between the 2 is not significant (*p*-value = 0.28).

**Discussion**

***Types of microplastic***

Our results affirmed that fish in the Pangempang Estuary indeed ingest microplastics, with fibers being the dominant type of microplastic observed. Previous research in aquatic environments has identified fibers as the most prevalent form of microplastics [44-47]. Sources of fibers are typically textile goods, such as clothes [48]. Degradation of synthetic fabrics was predicted as major contributor for microplastic fiber abundance in the environment [48,49]. During the lifespan of the fabric, these fibers are naturally released through regular usage and cleaning. In fact, a typical piece of clothing can release over 1,900 fibers at size less than 1 mm in length per wash cycle [50]. After being washed, synthetic fibers from laundry water find their way into the municipal water systems. While a portion of these fibers is eliminated during wastewater treatment, the remaining is typically discharged into surface waters [50]. In our study, the urban areas along the river that flows to Pangempang Estuary do not have municipal wastewater treatment facilities, consequently raw untreated urban wastewater discharge to this estuary. The microplastic of film was found as the second most abundant microplastic type in all fish species we examined, except in Jacks fish. The source of film microplastics may potentially come from abrasion of plastic bags that easily encountered as macro debris in the Pangempang Estuary waters. A microplastic study on commercially important marine fish species in Fiji documented that 50 % of sampled microplastic particles were microplastic film with some observed fibers and fragments but no observed beads [51]. We observed fragment particles was the lowest in number and only found in 3 of 5 fish species we examined (jack, blackspot snapper and coral grouper). In contrast, Karbalaei *et al.* [52] documented that among commercial marine fish in the adjacent waters of Malaysia, fragment-type microplastics were the most prevalent. Source of fragment microplastic mainly come from degraded larger plastic debris due to continuous weathering includes photo, biological, physical and chemical processes in the terrestrial and aquatic environment [53]. Recent investigations into the impact of microplastic fibers on aquatic organisms have unveiled various harmful consequences. According to Liang *et al.* [54] goldfish exposed to a concentration of 1,000 items per L of microplastic fibers displayed increased coughing behavior, reduced predatory activities, diminished daily food intake and stimulated dynamic mucus excretion during ventilation, feeding and swimming processes. Similarly, a study by Zhao *et al.* [55] demonstrated that following exposure, microplastic fibers were found in the digestive organs of zebrafish, even in their early life stages, resulting in intestinal damage and toxic effects indicated by observable histopathological changes and biomarker responses. Another study by Xie *et al.* [56] noted oxidative stress, dysbiosis in the intestine microbiome, and histological damage as significant symptoms in barramundi exposed to microplastic fibers.

***Abundance of microplastic in digestive organs***

Our finding revealed that jack consumed the highest average of microplastic particles, 41 (SE: 5.98) while golden snapper consumed the least, only 11 (SE: 0.97). These values are higher than those reported for fish in previous estuary studies, for instance Arias *et al.* [57] documented microplastic particle abundance ranged from 9 - 14 in digestives organs per individual across multiple fish species caught the Bahía Blanca Estuary in Argentina, up to 4 particles in semi pelagic boop boops in the Mediteranian Sea [45], and 1.1 - 7.2 microplastic particles per fish collected from China marine waters [44]. Interestingly, in comparison to microplastic presence in freshwater river fish, our result was also higher than the microplastic abundance reported in the Han River and its tributaries, South Korea with mean only 22.0 ± 16.0 particles per fish individual [58].

When accounting for size, the blackspot snapper has the highest amount of microplastics per kg of fish, 501.5 ± 198.8 SE, while the pickhandle barracuda has the smallest amount (40.2 per kg ± 25.0 SE). The average individual fish weight was the key determinant of this abundance. An interesting point is that the results of the regression test on the average number of microplastic particles with the average fish weight per species show a negative relationship ($R^2 = 0.36$), however the relationship between the 2 is not significant ($p$-value = 0.28). Similarly, Chen *et al.* [59] reported that fish size did not affect microplastic abundance in the fish digestive organs.

***Foraging ecology***

Microplastic ingestion is potentially influenced by 2 crucial factors: The type of habitat and the feeding behavior/strategy of organisms [60]. Within marine environments, scientists have noted markedly greater quantities of microplastics in demersal fish species compared to pelagic species [44,46]. This trend could be linked to the prevalence of plastic waste near the seabed, which is often regarded as the final zone of accumulation for plastics within marine ecosystems.

Microplastics could potentially be present in fish across different trophic levels and could be transferred via predation as secondary ingestion. However, current data are insufficient to make definitive conclusions about the absorption or transfer of microplastics [61]. In fact, distinguishing between directly and indirectly ingested microplastics is exceedingly challenging [62]. In 2020, Walkinshaw *et al.* [63] performed a semi-systematic review on research examining the presence of microplastics in commercially significant organisms across various trophic levels. The findings indicate that microplastics do not exhibit biomagnification, and organisms at lower trophic levels are more prone to contamination by microplastic pollution compared to apex predators.

In the present study, all collected species were adult predator fish, 4 fish species were demersal, and one was pelagic (**Table 1**). The microplastic abundances in the 3 species of demersal fish (jacks, blackspot snapper and coral grouper) were higher than the only pelagic fish of pickhandle barracuda. However, golden snapper that identified as demersal fish showing lower microplastic abundance than the pickhandle barracuda. It is hypothesized that there will be substantial variations in the consumption rates of microplastics among different species in estuaries. This variation is expected because estuaries are dynamic environments influenced by multiple factors such as tides, wind and residence time [64]. This study did not identify any herbivorous species, resulting in a lack of information regarding the effect of feeding habits on microplastic consumption. However, a study conducted along the Mediterranean coasts of Israel found that 2 herbivorous fish species consistently ingest significant quantities of microplastic particles as much as 10 - 99 microplastic particles per fish gut [65].

## Conclusions

This study is the first to document the presence of microplastic particles in commercially important marine fishes of Kalimantan Island in Indonesia. The average abundance per fish individual were higher than those observed for commercial fish in previous marine estuary studies. The relationship between the average weight of species and the average abundance of microplastics was negative, but it did not reach statistical significance. Microplastic fibers constitute the largest portion of the microplastic pollution found in the digestive systems of sampled fish from the Pangempang Estuary. Our research suggests that demersal fish may have ingested a higher quantity of microplastic particles compared to pelagic species.

Our findings also reaffirm the need for continued investigation into microplastic ingestion by commercial fish species, as there is potential for negative health impacts to these populations (source) and potential for transfer of these microplastics into humans that consume these fishes (source). Future studies should seek to identify potential differences in microplastic ingestion across a variety of habitat types and foraging strategies. Future microplastic studies in Indonesia should also include shellfish in their analyses, as there are very few studies on this topic. Given the prevalence and high abundance of these microplastic fibers, we recommend to include them in assessments of the risks associated with microplastic pollution, particularly regarding their potential toxicity as these species are considered as important commercial fish in this region.

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