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Microplastic contamination in fish harvested from the estuarine mangrove forest of Banda Aceh City, Indonesia

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Abstract: Mullet fish (*Mugil cephalus*), glassfish (*Ambassis nalua*), and mudskipper (*Periophthalmus* sp.) dominate the mangrove ecosystem waters of Banda Aceh City, Indonesia. These fish are potentially contaminated with microplastics from domestic and industrial waste. This study aimed to analyze microplastic contamination in the digestive tracts and flesh of fish from the mangrove area of Banda Aceh City, Indonesia. Sampling was conducted at 3 stations: Alue Naga in Syiah Kuala District, Pande in Kuta Raja District, and Blang in Meuraxa District, from December 2023 to February 2024. A total of 478 mullets, 462 glassfish, and 435 mudskippers were sampled. Based on fish species and sampling location, glassfish and the Alue Naga station exhibited the highest abundance of microplastics, with values of 1.55 particles/fish and 1.77 particles/fish, respectively. Black was the most dominant color of microplastics found in all fish samples, with the predominant size groups being <20 µm and 21–40 µm. Film was the predominant shape of microplastics in all fish species. FTIR analysis confirmed the presence of nylon and polypropylene microplastic polymers in the fish flesh. Mullet fish, glassfish, and mudskippers from the mangrove forest waters of Banda Aceh City, Indonesia, have been contaminated by microplastics.

Introduction

Plastic waste pollution in water is a crucial environmental issue that has garnered significant attention and concern. In 2018, approximately 360 million metric tonnes of plastic products were produced in the world, with 2–5% having the potential to pollute the world's marine waters (Jambeck et al. 2015). Plastic waste is a non-organic material that resists natural decomposition due to its long carbon chains. Microplastics pose a significant threat to aquatic environment, including mangrove ecosystems in coastal areas. These particles can be ingested by aquatic biota inhabiting mangrove ecosystems close to residential areas or coastal industries, leading to digestive disorders, damage to digestive organs, and even the death of fish (Zaman et al. 2023). Furthermore, the consumption of fish contaminated with microplastics negatively impacts human health (Hernandez et al. 2017).

Previous studies have examined microplastic contamination in waters worldwide. For instance, Hou et al. (2021) reported an increase in plastic waste in Chicago waters, rising from 2.8 million tonnes in 2010 to 9.8 million tonnes in 2017. Similarly, Brandon et al. (2019) documented an exponential increase in the accumulation of microplastics California's coastal waters from 1945 to 2009. Approximately 65% of marine debris in the Atlantic Ocean is plastic (Waring et al. 2018). Microplastics have also been discovered in the coastal waters of Kenya, Africa (Kishore et al. 2022) and the South China Sea, where 187-1,816 microplastic particles/m^3 were reported (Cui et al. 2018). In Indonesian waters, significant microplastic contamination has also been documented. Indonesia is ranked as the fourth largest contributor to plastic waste in global waters (Jambeck et al. 2015). For instance, waters in North Sumatra revealed microplastic contamination at approximately 2.30 particles/g in fish (Arisanti et al. 2023). Similarly, Sarasita et al. (2020) reported an abundance of 7 microplastic particles per individual fish in the Bali Strait.

Banda Aceh is a coastal city in Indonesia experiencing rapid population growth. According to the 2021 data from the Statistics Agency, the city has a population of 225,029 people

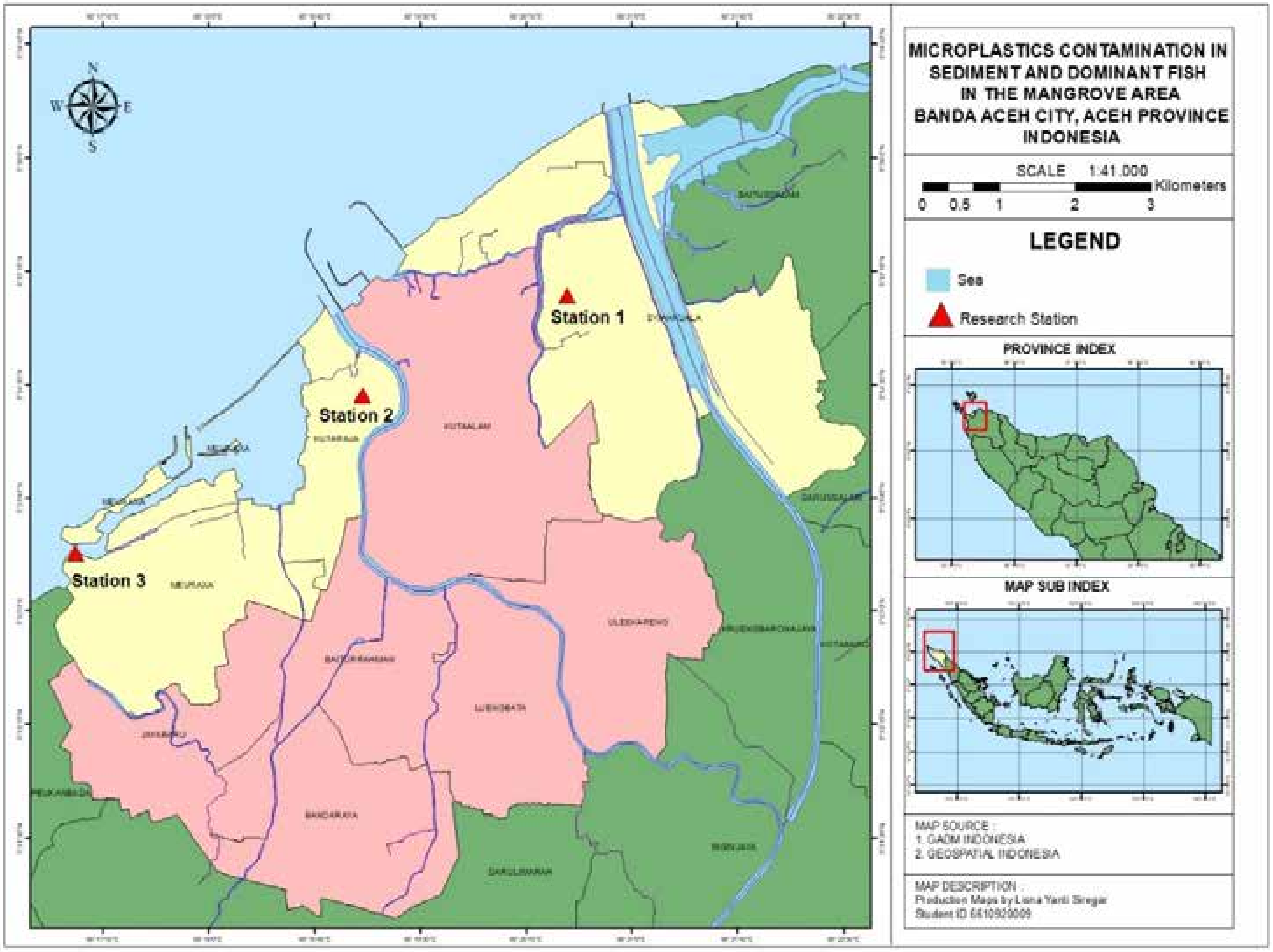

Figure 1. The map of Banda Aceh city showing the sampling location. The sampling location is indicate with red triangles.

and a density of around 4,156 people/km². Banda Aceh is the most densely populated area in Aceh Province, with the majority of its residents living in coastal regions. In 2020 and 2021, the city generated 6.94.88 and 8.419.412 tonnes of plastic waste, respectively, which poses a significant risk of water pollution (BPS Banda Aceh 2021). Studies on microplastic contamination in the waters of Aceh Province are limited. Maulana et al. (2023) reported microplastic contamination in mullet (*Mugil cephalus*) and bagok catfish (*Hexanematichthys sagor*) from the estuarine waters of the Krueng River. Similarly, Hamdan et al. (2022) found that the sediment in the Krueng Aceh River was contaminated with microplastics. However, research on microplastic contamination in fish from the coastal waters of Banda Aceh City, particularly in mangrove areas, has not yet been conducted. Mangrove areas play an important role as "green traps," preventing plastic waste from drifting from land into the open sea. These areas serve as habitats for various fish species, including mullet (*Mugil cephalus*), glassfish (*Ambassis nalua*), and mudskipper (*Periophthalmus* sp.). Furthermore, mullet and glassfish are commonly consumed by the local population. Hence, this study aims to analyze microplastic contamination in three predominant fish species - mullet, glassfish, and mudskipper - harvested from the mangrove areas of Banda Aceh city, Indonesia.

Materials and Methods

Site and time

Fish sampling was conducted from December 2023 to February 2024 at 3 locations within the mangrove forest of Banda Aceh City (Figure 1). The presence of microplastics in the digestive tract of the fish samples was analyzed at the Chemistry and Biotechnology Laboratory, Faculty of Marine Affairs and Fisheries, Syiah Kuala University in Banda Aceh City. Fourier Transform Infrared Spectroscopy (FTIR) analysis on the fish flesh was performed at the Chemistry Laboratory, Faculty of Engineering, Syiah Kuala University.

FIG.1

The sampling stations were purposefully selected based on their characteristics and coverage areas. A preliminary survey identified 3 mangrove areas, each covering a significant area of 4 - 8 ha. The first, second, and third stations were located in Alue Naga Village (Syiah Kuala District), Pande Village (Kuta Raja District), and Blang Village (Meuraxa District), respectively. The names, coordinates, and characteristics of the study locations are presented in Table 1. Initial sampling revealed 3 dominant fish species: mullet (*Mugil cephalus*), glassfish (*Ambassis nalua*), and mudskipper (*Periophthalmus* sp.). The taxonomic status of these fish was validated using the

Table 1. The sampling location name, GPS coordinate and characteristics of sampling location in mangrove forest of Banda Aceh city

Station	GPS coordinate	Characteristics
Alue Naga Village, Syiah Kuala sub district, Banda Aceh city	5°59'51.66"N 95°34'34.9"E	The mangrove area of Alue Naga Village has a covered area of 26 ha. This location has the characteristics that the mangrove plant is dominated by *Rhizophora* sp. and is close to residential and agricultural land, as well as fishing activities and jetties.
Pande Village, Kuta Raja sub district, Banda Aceh city	5°57'78.85"N 95°30'91.2"E	The mangrove area in Pande Village has an area of 48 ha which is dominated by *Rhizophora* sp. This location is close to densely populated residential areas, the Lampulo Ocean fishing port and fish market, and the waste disposal site in Banda Aceh City.
Blang Village, Meuraxa subdistrict, Banda Aceh city	5°54'89.60"N 95°28'12.2"E	The mangrove area in Alue Naga Village has an area of 4 ha. which is dominated by *Rhizophora* sp. This is a tidal area, close to residential areas and community farms.

references provided by Muchlisin and Siti-Azizah (2009), and Gomon et al. (2008).

Fish sample collection

Fish sampling was conducted twice a month from December 2023 to February 2024. The samples were collected using gill nets or casting nets during low tide between 07.00 AM and 09.00 AM. The sampled fish were then stored in an ice box (4°C) and transported to the Marine Chemistry and Biotechnology Laboratory, Faculty of Marine Affairs and Fisheries, Syiah Kuala University, for further analysis. To prevent contamination, all equipment used during sample collection and analysis was made from non-plastic materials.

Sample preparation and microplastic analysis

The sample preparation and analysis followed the standard procedure for microplastics analysis as outlined by Prata *et al.* (2019). Fish samples were dissected, and the digestive tracts were removed, opened, and their contents transferred to an Erlenmeyer flask. A 10% potassium hydroxide solution was added to submerge the contents. The flask was covered with aluminium foil and heated on a hot plate at 60°C until near boiling. The sample was then left to settle for 24 hours to allow the organic material to break down. Subsequently, the mixture was centrifuged at 7,000 rpm for 20 minutes and filtered using Whatman filter paper No. 42. The filter paper was dried in a furnace at 60°C for 180 minutes. Microplastics were observed under a microscope with a magnification of 400–600X. Finally, their shape, color, size, and abundance were analyzed based on the methodology described by Boerger et al. (2010). According to Bermúdez and Swarzenski (2021), microplastic sizes range from 20-200 μm.

FTIR analysis

Fourier-transform infrared spectroscopy (FTIR) analysis was performed on the fish flesh samples obtained from the identified locations to detect the presence of microplastic polymers. The sample preparation procedure for FTIR analysis followed the method described by Cunsolo et al. (2021). Flesh from each species was dried in a furnace at 90°C for 36 hours, ground into a fine powder, and sieved. A total of 2 g of the powdered fish flesh was mixed with a 10% potassium bromide solution. The analysis was conducted using an FTIR machine. The principle of the FTIR analysis is to identify the functional polymer groups of a compound based on infrared absorbance. The resulting wavelength values were plotted as a graph and compared with reference values reported by Jung et al. (2018) (Table 2).

Data analysis

Data on the color, shape, size and abundance of microplastics are presented in the tables and figure, and were analyzed descriptively by comparing them with relevant previous studies and reports. The mean values of microplastic abundance, based on fish species, location, and time, were subjected to

Table 2. The validation reference wavelength of FT-IR spectral (Jung *et al.*, 2018)

Plastic type	Wave length (cm^{-1})	Functional groups
Polypropylene	2950	C-H
	2915	C-H
	2838	C-H
	1455	C-H
	997	C-H
	1397	C-H
	1166	C-C
	972	C-H
	840	CH_2
	808	CH_2
Nylon	1634	C=O
	1538	N-H
	1464	C-H
	1372	C-H
	1274	N-H
	1199	C-H
	687	C-H
	1713	C=O stretch
	1241	C-O stretch
	1094	C-O stretch

Table 3. The total of microplastics abundance based on microplastics shapes in the digestive tract of fish samples harvested from mangrove forest of Banda Aceh coastal waters during December 2023 to February 2024

Fish species	Station	Total sample (ind.)	Microplastic shapes					Total	Average particles/ fish
			Fiber	Film	Fragment	Pellet	Foam		
Mullet (*Mugil cephalus*)	Alue Naga Village	176	79	155	26	37	67	364	2.06±0.29
	Pande Village	156	50	51	19	20	30	170	1.08±0.10
	Blang Village	146	38	96	10	13	49	206	1.41±0.24
Glassfish (*Ambassis nalua*)	Alue Naga Village	169	33	149	32	19	67	300	1.77±0.31
	Pande Village	158	26	120	40	18	50	254	1.60 ±0.26
	Blang Village	135	19	128	9	4	13	173	1.28 ±0.39
Mudskipper (*Periophthalmus* sp.)	Alue Naga Village	165	10	102	10	15	107	244	1.47±0.31
	Pande Village	141	41	32	12	35	24	144	1.02±0.08
	Blang Village	129	39	54	11	16	37	157	1.21±0.14
Total	-	1375	335	887	169	177	444	2012	-

Table 4. The total of microplastics abundance based on color in the digestive tract of fish samples harvested from mangrove forest of Banda Aceh coastal waters during December 2023 to February 2024

Fish species	Station	Total sample (ind.)	Microplastics color								Total	Average particles/fish
			BR	YL	BL	WH	PU	BK	GR	RE		
Mullet (*Mugil cephalus*)	Alue Naga Village	176	107	30	21	69	2	122	º0	13	364	2.06±0.27
	Pande Village	156	62	3	2	26	0	70	0	7	170	1.08±0.19
	Blang Village	146	54	2	2	11	0	131	0	6	206	1.41±0.32
Glassfish (*Ambassis nalua*)	Alue Naga Village	169	75	60	4	55	0	89	2	15	300	1.77±0.21
	Pande Village	158	57	14	4	49	0	116	2	12	254	1.60±0.26
	Blang Village	135	16	5	2	12	0	134	1	3	173	1.28±0.334
Mudskipper (*Periophthalmus* sp.)	Alue Naga Village	165	31	20	0	138	0	54	0	1	244	1.47±0.29
	Pande Village	141	30	15	6	16	1	71	1	4	144	1.02±0.17
	Blang Village	129	47	16	4	33	0	49	0	8	157	1.21±0.16
Total	-	1375	479	165	45	409	3	836	6	69	2012	-

*Note: BR= Brown, YE= Yellow, BL= Blue, WH= White, PU= Purple, BK= Black, GR= Green, RE= Red

the Duncan's post-hoc test to evaluate significant differences among the values using SPSS version 20.0 software.

Results

A total of 478 mullet fish, 462 glassfish, and 435 mudskipper samples were successfully collected during the 3 months of sampling. Among the mullet fish, the highest and lowest microplastic abundances were recorded at the Alue Naga and Pande Village stations, with 2.06 particles/fish and 1.08 particles/fish, respectively. For glass fish, the highest and lowest abundances were found at the Alue Naga and Blang Village stations, with 1.77 and 1.28 particles/fish, respectively. Similarly, mudskippers had the highest and lowest abundances at the Alue Naga and Pande Village stations, respectively, with 1.47 and 1.02 particles/fish, as presented in Table 3. Microplastics in the digestive tracts of mullet, glassfish, and mudskipper exhibited 8 colors: brown, yellow, blue, white, purple, black, green, and red. Black and brown were the dominant colors in the species from all study stations (Table 4).

Based on fish species from all sampling stations, the highest abundance of microplastics was discovered in glassfish (1.55 particles/fish), though it was not significantly different from mullet, which had an abundance of 1.52 particles/ fish (Figure 2a). Regarding sampling stations, the highest abundance of microplastics was observed in fish samples from Alue Naga (1.77 particles/fish), a value that was significantly different from the other two stations, as shown in Figure 2b. In terms of sampling time, the highest abundance of microplastics was discovered in Alue Naga Village in December 2023 (Figure 2c). The highest abundances from Pande and Blang Villages were observed in January 2024 and December 2023, respectively. Overall, however, the Alue Naga station had the higher microplastic abundance across all sampling months (Figure. 2c). Microplastic sizes in the digestive tracts of mullet

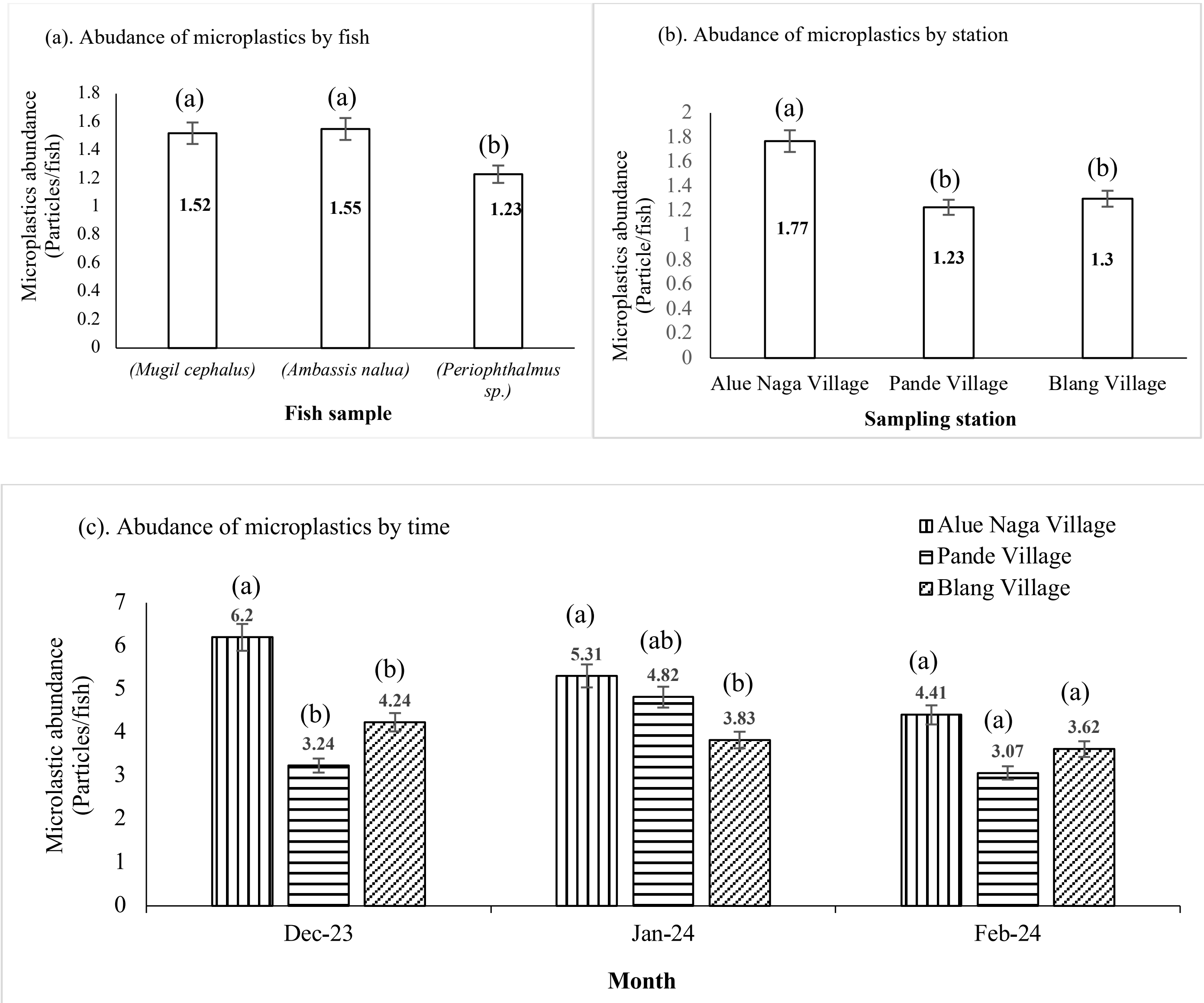

Figure 2. The abundance of microplastics based on (a) fish species, and (b) sampling location, and (c) sampling time. The average values followed by small letter in the brackets are significantly different (P<0.05)

and glassfish ranged from 20 to 140 µm and 20 to 180 µm, respectively, with the dominant sizes being 20–40 µm. In mudskipper, the microplastic size ranged from 20 to 140 µm, with the dominant sizes being 20–40 µm across all fish species (Table 5).

FTIR analysis of mullet samples from the Alue Naga Village station showed that the highest wavelengths and functional groups ranged from 2964.59 cm1 (C=H stretch) to 3016.67 cm1 (C=H stretch), indicating the presence of a polypropylene polymer. In mullet samples from Pande and Blang Villages, the highest wavelengths and functional groups ranged from 1541.12 and 1539.20 cm1 (NH bend, C-N stretch), indicating the presence of a nylon polymer, as shown in Figure 3a. For glassfish and mudskipper samples from all 3 stations, the highest wavelengths and functional groups ranged from 1543.05 to 1517.98 cm1 (NH bend, C-N stretch), also signifying the presence of a nylon polymer (Figures 3b and 3c).

Discussion

This study showed that fish samples from the stations were contaminated with microplastics, with an average abundance ranging from 1.02 to 2.06 particles/fish. However, these values were lower than those found in mullet and bagok catfish *Hexanemachthys sagor* from Krueng Aceh River estuary area, Banda Aceh City, which had an average of approximately 7 – 8 particles/fish (Maulana et al. 2023). Additionally, tilapia (*Oreochromis niloticus*) and *Hypostomus plecostomus* in eastern Javanese waters, Indonesia, were contaminated with microplastics, with average abundances of 18, and 468 particles/fish, respectively (Sandra and Radityaningrum, 2021). Among the fish species, the highest abundance of microplastics was discovered in mullet and glassfish. In terms of sampling stations, the highest abundance was observed in fish from the Alue Naga village station, likely due to several potential sources of plastic waste in the area, such as densely populated

Table 5. Microplastics size in the digestive tract of mullet Mugil cephalus, glassfish Ambassis nalua, and mudskipper Periophthalmus sp.

Fish species	Microplastics size (µm)	Sampling station			Total	Average Particle/fish
		Alue Naga	Pande	Blang		
Mullet (*Mugil cephalus*)	20-40	321	136	188	1.184	16.13
	41-60	17	18	6	41	2.05
	61-80	12	8	5	25	1.25
	81-120	13	7	6	26	0.65
	121-140	1	1	1	3	0.15
Glassfish (*Ambassis nalua*)	20-40	285	245	170	700	17.5
	41-60	3	4	1	8	0.4
	61-80	7	2	1	10	0.5
	81-120	4	3	-	7	0.17
	121-140	-	-	-	-	-
	141-160	-	-	-	-	-
	161-180	1	-	1	2	0.1
Mudskipper (*Periophthalmus* sp.)	20-40	236	133	194	563	14.08
	41-60	3	10	3	16	0.8
	61-80	2	-	2	4	0.2
	81-120	3	2	-	5	0.12
	121-140	-	2	-	2	0.1

settlements, tourist areas, and small-scale fishing ports. A river that flows into the area carries plastic waste from the mainland, which settles in the estuary when water recedes. This finding aligns with Supit et al. (2022), who stated that the abundance and distribution of microplastics on coastlines are influenced by oceanographic factors, specifically currents and tides.

Film was the dominant form of microplastics found in the digestive tracts of fish samples. These irregular sheets of plastic have a lighter mass, which facilitates their transportation by currents and their accidental ingestion by fish (Constant et al. 2022; Phaksopa et al. 2021). This is related to the habitats of species living in mangrove coastal waters (Mulfizar et al. 2012), where significant amounts of plastic waste accumulate among plants. Previous studies have shown that black and brown were the most dominant colors of microplastics found in fish stomachs. The black color indicates a longer presence of particles in water, during which they absorb more contaminants (Maulana et al., 2023). The color of microplastics tends to match the color of the surrounding water due to the absorption of materials (Issac and Kandasubramanian, 2021). Field observations revealed that the Alue Naga Village station has higher water turbidity, specifically during high tide when rubbish is washed away from nearby settlements.

The dominant and smallest size group of microplastics discovered in the 3 species was 20-40 µm. Similarly, Maulana et al. (2023) reported that these sizes were dominant among mullet and bagok catfish in the estuary of the Krueng River, Aceh, Indonesia. The size of microplastics is inversely proportional to their likelihood of being swallowed by fish. Furthermore, factors such as sunlight radiation intensity, microplastic density, water temperature, and wind contribute to size composition (de Vries et al. 2020; Yao et al. 2021). The warm temperature of tropical waters, such as those in Indonesia, accelerate the decomposition of plastic waste into smaller sizes. This is in line with the findings of Xiong et al. (2019), who reported that climate influences the decomposition process of microplastic particles in water.

In this study, FTIR analysis confirmed the presence of nylon and polypropylene polymers in the muscles of fish species. Nylon, often sourced from fabrics and domestic waste, is commonly used in the production of ropes and fishing nets (Halstead et al., 2018). Similarly, polypropylene polymers, characterized by the presence of CH absorption as a constituent bond, are derivrd from sources such as plastic food packaging, buckets, glasses, used automotive parts, medical waste, and plastic bottles (Alabi et al., 2019; Wright et al., 2013). Observations at the Alue Naga Village station, a river estuary near densely populated settlements, revealed large amounts of these polymers, primarily from food or drink packaging. Further research is needed to assess the concentration of microplastics in fish flesh.

This study confirmed that mullet, glassfish, and mudskipper from the tidal waters of the Banda Aceh City mangrove forest have been contaminated by microplastics. However, the level of concentration of microplastics in the fish flesh is not yet known. Therefore, further study is needed to assess the concentration of microplastics in the fish flesh remains unknown, necessitating further research. This information is crucial for ensuring food security for the community. Currently, no official quantitative standards exist for microplastic levels in

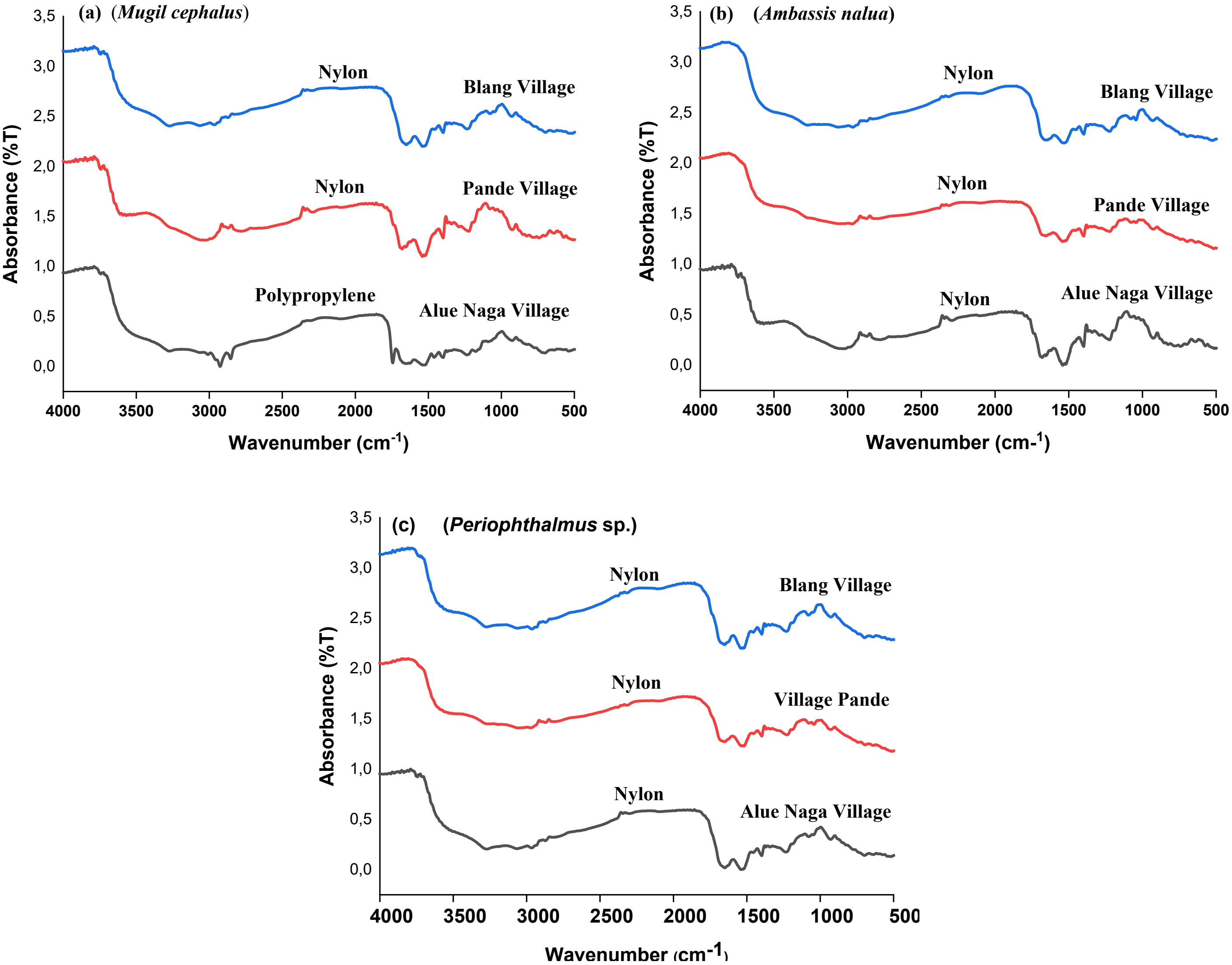

Figure 3. The electrogram of FTIR from three species of fish (a) mullet *Mugil cephalus*, glassfish *Ambassis nalua* and mudskipper *Periophtalmus* sp. harvest from Banda Aceh coastal water from December 2023 to February 2024

fish, highlighting the urgent need to establish safety standards to regulate microplastic content in fish.

In mammals, microplastic particles can migrate from the digestive tract into the circulatory system, with some crossing the placenta and impacting fetal development (Sharma and Kaushik, 2021). However, their effects on fish reproductive systems have not been extensively studied. Subaramaniyam et al. (2023) reported that microplastic exposure negatively affects reproductive success, reducing fertility and hatching rates. In humans, microplastic contamination can cause disorders such as inflammation of the digestive tract, constipation, colitis, and colon irritation due to increased cell death (Zhao et al., 2023; Qiao et al., 2019). It also contributes to digestive disorders, weakens the immune system, and increases the risk of chronic disease and pathogenic infections (Fackelmann and Sommer, 2019; Deng et al., 2020). Furthermore, microplastics in water can absorb various environmental chemicals, including endocrine-disrupting compounds (EDCs), which pose significant risk to human health. These compounds can damage the endocrine system, disrupt hormonal balance, and reproductive functions (Gallo et al., 2018).

Conclusions

The digestive tracts and flesh of mullet, glassfish, and mudskipper from the mangrove ecosystem waters of Banda Aceh City, Indonesia, were found to be contaminated with microplastics. Glassfish from the Alue Naga Village station in Syiah Kuala District showed the highest abundance of microplastic contamination. Five forms of microplastics were identified: fiber, film, fragments, pellets, and foam, with film being the most dominant. Observations revealed that black microplastic particles, ranging in size from 20 to 180 µm, were the most common. Finally, nylon and polypropylene polymers were detected in the flesh samples of the fish.

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