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

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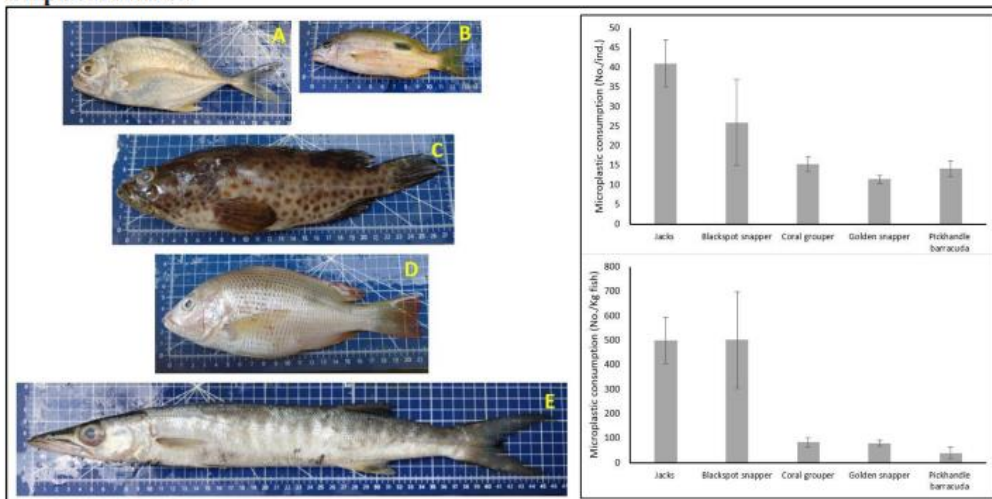
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Highlights

- This study demonstrates the presence of microplastic particles in five commercially important fish in a tropical estuary of Kalimantan Island in Indonesia.
- Fiber was the most dominant type of microplastic.
- Demersal fish may have ingested a higher quantity of microplastic particles compared to pelagic species.

Graphical abstract



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 caralligala*), golden snapper (*Lutjanus johnii*), and pickhandle barracuda (*Sphyrna jello*) from Pangemang Estuary in Indonesia. To separate the microplastic particles, each sample was digested using alkaline digestion solution, filtered on Whatman paper and then dried. Using a dissecting stereo microscope, microplastic particles in the processed digestive organ contents were identified and enumerated. Microplastics were found in 96% of fish digestive organs (n = 50). In fish samples of jacks, blackspot snapper, coral grouper, golden snapper, and pickhandle barracuda (n = 10 for each species), we found in average of 41 ± 6.0 SE, 26 ± 11.0 SE, 15 ± 2.0 SE, 11 ± 1.0 SE, 14 ± 2.0 SE microplastic particles per individual, respectively, and we found in average of 499.5 ± 94.4 SE, 501.5 ±

198.8 SE, 83.6 ± 18.4 SE, 79.8 ± 11.6 SE, 40.2 ± 25.0 SE microplastic particles per kg fish weight, respectively. Fiber was the most dominant type of microplastic. Microplastic beads were not detected. The relationship of the average consumed microplastic and the average fish weight per species show a negative relationship ($R^2 = 0.36$), but insignificant ($P\text{-value} = 0.28$). 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: *Pollution, Ingestion, Commercial fish, Borneo, Indonesia*

Introduction

After invented over 110 years ago, plastic materials become ubiquitous in the environment. Plastic material is made from polymers that can be shaped easily for many varieties of uses. The elevated desire to use plastic materials due to their affordability, bio-inertia, high strength to weight ratio, and resistance to degradation. These characteristics has made plastic materials become important materials for human life nowadays. By the year of 2017, the total global production of plastic was estimated to reach 8300 million metric tons [1], with the rate of production has reached 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 suspected responsible to bring 88%-95% of global marine debris load into ocean [4]. The proportion of plastics usually take about 70% of total marine debris [5].

Initially regarded as a safe material, several decades of plastic production have brought a wide range of environmental problems, particularly in marine ecosystems. In oceans, even though plastic is considered as durable material and resistant to natural biodegradation. However, 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) [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 from the surrounding [11]. Cole et al. reported that ingestion of microplastic particles by zooplanktons shows negative impacts on feeding, hatching, and their health [12]. 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 a type of marine ecosystems often utilized for fisheries purposes. [16,17], and these waters 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 estuary is problematic as estuaries also provide crucial habitat for a variety of commercially and recreationally important species such as crabs, fish, and shellfish [20]. Numerous studies investigating microplastics have placed their emphasis on ecosystems in both marine and inland environments [21,22,23]. However, there have been limited evaluation on microplastic contamination in transitional waters between the two 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 and asked the main following questions: what type of microplastic? And how is the abundance of microplastic in fish collected from this transitional waters?

Materials and methods

Site description and Sampling

The Pangempang Estuary ($0^{\circ}12'52.52''\text{S}$, $117^{\circ}25'30.65''\text{E}$) 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. The Pangempang River spans approximately 26 kilometers, originating from the Marangkayu reservoir. Its width ranges from 8 meters upstream to 140 meters downstream. The river passes through the Marangkayu Town settlement, situated roughly 20 kilometers upstream from the Pangempang Estuary. Additionally, about 7 kilometers 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.

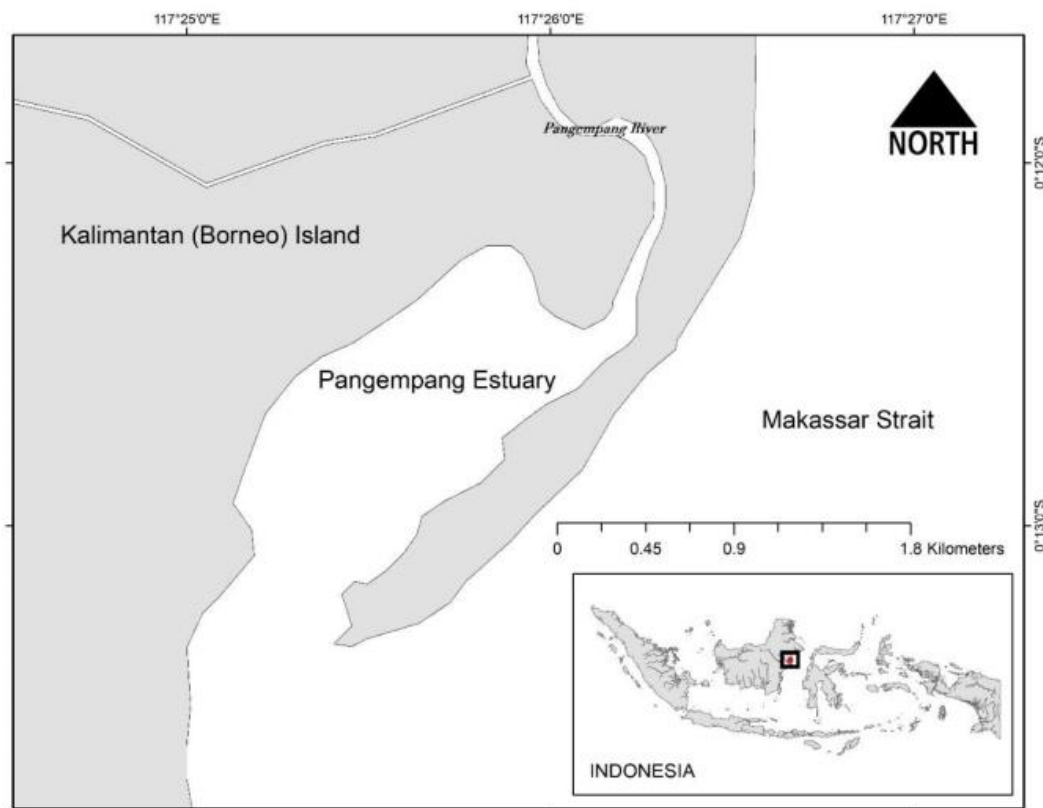


Figure 1 The Pangempang Estuary (East Kalimantan, Indonesia)

To evaluate the ingestion of microplastics by fish, we obtained fish specimens from local fishermen operating in the waters of the Pangempang Estuary. We were able to collect ten replicates of each five different species from these fishermen. The species we identified were jack (*Caranx* SP.), blackspot snapper (*Lutjanus fulvivlamma*), coral grouper (*Epinephelus carallicala*), golden snapper (*Lutjanus johnii*), and pickhandle barracuda (*Sphyræna jello*). We conducted the identification of these fish species and their habitats based on the information provided in the book titled "Marine Fishes and Crustaceans of the Southeast Asian Region" [24]. Each individual fish was placed in plastic bags, 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.

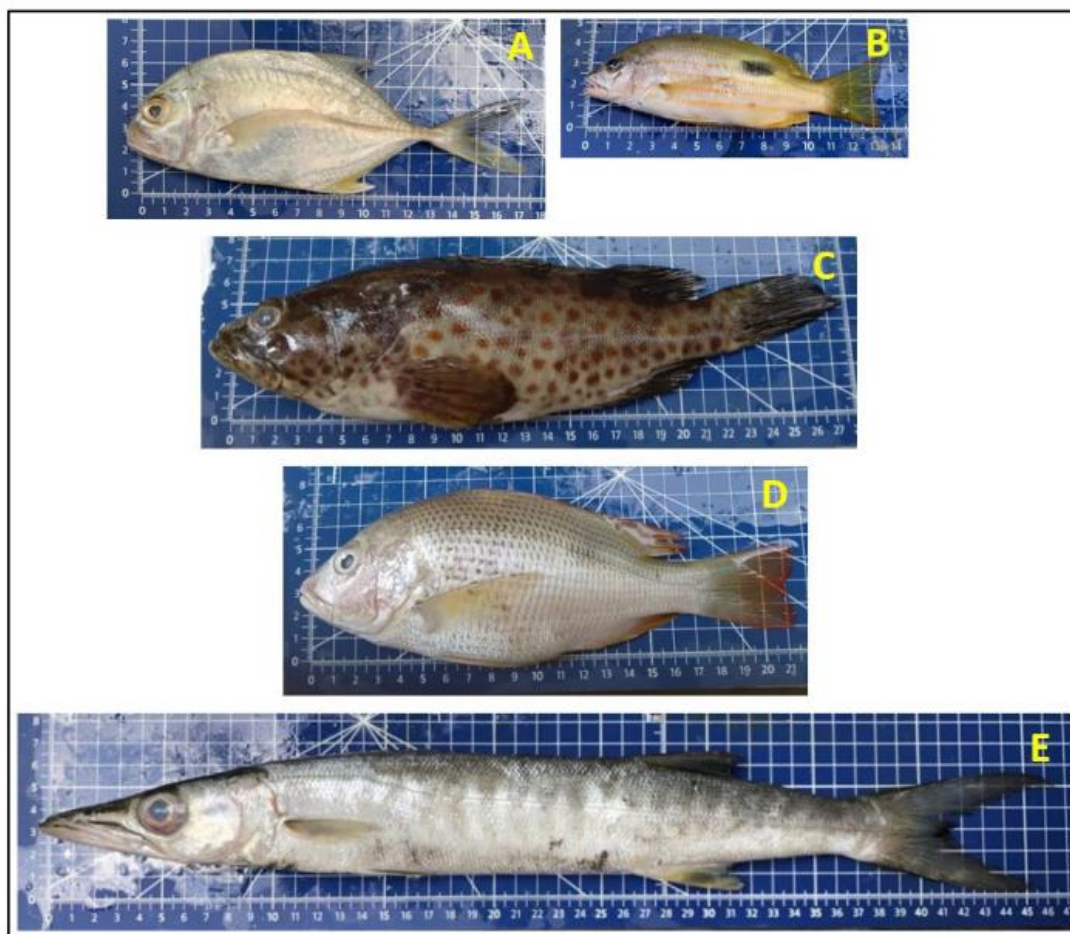


Figure 2 Photographs illustrating the observed fish species: (A) Jack (*Caranx* SP.), (B) blackspot snapper (*Lutjanus fulvivflamma*), (C) coral grouper (*Epinephelus carallicala*), (D) golden snapper (*Lutjanus johnii*), and (E) pickhandle barracuda (*Sphyræna jello*).

Laboratory processing

We measured fish weight (g) of each specimen. 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, each stomach content was digested using alkaline digestion solution of potassium hydroxide (KOH) [25,26]. Each stomach content was placed in a beaker and 10% KOH was added allowing submersion of stomach contents in the beaker. Each beaker was covered with aluminum foil. We stirred the samples in the beaker using magnetic spinner at 350 rpm for 2 hours. 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 hour stirring, samples were vacuum pump filtered on 1.6 μm pore size Whatman paper and then dried.

Using a 10–45 $\times$ magnification dissecting microscope, microplastic particles in the processed stomach contents were identified and enumerated. The microplastic particles were observed and categorized into four major types (fiber, fragment, film, and bead; [27]) following protocols from Hidalgo-Ruz et al., [28] and Eppehimer et al. [29]. 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 used. All these processes were conducted in the laboratory behind closed doors and windows.

Results

We observed microplastic shapes of fibers, film, fragments. Microplastic beads were not detected in any of our samples. In jacks, coral grouper, golden snapper, and pickhandle barracuda, we detected microplastic particles in 100% of samples, except for blackspot snapper only 80% of samples.

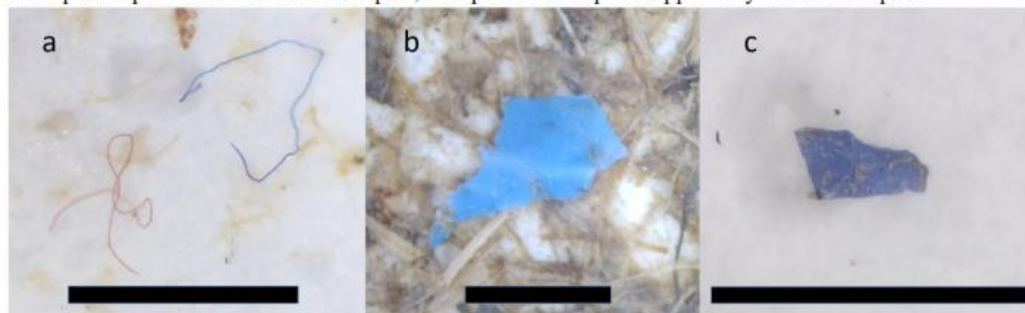


Figure 3 Photographs illustrating the observed microplastic types found in digestive organs from sample fishes collected in the Pangempang Estuary: **a.** fiber, **b.** film, and **c.** fragment. Horizontal scale bar ≈ 0.5 mm

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

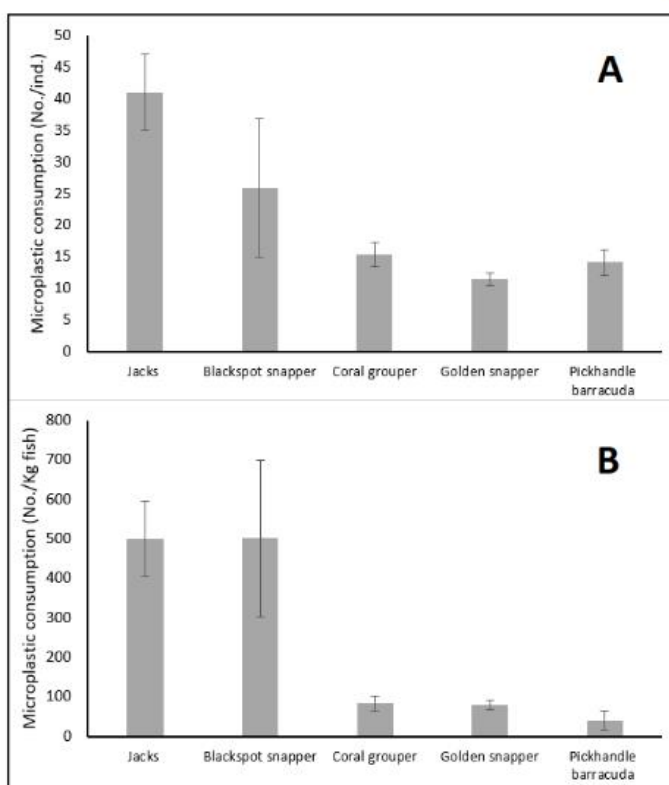


Figure 4 Average of microplastic particles per individual (A) and per kilogram fish weight (B)

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.

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%, 50% of all samples respectively.

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%, 40% of our samples respectively.

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.

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.

Among the five 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. The relationship of the average consumed microplastic particles and the average fish weight per species (**Figure 3**) show a negative relationship ($R^2 = 0.36$), but the relationship between the two is not significant ($P\text{-value} = 0.28$).

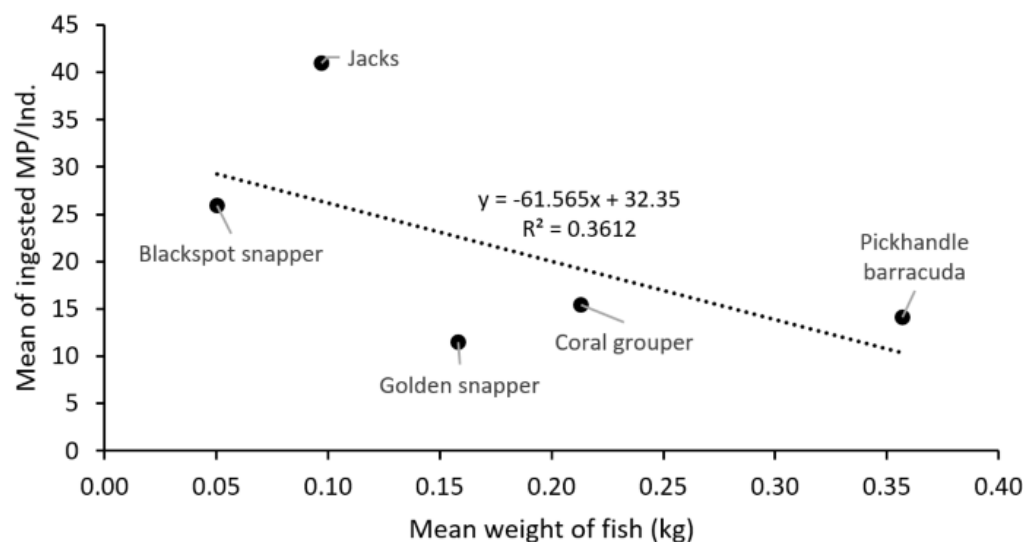


Figure 5 Relationship of mean weight of observed fish and mean number of ingested microplastic particles per individual

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 (fish, shrimps, crabs and cephalopods) generally smaller in size than their own bodies.

Table 1 Taxonomy, common name, foraging ecology

No.	Taxonomy and Common name	Foraging ecology
1.	Family: Carangidae Genus: Caranx Common names: Jacks or trevallies	Most species are demersal , or bottom dwelling, while others are pelagic , moving long distances in the upper water column [30]. All species are predatory fish, taking smaller fish, cephalopods as prey [31].

2.	Family: Lutjanidae Species: <i>Lutjanus fulvivflamma</i> (Forsskal, 1775) Common names: Blackspot snapper	Many species under this family are demersal and associated with coral reefs [32]. Some of the larger red snapper species descend into deeper waters, at least to 200 m in depth [33]. <i>Lutjanus</i> is predatory fish usually found in tropical and subtropical reefs, and mangrove forests.
3.	Family: Serranidae <i>Epinephelus carallicala</i> (Kuhl & Hasselt, 1828) 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 [34]. 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 [35].
4.	Family: Lutjanidae <i>Lutjanus johnii</i> (Bloch, 1792) Common names: Golden snapper, or John's snapper	<i>Lutjanus</i> exhibits a two-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) [36]. Feed on fishes and benthic invertebrates including shrimps, crabs and cephalopods. Adults probably frequent coral reef areas; juveniles found in mangrove estuaries [37].
5.	Family: Sphyrnidae <i>Sphyrna jello</i> Cuvier, 1829 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 [38]. Prey on fish, cephalopods, and shrimps [39].

Discussion

Types of microplastic

Our findings confirmed that fish in Pangempang Estuary do consume microplastics and fibers were the most dominant type of microplastic. In the aquatic environment, ample studies reported that fibers are the most encountered microplastic type [40-43]. Sources of fibers are predominantly textile goods, such as clothes [44]. Degradation of synthetic fabrics was predicted as major contributor for microplastic fiber abundance in our environment [44,45]. 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 1900 fibers at size less than 1 mm in length per wash cycle [46]. 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 discharged into surface waters [46]. 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 film microplastic portion was half of the total discovered microplastic particles, and the remaining shared between fiber and fragment [47]. We observed fragment particles was the lowest in number and only found in three of five fish species we examined. In contrast, Karbalaie et al. documented that among commercial marine fish in the adjacent waters of Malaysia, fragment-type microplastics were the most prevalent [48]. 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 [49].

Recent investigations into the impact of microplastic fibers on aquatic organisms have unveiled various harmful consequences. According to Liang et al., goldfish exposed to a concentration of 1000 items per liter 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 [50]. Similarly, a study by Zhao et al. 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 [51]. Another study by Xie et al. noted oxidative stress, dysbiosis in the intestine microbiome, and histological damage as significant symptoms in barramundi exposed to microplastic fibers [52].

Abundance of microplastic in digestive organs

Our finding revealed that jack as individual consumed the highest average of microplastic particles, 41 (SE: 5.98) while golden snapper was observed consuming 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. documented microplastic particle abundance ranged from 9-14 per gastrointestinal tract in multiple fish species caught the Bahía Blanca Estuary in Argentina [53], up to 4 particles in semi pelagic boop boops in the Mediterranean Sea [41], and ranged 1.1- 7.2 microplastic particles in fish collected from China marine waters [40]. On the other hand, the blackspot snapper has the highest amount of microplastics per kilogram of fish, 501.5 ± 198.8 SE, while the pickhandle barracuda has the smallest amount, only 40.2 ± 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 two is not significant ($P\text{-value} = 0.28$). Similarly, Chen et al. reported that fish size did not affect microplastic abundance in the fish digestive organs [54].

Foraging ecology

Microplastic ingestion is potentially influenced by two crucial factors: the type of habitat and the feeding habits of organisms [55]. Within marine environments, scientists have noted markedly greater quantities of microplastics in demersal fish species compared to pelagic species [40,42]. This trend could be linked to the prevalence of plastic waste near the seabed, which is often regarded as the final accumulation for plastics within the marine ecosystem.

Microplastics could potentially be present in fish across different trophic levels. However, current data are insufficient to make definitive conclusions about the absorption of microplastics [56]. In fact, distinguishing between directly and indirectly ingested microplastics is challenging [57]. Walkinshaw et al. performed a semi-systematic review on research examining the presence of microplastics in commercially significant organisms across various trophic levels [58]. 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 are adult predator fish, four fish species are demersal, and one is pelagic (Table 1). The microplastic abundances in the three 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 anticipated 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 [59]. 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 two herbivorous fish species consistently ingest significant quantities of microplastic particles as much as 10-99 microplastic particles per fish gut [60].

Conclusions

For the first time, this study demonstrates the presence of microplastic particles in five commercially important fish in a tropical estuary of Kalimantan Island in Indonesia. The average abundance per fish individual were higher than those documented 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 a significant 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.

This study result also confirms the urgency to observe wide range of fish species in term of their habitat within the water column and as well as the feeding habit. 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. Perhaps analysis based on muscles and internal organs should also be considered in the near future.

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Names of 5-7 referees

We encourage authors to submit the names of 5-7 referees (not from your own country) suitable to review the work to aid in the peer review process.

1. Paula Sobra, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Campus da Caparica, 2829-516 Caparica, Portugal, email: psobral@fct.unl.pt
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3. Gabriel Enrique De-La-Torre, Universidad San Ignacio de Loyola, Lima, Perú, e-mail: Gabriel.e.dltp@gmail.com
4. Pouran Makhdomi, Kermanshah University of Medical Sciences, Kermanshah, Iran, e-mail: pmakhdomi@gmail.com
5. Andrés H. Arias, Instituto Argentino de Oceanografía (IADO-CONICET/UNS), Bahía Blanca, Argentina, e-mail: aharias@iado-conicet.gob.ar

No.	Perihal	Tanggal	Bukti
2	Notifikasi Revisi Round 1	13 Desember 2023	a. Email konfirmasi dan korespondensi revisi b. Laman akun jurnal - Revisi 1
a.	Email konfirmasi dan korespondensi revisi 6/14/26, 11:28 AM Universitas Mulawarman Mail - [TiS] Manuscript Decision  Hamdhani Hamdhani <hamdhani@fpik.unmul.ac.id> [TiS] Manuscript Decision 1 message Trends in Sciences (TiS) <journal.wu@gmail.com> To: Hamdhani Hamdhani <hamdhani@fpik.unmul.ac.id>, Ghitarina Ghitarina <ghitarina@fpik.unmul.ac.id>, Ristiana Eryati <eryati.ristiana@gmail.com>, "Drew E. Eppehimer" <depprehimer@arizona.edu> Wed, Dec 13, 2023 at 6:13 AM Dear Dr. Hamdhani Hamdhani, Ghitarina Ghitarina, Ristiana Eryati, Drew E. Eppehimer: We have reached a decision regarding your submission to Trends in Sciences, "Occurrence of Microplastic Ingestion by Commercial Fish Species from the Pangempang Estuary in Indonesia". Please revise the manuscript carefully. The manuscript should be resubmitted along with point-by-point explanation according to reviewers' comments. If you disagree with any of the comments, please state your reasons. All corrections are mandatory and must be differentiated with red colour and submit it. Our decision is to: Resubmit for Review We request that you send a revised manuscript within 15 days, otherwise it may be considered withdrawn. PS. Please submit in Microsoft Word version with TiS template, the references should be in TiS format, please recheck. Thank you very much for your kind helps. STEP for upload revised version: Submissions > Workflow > My Assigned > ID Number > Review > Revisions > Upload file Author Guidelines: https://tis.wu.ac.th/index.php/tis/about/submissions Manuscript URL: https://tis.wu.ac.th/index.php/tis/authorDashboard/submission/7762 Reviewer A: Recommendation: Revisions Required Originality of the work Adequate Experimental design and methodology Adequate Adequacy of the discussion Adequate Technical accuracy Adequate https://mail.google.com/mail/u/3/?ik=35ace941da&view=pt&search=all&permthid=thread-f:1785115973736907738&simpl=msg-f:1785115973736907738 1/5		

Suitability of references

Good

Use of Tables and Figures

Adequate

Standard of English

Adequate

Clarity and conciseness

Adequate

Referee's Comments

See attachment file.

Reviewer C:
Recommendation: Accept Submission

Originality of the work

Good

Experimental design and methodology

Good

Adequacy of the discussion

Good

Technical accuracy

Adequate

Suitability of references

Good

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Use of Tables and Figures

Adequate

Standard of English

Good

Clarity and conciseness

Good

Referee's Comments

this study demonstrates the presence of microplastic particles in five commercially important fish in a tropical estuary of Kalimantan Island in Indonesia. The average abundance per fish individual were higher than those documented for commercial fish in previous marine estuary studies. Minor corrections are suggested. It is suitable for publication.

Reviewer D:

Recommendation: Resubmit for Review

Originality of the work

Good

Experimental design and methodology

Adequate

Adequacy of the discussion

Poor

Technical accuracy

Poor

Suitability of references

Good

Use of Tables and Figures

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Universitas Mulawarman Mail - [TIS] Manuscript Decision

Poor

Standard of English

Poor

Clarity and conciseness

Adequate

Referee's Comments

A major revision is needed:

1. Language : need proofreading; a lot of grammatical errors; the use of subjective pronouns
2. Methodology : missing some essential part : method to extract microplastics from fish's organ, no method of statistical analysis
3. Results: data of microplastic concentration from each type of fish can be more presentable in a graph; the graph presentation is not complete; there is no fair comparison (since the type of demersal fish is far more than pelagic fish); it will be beneficial if size of microplastic is presented and discussed, supporting the discussion regarding how the ingestion of microplastic by fish occurred.
4. Data of microplastic in the nearby river, the estuary water and the seawater might be added to have a clearer explanation

Reviewer I:

Recommendation: Revisions Required

Originality of the work

Good

Experimental design and methodology

Good

Adequacy of the discussion

Adequate

Technical accuracy

Adequate

Suitability of references

Adequate

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Use of Tables and Figures

Adequate

Standard of English

Adequate

Clarity and conciseness

Adequate

Referee's Comments

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6/14/26, 11:33 AM Hamdhani Hamdhani, Occurrence of Microplastic Ingestion by Commercial Fish Species from the Pangempang Estuary in Indonesia

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3	Reviewer Comments Round 1	13 Desember 2023	a. 21235- Reviewer 1 b. 21308- Reviewer 2 c. 21464- Reviewer 3 d. 21591- Reviewer 4
a.	21235- Reviewer 1	TRENDS IN SCIENCES 2023; xx(xx): xxxxxx https://doi.org/ RESEARCH ARTICLE Occurrence of Microplastic Ingestion by Commercial Fish Species from the Pangempang Estuary in Indonesia Highlights - This study demonstrates the presence of microplastic particles in five commercially important fish in a tropical estuary of Kalimantan Island in Indonesia. - Fiber was the most dominant type of microplastic. - Demersal fish may have ingested a higher quantity of microplastic particles compared to pelagic species. Graphical abstract 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 (<i>Carangus</i> SP.), blackspot snapper (<i>Lutjanus fulvus</i>), coral grouper (<i>Epinephelus corallicola</i>), golden snapper (<i>Lutjanus xanthurus</i>), and pickhandle barracuda (<i>Syngnathus</i>) 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. Using a dissecting stereo microscope, microplastic particles in the processed digestive organ contents were identified and enumerated. Microplastics were found in 96% of fish digestive organs ($n = 50$). In fish samples of jack, blackspot snapper, coral grouper, golden snapper, and pickhandle barracuda ($n = 10$ for each species), we found in average of 41 ± 6.0 SE, 26 ± 11.0 SE, 15 ± 2.0 SE, 11 ± 1.0 SE, 14 ± 2.0 SE microplastic particles per individual, respectively, and we found in average of 499.5 ± 94.4 SE, 501.5 ± 198.8 SE, 83.6 ± 18.4 SE, 79.4 ± 11.6 SE, 40.2 ± 25.0 SE microplastic particles per kg fish weight, respectively. Fiber was the most dominant type of microplastic. Microplastic beads were not detected. The relationship of the average consumed microplastic and the average fish weight per species show a negative relationship ($R^2 = 0.36$), but insignificant ($P\text{-value} = 0.29$). Our finding suggests that demersal fish like jack, blackspot snapper, and coral grouper may have ingested a higher quantity of microplastic particles compared to pelagic species like pickhandle barracuda. Keywords: Pollution, Ingestion, Commercial fish, Borneo, Indonesia	Trends Sci. 2023; xx(xx): xxxxxx 2 of 11 Introduction After invented over 110 years ago, plastic materials become ubiquitous in the environment. Plastic material is made from polymers that can be shaped easily for many varieties of uses. The elevated desire to use plastic materials due to their affordability, bio-inertia, high strength to weight ratio, and resistance to degradation. These characteristics has made plastic materials become important materials for human life nowadays. By the year of 2017, the total global production of plastic was estimated to reach \$300 million metric tons [1], with the rate of production has reached 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 suspected responsible to bring 88%-93% of global marine debris load into ocean [4]. The proportion of plastics usually take about 70% of total marine debris [5]. Initially regarded as a safe material, several decades of plastic production have brought a wide range of environmental problems, particularly in marine ecosystems. In oceans, even though plastic is considered as durable material and resistant to natural biodegradation. However, 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) [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 pollutants entering the food chain [8]. 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 from the surrounding [11]. Cole et al. reported that ingestion of microplastic particles by zooplanktons shows negative impacts on feeding, hatching, and their health [12]. 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 a type of marine ecosystems often utilized for fisheries purposes. [16,17], and these waters 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 estuary is problematic as estuaries also provide crucial habitat for a variety of commercially and recreationally important species such as crabs, fish, and shellfish [20]. Numerous studies investigating microplastics have placed their emphasis on ecosystems in both marine and inland environments [21,22,23]. However, there have been limited evaluation on microplastic contamination in transitional waters between the two ecosystems, such as estuarine waters, allowing analysis of microplastic concentrations ingested by some commercial fish. To address this knowledge gap, we evaluated the type and abundance of microplastic particles in available commercial fish samples collected from Pangempang Estuary and asked the main following questions: what type of microplastic? And how is the abundance of microplastic in fish collected from this transitional waters? Materials and methods Site description and Sampling The Pangempang Estuary (0°12'52.52"S, 117°23'30.65"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. The Pangempang River spans approximately 26 kilometers, originating from the Marangkayu Trends Sci. 2023; xx(xx): xxxxxx 3 of 11 reservoir. Its width ranges from 8 meters upstream to 140 meters downstream. The river passes through the Marangkayu settlement, situated roughly 20 kilometers upstream from the Pangempang Estuary. Additionally, about 7 kilometers 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. Figure 1 The Pangempang Estuary (East Kalimantan, Indonesia) To evaluate the ingestion of microplastics by fish, we obtained fish specimens from local fishermen operating in the waters of the Pangempang Estuary. We were able to collect ten replicates of each five different species from these fishermen. The species we identified were jack (<i>Carangus</i> SP.), blackspot snapper (<i>Lutjanus fulvus</i>), coral grouper (<i>Epinephelus corallicola</i>), golden snapper (<i>Lutjanus xanthurus</i>), and pickhandle barracuda (<i>Syngnathus</i>). We conducted the identification of these fish species and their habitats based on the information provided in the book titled "Marine Fishes and Crustaceans of the Southeast Asian Region" [24]. Each individual fish was placed in plastic bags, 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. Several times used "we", better to avoid much



Figure 2 Photographs illustrating the observed fish species: (A) Jack (*Caranx* SP.), (B) blackspot snapper (*Lutjanus chrysopus*), (C) coral grouper (*Epinephelus corallicola*), (D) golden snapper (*Lutjanus johni*), and (E) pickhandle barracuda (*Sphyrna tiburo*).

Laboratory processing

We measured fish weight (g) of each specimen. 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, each stomach content was digested using alkaline digestion solution of potassium hydroxide (KOH) [25,26]. Each stomach content was placed in a beaker and 10% KOH was added allowing submersion of stomach contents in the beaker. Each beaker was covered with aluminum foil. We stirred the samples in the beaker using magnetic stirrer at 350 rpm for 2 hours. 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% H₂O₂ was added and then stirred. After 1 hour stirring, samples were vacuum pump filtered on 1.6 µm pore size Whatman paper and then dried.

Using a 10–45× magnification dissecting microscope, microplastic particles in the processed stomach contents were identified and enumerated. The microplastic particles were observed and categorized into four major types (fiber, fragment, film, and bead; [27]) following protocols from Hidalgo-Bus et al. [28] and Eppelbaum et al. [29]. 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 used. All these processes were conducted in the laboratory behind closed doors and windows.

Results

We observed microplastic shapes of fibers, film, fragments. Microplastic beads were not detected in any of our samples. In jacks, coral grouper, golden snapper, and pickhandle barracuda, we detected microplastic particles in 100% of samples, except for blackspot snapper only 80% of samples.

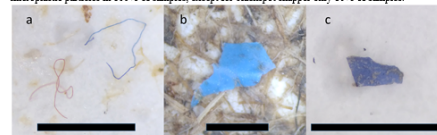


Figure 3 Photographs illustrating the observed microplastic types found in digestive organs from sample fishes collected in the Pangasinan Estuary: a. fiber, b. film, and c. fragment. Horizontal scale bar ≈ 0.5 mm

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

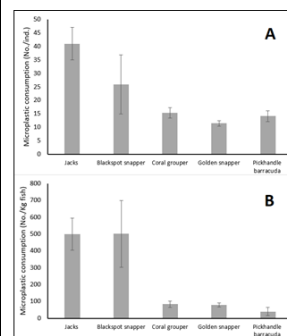


Figure 4 Average of microplastic particles per individual (A) and per kilogram fish weight (B)

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.

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%, 50% of all samples respectively.

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%, 40% of our samples respectively.

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.

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.

Among the five 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. The relationship of the average consumed microplastic particles and the average fish weight per species (Figure 5) shows negative relationship ($R^2 = 0.36$), but the relationship between the two is not significant ($P\text{-value} = 0.28$).

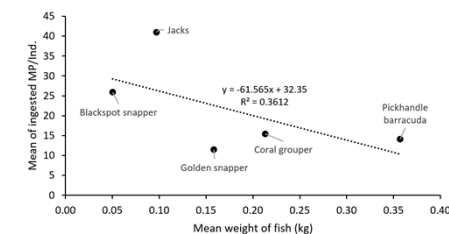


Figure 5 Relationship of mean weight of observed fish and mean number of ingested microplastic particles per individual

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 (fish, shrimps, crabs and cephalopods) generally smaller in size than their own bodies.

Satish Singampalli
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Trends Sci. 2024; xx(x): xxxxxx

7 of 11

Table 1 Taxonomy, common name, foraging ecology

No.	Taxonomy and Common name	Foraging ecology
1.	Family: <i>Carangidae</i> Genus: <i>Caranx</i> Common names: Jacks or trevallies	Most species are demersal , or bottom dwelling, while others are pelagic , moving long distances in the upper water column [50]. All species are predatory fish, taking smaller fish, cephalopods as prey [31].
2.	Family: <i>Lutjanidae</i> Species: <i>Lutjanus fulvus</i> (Forsk., 1775) Common names: Blackspot snapper	Many species under this family are demersal and associated with coral reefs [32]. Some of the larger red snapper species descend into deeper waters, at least to 200 m in depth [33]. <i>Lutjanus</i> is predatory fish usually found in tropical and subtropical reefs, and mangrove forests.
3.	Family: <i>Serranidae</i> <i>Epinephelus microdon</i> (Kuhle, Hasselt, 1828) 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 habitat during its juvenile phase [54]. 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 [55].
4.	Family: <i>Lutjanidae</i> <i>Lutjanus fulvus</i> (Bloch, 1792) Common names: Golden snapper, or John's snapper	<i>Lutjanus</i> exhibits a two-part life cycle: an initial stage with pelagic eggs and larvae , succeeded by a later stage with juveniles and adults that are oriented towards the seafloor (demersal) [36]. Feed on fishes and benthic invertebrates including shrimps, crabs and cephalopods. Adults probably frequent coral reef areas; juveniles found in mangrove estuaries [37].
5.	Family: <i>Sphyraenidae</i> <i>Sphyraenidae</i> Cuvier, 1829 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 [38]. Prey on fish, cephalopods, and shrimps [39].

Discussion

Types of microplastic

Our findings confirmed that fish in **Pangkepang** Estuary do consume microplastics and fibers were the most dominant type of microplastic. In the aquatic environment, ample studies reported that fibers are the most encountered microplastic type [40-43]. Sources of fibers are predominantly textile goods, such as clothes [44]. Degradation of synthetic fabrics was predicted as major contributor for microplastic fiber abundance in our environment [44-45]. 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 1900 fibers at size less than 1mm in length per wash cycle [46]. 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 discharged into surface waters [46]. In our study, the urban areas along the river that flows to **Pangkepang** 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 **Pangkepang** Estuary waters. A microplastic study on commercially important marine fish species in Fiji documented that film microplastic portion was half of the total discovered microplastic particles, and the remaining shared between fiber and fragment [47]. We observed fragment particles was the lowest in number and only found in three of five fish species we examined. In contrast, Kijboon et al. documented that among commercial marine fish in the adjacent waters of Malaysia, fragment-type microplastics were the most prevalent [48]. Source of fragment microplastic mainly come from degraded larger plastic debris due to continuous weathering

Trends Sci. 2024; xx(x): xxxxxx

8 of 11

includes photo, biological, physical and chemical processes in the terrestrial and aquatic environment [49].

Recent investigations into the impact of microplastic fibers on aquatic organisms have unveiled various harmful consequences. According to Liang et al., goldfish exposed to a concentration of 1000 items per liter 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 [50]. Similarly, a study by Zhao et al. 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 [51]. Another study by Xie et al. noted oxidative stress, *dyshome*, in the intestine microbiome, and histological damage as significant symptoms in barramundi exposed to microplastic fibers [52].

Abundance of microplastic in digestive organs

Our finding revealed that jack as individual consumed the highest average of microplastic particles, 41 (SE: 5.98) while golden snapper was observed consuming 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. documented microplastic particle abundance ranged from 9-14 per gastrointestinal tract in multiple fish species caught the Bahía Blanca Estuary in Argentina [53], up to 4 particles in semi pelagic hoploids in the Mediterranean Sea [41], and ranged 1.1- 7.2 microplastic particles in fish collected from China marine waters [40]. On the other hand, the blackspot snapper has the highest amount of microplastics per kilogram of fish, 501.5 ± 198.8 SE, while the **pickhandle** barracuda has the smallest amount, only 40.2 ± 25.0 SE. The average individual fish weight was the key determinant of fish 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 two is not significant ($P\text{-value} = 0.28$). Similarly, Chen et al. reported that fish size did not affect microplastic abundance in the fish digestive organs [54].

Foraging ecology

Microplastic ingestion is potentially influenced by two crucial factors: the type of habitat and the feeding habits of organisms [55]. Within marine environments, scientists have noted markedly greater quantities of microplastics in demersal fish species compared to pelagic species [40,42]. This trend could be linked to the prevalence of plastic waste near the seabed, which is often regarded as the final accumulation for plastics within the marine ecosystem.

Microplastics could potentially be present in fish across different trophic levels. However, current data are insufficient to make definitive conclusions about the absorption of microplastics [56]. In fact, distinguishing between directly and indirectly ingested microplastics is challenging [57]. Walkinshaw et al. performed a semi-systematic review on research examining the presence of microplastics in commercially significant organisms across various trophic levels [58]. 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 are adult predator fish, four fish species are demersal, and one is pelagic (Table 1). The microplastic abundances in the three 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 anticipated 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 [59]. 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 two herbivorous fish species consistently ingest significant quantities of microplastic particles as much as 10-99 microplastic particles per fish gut [60].

Conclusions

For the first time, this study demonstrates the presence of microplastic particles in five commercially important fish in a tropical estuary of Kalimantan Island in Indonesia. The average abundance per fish individual were higher than those documented for commercial fish in previous marine

Trends Sci. 2024; xx(x): xxxxxx

9 of 11

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 a significant portion of the microplastic pollution found in the digestive systems of sampled fish from the **Pangkepang** Estuary. Our research suggests that demersal fish may have ingested a higher quantity of microplastic particles compared to pelagic species.

This study result also confirms the urgency to observe wide range of fish species in term of their habitat within the water column and as well as the feeding habit. 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. Perhaps analysis based on muscles and internal organs should also be considered in the near future.

Acknowledgements

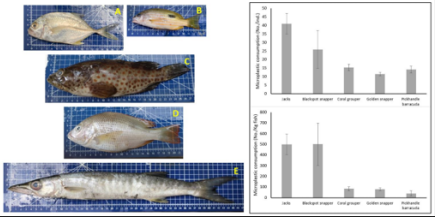
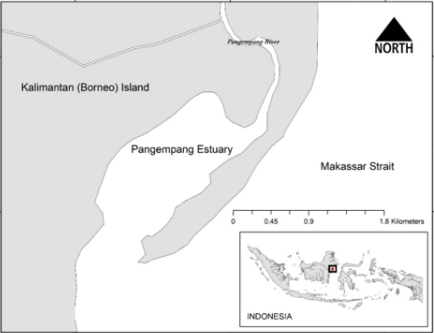
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	TRENDS IN SCIENCES 202x; xx(xx): xxxxxx https://doi.org/ RESEARCH ARTICLE Occurrence of Microplastic Ingestion by Commercial Fish Species from the Pangempang Estuary in Indonesia Highlights - This study demonstrates the presence of microplastic particles in five commercially important fish in a tropical estuary of Kalimantan Island in Indonesia. - Fiber was the most dominant type of microplastic. - Demersal fish may have ingested a higher quantity of microplastic particles compared to pelagic species. Graphical abstract  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 (<i>Caesox SF.</i>), blackspot snapper (<i>Lutjanus fulvus</i>), coral grouper (<i>Epinephelus corallicola</i>), golden snapper (<i>Lutjanus johnii</i>), and pickhandle barracuda (<i>Sphyrna jello</i>) 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. Using a dissecting stereo microscope, microplastic particles in the processed digestive organ contents were identified and enumerated. Microplastics were found in 96% of fish digestive organs ($n = 50$). In fish samples of jacks, blackspot snapper, coral grouper, golden snapper, and pickhandle barracuda ($n = 10$ for each species), we found in average of 41 ± 6.0 SE, 26 ± 11.0 SE, 15 ± 2.0 SE, 11 ± 1.0 SE, 14 ± 2.0 SE microplastic particles per individual, respectively, and we found in average of 499.5 ± 94.4 SE, 501.5 ± 198.8 SE, 83.6 ± 13.4 SE, 79.8 ± 11.6 SE, 40.2 ± 25.0 SE microplastic particles per kg fish weight, respectively. Fiber was the most dominant type of microplastic. Microplastic that beads were not detected. The relationship of the average consumed microplastic and the average fish weight per species show a negative relationship ($R^2 = 0.36$), but insignificant ($P\text{-value} = 0.20$). 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: Pollution, Ingestion, Commercial fish, Borneo, Indonesia	Trends Sci. 202x; xx(xx): xxxxxx 2 of 11 Introduction After invented over 110 years ago, plastic materials become ubiquitous in the environment. Plastic material is made from polymer that can be shaped easily for many varieties of uses. The elevated desire to use plastic materials due to their affordability, bio-inertia, high strength to weight ratio, and resistance to degradation. These characteristics has made plastic materials become important materials for human life nowadays. By the year of 2017, the total global production of plastic was estimated to reach 8300 million metric tons [1], with the rate of production has reached over 500 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 suspected responsible to bring 88%-95% of global marine debris load into ocean [4]. The proportion of plastics usually take about 70% of total marine debris [5]. Initially regarded as a safe material, several decades of plastic production have brought a wide range of environmental problems, particularly in marine ecosystems. In oceans, even though plastic is considered as durable material and resistant to natural biodegradation. However, 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) [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 from the surrounding [11]. Cole et al., reported that ingestion of microplastic particles by zooplankton shows negative impacts on feeding, hatching, and their health [12]. 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 a type of marine ecosystems often utilized for fisheries purposes. [16,17], and these waters are a socio-cultural 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 estuary is problematic as estuaries also provide crucial habitats for a variety of commercially and recreationally important species such as crabs, fish, and shellfish [20]. Numerous studies investigating microplastics have placed their emphasis on ecosystems in both marine and inland environments [11,22,23]. However, there have been limited evaluation on microplastic contamination in transitional waters between the two 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 and asked the main following questions: what type of microplastic? And how is the abundance of microplastic in fish collected from this transitional waters? Materials and methods Site description and Sampling The Pangempang Estuary (0°12'52.52"S, 117°2'30.65"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. The Pangempang River spans approximately 26 kilometers, originating from the Marangkapu reservoir. Its width ranges from 8 meters upstream to 140 meters downstream. The river passes through	Trends Sci. 202x; xx(xx): xxxxxx 3 of 11 Yayan We don't see any commercial fish species in your introduction If commercial fish is contaminated with microplastics, why many people eat it? And we should replace with other food products that are not contaminated. the Marangkapu Town settlement, situated roughly 20 kilometers upstream from the Pangempang Estuary. Additionally, about 7 kilometers 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.  Yayan In what month and year did you take the sample? Season is a factor that influence on fish in the sea biomass, in what season do you take the sample? Why not the total length measured? Figure 1 The Pangempang Estuary (East Kalimantan, Indonesia) To evaluate the ingestion of microplastics by fish, we obtained fish specimens from local fishermen operating in the waters of the Pangempang Estuary. We were able to collect ten replicates of each five different species from these fishermen. The species we identified were jack (<i>Caesox SF.</i>), blackspot snapper (<i>Lutjanus fulvus</i>), coral grouper (<i>Epinephelus corallicola</i>), golden snapper (<i>Lutjanus johnii</i>), and pickhandle barracuda (<i>Sphyrna jello</i>). We conducted the identification of these fish species and their habitats based on the information provided in the book titled "Marine Fishes and Crustaceans of the Southeast Asian Region" [24]. Each individual fish was placed in plastic bags, 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.

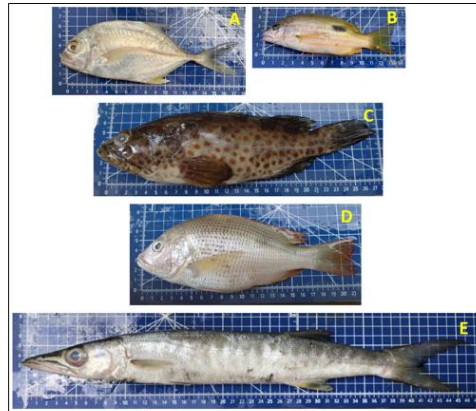


Figure 2 Photographs illustrating the observed fish species: (A) Jack (*Canary SP.*), (B) blackspot snapper (*Lutjanus fulvus*), (C) coral grouper (*Epinephelus corallicola*), (D) golden snapper (*Lutjanus johni*), and (E) pickhandle barracuda (*Sphyrna tiburo*).

Laboratory processing

We measured fish weight (g) of each specimen. 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, each stomach content was digested using alkaline digestion solution of potassium hydroxide (KOH) [25,26]. Each stomach content was placed in a beaker and 10% KOH was added allowing submersion of stomach contents in the beaker. Each beaker was covered with aluminum foil. We stirred the samples in the beaker using magnetic spinner at 350 rpm for 2 hours. 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% H₂O₂ was added and then stirred. After 1 hour stirring, samples were vacuum pump filtered on 1.6 µm pore size Whatman paper and then dried.

Using a 10-45x magnification dissecting microscope, microplastic particles in the processed stomach contents were identified and enumerated. The microplastic particles were observed and categorized into four major types (fiber, fragment, film, and bead; [27]) following protocols from Hidalgo-Ruz, et al., [28] and Eppehimer, et al. [29]. 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. All these processes were conducted in the laboratory behind closed doors and windows.

How do you calculate the microplastics in the sample? And what's the unit it? Describe please the equations based on percent, number/individual, weight and correlation.

Results

We observed microplastic shapes of fibers, film, fragments. Microplastic-beads were not detected in any of our samples. In jacks, coral grouper, golden snapper, and pickhandle barracuda, we detected microplastic particles in 100% of samples, except for blackspot snapper only 80% of samples.

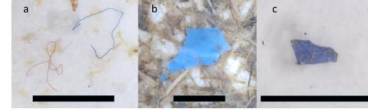
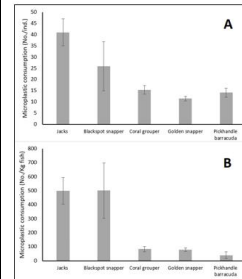


Figure 3 Photographs illustrating the observed microplastic types found in digestive organs from sample fishes collected in the Paagoupang Estuary: a. fiber, b. film, and c. fragment. Horizontal scale bar = 0.3 mm.

In fish samples of jacks, blackspot snapper, coral grouper, golden snapper, and pickhandle barracuda, we found in average of 41 ± 6.0 SE, 26 ± 11.0 SE, 15 ± 2.0 SE, 11 ± 1.0 SE, 14 ± 2.0 SE microplastic particles per individual, respectively, and we found in average of 499.2 ± 94.4 SE, 501.5 ± 198.8 SE, 83.6 ± 10.4 SE, 79.8 ± 11.6 SE, 40.2 ± 25.0 SE microplastic particles per kg fish weight, respectively. The blackspot snapper had the highest number of microplastic particles per kilogram of fish weight, reaching 501.5 ± 198.8 SE microplastic particles. The lowest number of particles per kilogram of fish was found in the pickhandle barracuda, which amounted to 40.2 ± 25.0 SE microplastic particles.



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Figure 4 Average of microplastic particles per individual (A) and per kilogram fish weight (B) 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 80% of our jack fish samples.

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%, 50% of all samples respectively.

The coral grouper samples showed that fiber particles were the most abundant with 80.3%, as compared to percentages found for film (16.3%) and fragments (3.2%). In this species, fibers, film, and fragments were detected in 100%, 80%, 40% of our samples respectively.

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. 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.

Among the five 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. The relationship of average consumed microplastic particles and the average fish weight per species (Figure 3) show a negative relationship ($R^2 = 0.36$), but the relationship between the two is not significant (P -value = 0.28).

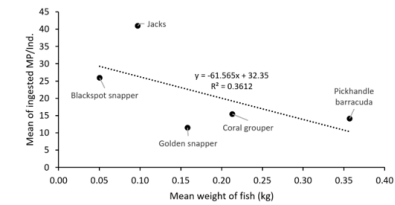


Figure 5 Relationship of mean weight of observed fish and mean number of ingested microplastic particles per individual

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 (fish, shrimps, crabs and cephalopods) generally smaller in size than their own bodies.

No.	Taxonomy and Common name	Foraging ecology
1.	Family: <i>Canapidae</i> Genus: <i>Canax</i> Common names: Jacks or	Most species are demersal, or bottom dwelling, while others are pelagic, moving long distances in the upper water column [10]. All species are predatory fish, taking smaller

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Trends Sci. 202x; xx(xx): xxxxxx

7 of 11

	trevalies	fish, cephalopods as prey [31]
2.	Family: Lutjanidae Species: <i>Lutjanus fulviflamma</i> (Forsskal, 1775) Common names: Blackspot snapper	Many species under this family are demersal and associated with coral reefs [32]. Some of the larger red snapper species descend into deeper waters, at least to 200 m in depth [33]. <i>Lutjanus</i> is predatory fish usually found in tropical and subtropical reefs, and mangrove forests.
3.	Family: Serranidae Species: <i>Epinephelus corallivola</i> (Kuhl & Hasselt, 1828) 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 [34]. 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 [35].
4.	Family: Lutjanidae Species: <i>Lutjanus johni</i> (Bloch, 1792) Common names: Golden snapper, or John's snapper	<i>Lutjanus</i> exhibits a two-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) [36]. Feed on fishes and benthic invertebrates including shrimps, crabs and cephalopods. Adults probably frequent coral reef areas; juveniles found in mangrove estuaries [37].
5.	Family: Sparidae Species: <i>Sparus luteus</i> Cuvier, 1829 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 [38]. Prey on fish, cephalopods, and shrimps [39].

Discussion

Types of microplastic

Our findings confirmed that fish in Pangempang Estuary do consume microplastics and fibers were the most dominant type of microplastic. In the aquatic environment, ample studies reported that fish are the most encountered microplastic type [40-43]. Sources of fibers are predominantly textile goods such as clothes [44]. Degradation of synthetic fabrics was predicted as major contributor for microplastic fiber abundance in our environment [44,45]. 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 1900 fibers at size less than 1mm in length per wash cycle [46]. After being washed, synthetic fibers from laundry waste find their way into the municipal water systems. While a portion of these fibers is eliminated during wastewater treatment, the remaining is discharged into surface waters [46]. 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 is predominantly from discarded 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 film microplastic portion was half of the total discovered microplastic particles, and the remaining shared between fiber and fragment [47]. We observed fragment particles was the lowest in number and only found in three of five fish species we examined. In contrast, Khabibaei et al. documented that among commercial marine fish in the adjacent waters of Malaysia, fragment-type microplastics were the most prevalent [48]. 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 [49].

Recent investigations into the impact of microplastic fibers on aquatic organisms have unveiled various harmful consequences. According to Liang et al., goldfish exposed to a concentration of 1000 items per liter 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 [50]. Similarly, a study by Zhao et al. demonstrated that following exposure, microplastic fibers were found in the digestive organs of zebrafish, even in their early life stages,

Trends Sci. 202x; xx(xx): xxxxxx

8 of 11

resulting in intestinal damage and toxic effects indicated by observable histopathological changes and biomarker responses [51]. Another study by Xie et al. noted oxidative stress, dysbiosis in the intestine microbiome, and histological damage as significant symptoms in barramundi exposed to microplastic fibers [52].

Abundance of microplastic in digestive organs

Our finding revealed that jack as individual consumed the highest average of microplastic particles, 41 (SE: 5.98) while golden snapper was observed consuming 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. documented microplastic particle abundance ranged from 9-14 per gastrointestinal tract in multiple fish species caught the Bahía Blanca Estuary in Argentina [53], up to 4 particles in semi pelagic hoop hoppers in the Mediterranean Sea [41], and ranged 1.1- 7.2 microplastic particles in fish collected from China marine waters [40]. On the other hand, the blackspot snapper has the highest amount of microplastics per kilogram of fish, 501.5 ± 198.8 SE, while the pickhandle barracuda has the smallest amount, only 40.2 ± 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 two is not significant (P -value = 0.26). Similarly, Chen et al. reported that fish size did not affect microplastic abundance in the fish digestive organs [54].

Foraging ecology

Microplastic ingestion is potentially influenced by two crucial factors: the type of habitat and the feeding habits of organisms [55]. Within marine environments, scientists have noted markedly greater quantities of microplastics in demersal fish species compared to pelagic species [40,42]. This trend could be linked to the prevalence of plastic waste near the seabed, which is often regarded as the final accumulation for plastics within the marine ecosystem.

Microplastics could potentially be present in fish across different trophic levels. However, current data are insufficient to make definitive conclusions about the absorption of microplastics [56]. In fact, distinguishing between directly and indirectly ingested microplastics is challenging [57]. Walkinshaw et al. performed a semi-systematic review on research examining the presence of microplastics in commercially significant organisms across various trophic levels [58]. The findings indicate that microplastics do not exhibit bioaccumulation, 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 are adult predator fish, four fish species are demersal, and one is pelagic (Table 1). The microplastic abundances in the three 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 anticipated 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 [59]. 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 two herbivorous fish species consistently ingest significant quantities of microplastic particles as much as 10-99 microplastic particles per fish gut [60].

Conclusions

For the first time, this study demonstrates the presence of microplastic particles in five commercially important fish in a tropical estuary of Kalimantan Island in Indonesia. The average abundance per fish individual were higher than those documented 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 a significant 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.

This study result also confirms the urgency to observe wide range of fish species in term of their habitat within the water column and as well as the feeding habit. Given the prevalence and high abundance of these microplastic fibers, we recommend to include them in assessments of the risks

Trends Sci. 202x; xx(xx): xxxxxx

9 of 11

associated with microplastic pollution, particularly regarding their potential toxicity as these species are considered as important commercial fish in this region. Perhaps analysis based on muscles and internal organs should also be considered in the near future.

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	TRENDS IN SCIENCE 2024; XXXX XXXX RESEARCH ARTICLE https://doi.org/ Occurrence of Microplastic Ingestion by Commercial Fish Species from the Pangempang Estuary in Indonesia Highlights - This study demonstrates the presence of microplastic particles in five commercially important fish in a tropical estuary of Kalimantan Island in Indonesia. - Fiber was the most dominant type of microplastic. - Demersal fish may have ingested a higher quantity of microplastic particles compared to pelagic species. Graphical abstract Abstract The aim of this study was to assess the occurrence of microplastic in digestive organs (neophages to intestines) of popular marine commercial fish species: jack (<i>Caranx SP.</i>), blackspot snapper (<i>Lutjanus fulvus</i>), coral grouper (<i>Epinephelus corallificus</i>), golden snapper (<i>Lutjanus johnii</i>), and pickhandle barracuda (<i>Sphyrna zello</i>) 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. Using a dissecting stereo-microscope, microplastic particles in the processed digestive organs contents were identified and enumerated. Microplastic particles in the processed digestive organs contents were identified and enumerated using a dissecting stereo-microscope. Microplastics were found in 96% of fish digestive organs ($n = 50$). In fish samples of jacks, blackspot snapper, coral grouper, golden snapper, and pickhandle barracuda ($n = 10$ for each species), we found an average of 41 ± 6.0 SE, 26 ± 11.0 SE, 17 ± 1.0 SE, 11 ± 1.0 SE, 14 ± 2.0 SE microplastic particles per individual, respectively, and we found an average of 499.5 ± 94.4 SE, 501.5 ± 198.8 SE, 83.6 ± 18.4 SE, 79.8 ± 11.6 SE, 40.2 ± 22.0 SE microplastic particles per kg fish weight, respectively. Fiber was the most dominant type of microplastic. Microplastic beads were not detected. The relationship of the average consumed microplastic and the average fish weight per species shows a negative relationship ($R^2 = 0.40$), but insignificant (P-value = 0.20). 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: Pollution, Ingestion, Commercial fish, Borneo, Indonesia	Trends Sci. 2024; xx(xx): XXXX 2 of 11 Introduction After invented over 110 years ago, plastic materials become ubiquitous in the environment. Plastic material is made from polymers that can be shaped easily for many varieties of uses. The elevated desire to use plastic materials due to their affordability, bio-inertia, high strength to weight ratio, and resistance to degradation. These characteristics have made plastic materials become important materials for human life nowadays. By the year of 2017, the total global production of plastic was estimated to reach \$300 million metric tons [1], with the rate of production has reached 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 suspected responsible to bring 88%-95% of global marine debris load into ocean [4]. The proportion of plastics usually take about 70% of total marine debris [5]. Initially regarded as a safe material, several decades of plastic production have brought a wide range of environmental problems, particularly in marine ecosystems. In oceans, even though plastic is considered as durable material and resistant to natural biodegradation. However, 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) [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 from the surrounding [11]. Cole et al. reported that ingestion of microplastic particles by zooplanktons shows negative impacts on feeding, hatching, and their health [12]. 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 a type of marine ecosystems often utilized for fisheries purposes. [16,17], and these waters are a socio-cultural 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 estuary is problematic as estuaries also provide crucial habitat for a variety of commercially and recreationally important species such as crabs, fish, and shellfish [20]. Numerous studies investigating microplastics have placed their emphasis on ecosystems in both marine and inland environments [21,22,23]. However, there have been limited evaluation on microplastic contamination in transitional waters between the two ecosystems, such as estuarine waters, allowing analysis of microplastic concentrations ingested by some commercial fish. To address this knowledge gap, we evaluated evaluation of the types and abundance of microplastic particles in available commercial fish samples collected from Pangempang Estuary. was important, and asked the main following questions i.e., what is type of microplastic? And how is the abundance of microplastic in fish collected from this transitional waters? Materials and methods Site description and Sampling 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. The Pangempang River spans approximately 26 kilometers, originating from the Marangkayu reservoir. Its	Trends Sci. 2024; xx(xx): XXXX 3 of 11 width ranges from 8 meters upstream to 140 meters downstream. The river passes through the Marangkayu Town settlement, situated roughly 20 kilometers upstream from the Pangempang Estuary. Additionally, about 7 kilometers 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. Figure 1 The Pangempang Estuary (East Kalimantan, Indonesia) To evaluate the ingestion of microplastics by fish, we obtained fish specimens from local fishermen operating in the waters of the Pangempang Estuary. We were able to collect ten replicates of each five different species from these fishermen. The species we identified were jack (<i>Caranx SP.</i>), blackspot snapper (<i>Lutjanus fulvus</i>), coral grouper (<i>Epinephelus corallificus</i>), golden snapper (<i>Lutjanus johnii</i>), and pickhandle barracuda (<i>Sphyrna zello</i>). We conducted the identification of these fish species and their habitats based on the information provided in the book titled "Marine Fishes and Crustaceans of the Southeast Asian Region" [24]. Each individual fish was placed in plastic bags, and then stored in a freezer during transport to the Water Quality Laboratory at Mulawarman University. The samples were preserved in a freezer before processing and analysis.

Table 1 Taxonomy, common name, foraging ecology

No.	Taxonomy and Common name	Foraging ecology
1.	Family: Carangidae Genus: <i>Caranx</i> Common names: Jacks or trevallies	Most species are demersal , or bottom dwelling, while others are pelagic , moving long distances in the upper water column [30]. All species are predatory fish, taking smaller fish, cephalopods as prey [31].
2.	Family: Lutjanidae Species: <i>Lutjanus fulviflamma</i> (Forsk., 1775) Common names: Blackspot snapper	Many species under this family are demersal and associated with coral reefs [32]. Some of the larger red snapper species descend into deeper waters, at least to 200 m in depth [33]. <i>Lutjanus</i> is predatory fish usually found in tropical and subtropical reefs, and mangrove forests.
3.	Family: Serranidae <i>Epinephelus caralligala</i> (Kuhl & Hasselt, 1828) 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 [34]. 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 [35].
4.	Family: Lutjanidae <i>Lutjanus johni</i> (Bloch, 1792) Common names: Golden snapper, or John's snapper	<i>Lutjanus</i> exhibits a two-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) [36]. Feed on fishes and benthic invertebrates including shrimps, crabs and cephalopods. Adults probably frequent coral reef areas; juveniles found in mangrove estuaries [37].
5.	Family: Sphyraenidae <i>Sphyraena jello</i> Cuvier, 1829 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 [38]. Prey on fish, cephalopods, and shrimps [39].

Discussion

Types of microplastic

Our findings confirmed that fish in Pangempang Estuary do consume microplastics and fibers were the most dominant type of microplastic. In the aquatic environment, ample studies reported that fibers are the most encountered microplastic type [40-43]. Sources of fibers are predominantly textile goods, such as clothes [44]. Degradation of synthetic fabrics was predicted as major contributor for microplastic fiber abundance in our environment [44,45]. 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 1900 fibers at size less than 1mm in length per wash cycle [46]. 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 discharged into surface waters [46]. 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 film microplastic portion was half of the total discovered microplastic particles, and the remaining shared between fiber and fragment [47]. We observed fragment particles was the lowest in number and only found in three of five fish species we examined. In contrast, Karbalaei et al. documented that among commercial marine fish in the adjacent waters of Malaysia, fragment-type microplastics were the most prevalent [48]. 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 [49].

Recent investigations into the impact of microplastic fibers on aquatic organisms have unveiled various harmful consequences. According to Ling et al., goldfish exposed to a concentration of 1000 items per liter 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 [50]. Similarly, a study by Zhao et al. 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 [51]. Another study by Xie et al. noted oxidative stress, dysbiosis in the intestinal microbiome, and histological damage as significant symptoms in barramundi exposed to microplastic fibers [52].

Abundance of microplastic in digestive organs

Our finding revealed that jack as individual consumed the highest average of microplastic particles, 41 (SE = 5.8) while golden snapper was observed consuming the least, only 11 (SE: 0.9). These values are higher than those reported for fish in previous estuary studies, for instance Kiani et al. documented microplastic particle abundance ranged from 9-14 per gastrointestinal tract in multiple fish species caught in the Balika Banta Estuary in Argentina [53], up to 14 particles in semi pelagic loop hoops in the Mediterranean Sea [41], and ranged **10-20 microplastic particles** in fish collected from China marine waters [40]. On the other hand, the blackspot snapper has the highest amount of microplastics per kilogram of fish, 301.5 ± 191.8 SE, while the pickhandle barracuda has the smallest amount, only 40.2 ± 23.9 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 ($R^2 = 0.56$), however the relationship between the two is not significant ($P\text{-value} = 0.26$). Similarly, Chen et al. reported that fish size did not affect microplastic abundance in the fish digestive organs [54].

Foraging ecology

Microplastic ingestion is potentially influenced by two crucial factors: the type of habitat and the feeding habits of organisms [55]. Within marine environments, scientists have noted markedly greater quantities of microplastics in demersal fish species compared to pelagic species [40,42]. This trend could be linked to the prevalence of plastic waste near the seabed, which is often regarded as the final accumulation for plastics within the marine ecosystem.

Microplastics could potentially be present in fish across different trophic levels. However, current data are insufficient to make definitive conclusions about the absorption of microplastics [56]. In fact, distinguishing between directly and indirectly ingested microplastics is challenging [57]. Walikowski et al. performed a semi-systematic review on research examining the presence of microplastics in commercially significant organisms across various trophic levels [58]. The findings indicate that microplastics do not exhibit bioaccumulation, 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 are **adult predator fish**, **four fish species are demersal**, and one is **pelagic (Table 1)**. The microplastic abundances in the three 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 anticipated 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 [59]. 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 two herbivorous fish species consistently ingest significant quantities of microplastic particles as much as 10-99 microplastic particles per fish gut [60].

Conclusions

For the first time, this study demonstrates the presence of microplastic particles in five commercially important fish in a tropical estuary of Kalimantan Island in Indonesia. The average abundance per fish individual were higher than those documented 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 a significant 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.

This study result also confirms the urgency to observe wide range of fish species in term of their habitat within the water column and as well as the feeding habit. 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. Perhaps analysis based on muscles and internal organs should also be considered in the near future.

Acknowledgements

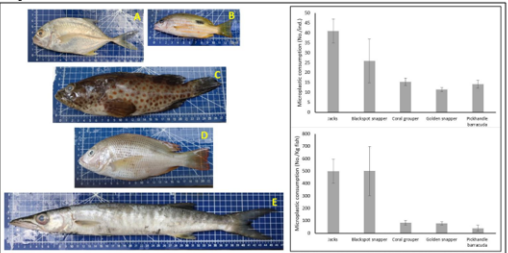
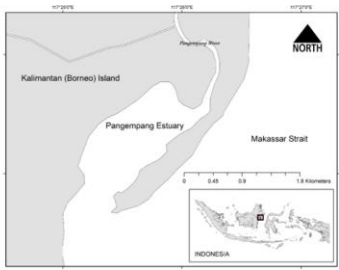
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	TRENDS IN SCIENCES 2024; xx(xx): xxxxxx https://doi.org/ Occurrence of Microplastic Ingestion by Commercial Fish Species from the Pangempang Estuary in Indonesia Highlights - This study demonstrates the presence of microplastic particles in five commercially important fish in a tropical estuary of Kalimantan Island in Indonesia. - Fiber was the most dominant type of microplastic. - Demersal fish may have ingested a higher quantity of microplastic particles compared to pelagic species. Graphical abstract  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 (<i>Caranx</i> sp.), blackspot snapper (<i>Lutjanus fulvus</i>), coral grouper (<i>Epinephelus carallaica</i>), golden snapper (<i>Lutjanus johni</i>), and pickhandle barracuda (<i>Sphyrna tiburo</i>) 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. Using a dissecting stereo microscope, microplastic particles in the processed digestive organ contents were identified and enumerated. Microplastics were found in 96% of fish digestive organs ($n = 50$). In fish samples of jacks, blackspot snapper, coral grouper, golden snapper, and pickhandle barracuda ($n = 10$ for each species), we found in average of 41 ± 6.0 SE, 36 ± 11.0 SE, 15 ± 2.0 SE, 11 ± 1.0 SE, 14 ± 2.0 SE microplastic particles per individual, respectively, and we found in average of 499.5 ± 94.4 SE, 501.5 ± 198.8 SE, 83.6 ± 18.4 SE, 79.8 ± 11.6 SE, 40.2 ± 25.0 SE microplastic particles per kg fish weight, respectively. Fiber was the most dominant type of microplastic. Microplastic beads were not detected. The relationship of the average consumed microplastic and the average fish weight per species show a negative relationship ($R^2 = 0.36$), but insignificant ($P > 0.05 = 0.26$). 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: Pollution, Ingestion, Commercial fish, Borneo, Indonesia	Trends Sci 2024; xx(xx): xxxxxx 3 of 11 Introduction After invented over 110 years ago, plastic materials become ubiquitous in the environment. Plastic material is made from polymers that can be shaped easily for many varieties of uses. The elevated desire to use plastic materials due to their affordability, low-cost, high strength to weight ratio, and resistance to degradation. These characteristics has made plastic materials become important materials for human life nowadays. By the year of 2017, the total global production of plastic was estimated to reach 350 million metric tons [1], with the rate of production has reached 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 suspected responsible to bring 85%-97% of global marine debris load into ocean [4]. The proportion of plastics usually take about 70% of total marine debris [5]. Initially regarded as a safe material, several decades of plastic production have brought a wide range of environmental problems, particularly in marine ecosystems. In oceans, even though plastic is considered as durable material and resistant to natural biodegradation. However, continuous exposure to weathering factors such as sunlight, wind, and waves, along with the process of oxidation, cause 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) [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 from the surrounding [11]. Cole et al. reported that ingestion of microplastic particles by zooplankton shows negative impacts on feeding, hatching, and their health [12]. Similarly, the ingestion of microplastics by crustaceans 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 a type of marine ecosystems often utilized for fisheries purposes [16,17], and these waters are a socio-cultural 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 island 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 estuary is problematic as estuaries also provide crucial habitat for a variety of commercially and recreationally important species such as crabs, fish, and shellfish [20]. Numerous studies investigating microplastics have placed their emphasis on ecosystems in both marine and inland environments [21,22,23]. However, there have been limited evaluation on microplastic contamination in transitional waters between the two 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 and asked the main following questions: what type of microplastic? And how is the abundance of microplastic in fish collected from this transitional waters? Materials and methods Site description and sampling The Pangempang Estuary (0°12'52.52"N, 117°22'30.65"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 kilometers, originating from the Manangkaya reservoir. Its width ranges from 5 meters upstream to 140 meters downstream. The river passes through the	Trends Sci 2024; xx(xx): xxxxxx 3 of 11 Town settlement, situated roughly 20 kilometers upstream from the Pangempang Estuary. Additionally, about 7 kilometers upstream from the estuary, there is a small fishing 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.  Figure 1 The Pangempang Estuary (East Kalimantan, Indonesia) To evaluate the ingestion of microplastics by fish, we obtained fish specimens from local fishermen operating in the waters of the Pangempang Estuary. We were able to collect ten replicates of each five different species from these fishermen. The species we identified were jack (<i>Caranx</i> sp.), blackspot snapper (<i>Lutjanus fulvus</i>), coral grouper (<i>Epinephelus carallaica</i>), golden snapper (<i>Lutjanus johni</i>), and pickhandle barracuda (<i>Sphyrna tiburo</i>). We conducted the identification of these fish species and their habitats based on the information provided in the book titled "Marine Fishes and Crustaceans of the Southeast Asian Region" [24]. Each individual fish was placed in plastic bags, 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.

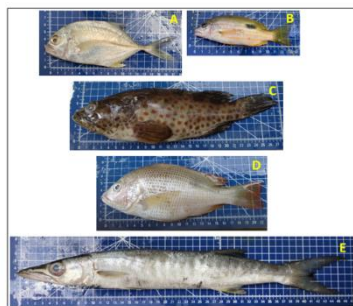


Figure 2 Photographs illustrating the observed fish species: (A) Jack (*Caranx* SP.), (B) blackspot snapper (*Lutjanus fulvus*), (C) coral grouper (*Epinephelus corallicolus*), (D) golden snapper (*Lutjanus johni*), and (E) pickhandle barracuda (*Spharxena retio*).

Laboratory processing

We measured fish weight (g) of each specimen using a digital scale. 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, each stomach content was digested using alkaline digestion solution of potassium hydroxide (KOH) [27,28]. Each stomach content was placed in a beaker and 10% KOH was added allowing solubilization of stomach contents in the beaker. Each beaker was covered with aluminum foil. We stirred the samples in the beaker using magnetic stirrer at 350 rpm for 2 hours. 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% H₂O₂ was added and then stirred. After 1 hour stirring, samples were vacuum pump filtered on 1.6 µm pore size Whatman paper and then dried.

Using a 10-45× magnification dissecting microscope, microplastic particles in the processed stomach contents were identified and enumerated. The microplastic particles were observed and categorized into four major types (fiber, fragment, film, and bead, [27]) following protocols from Hidalgo-Paez et al., [28] and Bredenoord et al. [29]. 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 used. All these processes were conducted in the laboratory behind closed doors and windows.

Resubmission Requirement

The author can rewrite this sentence by using this example "The specimens were weighed using an analytical balance. In addition, the fish were measured the body length of each fish or not? How many fish from each species were used in this study?"

Resubmission Requirement

In this study considering the microplastic contents in fish stomach only not other part of fish organ?

Results

We observed microplastic shapes of fiber, film, fragment. Microplastic beads were not detected in any of our samples. In jacks, coral grouper, golden snapper, and pickhandle barracuda, we detected microplastic particles in 100% of samples, except for blackspot snapper only 80% of samples.

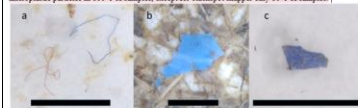


Figure 3 Photographs illustrating the observed microplastic types found in digestive organs from sample fishes collected in the Pangasinan Estuary: a. fiber, b. film, and c. fragment. Horizontal scale bar = 0.5 mm.

In fish samples of jacks, blackspot snapper, coral grouper, golden snapper, and pickhandle barracuda, we found in average of 41 ± 6.0 SE, 26 ± 11.0 SE, 15 ± 2.0 SE, 11 ± 1.0 SE, 14 ± 2.0 SE microplastic particles per individual, respectively, and we found in average of 499.3 ± 94.4 SE, 501.5 ± 198.8 SE, 83.6 ± 14.4 SE, 79.8 ± 11.4 SE, 40.2 ± 25.0 SE microplastic particles per kg fish weight, respectively. The blackspot snapper had the highest number of microplastic particles per kilogram of fish weight, reaching 501.5 ± 198.8 SE microplastic particles. The lowest number of particles per kilogram of fish was found in the pickhandle barracuda, which amounted to 40.2 ± 25.0 SE microplastic particles.

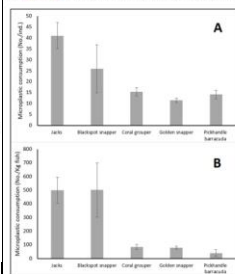


Figure 4 Average of microplastic particles per individual (A) and per kilogram fish weight (B).

Resubmission Requirement

The first part of your result the author should add a table to explain the characteristics of different fish species which include common name, species name, number of samples, feeding mode, habitat, class, length etc.

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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. 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%, 50% of all samples respectively.

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%, 40% of our samples respectively. 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.

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. Among the five 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. The relationship of the average consumed microplastic particles and the average fish weight per species (Figure 5) shows a negative relationship ($P = 0.16$), but the relationship between the two is not significant (P -value = 0.28).

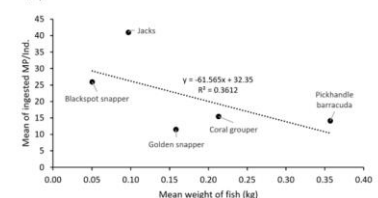


Figure 5 Relationship of mean weight of observed fish and mean number of ingested microplastic particles per individual.

In this study, the majority of observed fish species are demersal (Table 1), or at least in part of their life phase, depending on 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 (fish, shrimp, crabs and cephalopods) generally smaller in size than their own bodies.

Resubmission Requirement

I recommend to use pie chart to illustrate different type of microplastic contamination in each fish species. Please document that you can use in improving your writing: <https://doi.org/10.1371/journal.pone.0273189>

Resubmission Requirement

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<i>Trends Sci. 202x; xx(xx): xxxxx.</i> 7 of 11 Table 1 Taxonomy, common name, foraging ecology <table border="1"> <thead> <tr> <th>No.</th> <th>Taxonomy and Common name</th> <th>Foraging ecology</th> </tr> </thead> <tbody> <tr> <td>1.</td> <td>Family: <i>Carangidae</i> Genus: <i>Caranx</i> Common names: Jacks or trevallies</td> <td>Most species are demersal, or bottom dwelling, while others are pelagic, moving long distances in the upper water column [50]. All species are predatory fish, taking smaller fish, cephalopods or prey [51].</td> </tr> <tr> <td>2.</td> <td>Family: <i>Lutjanidae</i> Species: <i>Lutjanus fulvus</i> (Forsskal, 1775) Common names: Blackspot snapper</td> <td>Many species under this family are demersal and associated with coral reefs [52]. Some of the larger red snapper species descend into deeper waters, or least to 200 m in depth [53]. <i>Lutjanus</i> is predatory fish usually found in tropical and subtropical reefs, and mangrove forests.</td> </tr> <tr> <td>3.</td> <td>Family: <i>Serranidae</i> <i>Lateolabrax temminckii</i> (Kohli & Hansel, 1928) Common names: Coral grouper</td> <td>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 [54]. 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 [55].</td> </tr> <tr> <td>4.</td> <td>Family: <i>Lutjanidae</i> <i>Lutjanus johni</i> (Bleek, 1792) Common names: Golden snapper, or John's snapper</td> <td><i>Lutjanus</i> exhibits a two-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) [56]. Feed on fishes and benthic invertebrates including shrimps, crabs and cephalopods. Adults probably frequent coral reef areas; juveniles found in mangrove estuaries [57].</td> </tr> <tr> <td>5.</td> <td>Family: <i>Sphyrnidae</i> <i>Sphyrna tiburo</i> (Cuvier, 1829) Common names: Pickhandle barracuda or Banded barracuda</td> <td>They are migratory pelagic predators and usually be found in schools swimming in the tropical waters, predominantly nearby coral reefs [58]. Prey on fish, cephalopods, and shrimps [59].</td> </tr> </tbody> </table> Discussion Types of microplastic Our findings confirmed that fish in Panganggang Estuary do consume microplastics and fibers over the most dominant type of microplastic. In the aquatic environment, sample studies reported that fibers are the most encountered microplastic type [40-43]. Sources of fibers are predominantly textile goods, such as clothes [44]. Degradation of synthetic fibers was predicted as major contributor for microplastic fiber abundance in our environment [44,45]. 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 1900 fibers at size less than 5mm in length per wash cycle [46]. 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 discharged into surface waters [46]. In our study, the urban areas along the river that flows to Panganggang 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 Panganggang Estuary waters. A microplastic study on commercially important marine fish species in Fiji documented that film microplastic portion was half of the total discovered microplastic particles, and the remaining shared between fibers and fragment [47]. We observed fragment particles was the lowest in number and only found in three of five fish species we examined. In contrast, <i>Kabaliha et al.</i> documented that among commercial marine fish in the adjacent waters of Malaysia, fragment type microplastics were the most prevalent [48]. 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 [49].	No.	Taxonomy and Common name	Foraging ecology	1.	Family: <i>Carangidae</i> Genus: <i>Caranx</i> Common names: Jacks or trevallies	Most species are demersal , or bottom dwelling, while others are pelagic , moving long distances in the upper water column [50]. All species are predatory fish, taking smaller fish, cephalopods or prey [51].	2.	Family: <i>Lutjanidae</i> Species: <i>Lutjanus fulvus</i> (Forsskal, 1775) Common names: Blackspot snapper	Many species under this family are demersal and associated with coral reefs [52]. 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Family: <i>Lutjanidae</i> <i>Lutjanus johni</i> (Bleek, 1792) Common names: Golden snapper, or John's snapper	<i>Lutjanus</i> exhibits a two-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) [56]. Feed on fishes and benthic invertebrates including shrimps, crabs and cephalopods. Adults probably frequent coral reef areas; juveniles found in mangrove estuaries [57].	5.	Family: <i>Sphyrnidae</i> <i>Sphyrna tiburo</i> (Cuvier, 1829) 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 [58]. Prey on fish, cephalopods, and shrimps [59].	<i>Trends Sci. 202x; xx(xx): xxxxx.</i> 8 of 11 Recent investigations into the impact of microplastic fibers on aquatic organisms have unveiled various harmful consequences. According to Liang et al., goldfish exposed to a concentration of 1000 items per liter 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 [50]. Similarly, a study by Zhao et al. 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 [51]. Another study by Xie et al. noted oxidative stress, dysbiosis in the intestine microbiome, and histological damage as significant symptoms in barramundi exposed to microplastic fibers [52]. Abundance of microplastic in digestive organs Our finding revealed that jack as individual consumed the highest average of microplastic particles, 41 (SE: 5.98) while golden snapper was observed consuming 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. documented microplastic particle abundance ranged from 9-14 per gastrointestinal tract in multiple fish species caught the Bahía Blanca Estuary in Argentina [53], up to 4 particles in semi pelagic hoop hoops in the Mediterranean Sea [41], and ranged 1.1- 7.2 microplastic particles in fish collected from China marine waters [40]. On the other hand, the blackspot snapper has the highest amount of microplastics per kilogram of fish, 501.5 ± 198.8 SE, while the pickhandle barracuda has the smallest amount, only 40.2 ± 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 two is not significant (P-value = 0.28). Similarly, Chen et al. reported that fish size did not affect microplastic abundance in the fish digestive organs [54]. Foraging ecology Microplastic ingestion is potentially influenced by two crucial factors: the type of habitat and the feeding habits of organisms [55]. Within marine environments, scientists have noted markedly greater quantities of microplastics in demersal fish species compared to pelagic species [40,42]. This trend could be linked to the prevalence of plastic waste near the seabed, which is often regarded as the final accumulation for plastics within the marine ecosystem. Microplastics could potentially be present in fish across different trophic levels. However, current data are insufficient to make definitive conclusions about the absorption of microplastics [56]. In fact, distinguishing between directly and indirectly ingested microplastics is challenging [57]. Walkinshaw et al. performed a semi-systematic review on research examining the presence of microplastics in commercially significant organisms across various trophic levels [58]. 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 are adult predator fish, four fish species are demersal, and one is pelagic (Table 1). The microplastic abundances in the three 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 anticipated 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 [59]. 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 two herbivorous fish species consistently ingest significant quantities of microplastic particles as much as 10-99 microplastic particles per fish gut [60]. Conclusions For the first time, this study demonstrates the presence of microplastic particles in five commercially important fish in a tropical estuary of Kalimantan Island in Indonesia. The average abundance per fish individual were higher than those documented 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 a significant portion of the microplastic pollution found in the digestive systems of sampled fish from the Panganggang Estuary.	<i>Trends Sci. 202x; xx(xx): xxxxx.</i> 9 of 11 Our research suggests that demersal fish may have ingested a higher quantity of microplastic particles compared to pelagic species. This study result also confirms the urgency to observe wide range of fish species in term of their habitat within the water column and as well as the feeding habit. 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. Perhaps analysis based on muscles and internal organs should also be considered in the near future. Acknowledgements We thank Baitur Rohmah, Desvi Anjani, Cicilia Imelda Artonang dan Dinda Qurata Ayun for their help during laboratory processing. References [1] Geyer, R., Jambeck, J. R., & Law, K. L. (2017). Production, use, and fate of all plastics ever made. <i>Science advances</i>, 3(7), e1700782. [2] Worm, B., Lotze, H. K., Jubinville, I., Wilcox, C., & Jambeck, J. (2017). Plastic as a persistent marine pollutant. <i>Annual Review of Environment and Resources</i>, 42, 1-26. [3] Baig, O. (2021). From plastics to microplastics and organisms. <i>FEBS Open bio</i>, 11(4), 954-966. 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No.	Taxonomy and Common name	Foraging ecology																		
1.	Family: <i>Carangidae</i> Genus: <i>Caranx</i> Common names: Jacks or trevallies	Most species are demersal , or bottom dwelling, while others are pelagic , moving long distances in the upper water column [50]. All species are predatory fish, taking smaller fish, cephalopods or prey [51].																		
2.	Family: <i>Lutjanidae</i> Species: <i>Lutjanus fulvus</i> (Forsskal, 1775) Common names: Blackspot snapper	Many species under this family are demersal and associated with coral reefs [52]. Some of the larger red snapper species descend into deeper waters, or least to 200 m in depth [53]. <i>Lutjanus</i> is predatory fish usually found in tropical and subtropical reefs, and mangrove forests.																		
3.	Family: <i>Serranidae</i> <i>Lateolabrax temminckii</i> (Kohli & Hansel, 1928) 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 [54]. 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 [55].																		
4.	Family: <i>Lutjanidae</i> <i>Lutjanus johni</i> (Bleek, 1792) Common names: Golden snapper, or John's snapper	<i>Lutjanus</i> exhibits a two-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) [56]. Feed on fishes and benthic invertebrates including shrimps, crabs and cephalopods. Adults probably frequent coral reef areas; juveniles found in mangrove estuaries [57].																		
5.	Family: <i>Sphyrnidae</i> <i>Sphyrna tiburo</i> (Cuvier, 1829) 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 [58]. Prey on fish, cephalopods, and shrimps [59].																		

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3	Hasil Revisi Round 1	21 Desember 2023	a. 21753-1 - Responses to Reviewer, Hamdhani et al Responses the Reviewer's Comment(s) b. 21760-1 - Responses to Reviewer, Reviewer 1 c. 21757-1 - Responses to Reviewer, Reviewer 2 d. 21758-1 - Responses to Reviewer, Reviewer 3 e. 21759-1 - Responses to Reviewer, Reviewer 4																																	
a.	21753-1 - Responses to Reviewer, Hamdhani et al Responses the Reviewer's Comment(s)																																			
 Trends in Sciences <i>Responses the referee's comments</i> Manuscript ID: <u>7762</u> Title: <u>Occurrence of Microplastic Ingestion by Commercial Fish Species from the Pangempang Estuary in Indonesia</u> Reviewer A <table><tr><th>No.</th><th>Referee's Comments</th><th>Responses (for author)</th></tr><tr><td>1.</td><td>Do they have any report of microplastic contamination in this Island or in other places in Indonesia. If there is some article or report available, the author should provide this information.</td><td>A microplastic study in the nearby area has been cited</td></tr><tr><td>2.</td><td>The author should add more information about climate data for this study site such as average air temperature, precipitation data</td><td>The suggested information has been added.</td></tr><tr><td>3.</td><td>The author should reference Figure 1 into the text. I put it after this sentence. Please recheck the reference for figure 1 again.</td><td>Good point, we have fixed this issue. Thank you</td></tr><tr><td>4.</td><td>It's should be great if you can add the location of this coal loading port into the map (figure 1)</td><td>Unfortunately, the coal loading port is situated outside the presented map scope</td></tr><tr><td>5.</td><td>Add more detail about fish characteristic such as feeding mode, habitat (pelagic, benthic-pelagic, or benthos)</td><td>The fish characteristic is presented in Table 1.</td></tr><tr><td>6.</td><td>Rephrase this sentence again.</td><td>We have modified the sentence as suggested.</td></tr><tr><td>7.</td><td>The author can rewrite this sentence by using this example "The specimens were weighed using an analytical balance....." In addition, Do you measure the body length of each fish or not? How many fish from each species have been used in this study?</td><td>We have modified the sentence. We measured the length as well but in this study we focus on the fish weight only We used 10 fish for each species. This have mentioned earlier.</td></tr><tr><td>8.</td><td>Is this study considering the microplastic contaminate in fish stomach only not other part of fish organ?</td><td>Yes, only ingested microplastics</td></tr><tr><td>9.</td><td>The first part of your result the author should add a table to explain the characteristics of different five fish species which include common name, species name, number of samples, feeding mode, habitat, mass, length etc.</td><td>The suggested table has been added</td></tr><tr><td>10.</td><td>The author should move Table 1 into material and</td><td>Thank you for the</td></tr></table>				No.	Referee's Comments	Responses (for author)	1.	Do they have any report of microplastic contamination in this Island or in other places in Indonesia. If there is some article or report available, the author should provide this information.	A microplastic study in the nearby area has been cited	2.	The author should add more information about climate data for this study site such as average air temperature, precipitation data	The suggested information has been added.	3.	The author should reference Figure 1 into the text. I put it after this sentence. Please recheck the reference for figure 1 again.	Good point, we have fixed this issue. Thank you	4.	It's should be great if you can add the location of this coal loading port into the map (figure 1)	Unfortunately, the coal loading port is situated outside the presented map scope	5.	Add more detail about fish characteristic such as feeding mode, habitat (pelagic, benthic-pelagic, or benthos)	The fish characteristic is presented in Table 1.	6.	Rephrase this sentence again.	We have modified the sentence as suggested.	7.	The author can rewrite this sentence by using this example "The specimens were weighed using an analytical balance....." In addition, Do you measure the body length of each fish or not? How many fish from each species have been used in this study?	We have modified the sentence. We measured the length as well but in this study we focus on the fish weight only We used 10 fish for each species. This have mentioned earlier.	8.	Is this study considering the microplastic contaminate in fish stomach only not other part of fish organ?	Yes, only ingested microplastics	9.	The first part of your result the author should add a table to explain the characteristics of different five fish species which include common name, species name, number of samples, feeding mode, habitat, mass, length etc.	The suggested table has been added	10.	The author should move Table 1 into material and	Thank you for the
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10.	The author should move Table 1 into material and	Thank you for the																																		

	method section.	suggestion, we have moved the table to the beginning of result section
11.	I recommend to use pie chart to illustrate different type of microplastic contaminate in each fish species. More document that you can use to improving your writing: https://tis.wu.ac.th/index.php/tis/article/view/3073/189	The pie charts have been made and added
12.	This figure does not show a clear illustration to me. Considering to modify this graph if possible.	We believe that this figure is informative, telling the relationship of mean weight of observed fish and mean number of ingested microplastic particles per species individual
13.	Rephrase these sentences.	We have rephrased the sentences
14.	Why? Any explanation about this?	For sure, we do not know why this happened
15.	Add three five species	We have added the three fish names.

Reviewer B

No.	Referee's Comments	Responses (for author)
1.	space	Thank you for spotting this issue, we have fixed this issue as suggested.
2.	space	Thank you for spotting this issue, we have fixed this issue as suggested.
3.	several times used "we", better to avoid much	Thank you for pointing this out, but we believe that "we" should not be a problem. This way is also used in many international journals with high reputation.
4.	fig no wrong	Yes, we have fixed the numbering

Reviewer C

No.	Referee's Comments	Responses (for author)
1.	It better to mention name of the methods for microplastic quantification	Thank you, we have added the method name
2.	Avoid using subject	Thank you for the suggestion, but recently active voice is equally used in many journals with high reputation. For

	pronoun, use passive voice	example: https://onlinelibrary.wiley.com/doi/epdf/10.1111/fw.13831
3.	What does it mean by consumed here,...number of microplastic found ?	We have replaced into ingested
4.	Avoid subject pronoun, using passive voice	Similar response as above
5.	This conclusions must be adjusted , since only one type of pelagic fish was observed	Thank you for pointing out this issues. We will adjust as necessary. In this study, we only observed whatever fish species that the encountered fishermen caught in this estuary without considering pelagic or demersal. This is the reason we do not have equal representation of pelagic and demersal fish.
6.	Put in alphabetical order	We have ordered alphabetically
7.	Mention the method, and the reference	We used digestive method to separate MP and organic material. The reference is already cited (You may not realize as this publication is using numbers for cited references)
8.	Please mention how to extract microplastics from fish organ	Using digestive method as explained
9.	The used statistical analysis is not mention in the Method yet	Thank you for pointing this issue out. We have added the "Data analysis" section
10.	Don't need to put words SE after +	We put SE to indicated that as a Standard Error, and not as a Standard Deviation (SD)
11.	X axis --> type of fish	We have modified the graph as suggested.
12.	Confusing sentence	We have added some pie chart graphs, as also suggested by other reviewer.
13.	The yellow highlighted data can also be presented as graph	We have added some pie chart graphs, as also suggested by other reviewer.
14.	The 0.36R2 sounds is not a confidence value to sum-up the relationship between the two parameters	Yes, we agree.
15.	???	This is referred to a study conducted in an estuary in Argentina
16.	1.1-7.2 microplastics per fish ?? Or per kg ??	Average per fish
17.	Year ?	The year has been added as suggested
18.	No fair comparison for conclusion, since 4 type of demersal, but only 1 typt of pelagic fish	Yes, that is true. This is because our sample was gained from what the fishermen caught only. In fact from the catch, the two type of fish was not balance.
19.	Discussion regarding the comprison between the estary's fish and	In the " Abundance of microplastic in digestive organs " section, we have added more discussion as suggested to compared with microplastic in river fish.

	river-fish or sea-fish will be beneficial, and add enrich the discussion, as well as the river and sea microplastic data. It will be better if the size of ingested microplastic is also discussed	Unfortunately, we did not measure the individual microplastic particle size
20.	Language : need proofreading; a lot of grammatical errors; the use of subjective pronouns	We have proofread this revised version by an American colleague who also a co-author in this paper
21.	Methodology : missing some essential part : method to extract microplastics from fish's organ, no method of statistical analysis	The methodology has been revised as suggested
22.	Results: data of microplastic concentration from each type of fish can be more presentable in a graph; the graph presentation is not complete; there is no fair comparison (since the type of demersal fish is far more than pelagic fish); it will be beneficial if size of microplastic is presented and discussed, supporting the discussion regarding how the ingestion of microplastic by fish occurred.	We observed the fish species caught by the fishermen so we had no control to balance between pelagic and demersal fish. Unfortunately we did not observe the microplastic particle size.
23.	Data of microplastic in the nearby river, the estuary water and the seawater might be added to have a clearer explanation	We have added one study of microplastic in water from nearby river (Mahakam River)
Reviewer D		
No.	Referee's Comments	Responses (for author)
1.	Check sp or spp?	We use "sp" because it is only one kind and we only able to

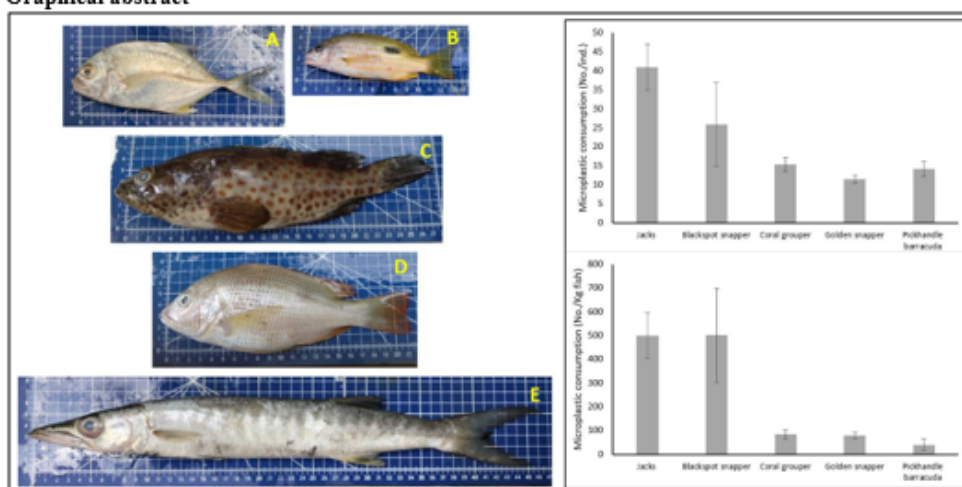
		identified up to the genus.
2.	Check	Good catch. Thank you
3.	We don't see any commercial fish issues in your introduction If commercial fish is contaminated with microplastics, why many people eat it? And we should replaced with other food products that are not contaminated	The effect of consuming fish that ingested microplastics to human need more studies. In other word we do not really know, yet.
4.	In what month and year did you take the sample? Season is a factor that influences MPs in the environment, in what season do you take the samples? Why isn't the total length measured?	We have added the information when the sampling was conducted.
5.	Using latin name is better. For all in results and discussions	We prefer to use common name as this way is also used in other international publications that we cited
6.	There is no figure or table for percent	We just confirming that only blackspot snapper that had specimen (20%) with no microplastic particle in the digestive organs, so we think that mentioning in the text is sufficient.
7.	Each paragraph consists of 3 sentences (minimum) Check at all paragraph	Thank you for pointing the issue. We have gathered these sentences into one paragraph.

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

Highlights

- This study demonstrates the presence of microplastic particles in five commercially important fish in a tropical estuary of Kalimantan Island in Indonesia.
- Fiber was the most dominant type of microplastic.
- Demersal fish may have ingested a higher quantity of microplastic particles compared to pelagic species.

Graphical abstract



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 corallicola*), 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. Using a dissecting stereo microscope, microplastic particles in the processed digestive organ contents were identified and enumerated. Microplastics were found in 96% of fish digestive organs ($n = 50$). In fish samples of jacks, blackspot snapper, coral grouper, golden snapper, and pickhandle barracuda ($n = 10$ for each species), we found in average of 41 ± 6.0 SE, 26 ± 11.0 SE, 15 ± 2.0 SE, 11 ± 1.0 SE, 14 ± 2.0 SE microplastic particles per individual, respectively, and we found in average of 499.5 ± 94.4 SE, 501.5 ± 198.8 SE, 83.6 ± 18.4 SE, 79.8 ± 11.6 SE, 40.2 ± 25.0 SE microplastic particles per kg fish weight, respectively. Fiber was the most dominant type of microplastic. Microplastic beads were not detected. The relationship of the average consumed microplastic and the average fish weight per species show a negative relationship ($R^2 = 0.36$), but insignificant ($P\text{-value} = 0.28$). 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: Pollution, Ingestion, Commercial fish, Borneo, Indonesia

Introduction

After invented over 110 years ago, plastic materials become ubiquitous in the environment. Plastic material is made from polymers that can be shaped easily for many varieties of uses. The elevated desire to use plastic materials due to their affordability, bio-inertia, high strength to weight ratio, and resistance to degradation. These characteristics has made plastic materials become important materials for human life nowadays. By the year of 2017, the total global production of plastic was estimated to reach 8300 million metric tons [1], with the rate of production has reached 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 suspected responsible to bring 88%-95% of global marine debris load into ocean [4]. The proportion of plastics usually take about 70% of total marine debris [5].

Initially regarded as a safe material, several decades of plastic production have brought a wide range of environmental problems, particularly in marine ecosystems. In oceans, even though plastic is considered as durable material and resistant to natural biodegradation. However, 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) [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 from the surrounding [11]. Cole et al. reported that ingestion of microplastic particles by zooplanktons shows negative impacts on feeding, hatching, and their health [12]. 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 a type of marine ecosystems often utilized for fisheries purposes [16,17], and these waters 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 estuary is problematic as estuaries also provide crucial habitat for a variety of commercially and recreationally important species such as crabs, fish, and shellfish [20]. Numerous studies investigating microplastics have placed their emphasis on ecosystems in both marine and inland environments [21,22,23]. In fact, Mejcabayanti et al. reported the presence of microplastics in waters of Mahakam River located in the East Kalimantan Island [61]. However, there have been limited evaluation on microplastic contamination in transitional waters between the two 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 and asked the main following questions: what type of microplastic? And how is the abundance of microplastic in fish collected from this transitional waters?

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 two periods of intense rainfall and two periods of reduced rainfall, with a total annual precipitation of approximately 97.2 inches (3.83 inches) [62]. The

Rwatcharee Ruairuen

Do they have any report of microplastic contamination in this Island or in other places in Indonesia. If there is some article or report available, the author should provide this information.

Hamdhani Hamdhani

A microplastic study in the nearby area has been cited

Rwatcharee Ruairuen

The author should add more information about climate data for this study site such as average air temperature, precipitation data.

Hamdhani Hamdhani

The suggested information has been added.

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 kilometers, originating from the Marangkayu reservoir. Its width ranges from 8 meters upstream to 140 meters downstream. The river passes through the Marangkayu Town settlement, situated roughly 20 kilometers upstream from the Pangempang Estuary. Additionally, about 7 kilometers 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.

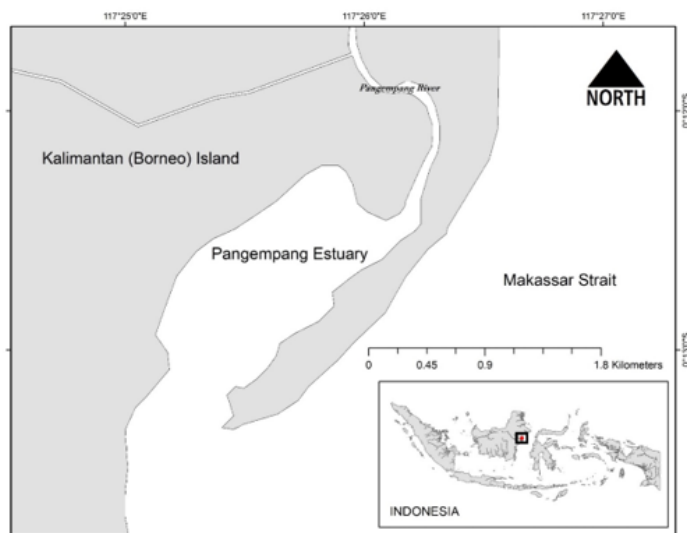


Figure 1 The Pangempang Estuary (East Kalimantan, Indonesia)

To evaluate the ingestion of microplastics by fish, we obtained fish specimens from local fishermen operating in the waters of the Pangempang Estuary. We were able to collect ten replicates of each five different species from these fishermen. The species we identified were jack (*Caranx* SP.), blackspot snapper (*Lutjanus fulviflavus*), coral grouper (*Epinephelus corallicola*), golden snapper (*Lutjanus johnii*), and pickhandle barracuda (*Sphyræna jello*). We identified the fish species based on the information provided in the book titled "Marine Fishes and Crustaceans of the Southeast Asian Region" [24]. Each individual fish was placed in plastic bags, 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.

Rwatcharee Ruairuen

The author should reference Figure 1 into the text. I put it after this sentence. Please recheck the reference for figure 1 again.

Comment

Good point, thank you

Rwatcharee Ruairuen

It's should be great if you can add the location of this coal loading port into the map (figure 1).

Comment

Unfortunately, the coal loading port is situated outside the presented map scope

Rwatcharee Ruairuen

Add more detail about fish characteristic such as feeding mode, habitat (pelagic, ~~benthic~~-pelagic, or benthos)

Comment

The fish characteristic is presented in Table 1.

Rwatcharee Ruairuen

Rephrase this sentence again.

Comment

We have modified the sentence as suggested.

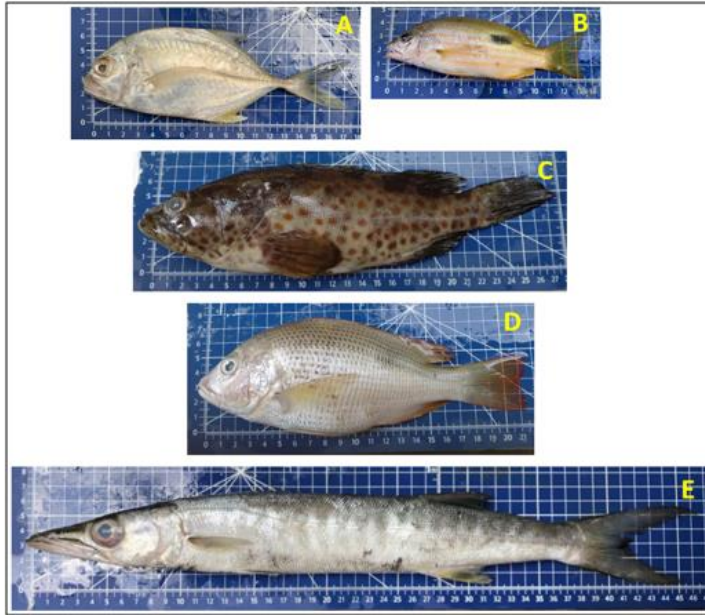


Figure 2 Photographs illustrating the observed fish species: (A) jack (*Caranx* SP.), (B) blackspot snapper (*Lutjanus fulvivittatus*), (C) coral grouper (*Epinephelus caralligala*), (D) golden snapper (*Lutjanus johnii*), and (E) pickhandle barracuda (*Sphyræna jello*).

Laboratory processing

We measured fish weight (g) of each specimen using [redacted]. The specimens were weighed using an analytical balance. 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, each stomach content was digested using alkaline digestion solution of potassium hydroxide (KOH) [25,26]. Each stomach content was placed in a beaker and 10% KOH was added allowing submersion of stomach contents in the beaker. Each beaker was covered with aluminum foil. We stirred the samples in the beaker using magnetic spinner at 350 rpm for 2 hours. 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% H₂O₂ was added and then stirred. After 1 hour stirring, samples were vacuum pump filtered on 1.6 µm pore size Whatman paper and then dried.

Using a 10-45× magnification dissecting microscope, microplastic particles in the processed stomach contents were identified and enumerated. The microplastic particles were observed and categorized into four major types (fiber, fragment, film, and bead; [27]) following protocols from Hidalgo-Ruz et al., [28] and Eppenhauer et al. [29]. 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 used. All these processes were conducted in the laboratory behind closed doors and windows.

Rwatcharee Ruairuen

The author can rewrite this sentence by using this example "The specimens were weighed using an analytical balance....." In addition, Do you measure the body length of each fish or not? How many fish from each species have been used in this study?

Comment

We have modified the sentence.
We measured the length as well but in this study we focus on the fish weight only.
We used 10 fish for each species. This have mentioned earlier.

Rwatcharee Ruairuen

Is this study considering the microplastic contaminate in fish stomach only not other part of fish organ?

Comment

Yes, only ingested microplastics

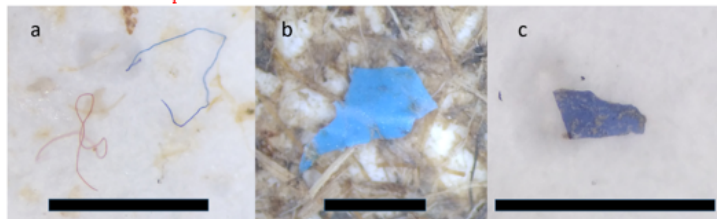
Results

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 (fish, shrimps, crabs and cephalopods) generally smaller in size than their own bodies.

Table 1 | taxonomy, common name, foraging ecology

No.	Taxonomy and Common name	Foraging ecology
1.	Family: Caraugidae Genus: Caraux Common names: Jacks or trevallies	Most species are demersal , or bottom dwelling, while others are pelagic , moving long distances in the upper water column [30]. All species are predatory fish, taking smaller fish, cephalopods as prey [31].
2.	Family: Lutjanidae Species: Lutjanus <i>Lutjanus fulvus</i> (Forsskal, 1775) Common names: Blackspot snapper	Many species under this family are demersal and associated with coral reefs [32]. Some of the larger red snapper species descend into deeper waters, at least to 200 m in depth [33]. <i>Lutjanus</i> is predatory fish usually found in tropical and subtropical reefs, and mangrove forests.
3.	Family: Serranidae <i>Epinephelus</i> catalisala (Kuhl & Hasselt, 1828) 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 [34]. 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 [35].
4.	Family: Lutjanidae <i>Lutjanus</i> johni (Bloch, 1792) Common names: Golden snapper, or John's snapper	<i>Lutjanus</i> exhibits a two-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) [36]. Feed on fishes and benthic invertebrates including shrimps, crabs and cephalopods. Adults probably frequent coral reef areas; juveniles found in mangrove estuaries [37].
5.	Family: Sphyraenidae <i>Sphyraena</i> jella Cuvier, 1829 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 [38]. Prey on fish, cephalopods, and shrimps [39].

We observed microplastic shapes of fibers, film, fragments (Figure 3). Microplastic beads were not detected in any of our samples. In jacks, coral grouper, golden snapper, and pickhandle barracuda, microplastic particles were identified in 100% of samples, except for blackspot snapper, where they were found in 80% of the samples.



Rwatcharee Ruairuen

The first part of your result the author should add a table to explain the characteristics of different five fish species which include common name, species name, number of samples, feeding mode, habitat, mass, length etc.

Comment

The suggested table has been added

Rwatcharee Ruairuen

The author should move Table 1 into material and method section.

Hamdhani Hamdhani

Thank you for the suggestion, we have moved the table to the beginning of result section

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 SE, 26 ± 11.0 SE, 15 ± 2.0 SE, 11 ± 1.0 SE, 14 ± 2.0 SE microplastic particles per individual, respectively (**Figure 4A**), and we found in average of 499.5 ± 94.4 SE, 501.5 ± 198.8 SE, 83.6 ± 18.4 SE, 79.8 ± 11.6 SE, 40.2 ± 25.0 SE microplastic particles per kg fish weight, respectively (**Figure 4B**). The blackspot snapper had the highest number of microplastic particles per kilogram of fish weight, reaching 501.5 ± 198.8 SE microplastic particles. The lowest number of particles per kilogram of fish was found in the pickhandle barracuda, which amounted to 40.2 ± 25.0 SE microplastic particles.

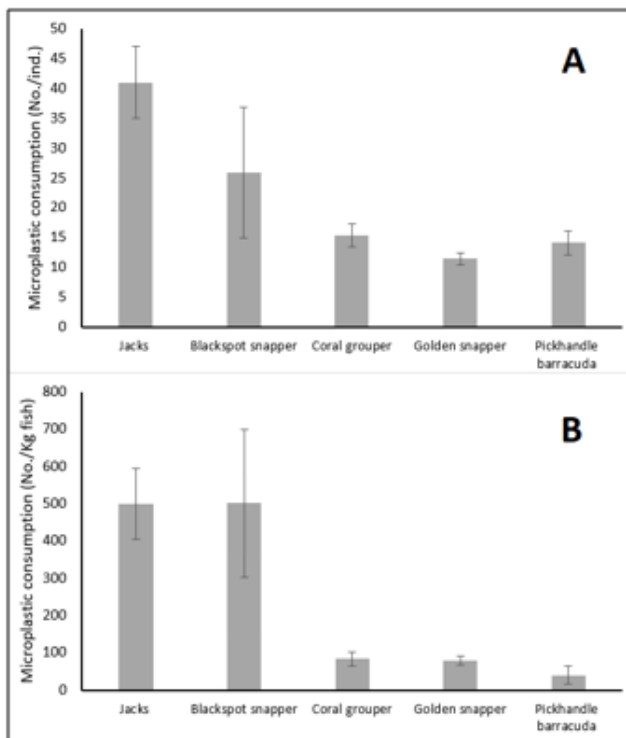


Figure 4 Average of microplastic particles per individual (A) and per kilogram fish weight (B)

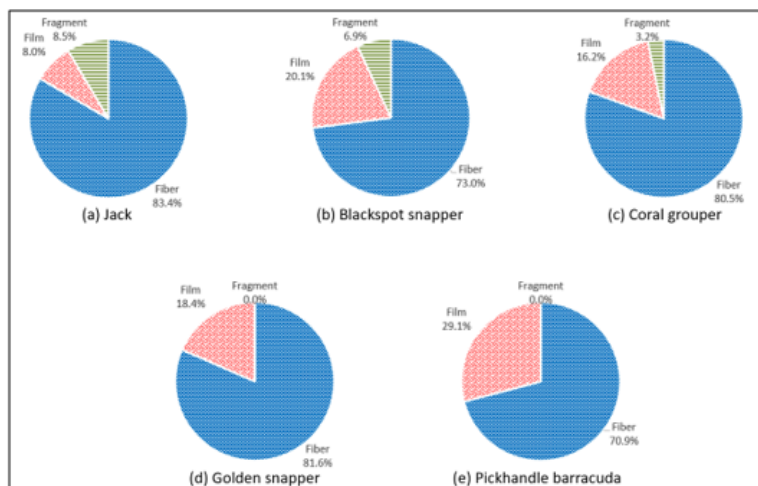


Figure 5 Percentages of the observed microplastic types found in digestive organs from sample fishes collected in the Pangkep Estuary: (a) Jack, (b) Blackspot snapper, (c) Coral grouper, (d) Golden snapper, and (e) Pickhandle barracuda.

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 5a). 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%, 50% of all samples respectively (Figure 5b). 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%, 40% of our samples respectively (Figure 5c). 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 5d). 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 5e).

Rwatcharee Ruairuen

I recommend to use pie chart to illustrate different type of microplastic contaminate in each fish species. More document that you can use to improving your writing: <https://tis.wu.ac.th/index.php/tis/article/view/3073/189>

Hamdhani Hamdhani

The pie charts have been made and added

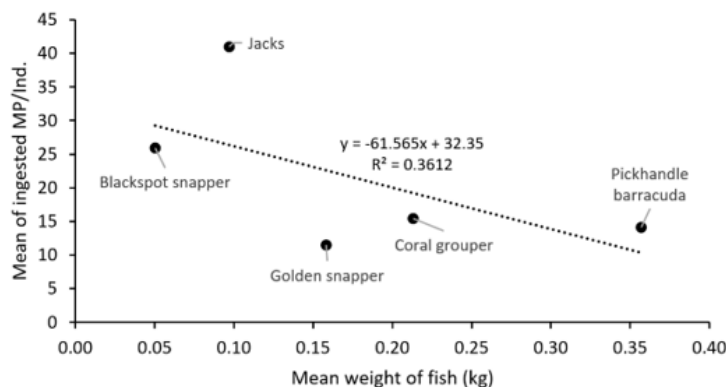


Figure 6 Relationship of mean weight of observed fish and mean number of ingested microplastic particles per species individual

Among the five 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. 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 two 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 predominant type of microplastic. Previous research in aquatic environments has identified fibers as the most prevalent form of microplastics [40–43]. Sources of fibers are predominantly textile goods, such as clothes [44]. Degradation of synthetic fabrics was predicted as major contributor for microplastic fiber abundance in our environment [44,45]. 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 1900 fibers at size less than 1mm in length per wash cycle [46]. 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 discharged into surface waters [46]. 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 jack 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 film microplastic portion was half of the total discovered microplastic particles, and the remaining shared between fiber and fragment [47]. We observed fragment particles was the lowest in number and only found in three of five fish species we examined (jack, blackspot snapper, and coral grouper). In contrast, Karbalaei et al. documented that among commercial marine fish in the adjacent waters of Malaysia, fragment-type microplastics were the most prevalent [48]. 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 [49].

Recent investigations into the impact of microplastic fibers on aquatic organisms have unveiled various harmful consequences. According to Liang et al., goldfish exposed to a concentration of 1000 items

Rwatcharee Ruairuen

This figure does not show a clear illustration to me. Considering to modify this graph if possible.

Hamdhani Hamdhani

We believe that this figure is informative, telling the relationship of mean weight of observed fish and mean number of ingested microplastic particles per species individual

Rwatcharee Ruairuen

Rephrase these sentences.

Hamdhani Hamdhani

We have rephrased the sentences

Rwatcharee Ruairuen

Why? Any explanation about this?

Hamdhani Hamdhani

Certainly we do not know why this happened

Rwatcharee Ruairuen

Add three five species

Hamdhani Hamdhani

We have added the three fish names.

per liter 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 [50]. Similarly, a study by Zhao et al. 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 [51]. Another study by Xie et al. noted oxidative stress, dysbiosis in the intestine microbiome, and histological damage as significant symptoms in barramundi exposed to microplastic fibers [52].

Abundance of microplastic in digestive organs

Our finding revealed that jack as individual consumed the highest average of microplastic particles, 41 (SE: 5.98) while golden snapper was observed consuming 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. documented microplastic particle abundance ranged from 9-14 per gastrointestinal tract in multiple fish species caught the Bahía Blanca Estuary in Argentina [53], up to 4 particles in semi pelagic boop boops in the Mediterranean Sea [41], and ranged 1.1- 7.2 microplastic particles in fish collected from China marine waters [40]. On the other hand, the blackspot snapper has the highest amount of microplastics per kilogram of fish, 501.5 ± 198.8 SE, while the pickhandle barracuda has the smallest amount, only 40.2 ± 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 two is not significant ($P\text{-value} = 0.28$). Similarly, Chen et al. reported that fish size did not affect microplastic abundance in the fish digestive organs [54].

Foraging ecology

Microplastic ingestion is potentially influenced by two crucial factors: the type of habitat and the feeding habits of organisms [55]. Within marine environments, scientists have noted markedly greater quantities of microplastics in demersal fish species compared to pelagic species [40,42]. This trend could be linked to the prevalence of plastic waste near the seabed, which is often regarded as the final accumulation for plastics within the marine ecosystem.

Microplastics could potentially be present in fish across different trophic levels. However, current data are insufficient to make definitive conclusions about the absorption of microplastics [56]. In fact, distinguishing between directly and indirectly ingested microplastics is challenging [57]. Walkinshaw et al. performed a semi-systematic review on research examining the presence of microplastics in commercially significant organisms across various trophic levels [58]. 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 are adult predator fish, four fish species are demersal, and one is pelagic (Table 1). The microplastic abundances in the three 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 anticipated 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 [59]. 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 two herbivorous fish species consistently ingest significant quantities of microplastic particles as much as 10-99 microplastic particles per fish gut [60].

Conclusions

For the first time, this study demonstrates the presence of microplastic particles in five commercially important fish in a tropical estuary of Kalimantan Island in Indonesia. The average abundance per fish individual were higher than those documented 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 a significant 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.

This study result also confirms the urgency to observe wide range of fish species in term of their habitat within the water column and as well as the feeding habit. 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. Perhaps analysis based on muscles and internal organs should also be considered in the near future.

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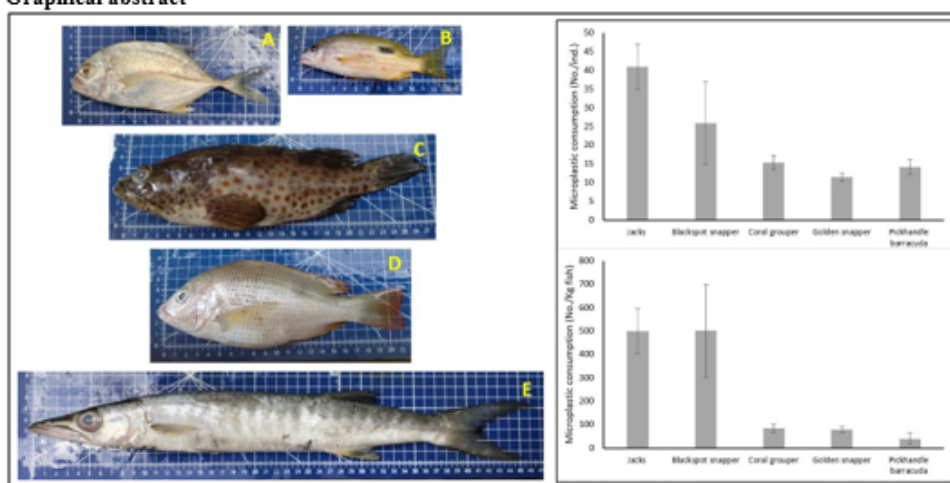
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Occurrence of Microplastic Ingestion by Commercial Fish Species from the Pangempang Estuary in Indonesia

Highlights

- This study demonstrates the presence of microplastic particles in five commercially important fish in a tropical estuary of Kalimantan Island in Indonesia.
- Fiber was the most dominant type of microplastic.
- Demersal fish may have ingested a higher quantity of microplastic particles compared to pelagic species.

Graphical abstract



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 corallicola*), golden snapper (*Lutjanus johnii*), and pickhandle barracuda (*Sphyræna jella*) 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. Using a dissecting stereo microscope, microplastic particles in the processed digestive organ contents were identified and enumerated. Microplastics were found in 96% of fish digestive organs ($n = 50$). In fish samples of jacks, blackspot snapper, coral grouper, golden snapper, and pickhandle barracuda ($n = 10$ for each species), we found in average of 41 ± 6.0 SE, 26 ± 11.0 SE, 15 ± 2.0 SE, 11 ± 1.0 SE, 14 ± 2.0 SE microplastic particles per individual, respectively, and we found in average of 499.5 ± 94.4 SE, 501.5 ± 198.8 SE, 83.6 ± 18.4 SE, 79.8 ± 11.6 SE, 40.2 ± 25.0 SE microplastic particles per kg fish weight, respectively. Fiber was the most dominant type of microplastic. Microplastic beads were not detected. The relationship of the average consumed microplastic and the average fish weight per species show a negative relationship ($R^2 = 0.36$), but insignificant ($P\text{-value} = 0.28$). 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: Pollution, Ingestion, Commercial fish, Borneo, Indonesia

Introduction

After invented over 110 years ago, plastic materials become ubiquitous in the environment. Plastic material is made from polymers that can be shaped easily for many varieties of uses. The elevated desire to use plastic materials due to their affordability, bio-inertia, high strength to weight ratio, and resistance to degradation. These characteristics has made plastic materials become important materials for human life nowadays. By the year of 2017, the total global production of plastic was estimated to reach 8300 million metric tons [1], with the rate of production has reached 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 suspected responsible to bring 88%-95% of global marine debris load into ocean [4]. The proportion of plastics usually take about 70% of total marine debris [5].

Initially regarded as a safe material, several decades of plastic production have brought a wide range of environmental problems, particularly in marine ecosystems. In oceans, even though plastic is considered as durable material and resistant to natural biodegradation. However, 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) [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 from the surrounding [11]. Cole et al. reported that ingestion of microplastic particles by zooplanktons shows negative impacts on feeding, hatching, and their health [12]. 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 a type of marine ecosystems often utilized for fisheries purposes [16,17], and these waters 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 estuary is problematic as estuaries also provide crucial habitat for a variety of commercially and recreationally important species such as crabs, fish, and shellfish [20]. Numerous studies investigating microplastics have placed their emphasis on ecosystems in both marine and inland environments [21,22,23]. In fact, Meicahayanti et al. reported the presence of microplastics in waters of Mahakam River located in the East Kalimantan Island [61]. However, there have been limited evaluation on microplastic contamination in transitional waters between the two 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 and asked the main following questions: what type of microplastic? And how is the abundance of microplastic in fish collected from this transitional waters?

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 two periods of intense rainfall and two periods of reduced rainfall, with a total annual precipitation of approximately 97.22 millimeters (3.83 inches) [62]. The

Rwatcharee Ruairuen

Do they have any report of microplastic contamination in this Island or in other places in Indonesia. If there is some article or report available, the author should provide this information.

Hamdhani Hamdhani

A microplastic study in the nearby area has been cited

Rwatcharee Ruairuen

The author should add more information about climate data for this study site such as average air temperature, precipitation data.

Hamdhani Hamdhani

The suggested information has been added.

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 kilometers, originating from the Marangkayu reservoir. Its width ranges from 8 meters upstream to 140 meters downstream. The river passes through the Marangkayu Town settlement, situated roughly 20 kilometers upstream from the Pangempang Estuary. Additionally, about 7 kilometers 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.

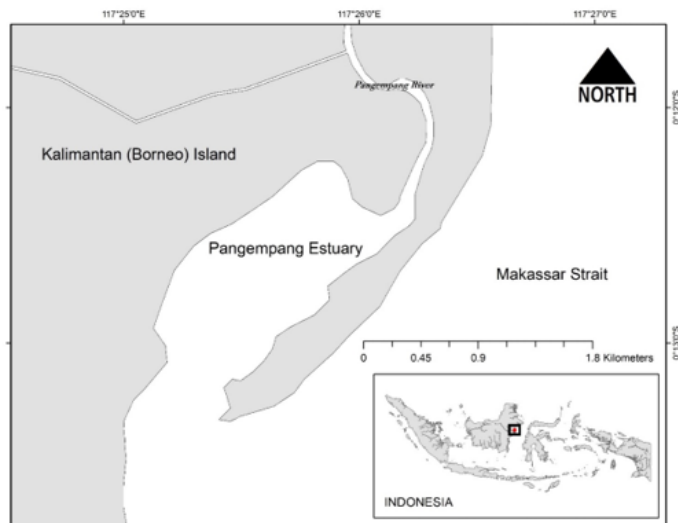


Figure 1 The Pangempang Estuary (East Kalimantan, Indonesia)

To evaluate the ingestion of microplastics by fish, we obtained fish specimens from local fishermen operating in the waters of the Pangempang Estuary. We were able to collect ten replicates of each five different species from these fishermen. The species we identified were jack (*Caranx* SP.), blackspot snapper (*Lutjanus fulvivittatus*), coral grouper (*Epinephelus corallicola*), golden snapper (*Lutjanus johni*), and pickhandle barracuda (*Sphyrna jello*). We identified the fish species based on the information provided in the book titled "Marine Fishes and Crustaceans of the Southeast Asian Region" [24]. Each individual fish was placed in plastic bags, 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.

Rwatharee Ruairuen

The author should reference Figure 1 into the text. I put it after this sentence. Please recheck the reference for figure 1 again.

Comment

Good point, thank you

Rwatharee Ruairuen

It's should be great if you can add the location of this coal loading port into the map (figure 1).

Comment

Unfortunately, the coal loading port is situated outside the presented map scope

Rwatharee Ruairuen

Add more detail about fish characteristic such as feeding mode, habitat (pelagic, ~~benthic~~-pelagic, or benthos)

Comment

The fish characteristic is presented in Table 1.

Rwatharee Ruairuen

Rephrase this sentence again.

Comment

We have modified the sentence as suggested.

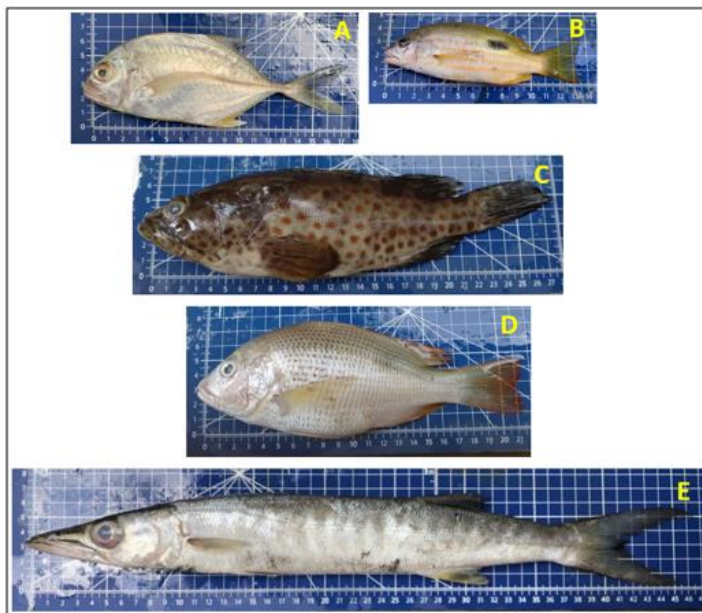


Figure 2 Photographs illustrating the observed fish species: (A) jack (*Caranx* SP.), (B) blackspot snapper (*Lutjanus fulviflavus*), (C) coral grouper (*Epinephelus corallicola*), (D) golden snapper (*Lutjanus johni*), and (E) pickhandle barracuda (*Sphyræna jello*).

Laboratory processing

We measured fish weight (g) of each specimen using an analytical balance. 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, each stomach content was digested using alkaline digestion solution of potassium hydroxide (KOH) [25,26]. Each stomach content was placed in a beaker and 10% KOH was added allowing submersion of stomach contents in the beaker. Each beaker was covered with aluminum foil. We stirred the samples in the beaker using magnetic spinner at 350 rpm for 2 hours. 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% H₂O₂ was added and then stirred. After 1 hour stirring, samples were vacuum pump filtered on 1.6 µm pore size Whatman paper and then dried.

Using a 10-45× magnification dissecting microscope, microplastic particles in the processed stomach contents were identified and enumerated. The microplastic particles were observed and categorized into four major types (fiber, fragment, film, and bead; [27]) following protocols from Hidalgo-Ruz et al., [28] and Eppenhumer et al. [29]. 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 used. All these processes were conducted in the laboratory behind closed doors and windows.

Rwatcharee Ruairuen

The author can rewrite this sentence by using this example "The specimens were weighed using an analytical balance.....". In addition, Do you measure the body length of each fish or not? How many fish from each species have been used in this study?

Comment

We have modified the sentence.
We measured the length as well but in this study we focus on the fish weight only.
We used 10 fish for each species. This have mentioned earlier.

Rwatcharee Ruairuen

Is this study considering the microplastic contaminate in fish stomach only not other part of fish organ?

Comment

Yes, only ingested microplastics

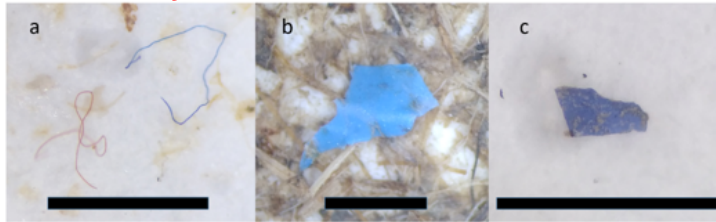
Results

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 (fish, shrimps, crabs and cephalopods) generally smaller in size than their own bodies.

Table 1 Taxonomy, common name, foraging ecology

No.	Taxonomy and Common name	Foraging ecology
1.	Family: Caraugidae Genus: Caraugus Common names: Jacks or trevallies	Most species are demersal , or bottom dwelling, while others are pelagic , moving long distances in the upper water column [30]. All species are predatory fish, taking smaller fish, cephalopods as prey [31].
2.	Family: Lutjanidae Species: Lutjanus fulviflavus (Forsk., 1775) Common names: Blackspot snapper	Many species under this family are demersal and associated with coral reefs [32]. Some of the larger red snapper species descend into deeper waters, at least to 200 m in depth [33]. <i>Lutjanus</i> is predatory fish usually found in tropical and subtropical reefs, and mangrove forests.
3.	Family: Serranidae Epinephelus caralliscala (Kuhl & Hasselt, 1828) 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 [34]. 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 [35].
4.	Family: Lutjanidae Lutjanus johnii (Bloch, 1792) Common names: Golden snapper, or John's snapper	<i>Lutjanus</i> exhibits a two-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) [36]. Feed on fishes and benthic invertebrates including shrimps, crabs and cephalopods. Adults probably frequent coral reef areas; juveniles found in mangrove estuaries [37].
5.	Family: Sphyraenidae Sphyraena isida Cuvier, 1829 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 [38]. Prey on fish, cephalopods, and shrimps [39].

We observed microplastic shapes of fibers, film, fragments (Figure 3). Microplastic beads were not detected in any of our samples. In jacks, coral grouper, golden snapper, and pickhandle barracuda, microplastic particles were identified in 100% of samples, except for blackspot snapper, where they were found in 80% of the samples.



Rwatharee Ruairuen

The first part of your result the author should add a table to explain the characteristics of different five fish species which include common name, species name, number of samples, feeding mode, habitat, mass, length etc.

Comment

The suggested table has been added

Rwatharee Ruairuen

The author should move Table 1 into material and method section.

Hamdhani Hamdhani

Thank you for the suggestion, we have moved the table to the beginning of result section

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 SE, 26 ± 11.0 SE, 15 ± 2.0 SE, 11 ± 1.0 SE, 14 ± 2.0 SE microplastic particles per individual, respectively (**Figure 4A**), and we found in average of 499.5 ± 94.4 SE, 501.5 ± 198.8 SE, 83.6 ± 18.4 SE, 79.8 ± 11.6 SE, 40.2 ± 25.0 SE microplastic particles per kg fish weight, respectively (**Figure 4B**). The blackspot snapper had the highest number of microplastic particles per kilogram of fish weight, reaching 501.5 ± 198.8 SE microplastic particles. The lowest number of particles per kilogram of fish was found in the pickhandle barracuda, which amounted to 40.2 ± 25.0 SE microplastic particles.

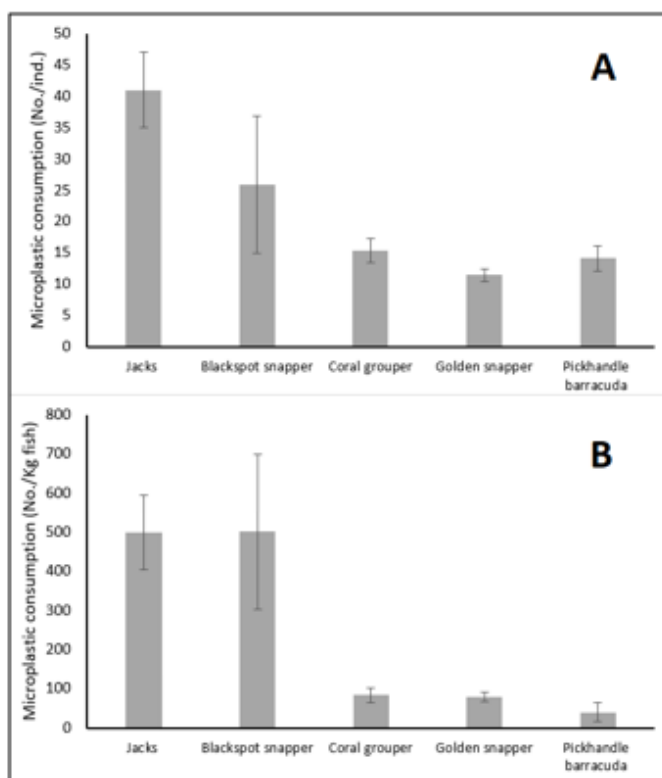


Figure 4 Average of microplastic particles per individual (A) and per kilogram fish weight (B)

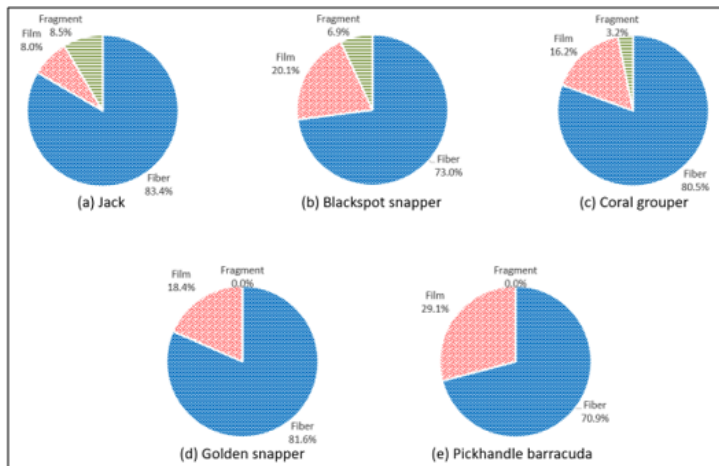


Figure 5 Percentages of the observed microplastic types found in digestive organs from sample fishes collected in the Pangsapang Estuary: (a) Jack, (b) Blackspot snapper, (c) Coral grouper, (d) Golden snapper, and (e) Pickhandle barracuda.

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 5a). 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%, 50% of all samples respectively (Figure 5b). 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%, 40% of our samples respectively (Figure 5c). 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 5d). 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 5e).

Rwatcharee Ruairuen

I recommend to use pie chart to illustrate different type of microplastic contaminate in each fish species. More document that you can use to improving your writing: <https://tis.wu.ac.th/index.php/tis/article/view/3073/189>

Hamdhani Hamdhani

The pie charts have been made and added

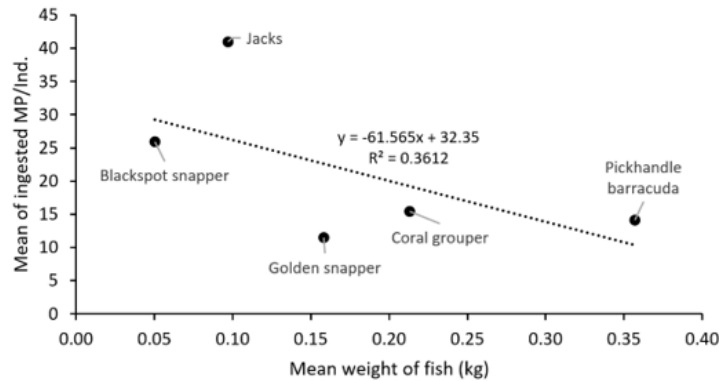


Figure 6 Relationship of mean weight of observed fish and mean number of ingested microplastic particles per species individual

Among the five 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. 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 two is not significant ($P\text{-value} = 0.28$).

Discussion

Types of microplastic

Our results affirmed that fish in the Pangempang Estuary indeed ingest microplastics, with fibers being the predominant type of microplastic. Previous research in aquatic environments has identified fibers as the most prevalent form of microplastics [40-43]. Sources of fibers are predominantly textile goods, such as clothes [44]. Degradation of synthetic fabrics was predicted as major contributor for microplastic fiber abundance in our environment [44,45]. 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 1900 fibers at size less than 1mm in length per wash cycle [46]. 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 discharged into surface waters [46]. 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 jack 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 film microplastic portion was half of the total discovered microplastic particles, and the remaining shared between fiber and fragment [47]. We observed fragment particles was the lowest in number and only found in three of five fish species we examined (jack, blackspot snapper, and coral grouper). In contrast, Karbalas et al. documented that among commercial marine fish in the adjacent waters of Malaysia, fragment-type microplastics were the most prevalent [48]. 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 [49].

Recent investigations into the impact of microplastic fibers on aquatic organisms have unveiled various harmful consequences. According to Liang et al., goldfish exposed to a concentration of 1000 items

Rwatcharee Ruairuen

This figure does not show a clear illustration to me. Considering to modify this graph if possible.

Hamdhani Hamdhani

We believe that this figure is informative, telling the relationship of mean weight of observed fish and mean number of ingested microplastic particles per species individual

Rwatcharee Ruairuen

Rephrase these sentences.

Hamdhani Hamdhani

We have rephrased the sentences

Rwatcharee Ruairuen

Why? Any explanation about this?

Hamdhani Hamdhani

Certainly we do not know why this happened

Rwatcharee Ruairuen

Add three five species

Hamdhani Hamdhani

We have added the three fish names.

per liter 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 [50]. Similarly, a study by Zhao et al. 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 [51]. Another study by Xie et al. noted oxidative stress, dysbiosis in the intestine microbiome, and histological damage as significant symptoms in barramundi exposed to microplastic fibers [52].

Abundance of microplastic in digestive organs

Our finding revealed that jack as individual consumed the highest average of microplastic particles, 41 (SE: 5.98) while golden snapper was observed consuming 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. documented microplastic particle abundance ranged from 9-14 per gastrointestinal tract in multiple fish species caught the Bahía Blanca Estuary in Argentina [53], up to 4 particles in semi pelagic boop boops in the Mediterranean Sea [41], and ranged 1.1- 7.2 microplastic particles in fish collected from China marine waters [40]. On the other hand, the blackspot snapper has the highest amount of microplastics per kilogram of fish, 501.5 ± 198.8 SE, while the pickhandle barracuda has the smallest amount, only 40.2 ± 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 two is not significant ($P\text{-value} = 0.28$). Similarly, Chen et al. reported that fish size did not affect microplastic abundance in the fish digestive organs [54].

Foraging ecology

Microplastic ingestion is potentially influenced by two crucial factors: the type of habitat and the feeding habits of organisms [55]. Within marine environments, scientists have noted markedly greater quantities of microplastics in demersal fish species compared to pelagic species [40,42]. This trend could be linked to the prevalence of plastic waste near the seabed, which is often regarded as the final accumulation for plastics within the marine ecosystem.

Microplastics could potentially be present in fish across different trophic levels. However, current data are insufficient to make definitive conclusions about the absorption of microplastics [56]. In fact, distinguishing between directly and indirectly ingested microplastics is challenging [57]. Walkinshaw et al. performed a semi-systematic review on research examining the presence of microplastics in commercially significant organisms across various trophic levels [58]. 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 are adult predator fish, four fish species are demersal, and one is pelagic (Table 1). The microplastic abundances in the three 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 anticipated 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 [59]. 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 two herbivorous fish species consistently ingest significant quantities of microplastic particles as much as 10-99 microplastic particles per fish gut [60].

Conclusions

For the first time, this study demonstrates the presence of microplastic particles in five commercially important fish in a tropical estuary of Kalimantan Island in Indonesia. The average abundance per fish individual were higher than those documented 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 a significant 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.

This study result also confirms the urgency to observe wide range of fish species in term of their habitat within the water column and as well as the feeding habit. 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. Perhaps analysis based on muscles and internal organs should also be considered in the near future.

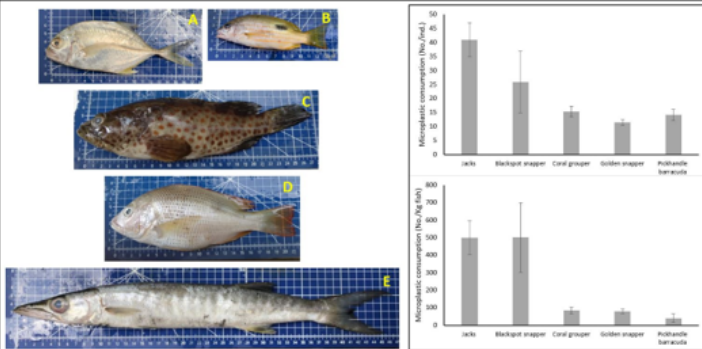
Acknowledgements

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d.	21758-1 - Responses to Reviewer, Reviewer 3 TRENDS IN SCIENCES 202x; xx(xx): xxxxx https://doi.org/ RESEARCH ARTICLE Occurrence of Microplastic Ingestion by Commercial Fish Species from the Pangempang Estuary in Indonesia Highlights • This study demonstrates the presence of microplastic particles in five commercially important fish in a tropical estuary of Kalimantan Island in Indonesia. • Fiber was the most dominant type of microplastic. • Demersal fish may have ingested a higher quantity of microplastic particles compared to pelagic species. Graphical abstract  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 (<i>Caranx</i> SP.), blackspot snapper (<i>Lutjanus fulviflamma</i>), coral grouper (<i>Epinephelus caralligala</i>), golden snapper (<i>Lutjanus johnii</i>), and pickhandle barracuda (<i>Sphyræna jello</i>) 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. <u>Using a dissecting stereo-microscope, microplastic particles in the processed digestive organ contents were identified and enumerated.</u> Microplastic particles in the processed digestive organ contents were identified and enumerated using a dissecting stereo microscope. Microplastics were found in 96% of fish digestive organs (n = 50). In fish samples of jacks, blackspot snapper, coral grouper, golden snapper, and pickhandle barracuda (n = 10 for each species), using digesting method we found in average of 41 ± 6.0 SE, 26 ± 11.0 SE, 15 ± 2.0 SE, 11 ± 1.0 SE, 14 ± 2.0 SE microplastic particles per individual, respectively, and we found in average of 499.5 ± 94.4 SE, 501.5 ± 198.8 SE, 83.6 ± 18.4 SE, 79.8 ± 11.6 SE, 40.2 ± 25.0 SE microplastic particles per kg fish weight, respectively. Fiber was the most dominant type of microplastic. Microplastic beads were not detected. The relationship of the average ingested microplastic and the average fish weight per species show a negative relationship ($R^2 = 0.36$), but insignificant ($P\text{-value} = 0.28$). 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: Borneo, commercial fish, Indonesia, ingestion, pollution	Budhi P It better to mention name of the methods for microplastic quantification Hamdhani Hamdhani Thank you, we have added the method name Budhi P Avoid using subject pronoun, use passive voice Hamdhani Hamdhani Thank you for the suggestion, but recently active voice is equally used in many journals with high reputation. For example: https://onlinelibrary.wiley.com/doi/epdf/10.1111/fwb.13831 Budhi P What does it mean by consumed here, number of microplastic found ? Hamdhani Hamdhani We have replaced into ingested Budhi P Avoid subject pronoun, using passive voice Hamdhani Hamdhani Similar response as above Budhi P This conclusions must be adjusted , since only one type of pelagic fish was observed Hamdhani Hamdhani Thank you for pointing out this issues. We will adjust as necessary. In this study, we only observed whatever fish species that the encountered fishermen caught in this estuary without considering pelagic or demersal. This is the reason we do not have equal representation of pelagic and demersal fish. Budhi P Put in alphabetical order Hamdhani Hamdhani We have ordered aphabetically
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Introduction

After invented over 110 years ago, plastic materials become ubiquitous in the environment. Plastic material is made from polymers that can be shaped easily for many varieties of uses. The elevated desire to use plastic materials due to their affordability, bio-inertia, high strength to weight ratio, and resistance to degradation. These characteristics has made plastic materials become important materials for human life nowadays. By the year of 2017, the total global production of plastic was estimated to reach 8300 million metric tons [1], with the rate of production has reached 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 suspected responsible to bring 88%-95% of global marine debris load into ocean [4]. The proportion of plastics usually take about 70% of total marine debris [5].

Initially regarded as a safe material, several decades of plastic production have brought a wide range of environmental problems, particularly in marine ecosystems. In oceans, even though plastic is considered as durable material and resistant to natural biodegradation. However, 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) [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 from the surrounding [11]. Cole et al. reported that ingestion of microplastic particles by zooplanktons shows negative impacts on feeding, hatching, and their health [12]. 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 a type of marine ecosystems often utilized for fisheries purposes. [16,17], and these waters 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 estuary is problematic as estuaries also provide crucial habitat for a variety of commercially and recreationally important species such as crabs, fish, and shellfish [20]. Numerous studies investigating microplastics have placed their emphasis on ecosystems in both marine and inland environments [21,22,23]. However, there have been limited evaluation on microplastic contamination in transitional waters between the two ecosystems, such as estuarine waters, allowing analysis of microplastic concentrations ingested by some commercial fish. To address this knowledge gap, ~~we evaluated-evaluation of~~ the types and abundance of microplastic particles in available commercial fish samples collected from Pangempang Estuary ~~was important~~ and ~~asked~~ the main following questions ~~is~~ : what ~~is~~ type of microplastic? And how is the abundance of microplastic in fish collected from this transitional waters?

Materials and methods

Site description and Sampling

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. The Pangempang River spans approximately 26 kilometers, originating from the Marangkayu reservoir. Its

width ranges from 8 meters upstream to 140 meters downstream. The river passes through the Marangkayu Town settlement, situated roughly 20 kilometers upstream from the Pangempang Estuary. Additionally, about 7 kilometers 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.

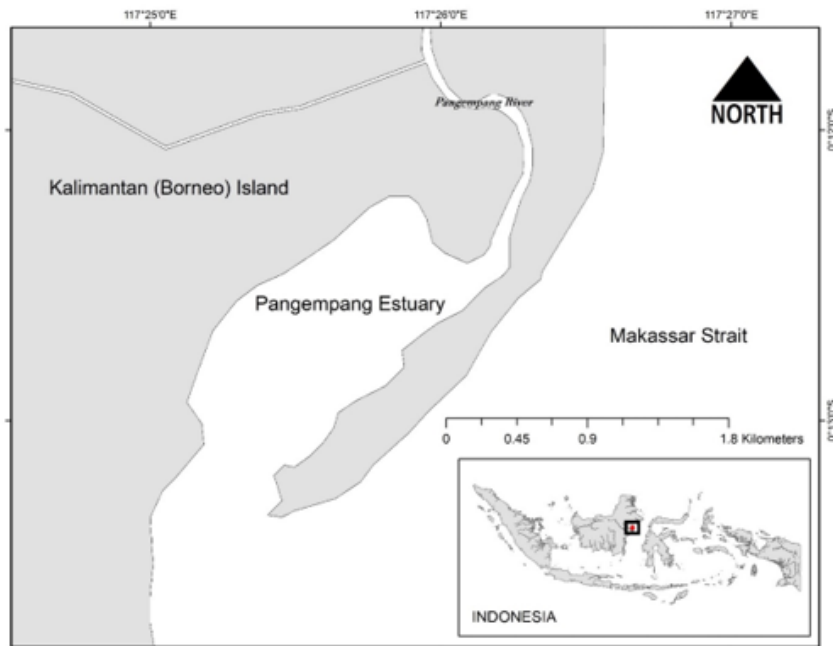


Figure 1 The Pangempang Estuary (East Kalimantan, Indonesia)

To evaluate the ingestion of microplastics by fish, we obtained fish specimens from local fishermen operating in the waters of the Pangempang Estuary. We were able to collect ten replicates of each five different species from these fishermen. The species we identified were jack (*Caranx* SP.), blackspot snapper (*Lutjanus fulvivlamma*), coral grouper (*Epinephelus corallicola*), golden snapper (*Lutjanus johnii*), and pickhandle barracuda (*Sphyræna jello*). We conducted the identification of these fish species and their habitats based on the information provided in the book titled "Marine Fishes and Crustaceans of the Southeast Asian Region" [24]. Each individual fish was placed in plastic bags, and then stored ~~on~~ in ice ~~cube~~ during transport to the Water Quality Laboratory at Mulawarman University. The samples were preserved in a freezer before processing and analysis.

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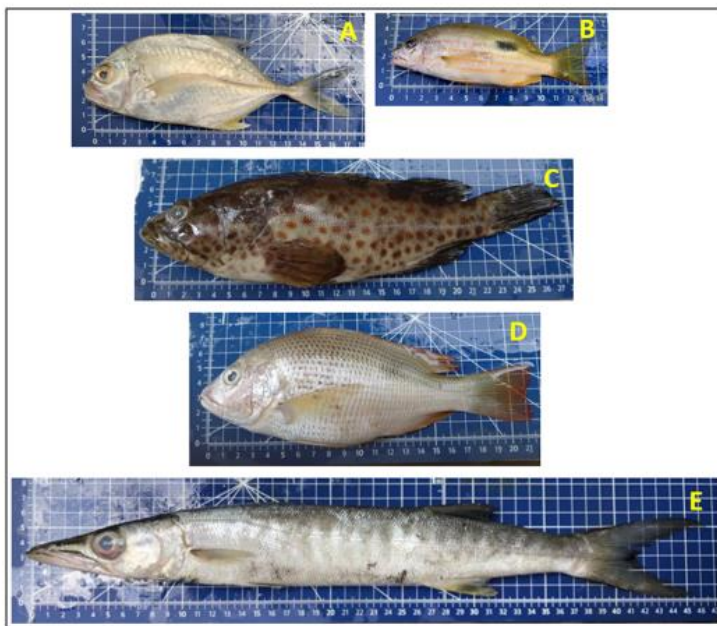


Figure 2 Photographs illustrating the observed fish species: (A) Jack (*Caranx* SP.), (B) blackspot snapper (*Lutjanus fulviflamma*), (C) coral grouper (*Epinephelus caralligala*), (D) golden snapper (*Lutjanus johnii*), and (E) pickhandle barracuda (*Sphyræna jello*).

Laboratory processing

We measured fish weight (g) of each specimen. 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, each stomach content was digested using alkaline digestion solution of potassium hydroxide (KOH) [25,26]. Each stomach content was placed in a beaker and 10% KOH was added allowing submersion of stomach contents in the beaker. Each beaker was covered with aluminum foil. We stirred the samples in the beaker using magnetic spinner at 350 rpm for 2 hours. 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% H₂O₂ was added and then stirred. After 1 hour stirring, samples were vacuum pump filtered on 1.6 µm pore size Whatman paper and then dried.

Using a 10-45× magnification dissecting microscope, microplastic particles in the processed stomach contents were identified and enumerated. The microplastic particles were observed and categorized into four major types (fiber, fragment, film, and bead; [27]) following protocols from Hidalgo-Ruz et al., [28] and Eppehimer et al. [29]. 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 used. All these processes were conducted in the laboratory behind closed doors and windows.

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Mention the method, and the reference

Hamdhani Hamdhani

We used **digestive method** to separate MP and organic material. The reference is already cited (You may not realize as this publication is using numbers for cited references)

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Please mention how to extract microplastics from fish organ

Hamdhani Hamdhani

Using digestive method as explained

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The used statistical analysis is not mention in the Method yet

Hamdhani Hamdhani

Thank you for pointing this issue out. We have added the "Data analysis" section

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) [62] to determine the trend and significance of fish body weight (independent variable) in affecting the abundance of ingested microplastics (dependent variable).

Results

We observed microplastic shapes of fibers, film, fragments. Microplastic beads were not detected in any of our samples. In jacks, coral grouper, golden snapper, and pickhandle barracuda, we detected microplastic particles in 100% of samples, except for blackspot snapper only 80% of samples.

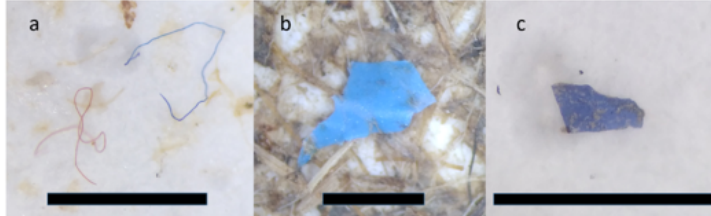


Figure 3 Photographs illustrating the observed microplastic types found in digestive organs from sample fishes collected in the Pangempang Estuary: a. fiber, b. film, and c. fragment. Horizontal scale bar ≈ 0.5 mm

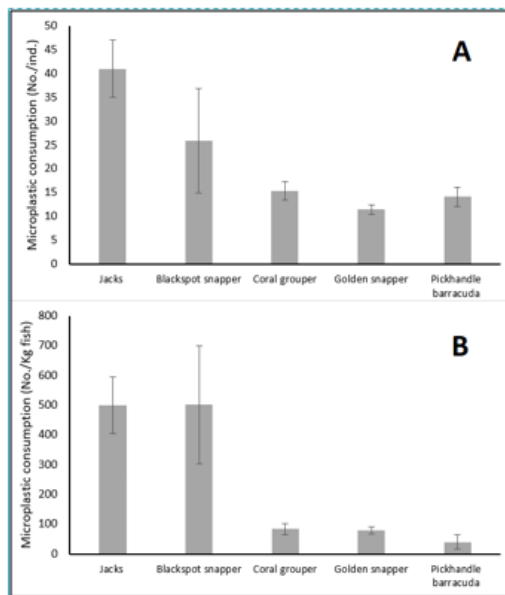
In fish samples of jacks, blackspot snapper, coral grouper, golden snapper, and pickhandle barracuda, we found in average of 41 ± 6.0 SE, 26 ± 11.0 SE, 15 ± 2.0 SE, 11 ± 1.0 SE, 14 ± 2.0 SE microplastic particles per individual, respectively, and we found in average of 499.5 ± 94.4 SE, 501.5 ± 198.8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

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Don't need to put words SE after +_

Hamdhani Hamdhani

We put SE to indicated that as a Standard Error, and not as a Standard Deviation (SD)



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Y axis --> Mp ingested

X axis --> type of fish

Hamdhani Hamdhani

We have modified the graph as suggested.

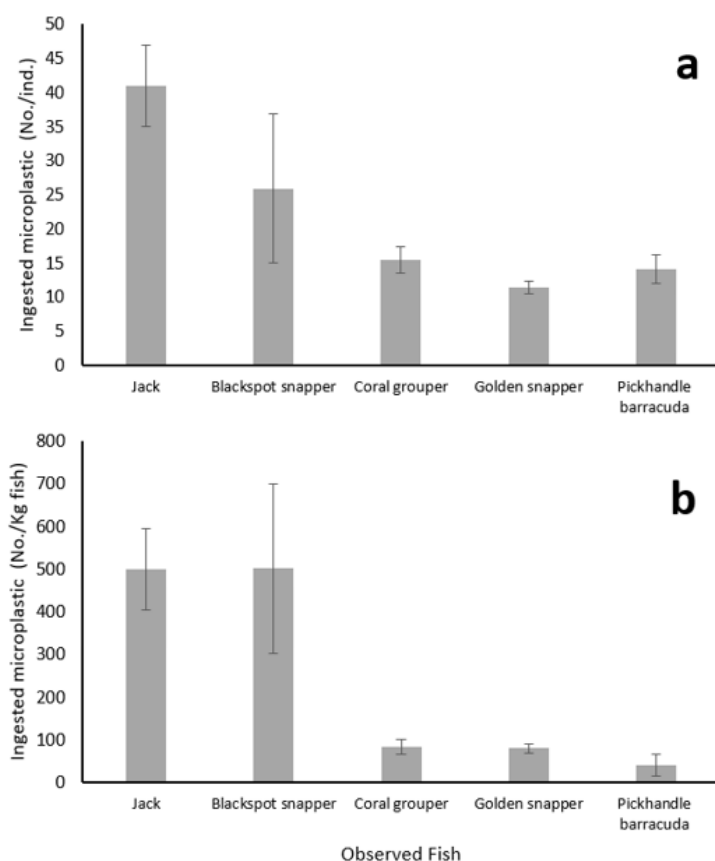


Figure 4 Average of microplastic particles per individual (a) and per kilogram fish weight (b)

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.

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%, 50% of all samples respectively.

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%, 40% of our samples respectively.

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Confusing sentence

Hamdhani Hamdhani
We have added some pie chart graphs, as also suggested by other reviewer.

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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.

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.

Among the five 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. The relationship of the average consumed microplastic particles and the average fish weight per species (Figure 3) show a negative relationship ($R^2 = 0.36$), but the relationship between the two is not significant (P -value = 0.28).

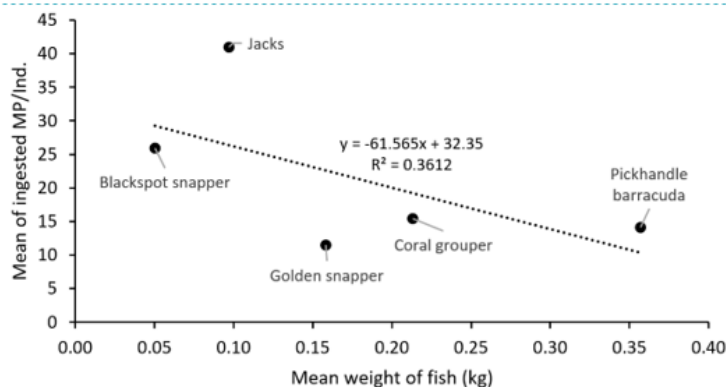


Figure 5 Relationship of mean weight of observed fish and mean number of ingested microplastic particles per individual

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 (fish, shrimps, crabs and cephalopods) generally smaller in size than their own bodies.

Table 1 Taxonomy, common name, foraging ecology

No.	Taxonomy and Common name	Foraging ecology
1.	Family: Carangidae Genus: Caranx Common names: Jacks or trevallies	Most species are demersal , or bottom dwelling, while others are pelagic , moving long distances in the upper water column [30]. All species are predatory fish, taking smaller fish, cephalopods as prey [31].

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The yellow highlighted data can also be presented as graph

Hamdhani Hamdhani

We have added some pie chart graphs, as also suggested by other reviewer.

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The 0.36R2 sounds is not a confidence value to sum-up the relationship between the two paramaters

Hamdhani Hamdhani

Yes, we agree.

2.	Family: Lutjanidae Species: <i>Lutjanus fulvivflamma</i> (Forsskal, 1775) Common names: Blackspot snapper	Many species under this family are demersal and associated with coral reefs [32]. Some of the larger red snapper species descend into deeper waters, at least to 200 m in depth [33]. <i>Lutjanus</i> is predatory fish usually found in tropical and subtropical reefs, and mangrove forests.
3.	Family: Serranidae <i>Epinephelus caralligala</i> (Kuhl & Hasselt, 1828) 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 [34]. 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 [35].
4.	Family: Lutjanidae <i>Lutjanus johnii</i> (Bloch, 1792) Common names: Golden snapper, or John's snapper	<i>Lutjanus</i> exhibits a two-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) [36]. Feed on fishes and benthic invertebrates including shrimps, crabs and cephalopods. Adults probably frequent coral reef areas; juveniles found in mangrove estuaries [37].
5.	Family: Sphyraenidae <i>Sphyraena jello</i> Cuvier, 1829 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 [38]. Prey on fish, cephalopods, and shrimps [39].

Discussion

Types of microplastic

Our findings confirmed that fish in Pangempang Estuary do consume microplastics and fibers were the most dominant type of microplastic. In the aquatic environment, ample studies reported that fibers are the most encountered microplastic type [40–43]. Sources of fibers are predominantly textile goods, such as clothes [44]. Degradation of synthetic fabrics was predicted as major contributor for microplastic fiber abundance in our environment [44,45]. 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 1900 fibers at size less than 1mm in length per wash cycle [46]. 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 discharged into surface waters [46]. 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 film microplastic portion was half of the total discovered microplastic particles, and the remaining shared between fiber and fragment [47]. We observed fragment particles was the lowest in number and only found in three of five fish species we examined. In contrast, Karbalaei et al. documented that among commercial marine fish in the adjacent waters of Malaysia, fragment-type microplastics were the most prevalent [48]. 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 [49].

Recent investigations into the impact of microplastic fibers on aquatic organisms have unveiled various harmful consequences. According to Liang et al., goldfish exposed to a concentration of 1000 items per liter 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 [50]. Similarly, a study by Zhao et al. 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 [51]. Another study by Xie et al. noted oxidative stress, dysbiosis in the intestine microbiome, and histological damage as significant symptoms in barramundi exposed to microplastic fibers [52].

Abundance of microplastic in digestive organs

Our finding revealed that jack as individual consumed the highest average of microplastic particles, 41 (SE: 5.98) while golden snapper was observed consuming 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. documented microplastic particle abundance ranged from 9 - 14 in digestive organs per individual of multiple fish species caught the Bahia Blanca Estuary in Argentina [53], up to 4 particles in semi pelagic boop boops in the Mediterranean Sea [41], and ranged 1.1 - 7.2 microplastic particles in fish collected from China marine waters [40]. 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/fish [62].

On the other hand, the blackspot snapper has the highest amount of microplastics per kilogram of fish, 501.5 ± 198.8 SE, while the pickhandle barracuda has the smallest amount, only 40.2 ± 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 two is not significant ($P\text{-value} = 0.28$). Similarly, Chen et al. reported that fish size did not affect microplastic abundance in the fish digestive organs [54].

Foraging ecology

Microplastic ingestion is potentially influenced by two crucial factors: the type of habitat and the feeding habits of organisms [55]. Within marine environments, scientists have noted markedly greater quantities of microplastics in demersal fish species compared to pelagic species [40,42]. This trend could be linked to the prevalence of plastic waste near the seabed, which is often regarded as the final accumulation for plastics within the marine ecosystem.

Microplastics could potentially be present in fish across different trophic levels. However, current data are insufficient to make definitive conclusions about the absorption of microplastics [56]. In fact, distinguishing between directly and indirectly ingested microplastics is challenging [57]. Walkinshaw et al. in the year of 2020 performed a semi-systematic review on research examining the presence of microplastics in commercially significant organisms across various trophic levels [58]. 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 are adult predator fish, four fish species are demersal, and one is pelagic (Table 1). The microplastic abundances in the three 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 anticipated 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 [59]. 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 two herbivorous fish species consistently ingest significant quantities of microplastic particles as much as 10-99 microplastic particles per fish gut [60].

Conclusions

For the first time, this study demonstrates the presence of microplastic particles in five commercially important fish in a tropical estuary of Kalimantan Island in Indonesia. The average abundance per fish individual were higher than those documented 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 a significant portion of the microplastic pollution found in the digestive systems of sampled fish from the Pangempang Estuary.

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Hamdhani Hamdhani

This is referred to a study conducted in an estuary in Argentina

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1.1-7.2 microplastics per fish ?? Or per kg ??

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Average per fish

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Year ?

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The year has been added as suggested

Budhi P

No fair comparison for conclusion, since 4 type of demersal, but only 1 type of pelagic fish

Hamdhani Hamdhani

Yes, that is true. This is because our sample was gained from what the fishermen caught only. In fact from the catch, the two type of fish was not balance.

Budhi P

Discussion regarding the comprison between the estary's fish and river-fish or sea-fih will be beneficial, and add enrich the discussion, as well as the river and se microplastic data.

It will be better if the size of ingested microplastic is also discussed

Hamdhani Hamdhani

In the "Abundance of microplastic in digestive organs" section, we have added more discussion as suggested to compared with microplastic in river fish.

Unfortunately, we did not measure the individual microplastic particle size

Our research suggests that demersal fish may have ingested a higher quantity of microplastic particles compared to pelagic species.

This study result also confirms the urgency to observe wide range of fish species in term of their habitat within the water column and as well as the feeding habit. 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. Perhaps analysis based on muscles and internal organs should also be considered in the near future.

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We thank Baitur Rohmah, Dewi Anjani, Cicilia Imelda Arintonang dan Dinda Qurrata Ayun for their help during laboratory processing.

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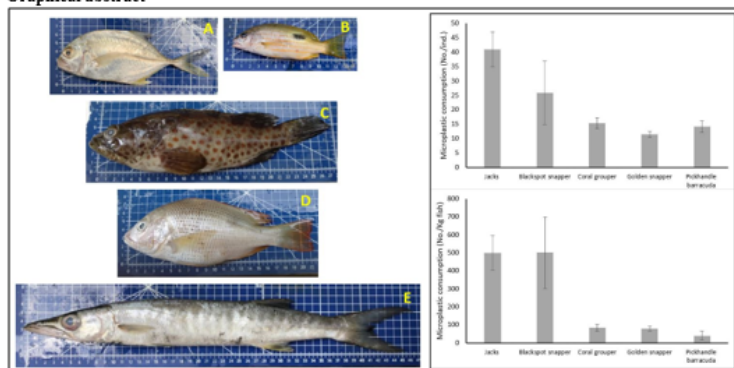
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Occurrence of Microplastic Ingestion by Commercial Fish Species from the Pangempang Estuary in Indonesia

Highlights

- This study demonstrates the presence of microplastic particles in five commercially important fish in a tropical estuary of Kalimantan Island in Indonesia.
- Fiber was the most dominant type of microplastic.
- Demersal fish may have ingested a higher quantity of microplastic particles compared to pelagic species.

Graphical abstract



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 fulvivlamma*), coral grouper (*Epinephelus caralligala*), 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. Using a dissecting stereo microscope, microplastic particles in the processed digestive organ contents were identified and enumerated. Microplastics were found in 96% of fish digestive organs (n = 50). In fish samples of jacks, blackspot snapper, coral grouper, golden snapper, and pickhandle barracuda (n = 10 for each species), we found in average of 41 ± 6.0 SE, 26 ± 11.0 SE, 15 ± 2.0 SE, 11 ± 1.0 SE, 14 ± 2.0 SE microplastic particles per individual, respectively, and we found in average of 499.5 ± 94.4 SE, 501.5 ± 198.8 SE, 83.6 ± 18.4 SE, 79.8 ± 11.6 SE, 40.2 ± 25.0 SE microplastic particles per kg fish weight, respectively. Fiber was the most dominant type of microplastic. Microplastic that beads were not detected. The correlation of the average consumed microplastic and the average fish weight per species show a negative relationship ($R^2 = 0.36$), but insignificant (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.

Yayan
Check
sp or spp?

Hamdhani Hamdhani
We use "sp" because it is only one kind and we only able to identified up to the genus.

Yayan
Check

Hamdhani Hamdhani
Good catch. Thank you

Introduction

After invented over 110 years ago, plastic materials become ubiquitous in the environment. Plastic material is made from polymers that can be shaped easily for many varieties of uses. The elevated desire to use plastic materials due to their affordability, bio-inertia, high strength to weight ratio, and resistance to degradation. These characteristics has made plastic materials become important materials for human life nowadays. By the year of 2017, the total global production of plastic was estimated to reach 8300 million metric tons [1], with the rate of production has reached 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 suspected responsible to bring 88%-95% of global marine debris load into ocean [4]. The proportion of plastics usually take about 70% of total marine debris [5].

Initially regarded as a safe material, several decades of plastic production have brought a wide range of environmental problems, particularly in marine ecosystems. In oceans, even though plastic is considered as durable material and resistant to natural biodegradation. However, 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) [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 from the surrounding [11]. Cole et al. reported that ingestion of microplastic particles by zooplanktons shows negative impacts on feeding, hatching, and their health [12]. 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 a type of marine ecosystems often utilized for fisheries purposes. [16,17], and these waters 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 estuary is problematic as estuaries also provide crucial habitat for a variety of commercially and recreationally important species such as crabs, fish, and shellfish [20]. Numerous studies investigating microplastics have placed their emphasis on ecosystems in both marine and inland environments [21,22,23]. However, there have been limited evaluation on microplastic contamination in transitional waters between the two 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 and asked the main following questions: what type of microplastic? And how is the abundance of microplastic in fish collected from this transitional waters?

Materials and methods

Site description and Sampling

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. The Pangempang River spans approximately 26 kilometers, originating from the Marangkayu

Yayan

We don't see any commercial fish issues in your introduction. If commercial fish is contaminated with microplastics, why many people eat it? And we should replace with other food products that are not contaminated.

Hamdhani Hamdhani

The effect of consuming fish that ingested microplastics to human need more studies. In other word we do not really know, yet.

Yayan

In what month and year did you take the sample? Season is a factor that influences MPs in the environment, in what season do you take the samples?

Why isn't the total length measured?

Hamdhani Hamdhani

We have added the information when the sampling was conducted.

reservoir. Its width ranges from 8 meters upstream to 140 meters downstream. The river passes through the Marangkayu Town settlement, situated roughly 20 kilometers upstream from the Pangempang Estuary. Additionally, about 7 kilometers 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.

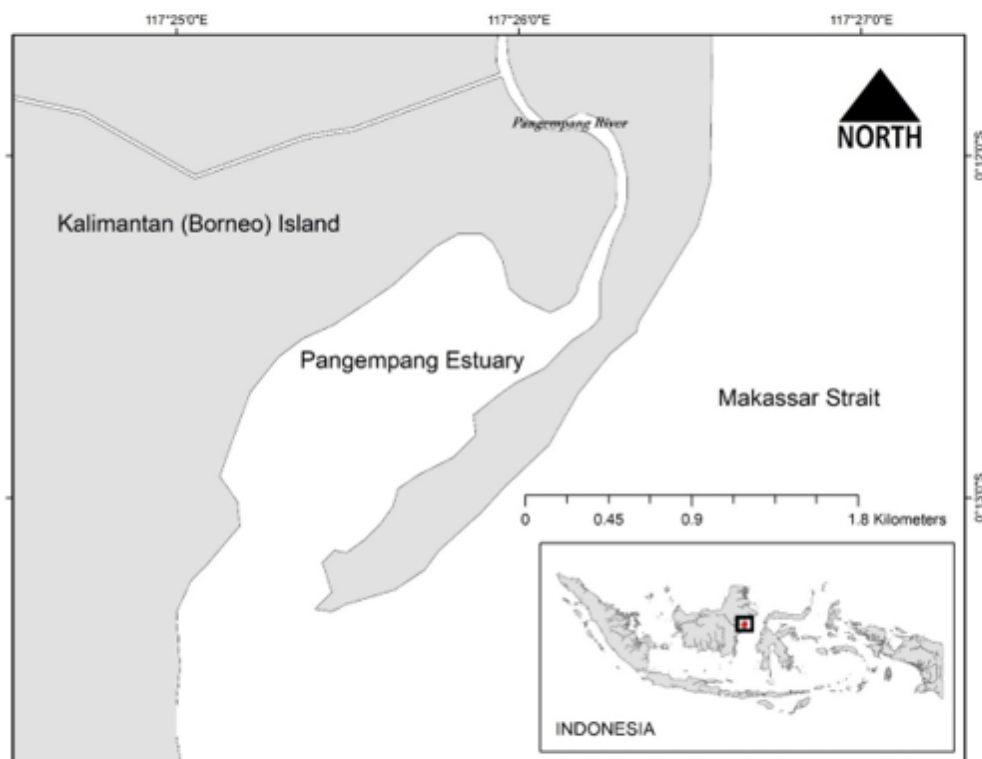


Figure 1 The Pangempang Estuary (East Kalimantan, Indonesia)

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 15th, 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 (Cleary, 2011). We were able to collect ten replicates of each five different species from these fishermen. The species we identified were jack (*Caranx* SP.), blackspot snapper (*Lutjanus fulviflamma*), coral grouper (*Epinephelus caralligala*), golden snapper (*Lutjanus johnii*), and pickhandle barracuda (*Sphyrna jello*). We conducted the identification of these fish species and their habitats based on the information provided in the book titled "Marine Fishes and Crustaceans of the Southeast Asian Region" [24]. Each individual fish was placed in plastic bags, 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.

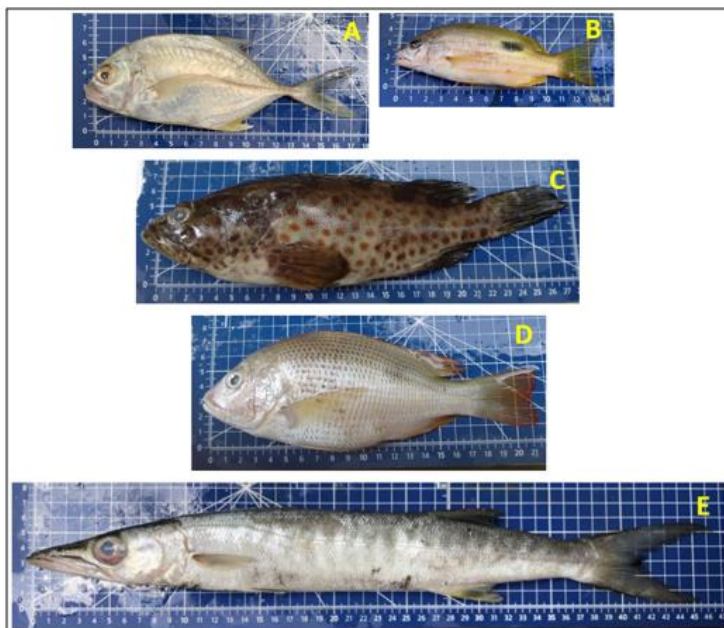


Figure 2 Photographs illustrating the observed fish species: (A) Jack (*Caranx* SP.), (B) blackspot snapper (*Lutjanus fulviflamma*), (C) coral grouper (*Epinephelus corallicola*), (D) golden snapper (*Lutjanus johnii*), and (E) pickhandle barracuda (*Sphyrna jello*).

Laboratory processing

We measured fish weight (g) of each specimen. 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, each stomach content was digested using alkaline digestion solution of potassium hydroxide (KOH) [25,26]. Each stomach content was placed in a beaker and 10% KOH was added allowing submersion of stomach contents in the beaker. Each beaker was covered with aluminum foil. We stirred the samples in the beaker using magnetic spinner at 350 rpm for 2 hours. 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 hour stirring, samples were vacuum pump filtered on 1.6 μm pore size Whatman paper and then dried.

Using a 10-45 $\times$ magnification dissecting microscope, microplastic particles in the processed stomach contents were identified and enumerated. The microplastic particles were observed and categorized into four major types (fiber, fragment, film, and bead; [27]) following protocols from Hidalgo-Ruz et al., [28] and Eppehimer et al. [29]. 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 used. All these processes were conducted in the laboratory behind closed doors and windows.

How do you calculate the microplastics in the sample? And what's the unit it? Describe please the equations based on percent, number/individual, weight and correlation.

Hamdhani Hamdhani

To address this question we have added a "Data analysis" section below

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) [62] to determine the trend and significance of fish body weight (independent variable) in affecting the abundance of ingested microplastics (dependent variable).

Results

We observed microplastic shapes of fibers, film, fragments. Microplastic beads were not detected in any of our samples. In jacks, coral grouper, golden snapper, and pickhandle barracuda, we detected microplastic particles in 100% of samples, except for blackspot snapper only 80% of samples.

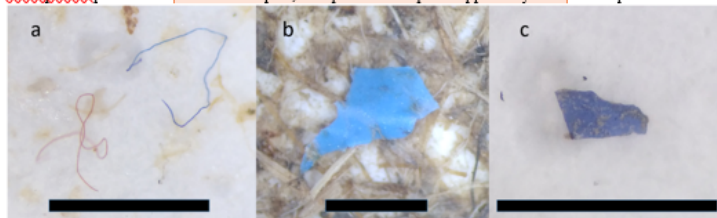


Figure 3 Photographs illustrating the observed microplastic types found in digestive organs from sample fishes collected in the Pongemang Estuary: a. fiber, b. film, and c. fragment. Horizontal scale bar ≈ 0.5 mm

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

Yayan

Using latin name is better. For all in results and discussions

Hamdhani Hamdhani

We prefer to use common name as this way is also used in other international publications that we cited.

Yayan

There is no figure or table for percent

Hamdhani Hamdhani

We just confirming that only blackspot snapper that had specimen (20%) with no microplastic particle in the digestive organs, so we think that mentioning in the text is sufficient.

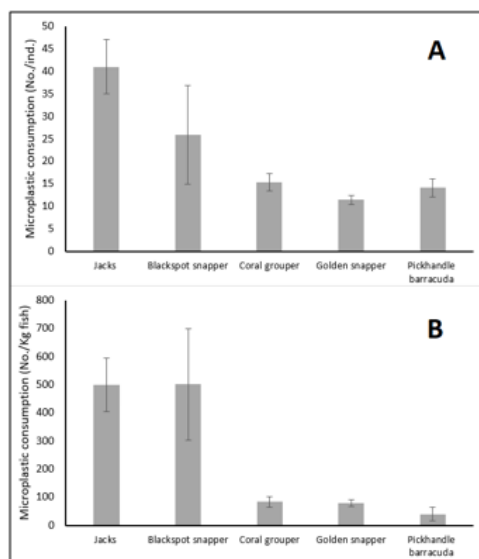


Figure 4 Average of microplastic particles per individual (A) and per kilogram fish weight (B)

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. 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%, 50% of all samples respectively. 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%, 40% of our samples respectively. 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. 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.

Among the five 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. The relationship of average consumed microplastic particles and the average fish weight per species (Figure 3) show a negative relationship ($R^2 = 0.36$), but the relationship between the two is not significant ($P\text{-value} = 0.28$).

Yayan

Each paragraph consists of 3 sentences (minimum)
Check at all paragraph

Hamdhani Hamdhani

Thank you for pointing the issue. We have gathered these sentences into one paragraph.

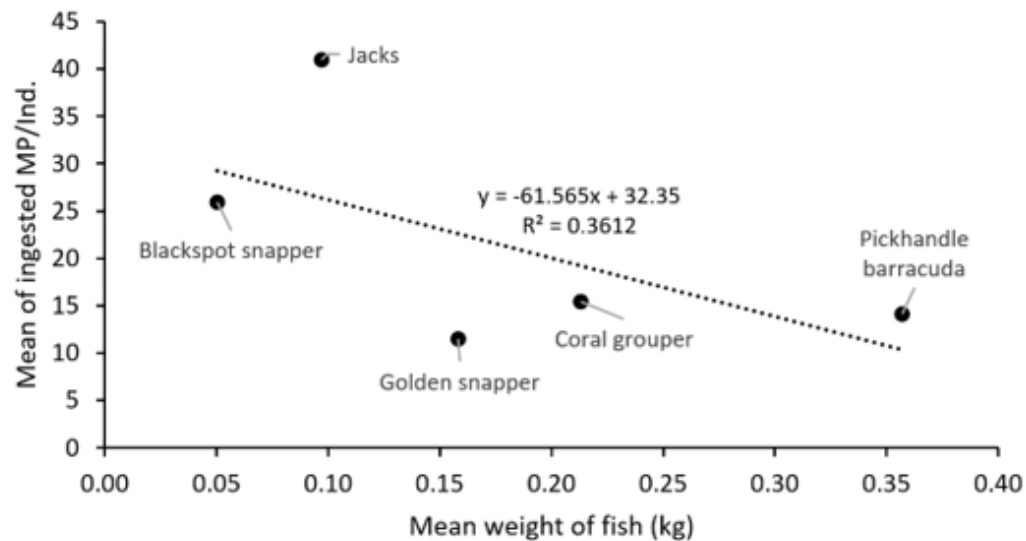


Figure 5 Relationship of mean weight of observed fish and mean number of ingested microplastic particles per individual

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 (fish, shrimps, crabs and cephalopods) generally smaller in size than their own bodies.

Table 1 Taxonomy, common name, foraging ecology

No.	Taxonomy and Common name	Foraging ecology
1.	Family: <i>Carangidae</i> Genus: <i>Caranx</i> Common names: Jacks or trevallies	Most species are demersal , or bottom dwelling, while others are pelagic , moving long distances in the upper water column [30]. All species are predatory fish, taking smaller fish, cephalopods as prey [31].
2.	Family: <i>Lutjanidae</i> Species: <i>Lutjanus fulviflamma</i> (Forsskal, 1775) Common names: Blackspot snapper	Many species under this family are demersal and associated with coral reefs [32]. Some of the larger red snapper species descend into deeper waters, at least to 200 m in depth [33]. <i>Lutjanus</i> is predatory fish usually found in tropical and subtropical reefs, and mangrove forests.
3.	Family: <i>Serranidae</i> <i>Epinephelus caralligala</i> (Kuhl & Hasselt, 1828) 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 [34]. 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 [35].
4.	Family: <i>Lutjanidae</i> <i>Lutjanus johnii</i> (Bloch, 1792) Common names: Golden snapper, or John's snapper	<i>Lutjanus</i> exhibits a two-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) [36]. Feed on fishes and benthic invertebrates including shrimps, crabs and cephalopods. Adults probably frequent coral reef areas; juveniles found in mangrove estuaries [37].
5.	Family: <i>Sphyraenidae</i>	They are migratory pelagic predators and usually be found

<i>Sphyrna jello</i> Cuvier, 1829 Common names: Pickhandle barracuda or Banded barracuda	in schools swimming in the tropical waters, predominantly nearby coral reefs [38]. Prey on fish, cephalopods, and shrimps [39].
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Discussion

Types of microplastic

Our findings confirmed that fish in Pangempang Estuary do consume microplastics and fibers were the most dominant type of microplastic. In the aquatic environment, ample studies reported that fibers are the most encountered microplastic type [40-43]. Sources of fibers are predominantly textile goods, such as clothes [44]. Degradation of synthetic fabrics was predicted as major contributor for microplastic fiber abundance in our environment [44,45]. 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 1900 fibers at size less than 1mm in length per wash cycle [46]. 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 discharged into surface waters [46]. 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 film microplastic portion was half of the total discovered microplastic particles, and the remaining shared between fiber and fragment [47]. We observed fragment particles was the lowest in number and only found in three of five fish species we examined. In contrast, Karbalaei et al. documented that among commercial marine fish in the adjacent waters of Malaysia, fragment-type microplastics were the most prevalent [48]. 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 [49].

Recent investigations into the impact of microplastic fibers on aquatic organisms have unveiled various harmful consequences. According to Liang et al., goldfish exposed to a concentration of 1000 items per liter 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 [50]. Similarly, a study by Zhao et al. 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 [51]. Another study by Xie et al. noted oxidative stress, dysbiosis in the intestine microbiome, and histological damage as significant symptoms in barramundi exposed to microplastic fibers [52].

Abundance of microplastic in digestive organs

Our finding revealed that jack as individual consumed the highest average of microplastic particles, 41 (SE: 5.98) while golden snapper was observed consuming 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. documented microplastic particle abundance ranged from 9-14 per gastrointestinal tract in multiple fish species caught the Bahía Blanca Estuary in Argentina [53], up to 4 particles in semi pelagic boop boops in the Mediterranean Sea [41], and ranged 1.1- 7.2 microplastic particles in fish collected from China marine waters [40]. On the other hand, the blackspot snapper has the highest amount of microplastics per kilogram of fish, 501.5 ± 198.8 SE, while the pickhandle barracuda has the smallest amount, only 40.2 ± 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 two is not significant ($P\text{-value} = 0.28$). Similarly, Chen et al. reported that fish size did not affect microplastic abundance in the fish digestive organs [54].

Foraging ecology

Microplastic ingestion is potentially influenced by two crucial factors: the type of habitat and the feeding habits of organisms [55]. Within marine environments, scientists have noted markedly greater quantities of microplastics in demersal fish species compared to pelagic species [40,42]. This trend could be linked to the prevalence of plastic waste near the seabed, which is often regarded as the final accumulation for plastics within the marine ecosystem.

Microplastics could potentially be present in fish across different trophic levels. However, current data are insufficient to make definitive conclusions about the absorption of microplastics [56]. In fact, distinguishing between directly and indirectly ingested microplastics is challenging [57]. Walkinshaw et al. performed a semi-systematic review on research examining the presence of microplastics in commercially significant organisms across various trophic levels [58]. 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 are adult predator fish, four fish species are demersal, and one is pelagic (Table 1). The microplastic abundances in the three 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 anticipated 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 [59]. 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 two herbivorous fish species consistently ingest significant quantities of microplastic particles as much as 10-99 microplastic particles per fish gut [60].

Conclusions

For the first time, this study demonstrates the presence of microplastic particles in five commercially important fish in a tropical estuary of Kalimantan Island in Indonesia. The average abundance per fish individual were higher than those documented 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 a significant 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.

This study result also confirms the urgency to observe wide range of fish species in term of their habitat within the water column and as well as the feeding habit. 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. Perhaps analysis based on muscles and internal organs should also be considered in the near future.

Acknowledgements

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4	Notifikasi Revisi Round 2	5 Januari 2024	a. Email konfirmasi b. Laman Revisi 2 di akun jurnal
a.	Email konfirmasi		
	6/14/26, 2:16 PM Universitas Mulawarman Mail - [TiS] Manuscript Decision  Hamdhani Hamdhani <hamdhani@fpik.unmul.ac.id> [TiS] Manuscript Decision 1 message Trends in Sciences (TiS) <journal.wu@gmail.com> To: Hamdhani Hamdhani <hamdhani@fpik.unmul.ac.id>, Ghitarina Ghitarina <ghitarina@fpik.unmul.ac.id>, Ristiana Eryati <eryati.ristiana@gmail.com>, "Drew E. Eppehimer" <deppehimer@arizona.edu> Fri, Jan 5, 2024 at 4:59 PM Dear Dr. Hamdhani Hamdhani, Ghitarina Ghitarina, Ristiana Eryati, Drew E. Eppehimer: We have reached a decision regarding your submission to Trends in Sciences, "Occurrence of Microplastic Ingestion by Commercial Fish Species from the Pangempang Estuary in Indonesia". Please revise the manuscript carefully. The manuscript should be resubmitted along with point-by-point explanation according to reviewers' comments. If you disagree with any of the comments, please state your reasons. All corrections are mandatory and must be differentiated with red colour and submit it. Our decision is to: Revise the Manuscript We request that you send a revised manuscript within 7 days, otherwise it may be considered withdrawn. PS. Please submit in Microsoft Word version with TiS template, the references should be in TiS format, please recheck. Thank you very much for your kind helps. STEP for upload revised version: Submissions > Workflow > My Assigned > ID Number > Review > Revisions > Upload file Author Guidelines: https://tis.wu.ac.th/index.php/tis/about/submissions Manuscript URL: https://tis.wu.ac.th/index.php/tis/authorDashboard/submission/7762 Reviewer A: Recommendation: Revisions Required Originality of the work Adequate Experimental design and methodology Adequate Adequacy of the discussion Good Technical accuracy Good https://mail.google.com/mail/u/3/?ik=35ace941da&view=pt&search=all&permthid=thread-f:1787240366595394382&simpl=msg-f:1787240366595394382 1/5		

Suitability of references

Good

Use of Tables and Figures

Good

Standard of English

Adequate

Clarity and conciseness

Good

Referee's Comments

I recommend the author consider Table 1 as one element in your material and methods section because these information is not your result. It's explain the fish characteristic for your study. However, in the result section an average of ten replicates of each five fish in term or lenght and weight should illustrated in the table.

Reviewer B:
Recommendation: Accept Submission

Originality of the work

Good

Experimental design and methodology

Good

Adequacy of the discussion

Good

Technical accuracy

Good

Suitability of references

<https://mail.google.com/mail/u/3/?ik=35ace941da&view=pt&search=all&permthid=thread-f:1787240366595394382&simpl=msg-f:1787240366595394382> 2/5

Good

Use of Tables and Figures

Good

Standard of English

Adequate

Clarity and conciseness

Good

Referee's Comments

The suggested comments were corrected.

Reviewer C:
Recommendation: Accept Submission

Originality of the work

Adequate

Experimental design and methodology

Adequate

Adequacy of the discussion

Adequate

Technical accuracy

Adequate

Suitability of references

Adequate

Use of Tables and Figures

Adequate

Standard of English

Adequate

Clarity and conciseness

Adequate

Referee's Comments

Most of the suggested comments from the reviewer have been accepted, and revised as suggested. I think we may recommend the articles be published in this journal.

Reviewer D:
Recommendation: Accept Submission

Originality of the work

Good

Experimental design and methodology

Good

Adequacy of the discussion

Good

Technical accuracy

Good

Suitability of references

Good

Use of Tables and Figures

Good

Standard of English

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Good

Clarity and conciseness

Good

Referee's Comments

There is no comments

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96 | Page

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5	Reviewer Round 2	Comments	5 Januari 2024	21807-1 - Reviewer Comments
21807-1 - Reviewer Comments				
TRENDS IN SCIENCES 202x; xx(xx): xxxxx RESEARCH ARTICLE https://doi.org/ Occurrence of Microplastic Ingestion by Commercial Fish Species from the Pangempang Estuary in Indonesia Highlights - This study demonstrates the presence of microplastic particles in five commercially important fish in a tropical estuary of Kalimantan Island in Indonesia. - Fiber was the most dominant type of microplastic. - Demersal fish may have ingested a higher quantity of microplastic particles compared to pelagic species. Graphical abstract 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 (<i>Caranx</i> SP.), blackspot snapper (<i>Lutjanus fulviflamma</i>), coral grouper (<i>Epinephelus caralla</i>), golden snapper (<i>Lutjanus johni</i>), and pickhandle barracuda (<i>Sphyrna jello</i>) 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. Using a dissecting stereo microscope, microplastic particles in the processed digestive organ contents were identified and enumerated. Microplastics were found in 96% of fish digestive organs (n = 50). In fish samples of jacks, blackspot snapper, coral grouper, golden snapper, and pickhandle barracuda (n = 10 for each species), we found in average of 41 ± 6.0 SE, 26 ± 11.0 SE, 15 ± 2.0 SE, 11 ± 1.0 SE, 14 ± 2.0 SE microplastic particles per individual, respectively, and we found in average of 499.5 ± 94.4 SE, 501.5 ± 198.8 SE, 83.6 ± 18.4 SE, 79.8 ± 11.6 SE, 40.2 ± 25.0 SE microplastic particles per kg fish weight, respectively. Fiber was the most dominant type of microplastic. Microplastic beads were not detected. The relationship of the average consumed microplastic and the average fish weight per species show a negative relationship ($R^2 = 0.36$), but insignificant ($P\text{-value} = 0.28$). 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: Pollution, Ingestion, Commercial fish, Borneo, Indonesia				

Introduction

After invented over 110 years ago, plastic materials become ubiquitous in the environment. Plastic material is made from polymers that can be shaped easily for many varieties of uses. The elevated desire to use plastic materials due to their affordability, bio-inertia, high strength to weight ratio, and resistance to degradation. These characteristics has made plastic materials become important materials for human life nowadays. By the year of 2017, the total global production of plastic was estimated to reach 8300 million metric tons [1], with the rate of production has reached 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 suspected responsible to bring 88%-95% of global marine debris load into ocean [4]. The proportion of plastics usually take about 70% of total marine debris [5].

Initially regarded as a safe material, several decades of plastic production have brought a wide range of environmental problems, particularly in marine ecosystems. In oceans, even though plastic is considered as durable material and resistant to natural biodegradation. However, 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) [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 from the surrounding [11]. Cole et al. reported that ingestion of microplastic particles by zooplanktons shows negative impacts on feeding, hatching, and their health [12]. 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 a type of marine ecosystems often utilized for fisheries purposes [16,17], and these waters 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 estuary is problematic as estuaries also provide crucial habitat for a variety of commercially and recreationally important species such as crabs, fish, and shellfish [20]. Numerous studies investigating microplastics have placed their emphasis on ecosystems in both marine and inland environments [21,22,23]. In fact, Meicabayanti et al. reported the presence of microplastics in waters of Mahakam River located in the East Kalimantan Island [61]. However, there have been limited evaluation on microplastic contamination in transitional waters between the two 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 and asked the main following questions: what type of microplastic? And how is the abundance of microplastic in fish collected from this transitional waters?

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 two periods of intense rainfall and two periods of reduced rainfall, with a total annual precipitation of approximately 97.22 millimeters (3.83 inches) [62]. The

Rwatcharee Ruairuen

Do they have any report of microplastic contamination in this Island or in other places in Indonesia. If there is some article or report available, the author should provide this information.

Hamdhani Hamdhani

A microplastic study in the nearby area has been cited

Rwatcharee Ruairuen

The author should add more information about climate data for this study site such as average air temperature, precipitation [data](#).

Hamdhani Hamdhani

The suggested information has been added.

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 kilometers, originating from the Marangkayu reservoir. Its width ranges from 8 meters upstream to 140 meters downstream. The river passes through the Marangkayu Town settlement, situated roughly 20 kilometers upstream from the Pangempang Estuary. Additionally, about 7 kilometers 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.

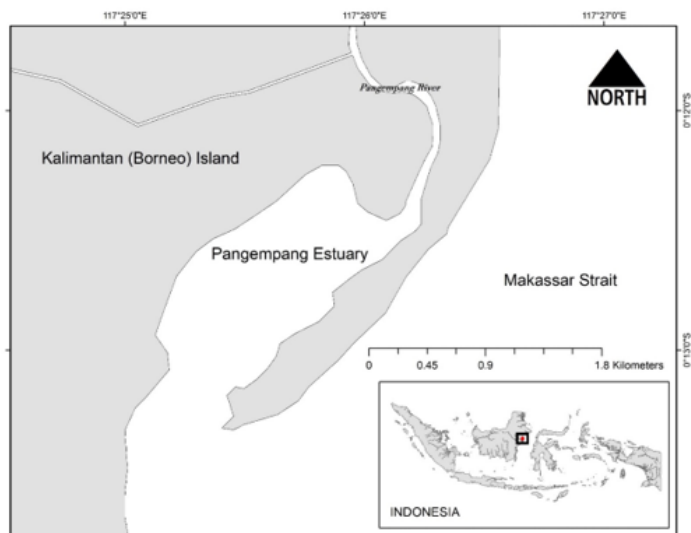


Figure 1 The Pangempang Estuary (East Kalimantan, Indonesia)

To evaluate the ingestion of microplastics by fish, we obtained fish specimens from local fishermen operating in the waters of the Pangempang Estuary. We were able to collect ten replicates of each five different species from these fishermen. The species we identified were jack (*Caranx* SP.), blackspot snapper (*Lutjanus fulviflamma*), coral grouper (*Epinephelus corallicola*), golden snapper (*Lutjanus johnii*), and pickhandle barracuda (*Sphyrna jella*). We identified the fish species based on the information provided in the book titled "Marine Fishes and Crustaceans of the Southeast Asian Region" [24]. Each individual fish was placed in plastic bags, 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.

Rwatcharee Ruairuen

The author should reference Figure 1 into the text. I put it after this sentence. Please recheck the reference for figure 1 again.

Comment

Good point, thank you

Rwatcharee Ruairuen

It's should be great if you can add the location of this coal loading port into the map (figure 1).

Comment

Unfortunately, the coal loading port is situated outside the presented map scope

Rwatcharee Ruairuen

Add more detail about fish characteristic such as feeding mode, habitat (pelagic, benthic-pelagic, or benthos)

Comment

The fish characteristic is presented in Table 1.

Rwatcharee Ruairuen

Rephrase this sentence again.

Comment

We have modified the sentence as suggested.

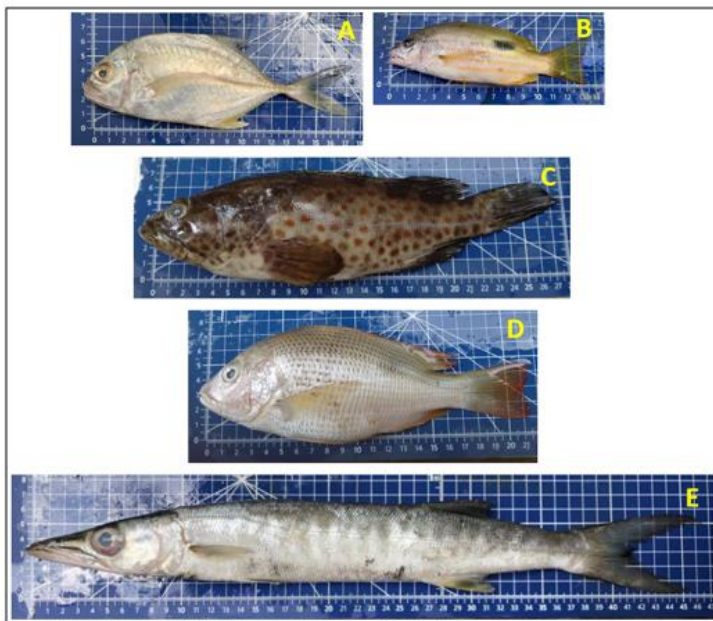


Figure 2 Photographs illustrating the observed fish species: (A) jack (*Caranx* SP.), (B) blackspot snapper (*Lutjanus fulviflamma*), (C) coral grouper (*Epimerus carallicola*), (D) golden snapper (*Lutjanus johnii*), and (E) pickhandle barracuda (*Sphyraena jello*).

Laboratory processing

We measured fish weight (g) of each specimen using [redacted]. [The specimens were weighed using an analytical balance.] 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, each stomach content was digested using alkaline digestion solution of potassium hydroxide (KOH) [25,26]. Each stomach content was placed in a beaker and 10% KOH was added allowing submersion of stomach contents in the beaker. Each beaker was covered with aluminum foil. We stirred the samples in the beaker using magnetic spinner at 350 rpm for 2 hours. 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 hour stirring, samples were vacuum pump filtered on 1.6 μm pore size Whatman paper and then dried.

Using a 10-45 $\times$ magnification dissecting microscope, microplastic particles in the processed stomach contents were identified and enumerated. The microplastic particles were observed and categorized into four major types (fiber, fragment, film, and bead; [27]) following protocols from Hidalgo-Ruz et al., [28] and Eppehimer et al. [29]. 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 used. All these processes were conducted in the laboratory behind closed doors and windows.

Rwatcharee Ruairuen

The author can rewrite this sentence by using this example "The specimens were weighed using an analytical balance.....". In addition, Do you measure the body length of each fish or not? How many fish from each species have been used in this study?

Comment

We have modified the sentence.
We measured the length as well but in this study we focus on the fish weight only.
We used 10 fish for each species. This have mentioned earlier.

Rwatcharee Ruairuen

Is this study considering the microplastic contaminate in fish stomach only not other part of fish organ?

Comment

Yes, only ingested microplastics

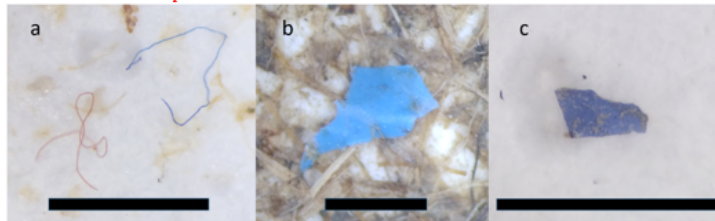
Results

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 (fish, shrimps, crabs and cephalopods) generally smaller in size than their own bodies.

Table 1 Taxonomy, common name, foraging ecology

No.	Taxonomy and Common name	Foraging ecology
1.	Family: Carangidae Genus: <i>Caranx</i> Common names: Jacks or trevallies	Most species are demersal , or bottom dwelling, while others are pelagic , moving long distances in the upper water column [30]. All species are predatory fish, taking smaller fish, cephalopods as prey [31].
2.	Family: Lutjanidae Species: <i>Lutjanus fulvivittatus</i> (Eggsakal, 1775) Common names: Blackspot snapper	Many species under this family are demersal and associated with coral reefs [32]. Some of the larger red snapper species descend into deeper waters, at least to 200 m in depth [33]. Lutjanus is predatory fish usually found in tropical and subtropical reefs, and mangrove forests.
3.	Family: Serranidae <i>Epinephelus caeruleus</i> (Kuhl & Hasselt, 1828) 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 [34]. 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 [35].
4.	Family: Lutjanidae <i>Lutjanus johnii</i> (Bloch, 1792) Common names: Golden snapper, or John's snapper	Lutjanus exhibits a two-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) [36]. Feed on fishes and benthic invertebrates including shrimps, crabs and cephalopods. Adults probably frequent coral reef areas; juveniles found in mangrove estuaries [37].
5.	Family: Sphyraenidae <i>Sphyraena jello</i> Cuvier, 1829 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 [38]. Prey on fish, cephalopods, and shrimps [39].

We observed microplastic shapes of fibers, film, fragments (Figure 3). Microplastic beads were not detected in any of our samples. In jacks, coral grouper, golden snapper, and pickhandle barracuda, microplastic particles were identified in 100% of samples, except for blackspot snapper, where they were found in 80% of the samples.



Rwatcharee Ruairuen

The first part of your result the author should add a table to explain the characteristics of different five fish species which include common name, species name, number of samples, feeding mode, habitat, mass, length etc.

Comment

The suggested table has been added

Rwatcharee Ruairuen

Table 1 should remove to material and method section because these information explain about five fish characteristics in your study. However, in the result section you should provide table that show data about an average of length $\pm$ SD, and weight $\pm$ SD of ten replicate for five fish.

Rwatcharee Ruairuen

The author should move Table 1 into material and method section.

Hamdhani Hamdhani

Thank you for the suggestion, we have moved the table to the beginning of result section

Rwatcharee Ruairuen

Table 1 should be depicted in material and method section because it not your result.

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 SE, 26 ± 11.0 SE, 15 ± 2.0 SE, 11 ± 1.0 SE, 14 ± 2.0 SE microplastic particles per individual, respectively (**Figure 4A**), and we found in average of 499.5 ± 94.4 SE, 501.5 ± 198.8 SE, 83.6 ± 18.4 SE, 79.8 ± 11.6 SE, 40.2 ± 25.0 SE microplastic particles per kg fish weight, respectively (**Figure 4B**). The blackspot snapper had the highest number of microplastic particles per kilogram of fish weight, reaching 501.5 ± 198.8 SE microplastic particles. The lowest number of particles per kilogram of fish was found in the pickhandle barracuda, which amounted to 40.2 ± 25.0 SE microplastic particles.

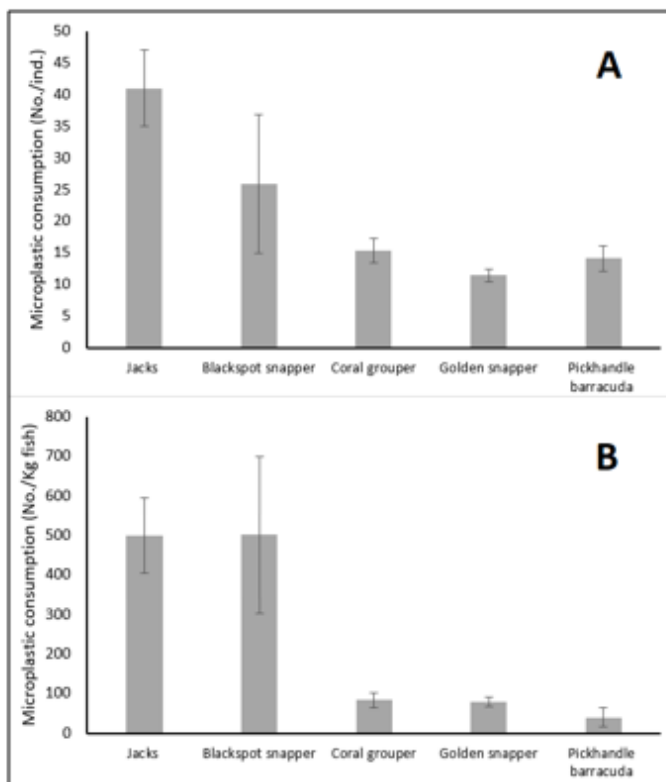


Figure 4 Average of microplastic particles per individual (A) and per kilogram fish weight (B)

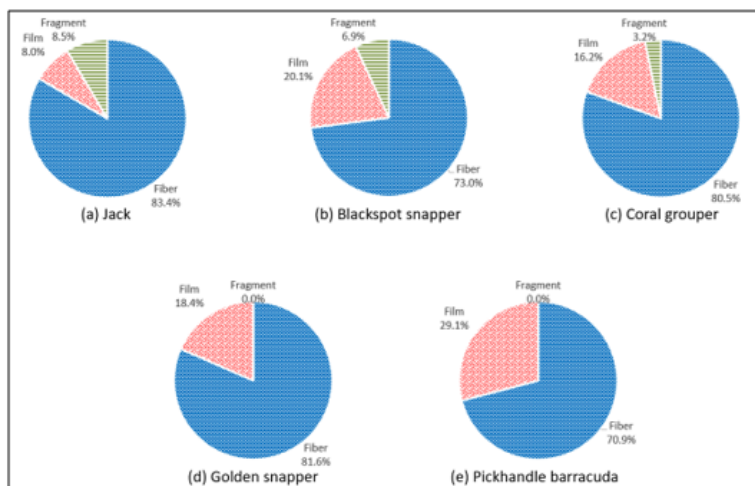


Figure 5 Percentages of the observed microplastic types found in digestive organs from sample fishes collected in the Pangkep Estuary: (a) Jack, (b) Blackspot snapper, (c) Coral grouper, (d) Golden snapper, and (e) Pickhandle barracuda.

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 5a). 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%, 50% of all samples respectively (Figure 5b). 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%, 40% of our samples respectively (Figure 5c). 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 5d). 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 5e).

Rwatcharee Ruairuen

I recommend to use pie chart to illustrate different type of microplastic contaminate in each fish species. More document that you can use to improving your writing: <https://tis.wu.ac.th/index.php/tis/article/view/3073/189>

Hamdhani Hamdhani

The pie charts have been made and added

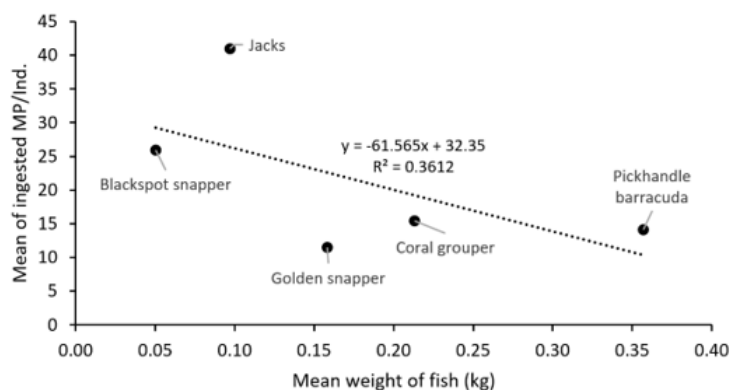


Figure 6 Relationship of mean weight of observed fish and mean number of ingested microplastic particles per species individual

Among the five 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. 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 two 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 predominant type of microplastic. Previous research in aquatic environments has identified fibers as the most prevalent form of microplastics [40-43]. Sources of fibers are predominantly textile goods, such as clothes [44]. Degradation of synthetic fabrics was predicted as major contributor for microplastic fiber abundance in our environment [44,45]. 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 1900 fibers at size less than 1mm in length per wash cycle [46]. 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 discharged into surface waters [46]. 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 jack 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 film microplastic portion was half of the total discovered microplastic particles, and the remaining shared between fiber and fragment [47]. We observed fragment particles was the lowest in number and only found in three of five fish species we examined (jack, blackspot snapper, and coral grouper). In contrast, Karbalas et al. documented that among commercial marine fish in the adjacent waters of Malaysia, fragment-type microplastics were the most prevalent [48]. 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 [49].

Recent investigations into the impact of microplastic fibers on aquatic organisms have unveiled various harmful consequences. According to Liang et al., goldfish exposed to a concentration of 1000 items

Rwatcharee Ruairuen

This figure does not show a clear illustration to me. Considering to modify this graph if possible.

Hamdhani Hamdhani

We believe that this figure is informative, telling the relationship of mean weight of observed fish and mean number of ingested microplastic particles per species individual

Rwatcharee Ruairuen

Rephrase these sentences.

Hamdhani Hamdhani

We have rephrased the sentences

Rwatcharee Ruairuen

Why? Any explanation about this?

Hamdhani Hamdhani

Certainly we do not know why this happened

Rwatcharee Ruairuen

Add three five species

Hamdhani Hamdhani

We have added the three fish names.

per liter 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 [50]. Similarly, a study by Zhao et al. 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 [51]. Another study by Xie et al. noted oxidative stress, dysbiosis in the intestine microbiome, and histological damage as significant symptoms in barramundi exposed to microplastic fibers [52].

Abundance of microplastic in digestive organs

Our finding revealed that jack as individual consumed the highest average of microplastic particles, 41 (SE: 5.98) while golden snapper was observed consuming 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. documented microplastic particle abundance ranged from 9-14 per gastrointestinal tract in multiple fish species caught the Bahía Blanca Estuary in Argentina [53], up to 4 particles in semi pelagic boop boops in the Mediterranean Sea [41], and ranged 1.1- 7.2 microplastic particles in fish collected from China marine waters [40]. On the other hand, the blackspot snapper has the highest amount of microplastics per kilogram of fish, 501.5 ± 198.8 SE, while the pickhandle barracuda has the smallest amount, only 40.2 ± 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 two is not significant ($P\text{-value} = 0.28$). Similarly, Chen et al. reported that fish size did not affect microplastic abundance in the fish digestive organs [54].

Foraging ecology

Microplastic ingestion is potentially influenced by two crucial factors: the type of habitat and the feeding habits of organisms [55]. Within marine environments, scientists have noted markedly greater quantities of microplastics in demersal fish species compared to pelagic species [40,42]. This trend could be linked to the prevalence of plastic waste near the seabed, which is often regarded as the final accumulation for plastics within the marine ecosystem.

Microplastics could potentially be present in fish across different trophic levels. However, current data are insufficient to make definitive conclusions about the absorption of microplastics [56]. In fact, distinguishing between directly and indirectly ingested microplastics is challenging [57]. Walkinshaw et al. performed a semi-systematic review on research examining the presence of microplastics in commercially significant organisms across various trophic levels [58]. 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 are adult predator fish, four fish species are demersal, and one is pelagic (Table 1). The microplastic abundances in the three 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 anticipated 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 [59]. 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 two herbivorous fish species consistently ingest significant quantities of microplastic particles as much as 10-99 microplastic particles per fish gut [60].

Conclusions

For the first time, this study demonstrates the presence of microplastic particles in five commercially important fish in a tropical estuary of Kalimantan Island in Indonesia. The average abundance per fish individual were higher than those documented 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 a significant 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.

This study result also confirms the urgency to observe wide range of fish species in term of their habitat within the water column and as well as the feeding habit. 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. Perhaps analysis based on muscles and internal organs should also be considered in the near future.

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a.	22127-1 - Manuskrip hasil Revisi 2																				
TRENDS IN SCIENCES 202x; xx(xx): xxxxx https://doi.org/ RESEARCH ARTICLE Occurrence of Microplastic Ingestion by Commercial Fish Species from the Pangempang Estuary in Indonesia Hamdhani Hamdhani¹, Ghitarina Ghitarina¹, Ristiana Eryati¹, Drew E. Eppehimer² ¹Department of Aquatic Resources Management, Faculty of Fisheries and Marine Science - Mulawarman University, Samarinda, Indonesia ²School of Natural Resources and the Environment, University of Arizona, Tucson, AZ, United States (Corresponding author's e-mail: hamdhani@fpik.unmul.ac.id) Received: xxx, Revised: xxx, Accepted: xxx, Published: xxx (Times New Roman, 9 pt, <i>Italic</i>) Highlights - This study demonstrates the presence of microplastic particles in five commercially important fish in a tropical estuary of Kalimantan Island in Indonesia. - Fiber was the most dominant type of microplastic. - Demersal fish may have ingested a higher quantity of microplastic particles compared to pelagic species. Graphical abstract <table border="1"><caption>Estimated data from Graphical Abstract charts</caption><thead><tr><th>Species</th><th>Ingested microplastic (No./Ind.) (Chart a)</th><th>Ingested microplastic (No./Kg fish) (Chart b)</th></tr></thead><tbody><tr><td>Jack</td><td>~40</td><td>~500</td></tr><tr><td>Blackspot snapper</td><td>~25</td><td>~500</td></tr><tr><td>Coral grouper</td><td>~15</td><td>~100</td></tr><tr><td>Golden snapper</td><td>~10</td><td>~100</td></tr><tr><td>Pickhandle barracuda</td><td>~15</td><td>~50</td></tr></tbody></table> 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 (<i>Caranx</i> SP.), blackspot snapper (<i>Lutjanus fulviflamma</i>), coral grouper (<i>Epinephelus corallicola</i>), golden snapper (<i>Lutjanus johnii</i>), and pickhandle barracuda (<i>Sphyræna jello</i>) 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 SE, 26 ± 11.0				Species	Ingested microplastic (No./Ind.) (Chart a)	Ingested microplastic (No./Kg fish) (Chart b)	Jack	~40	~500	Blackspot snapper	~25	~500	Coral grouper	~15	~100	Golden snapper	~10	~100	Pickhandle barracuda	~15	~50
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SE, 15 ± 2.0 SE, 11 ± 1.0 SE, 14 ± 2.0 SE microplastic particles per individual and an average of 499.5 ± 94.4 SE, 501.5 ± 198.8 SE, 83.6 ± 18.4 SE, 79.8 ± 11.6 SE, 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 ($P\text{-value} = 0.28$). 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 8300 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. reported that ingestion of microplastic particles by zooplanktons shows negative impacts on feeding, hatching, and overall health [12]. 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 [21,22,23]. In fact, Meicahayanti et al. reported the presence of microplastics in waters of Mahakam River located in the East Kalimantan Island [24]. However, there have been limited evaluation on microplastic contamination in transitional waters between the two 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 two periods of intense rainfall and two periods of reduced rainfall, with a total annual precipitation of approximately 97.22 millimeters (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 kilometers, originating from the Marangkayu reservoir. Its width ranges from 8 meters upstream to 140 meters downstream. The river passes through the Marangkayu Town settlement, situated roughly 20 kilometers upstream from the Pangempang Estuary. Additionally, about 7 kilometers 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.

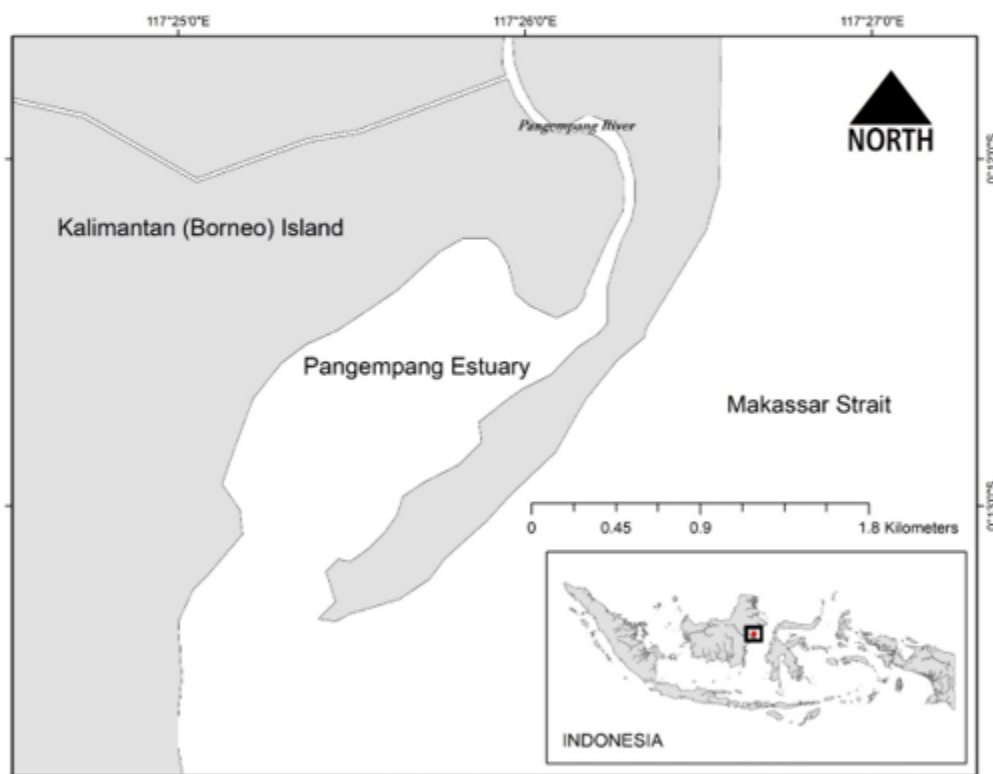


Figure 1 The Pangempang Estuary (East Kalimantan, Indonesia)

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 15th, 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 five different species from these fishermen. The species we analyzed were jack (*Caranx* SP.), blackspot snapper (*Lutjanus fulviflamma*), coral grouper (*Epinephelus corallicola*), golden snapper (*Lutjanus johni*), and pickhandle barracuda (*Spharxena 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.

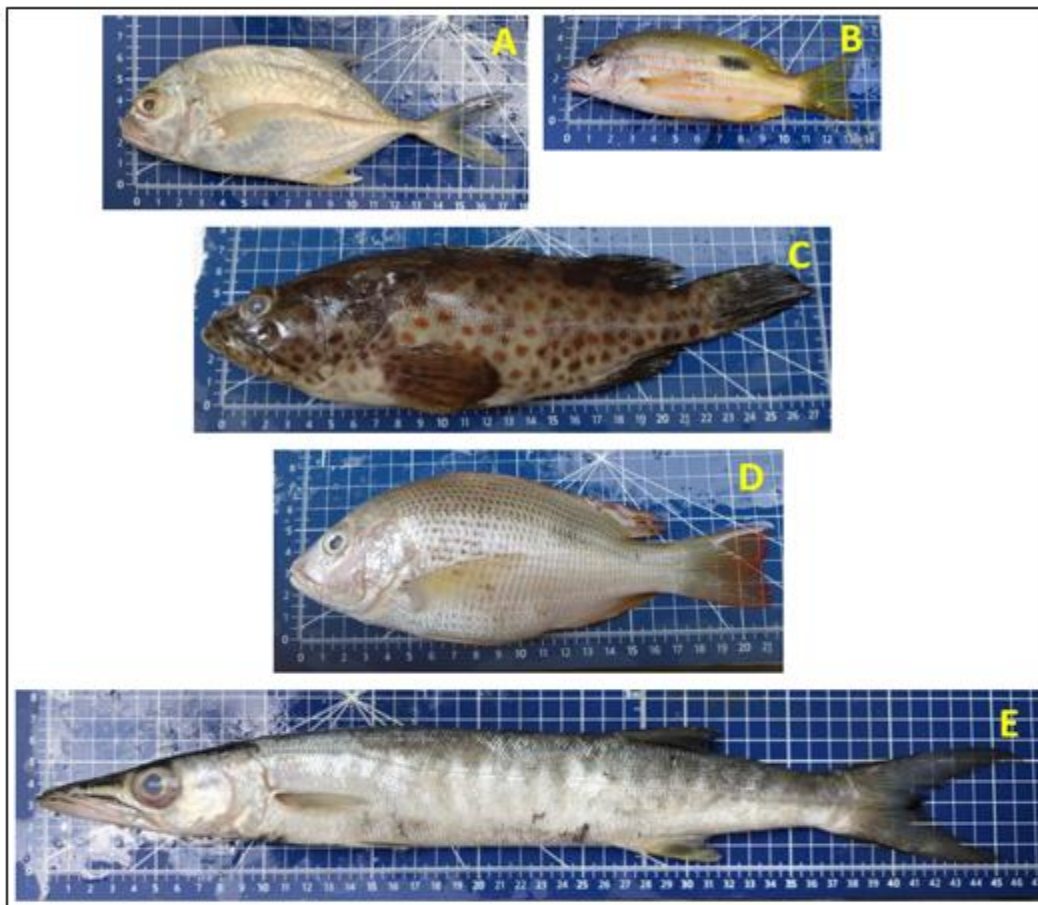


Figure 2 Photographs illustrating the observed fish species: (A) jack (*Caranx* SP.), (B) blackspot snapper (*Lutjanus fulvivittatus*), (C) coral grouper (*Epinephelus corallicola*), (D) golden snapper (*Lutjanus johnii*), and (E) pickhandle barracuda (*Sphyræna jello*).

Table 1 Taxonomy, common name, foraging ecology

No.	Taxonomy and Common name	Foraging ecology
1.	Family: <i>Carangidae</i> Genus: <i>Caranx</i> 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: <i>Lutjanidae</i> Species: <i>Lutjanus fulvivittatus</i> (Forsk., 1775) 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]. <i>Lutjanus</i> is predatory fish usually found in tropical and subtropical reefs, and mangrove forests.
3.	Family: <i>Serranidae</i> <i>Epinephelus corallicola</i> (Kuhl & Hasselt, 1828) 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: <i>Lutjanidae</i> <i>Lutjanus johnii</i> (Bloch, 1792) Common names: Golden snapper, or John's snapper	<i>Lutjanus</i> exhibits a two-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: <i>Sphyraenidae</i> <i>Sphyraena iella</i> Cuvier, 1829 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 hours. Then we collected the particles after the digestion using a sieve of 125 μ m and transferred to a previously cleaned beaker using distilled water. Into the beaker 30% H_2O_2 was added and then stirred. After 1 hour stirring, samples were vacuum pump filtered on 1.6 μ m pore size Whatman paper and then dried in an oven at 60°C for 24 hours.

Using a 10–45 $\times$ magnification dissecting microscope, microplastic particles in the processed digestive tract contents were identified and enumerated. The microplastic particles were observed and categorized into four major types (fiber, fragment, film, and bead; [40]) following protocols from Hidalgo-Ruz et al., [41] and 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

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. Eighty percent of blackspot snapper contained observable microplastics.

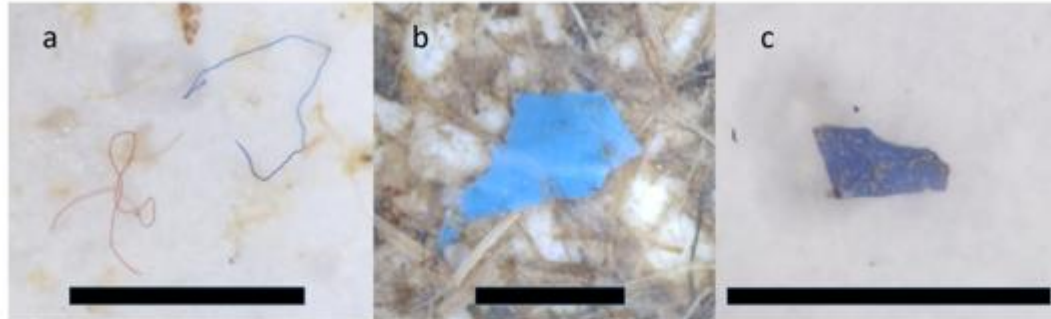


Figure 3 Photographs illustrating the observed microplastic types found in digestive organs from sample fishes collected in the Pangempang Estuary: a. fiber, b. film, and c. fragment. Horizontal scale bar ≈ 0.5 mm

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

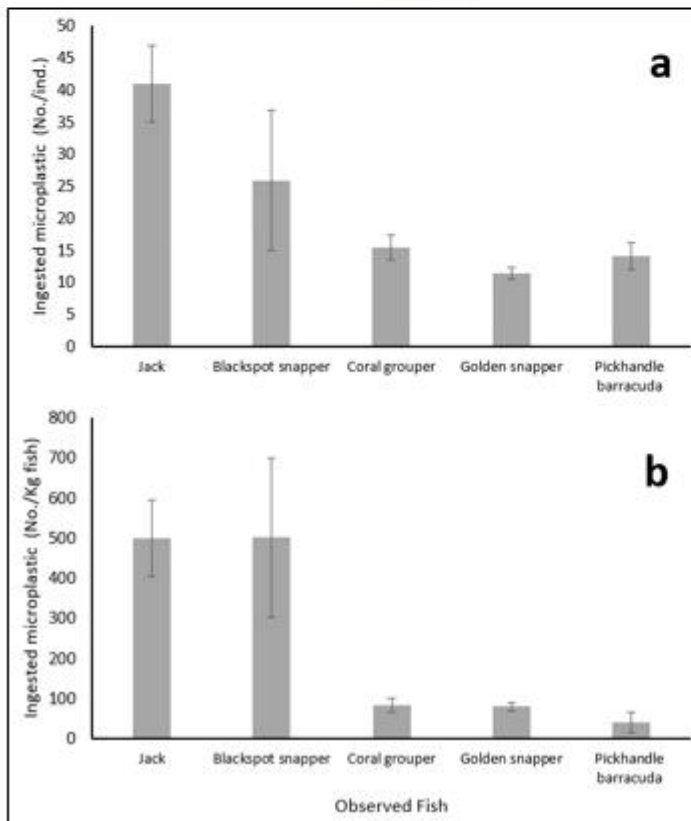


Figure 4 Average microplastic particles per individual (a) and per kilogram fish wet weight (b)

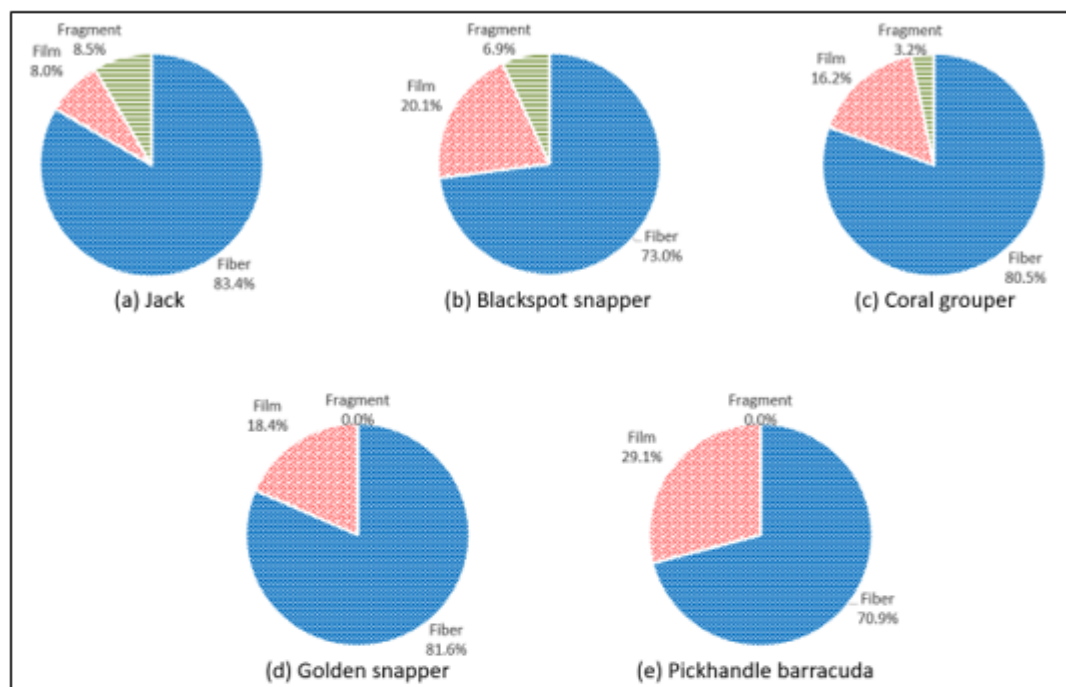


Figure 5 Percentages of the observed microplastic types found in digestive organs from sample fishes collected in the Pangempang Estuary

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 5a**). 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%, 50% of all samples respectively (**Figure 5b**). 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%, 40% of our samples respectively (**Figure 5c**). From our golden snapper samples, we observed that fibers were the most detected type (81.6%) 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 5d**). 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 5e**).

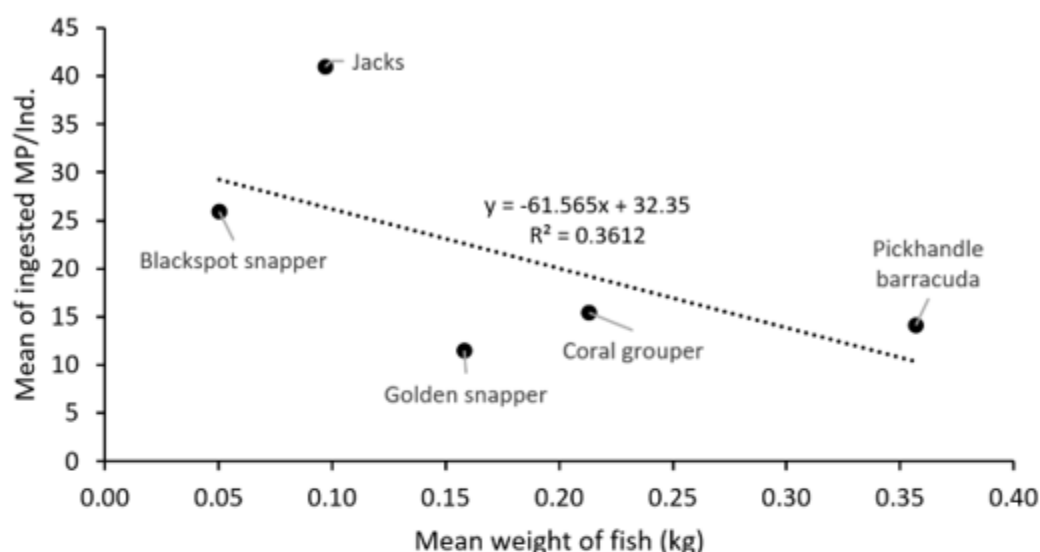


Figure 6 Relationship of mean weight of observed fish and mean number of ingested microplastic particles per species individual

Among the five 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 two is not significant ($P\text{-value} = 0.28$).

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 1900 fibers at size less than 1mm 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 three of five fish species we examined (jack, blackspot snapper, and coral grouper). In contrast, Karbalaei et al. documented that among commercial marine fish in the adjacent waters of Malaysia, fragment-type microplastics were the most prevalent [52]. 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., goldfish exposed to a concentration of 1000 items per liter 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 [54]. Similarly, a study by Zhao et al. 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 [55]. Another study by Xie et al. noted oxidative stress, dysbiosis in the intestine microbiome, and histological damage as significant symptoms in barramundi exposed to microplastic fibers [56].

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. documented microplastic particle abundance ranged from 9-14 in digestive organs per individual across multiple fish species caught the Bahía Blanca Estuary in Argentina [57], up to 4 particles in semi pelagic boop boops in the Mediterranean 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 kilogram of fish, 501.5 ± 198.8 SE, while the pickhandle barracuda has the smallest amount ($40.2 / \text{kg} \pm 25.0$ 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 two is not significant ($P\text{-value} = 0.28$). Similarly, Chen et al. reported that fish size did not affect microplastic abundance in the fish digestive organs [59].

Foraging ecology

Microplastic ingestion is potentially influenced by two 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. performed a semi-systematic review on research examining the presence of microplastics in commercially significant organisms across various trophic levels [63]. 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, four fish species were demersal, and one was pelagic (Table 1). The microplastic abundances in the three 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 two 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.

Acknowledgements

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b.

Respon kepada reviewer



Trends in Sciences

Responses the referee's comments (round 2)

Manuscript ID: 7762

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

Reviewer A

No.	Referee's Comments	Responses (for author)
1.	I recommend the author consider Table 1 as one element in your material and methods section because these information is not your result. It's explain the fish characteristic for your study. However, in the result section an average of ten replicates of each five fish in term or lenght and weight should illustrated in the table.	We have moved the Table 1 into the Materials and methods section as suggested (page 4-5). We have added a new table (Table 2) for average length and weight of our observed fish (page 5)

7	Konfirmasi final – artikel diterima	8 Januari 2024	Email konfirmasi
6/14/26, 11:40 AM Universitas Mulawarman Mail - [TiS] Manuscript Decision & Article Processing Charges  Hamdhani Hamdhani <hamdhani@fpik.unmul.ac.id> [TiS] Manuscript Decision & Article Processing Charges 7 messages Trends in Sciences (TiS) <journal.wu@gmail.com> Mon, Jan 8, 2024 at 9:29 AM To: Hamdhani Hamdhani <hamdhani@fpik.unmul.ac.id>, Ghitarina Ghitarina <ghitarina@fpik.unmul.ac.id>, Ristiana Eryati <eryati.ristiana@gmail.com>, "Drew E. Eppehimer" <depphimer@arizona.edu> Dear Professor Hamdhani Hamdhani, Ghitarina Ghitarina, Ristiana Eryati, Drew E. Eppehimer: We have reached a decision regarding your submission to Trends in Sciences, "Occurrence of Microplastic Ingestion by Commercial Fish Species from the Pangempang Estuary in Indonesia". The reviewers have no further comment. We are pleased to inform you that your manuscript is accepted for publication and is scheduled to be published in Trends in Sciences (TiS) in the Volume 21, 2024. Manuscript URL: https://tis.wu.ac.th/index.php/tis/authorDashboard/submission/7762 TiS Best Paper Awards 2023 https://tis.wu.ac.th/index.php/tis/announcement Regards, Managing Editor Trends in Sciences Important Notice: For any accepted manuscripts submitted from 1 October 2022, authors will be required to pay a one-time article processing charge (APC) upon formal manuscript acceptance. We advise prompt payment as we are unable to publish accepted articles until payment has been received. Payment can be made by the following instruction: Payment Instructions for Trends in Sciences Article Processing Charges (APC): URL: https://epay.wu.ac.th/en/register/conference Step 1: Please select > [2566-00043] Trends in Sciences Article Processing Charges (APC) "10,730.32 THB". Step 2: Fill out the information completely & then Confirm Step 3: Please fill in the debit or credit card information Thank you for submitting your payment and hope to receive your payment soon. We are looking forward to receiving high-quality articles from you in the future. Please note: 1) If your payment has been paid, please reply to us through email again. Thank you in advance for your cooperation. 2) Please pay as soon as you can, possibly within 15 days, otherwise it must be considered withdrawn. Trends in Sciences Formerly know as Walailak Journal of Science and Technology 2021 CiteScore (SCOPUS): 0.8 Hamdhani Hamdhani <hamdhani@fpik.unmul.ac.id> Mon, Jan 8, 2024 at 11:16 AM https://mail.google.com/mail/u/3/?ik=35ace941da&view=pt&search=all&permthid=thread-f:1787483801132284471&siml=msg-f:17874838011322844... 1/3			

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3/3

	Konfirmasi final – artikel terbit online (online published).	11 Juni 2024	Email konfirmasi
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