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Accumulation of Microplastics and Histological Analysis on Marine Fish from Coastal Waters of Baru and Trisik Beaches, Special Region of Yogyakarta

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ABSTRACT

River flow to the sea is regarded as a pathway for the dispersion and pollution of microplastics. The hydrodynamics of the coastal water of Baru and Trisik Beaches may increase microplastic concentrations in this estuarine-marine area. This research evaluated the microplastic concentration in surface seawater and microplastic accumulation in consumed marine fish and performed histological analysis on the demersal marine fish intestine under natural exposure. Sample collection was carried out at 12 stations with three replications. The microplastics analysis was performed on marine fish (dorsal muscle, gills, and gastrointestinal tract) and seawater, and microplastic characterization was based on physical and chemical characteristics. Environmental parameters for statistical analysis included air and water temperature, pH, dissolved oxygen, and salinity of seawater. Histological analysis of the intestine was performed on fish from three stations with two demersal fish species. The latter analysis included the length of the villi, the depth of the crypt cells, epithelial denudation with hematoxylin-eosin staining, and the number of goblet cells with periodic acid Schiff alcian blue staining. The results showed that the microplastic concentration was distributed throughout the fish sample for each of the stations. Microplastic concentrations for surface seawater showed the same pattern as marine fish. Microplastic accumulation in marine fish indicated the transfer of microplastic particles to various organs in the fish's body. The histological analysis indicated, microplastic internalization in the intestine tissue, damaging intestinal structures. Further research is needed as consuming marine fish contaminated by microplastics may present increasing health risks.

1. INTRODUCTION

Anthropogenic activities around rivers, estuaries, beaches, and coastal waters are the main sources of marine debris. Henceforward, plastic use will continue to increase and constitute 80 to 85% of marine debris (Auta et al., 2017). Global use of plastics is threatening marine life and human health (Lundebye et al., 2022). In the aquatic ecosystem, plastic waste is degraded by physical, chemical, and biological processes into smaller sizes (i.e., microplastics and nanoplastics) (Jovanović, 2017). Microplastics (MPs) size is less than 5 mm with different shapes, colors, and polymers (Barboza et al., 2020; Lundebye et al., 2022). Microplastics are transported from estuaries and beaches via ocean currents, gyres, and garbage patches (Lebreton et al., 2012; Wieczorek et al., 2018; van Sebille et al., 2020). Moreover, MPs can migrate and concentrate in surface seawaters, potentially accumulating in the estuaries and marine biota (Amelia et al., 2021; Auta et al., 2017; Zhang et al., 2020).

Marine fish, such as demersal and pelagic fish, act as a potential food source for humans, but can also accumulate and transfer MPs to other trophic levels (Carbery et al., 2018; Cedervall et al., 2012; Koongolla et al., 2020). Additive chemicals adsorbed onto the surface of MPs can also accumulate in marine fish organs (Woodall et al., 2014; Yu et al., 2019). Microplastics have been documented in marine fish organs such as the gastrointestinal tract (GIT), gills, muscle, skin, and liver (Abbasi et al., 2018; Barboza et al., 2020; Koongolla et al., 2020; Maaghloud et al.,

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2020). Demersal fish are bottom feeders and have the potential to swim and enter river-estuaries, which also accumulate MPs from water and benthic biota (Brodeur et al., 2021). Pelagic fish swimming near surface seawater, accumulate MPs from these waters and consume neritic organisms (Brodeur et al., 2021).

Several investigations have looked into the effects of MPs on marine fish from polluted areas, but histological analysis of marine fish organs is still limited (Haave et al., 2021). The controversial issue is the potential of MPs to affect the organs in the laboratory and natural conditions. In histopathology studies, MPs have been identified as having adverse effects, especially on fish intestines (Jovanović et al., 2018). Microplastic polymers cause inflammation due to leucocyte infiltration, and a loss of crypt and villi cells in the intestine of *Girella laevifrons* (Ahrendt et al., 2020). A study by Mbugani et al. (2022) reported that MPs caused histomorphological damage, such as induced intestinal wall degeneration, reactions on epithelial, goblet, and cryptic glandular cells, leucocytic infiltration, and blood congestion.

On the other hand, the histological section of the intestinal tract of zebrafish fed with different type and sizes of feed found MP particles in intestine tissues, but MPs did not induce any histopathological effects (Batel et al., 2020). The histological analysis of marine fish from natural habitats in the study of Haave et al. (2021) also did not reveal any tissue reaction that could be related to the accumulation of MPs. However, histological studies in fish tissue exposed under natural conditions clearly demonstrated the internalization or translocation of MPs. Histological information about marine fish from the natural state is important to determine the possible effects of MPs accumulation, which can be used as a reference to the effects of MPs on humans.

Baru and Trisik Beaches (BTB) are tourist destinations, famous fishing grounds, and sea turtle conservation areas important for local communities (Prakoso, 2018; Sahubawa et al., 2015). These two areas are also separated by the Progo River Estuary (PRE), which has become one of the hotspots of plastic pollution as described by Tasseron et al. (2020) and van Sebille et al. (2020). These coastal waters are also vulnerable to MPs contamination due to plastic waste disposal, mainly from tourist areas. Therefore, MPs can enter the ocean from rivers and coastline run-offs (Pequeno et al., 2021; Zhang et al., 2020), leading to the increase MPs in seawater and possibly accumulating in marine biota. However, to the authors' knowledge, no studies are available on MP pollution in the coastal waters of BTB.

This study evaluated MP concentrations in the coastal waters near the PRE, used as a local fishing ground for demersal-pelagic fish. The abundance and characteristics (sizes, colors, shapes, and polymers) of MPs were analyzed in the fish organs, including the dorsal muscle, gills, and gastrointestinal tract (GIT). In addition, we performed histological analysis of the fish intestines comprised of the paraffin method, hematoxylin eosin (HE), and periodic acid- Schiff-Alcian Blue (PAS-AB) staining. The correlation of MPs accumulation in the intestines by histological structure analysis was further studied to determine the structural damage on epithelium denudation, and villi, crypt, and goblet cells. Microplastic polymers were analyzed with Fourier transform infrared (FTIR) technique. The relationship between MP concentrations in surface seawaters, marine fish organs, and environmental parameters at each station was analyzed using principal component analysis (PCA). Our results provided vital information for the accumulation of MPs in demersal-pelagic fish that may be useful for long-term risk management of human health.

2. METHODOLOGY

2.1 Study area

This study was conducted at the coastal waters of BTB, Special Region of Yogyakarta, Indonesia. The BTB is located near a lowland consisting of a settlement area, field, sand mine, shrimp farm, tourist areas, and seafood restaurants. The PRE separates the BTB by black sand (Prakoso, 2018; Sahubawa et al., 2015). Surface seawater and marine fish were collected from October to November 2021 (west wind season). Twelve sampling stations with three replications each were selected in the BTB based on the visual level of plastic pollution and fishing activities related to the catchment of demersal and pelagic fish, according to a study by Koongolla et al. (2020).

The 12 sampling stations were divided into two lines: line 1 (stations 1 to 6), which was one mile away from the beach, and line 2 (stations 7 to 12), which was one mile away from line 1 (Figure 1). Sampling performed in line 1 evaluated the concentration of MPs in the tidal zone and estuary, while sampling in line 2 analyzed the concentration of MPs in the territorial sea. Stations 1 (7°59′55,917″ S 110°12′ 18,446″ E) to 6 (7°58′38,246″ S 110°9′57,561″ E) are classified as fishing grounds near the tourist location, which are densely populated. In addition, stations 3 and 4 are situated close to the PRE. Stations 7 (8°0′38,443″ S 110°11′47,511″ E) to 12 (7°59′19,893″ S 110°9′25,442″ E) are infamous fishing grounds.

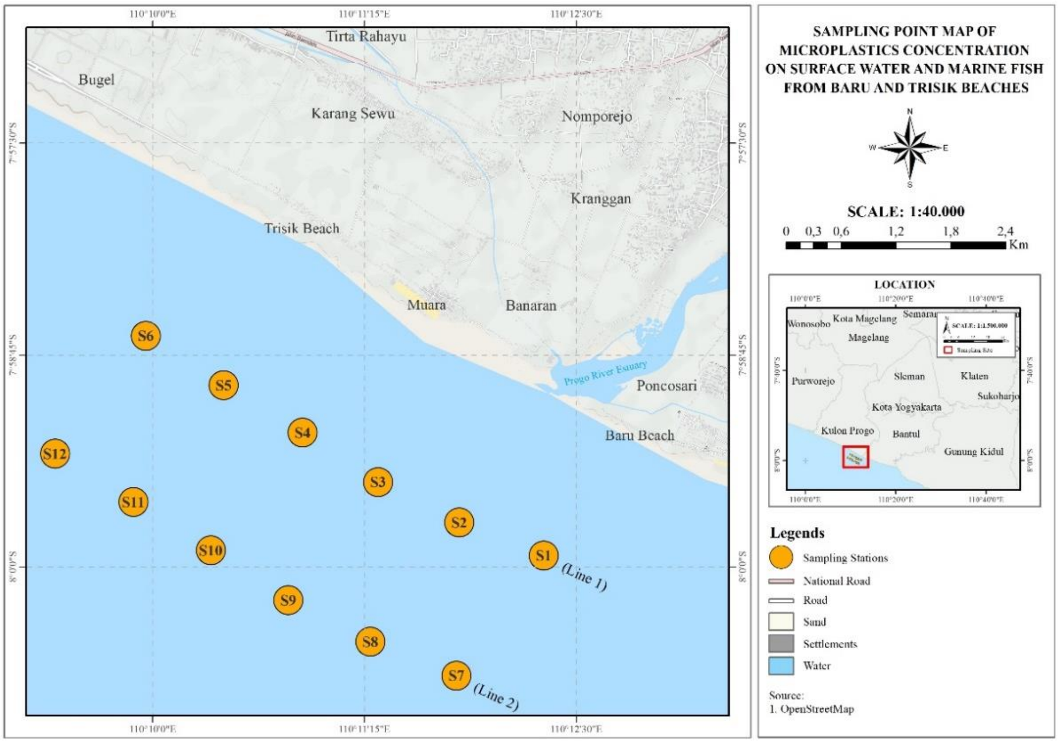

Figure 1. The sampling stations of surface seawater and marine fish collected in BTB

2.2 Sample collection

Surface seawater and marine fish samples from the 12 stations were collected from a fisherman's boat using a 15 PK capacity machine. Seawater was sampled in triplicate (at each station) at a depth of 0-60 cm from the surface referring to McNeish et al. (2018) and Sun et al. (2018), into a 5 L container and closed to avoid contamination (Yan et al., 2019; Zhang et al., 2020). Marine fish samples were collected using a gill net (length 50 m). Marine fish from all stations were identified and stored in an icebox. Marine fish species at each station were measured as the relative frequency (%) to the total number and recommended MPs biomonitoring species referring to Ali et al. (2020). Environmental factors measured in triplicate at each station included air and water temperature, pH, dissolved oxygen (DO), and salinity of seawater.

2.3 MPs extraction

Microplastics were extracted from surface seawater according to the methods described by Sun et al. (2018), Yan et al. (2019), and Zhang et al. (2020). For marine fish, MPs were extracted according to Karami et al. (2017), Koongolla et al. (2020), and Maaghloud et al. (2020). The surface seawater was filtered using a 0.45 µm filter paper (Whatman TM, UK) to filter MPs <5 mm (Zhang et al., 2020). The filter papers were stored in Petri dishes and labeled for identification and characterization.

Fish were captured and measured for length and weight. This research also identified the fish species using an identification book and database. Fish were dissected and separated into the gills, GIT, and dorsal muscles based on the recommendations of Barboza et al. (2020). Each organ was prepared, weighed, and analyzed in the laboratory to prevent contamination. Microplastics were extracted using 10% KOH (Barboza et al., 2020; Karami et al., 2017; Koongolla et al., 2020). The fish organ samples were dried in an oven at 60°C for 24 h to dissolve the organic compounds. After digestion, the solution was filtered on a 0.45 µm filter paper (WhatmanTM, UK). The filter paper was air-dried at room temperature and then stored in a Petri dish to identify the MPs. A control filter paper (i.e., filtered distilled water) was also included in MP identification. Extraction was performed as described above. The control sample was used against the laboratory samples to check for contamination (McNeish et al., 2018).

2.4 Microplastics characterization

Microplastics were observed using a Leica ICC50E and Optilab microscope. The characterization was based on size, shape, color, and polymer type according to GESAMP (2015), Hidalgo-Ruz et al. (2012), and McNeish et al. (2018). Microplastic sizes were classified into small (<1.5 mm), medium (1.5-3.3 mm), and large (>3.3 mm) and measured with Image Raster. The shape was classified into fragments, fibers, films, foams, and pellets. The color was classified into white, blue, green, red, yellow, brown, black, and transparent.

Polymers were characterized using Fourier transform infrared spectroscopy (FTIR) with a wavelength range of 4,000-400 cm^{-1}. The spectra were analyzed with OMNIC Software (Thermo Fisher Scientific Inc.) as described by Barboza et al. (2020) and Koongolla et al. (2020). The FTIR tests were based on the MPs shape and color. Analyzed spectrum data were compared with the reference database matching >85% to determine the type of polymers.

2.5 Histological analysis

The histological structure was analyzed in the intestine of two demersal species from stations S1, S3, and S6. Two demersal fish species were selected based on the highest number of demersal fish, commercial species, and amphidromous species. In addition, the two demersal species were used to evaluate MPs accumulation in coastal water of BTB and PRE. Three stations were selected based on the visual plastic pollution in line 1, station S1 was near Baru Beach, S3 was close to the PRE, and S6 was nearby Trisik Beach. Samples from these three stations were used to determine MPs accumulation on the beaches and estuary.

The MPs structure was analyzed in triplicate from fish intestine samples by the paraffin method as described elsewhere Ahrendt et al. (2020), Limonta et al. (2019), and Ratucoreh and Retnoaji (2018). The GIT samples were excised mid-intestinal for histological analysis, and the other sections were used for MPs extraction. Intestine samples were washed using 0.9% NaCl, fixed with a 10% neutral buffer formalin (NBF) solution, and then washed with 70% alcohol until clear. This intestine sample was dehydrated with an alcohol series starting from 70%, 80%, 90%, 96%, to 100%. It was cleared and infiltrated with toluene solution and paraffin at 60°C. This organ was sectioned with a rotary microtome at 5 µm. The coupe was pasted on object glass and stained with HE and PAS-AB. Before staining the coupes, all coupes of the intestine section were observed under the microscope to detect MPs particles. The MP particles were carefully collected from the coupes and separated with paraffin for FTIR tests. After MPs were retrieved from the intestinal tissue, paraffin blocks were cut again to obtain coupes for staining.

The coupes on object glass were deparaffinized with xylol for 30 min and washed in the alcohol series starting from 96%. The samples were dipped into hematoxylin for 10 s and washed with running water for 10 min. The samples were dipped into distilled water and the alcohol series starting from 30% to 70%. Afterward, the samples were stained with eosin Y for 1 min and washed in the alcohol series, starting from 70% to 96%. The samples were sealed with Canada balsam and a cover glass. The PAS-AB staining method commenced with deparaffinization and hydration using the alcohol series. The sample was dipped into alcian-blue for 5 min and washed with distilled water, slides were dipped into periodic acid 1% for 5 min, followed by Schiff reagent for 5 min. The slides were washed with warm distilled water, dehydrated with distilled water series, and mounted with xylol. The samples were sealed with Canada balsam and a cover glass. The histological analysis assessed the occurrence and localization of possible MPs particles in the tissues with a LEICA microscope (Haave et al., 2021).

2.6 Statistical analysis

Statistical analysis was conducted using Microsoft Excel 2013, SPSS v.25, and Xlstat. One-way ANOVA and Tukey test were performed to compare the MP concentrations in the surface seawater and marine fish at each station and the relationship between MPs in fish species. The relationship of MPs in surface seawater with environmental factors was analyzed with one-way ANOVA, Duncan, and Pearson correlation tests. The correlation of MPs in surface seawater and marine fish with environmental parameters was determined by PCA. One-way ANOVA and Kruskal-Wallis’s test analyzed the internalization of MPs in the intestine. Analysis of histology data (villi length, depth of crypt cells, and the number of goblet cells) of MPs in the GIT was tested by one-way ANOVA, Duncan, and Pearson correlation.

3. RESULTS AND DISCUSSION

3.1 Microplastics in surface seawater

Monitoring MPs in the BTB showed that the pollutant contaminated surface seawater at all stations. Microplastics in the surface seawater were significantly different at each station ($p<0.05$) (Figure 2(a)). Small particles (<1.5 mm) dominated the size of MPs (Figure 2(b)). Similar results have been reported by Zhang et al. (2020), which demonstrated MP pollution in the east coastal areas of Guangdong, South China, and the Pearl River estuary. The authors verified the degradation of plastics into MPs in the mentioned coastal waters and estuary. In this study, MPs potentially re-entered the coastal waters by ocean currents, waves, and wind, and may increase in the water column, and seafloor (Haave et al., 2021). Bastesen et al. (2021) reported similar results, where the Norwegian coastline over 100,000 km had a high concentration of MPs from the Norwegian Coastal Current and winds from the southwest.

The highest MP concentration (25 particles/L) was observed at station 3 (Figure 2(a)). This station is an estuary area receiving strong anthropogenic pressures from rivers, Rodrigues et al. (2020) on surface seawater at a Portuguese Estuary and Marine Park. In addition, fishing activities may increase the concentration of MPs from fishing lines or fishing tools.

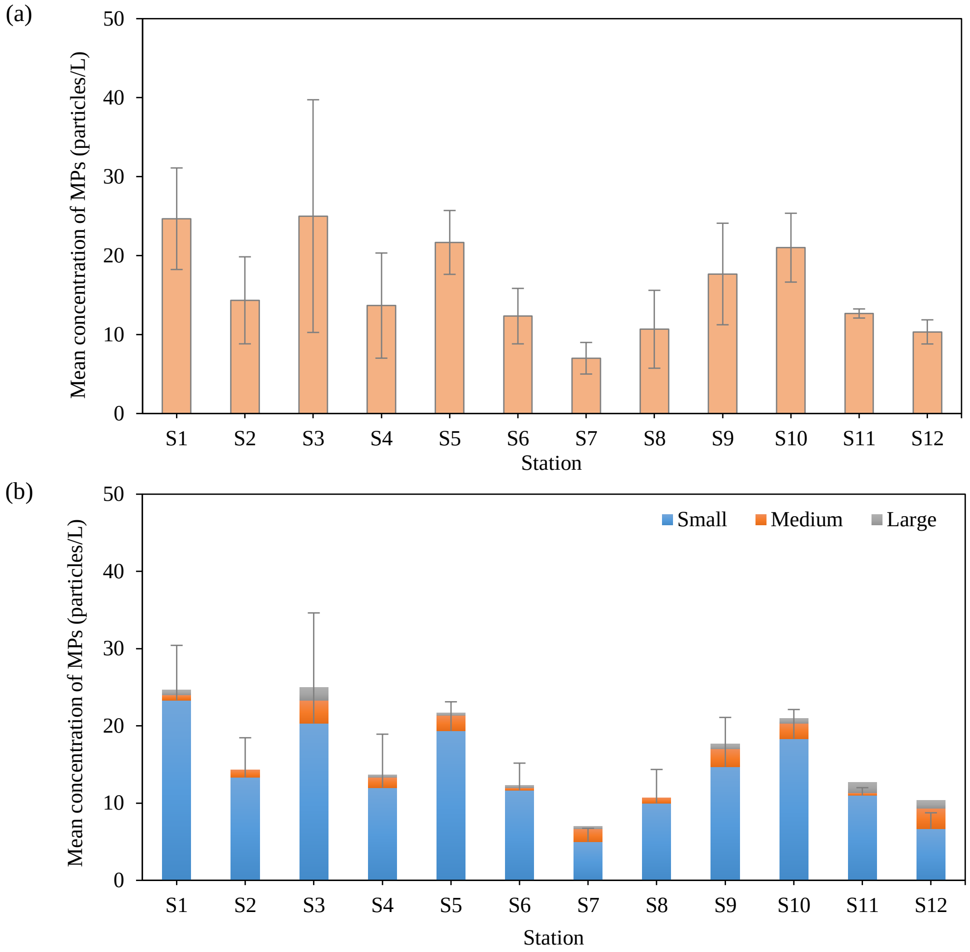

Figure 2. Concentration of MPs (a) and concentration of MPs in three size fractions (b) in surface seawater.

When comparing MPs concentration in surface seawaters at BTB, our results showed that lines 1 and 2 had 12.3-25.0 particles/L and 7-21 particles/L, respectively. The stations at line 1 had a higher concentration of MPs, suggesting MP pollution at these stations might be ascribed to their proximity to the shoreline (about 1 mile) and the mouth of the PRE. It also indicates that the high concentration of MPs in the estuary was dispersed by the currents, as reported by Haave et al. (2021).

3.2 Microplastics in marine fish

This study highlights the accumulation of MPs in all marine fish samples. A total of 21 species were divided into two habitat groups (10 demersal and 11 pelagic fish) (Table 1). Most marine fish were carnivores, except *Gazza minuta* and *Gerres oyena* which were omnivores. The MPs were found in the dorsal muscles, gills, and GIT of all fish samples, similar to the studies by Koongolla et al. (2020) and Maaghloud et al. (2020). In this study, the MP concentrations in the dorsal muscles, gills, and GIT were significantly different among stations ($p < 0.05$) (Figure 3(a)), commonly found in other studies Koongolla et al. (2020), Maaghloud et al. (2020), and Zhang et al. (2021).

The percentage of MPs accumulation was highest in the gill (37.49%) and GIT (37.38%), while the lowest was in the dorsal muscle (25.13%). These results indicate that MPs entered the fish mainly through the respiratory and digestive systems (Limonta et al., 2019). The gill-water interface causes the MPs to enter the fish's body readily. Our results are similar to those reported by Barboza et al. (2020) and Yona et al. (2022). Microplastic accumulation in the organs indicates MP pollution in the beach waters. Accumulation of MPs in gills can cause a decrease in respiratory efficiency and hypoxia (Movahedinia et al., 2012). In addition, MPs in the dorsal muscle can result from skin lesions and the bloodstream (Barboza et al., 2020).

Microplastics were accumulated in demersal and pelagic fish species 14.47-55.50 particles/fish (Table 1). The MP concentrations varied in marine fish with no standard threshold. The abundance of MPs and marine fish in the region might be influenced by swimming, cruising, and feeding habit (Koongolla et al., 2020; Maaghloud et al., 2020). The distribution of all marine fish species in this study may provide information on relative frequency values that can be used as a recommendation for microplastic biomonitoring. There were five species with high relative frequency (%), *Eleutheronema tetradactylum* (ET), *Eubleekeria splendens* (ES), *Leiognathus equula* (LE), *Scomberoides lysan* (SL), and *Scomberoides tala* (ST). Our results showed that the accumulation of MPs in the fish was higher than that detected by Mistri et al. (2021) in commercial marine fish from the Adriatic Sea (2.85-4.11 particles/fish), and marine fish from the coastal waters and estuary of the West China Sea (0.3-5.3 particles/fish) by Su et al. (2019).

For the demersal fish, we found a high MP accumulation of MPs was found in *E. tetradactylum*, *E. splendens*, *L. equula*, and *E. tetradactylum*, a demersal fish, had the highest number of individuals for 11 stations and accumulated the most MPs (Table 1). The highest MPs accumulation in pelagic fish was *S. lysan*, *S. tala*, and *S. lysan* the most common pelagic fish found at the research stations. These two fish species have high economic value at the research locations, and they are widely consumed by the surrounding community.

In previous studies, the highest MPs accumulation and distribution in estuarine-coastal waters area was observed in *E. tetradactylum*, suggesting its potential as an indicator species for MPs biomonitoring (Karbalaei et al., 2019; Mirad et al., 2020). This fish is an amphidromous species, so it has a high risk of accumulating MPs from the river, estuarine, and marine areas. The dominant pelagic fish family was Carangidae, this family is known to be present widely around the Atlantic, Indian, and Pacific Oceans. The Carangidae is also the fastest predator in the ocean (Maaghloud et al., 2020). Sawalman et al. (2021) reported that three species of the family of Carangidae from Barranglompo Island, Makassar, Indonesia, also accumulated MPs, similar to the results described for the Moroccan Central Atlantic Coast (Maaghloud et al., 2020). Furthermore, skipjack tuna (*K. pelamis*) and mackerel (*S. commerson*) are marine fish very popular for consumption in Indonesia that were also found to accumulate MPs (Chen et al., 2021; Syafitri et al., 2021; Yona et al., 2022).

Similar to surface seawater, MP concentrations in fish at the stations of line 1 were also higher than those of line 2. Marine fish at line 1 was more susceptible to ingesting MPs, while current velocity from surface waves can affect the ability of MPs to settle in seawater (Pequeno et al., 2021). Stations 3, 4, and 5 showed a similar pattern of MP accumulation in fish (Figure 3(a)), which was higher than the other stations. This indicates that the estuary is a protected area with a high diversity and abundance of marine fish. In addition, the estuary is a nursery area since many marine fish reproduce and spend the early part of their lives here (Pequeno et al., 2021). Current waves affect the distribution and accumulation of MPs in the estuary (Zhang et al., 2020). Accumulation of MPs in the marine fish at line 1 may be affected by hydrodynamic force, feeding habits, swimming range, and interaction with ecosystem components (Pequeno et al., 2021; Zhang et al., 2021). Species *E. tetradactylum*, *E. splendens*, *L. equula*, *S. lysan*, and *S. tala* are amphidromous fish crossing estuarine-marine ecosystems (Mirad et al., 2020). In this study, the amphidromous species have a high concentration of MPs (Figure 3(b)), which was also confirmed by Zhang et al. (2021).

Table 1. Accumulation of MPs in marine fish at all stations

Fish species	Family	Category	Body length (cm)	Body weight (g)	Number of fish at all stations	Average of MPs accumulation at all stations (particles/fish)	Relative frequency (%)
Demersal fish							
Eleutheronema tetradactylum (ET)	Polynemidae	Threadfins	14.10-30.33	70.12-257.95	144	20.31	15.49
Eubleekeria splendens (ES)	Leiognathidae	Ponyfish	12.20-18.50	40.21-60.83	100	20.54	9.86
Leiognathus equula (LE)	Leiognathidae	Ponyfish	13.36-18.80	47.12-64.31	113	18.54	9.86
Karalla daura (KD)	Leiognathidae	Ponyfish	14.18-16.35	50.36-68.51	32	14.47	2.82
Gazza minuta (GM)	Leiognathidae	Toothpony	15.13-18.02	43.70-61.11	47	14.47	5.63
Gerres oyena (GO)	Gerreidae	Silverbellies	14.12-15.28	40.15-51.02	22	17.77	2.82
Johnius belangerii (JB)	Sciaenidae	Croackers	17.90-21.30	54.02-115.00	47	17.36	5.63
Johnius dussumieri (JD)	Sciaenidae	Croackers	30.55-36.20	250.15-290.12	10	19.60	1.41
Drepane longimana (DL)	Drepaneidae	Concertina fish	17.50-18.00	50.35-55.05	2	16.00	1.41
Mystus gulio (GL)	Bagridae	Long whiskers catfish	28.20-33.70	140.93-253.78	9	22.44	9.86
Pelagic fish							
Scomberoides lysan (SL)	Carangidae	Carangids/Jack and pompanus	28.20-33.70	140.93-253.78	54	22.44	9.86
Scomberoides tala (ST)	Carangidae	Carangids/Jack and pompanus	23.70-34.70	115.12-255.15	42	19.02	7.04
Atule mate (AM)	Carangidae	Carangids/Jack and pompanus	16.35-23.41	70.35-205.35	36	17.72	4.23
Carangoides oblongus (CO)	Carangidae	Carangids/Jack and pompanus	16.80-24-50	120.25-140.12	21	20.71	4.23
Caranx ferdau (CF)	Carangidae	Carangids/Jack and pompanus	15.80-21.50	115.60-143.54	20	17.45	2.82
Caranx sexfasciatus (CS)	Carangidae	Carangids/Jack and pompanus	17.80-19.70	120.20-126-12	5	19.20	1.41
Caranx ignobilis (CI)	Carangidae	Carangids/Jack and pompanus	17.18-21.50	163.36-258.26	21	17.43	2.82
Katsuwonus pelamis (KP)	Scombridae	Mackerels	26.00-28.90	170.00-205.02	22	20.18	4.23
Scomberomorus commerson (SC)	Scombridae	Carangids/Jack and pompanus	35.52-36.10	180.50-208.60	5	28.20	1.41
Sphyraena barracuda (SB)	Sphyraenidae	Barracudas	45.00-47.20	320.20-360.20	2	55.50	2.82
Trichiurus lepturus (TL)	Trichiuridae	Largehead hair tall	51.25-52.26	142.50-145.50	2	21.50	1.41

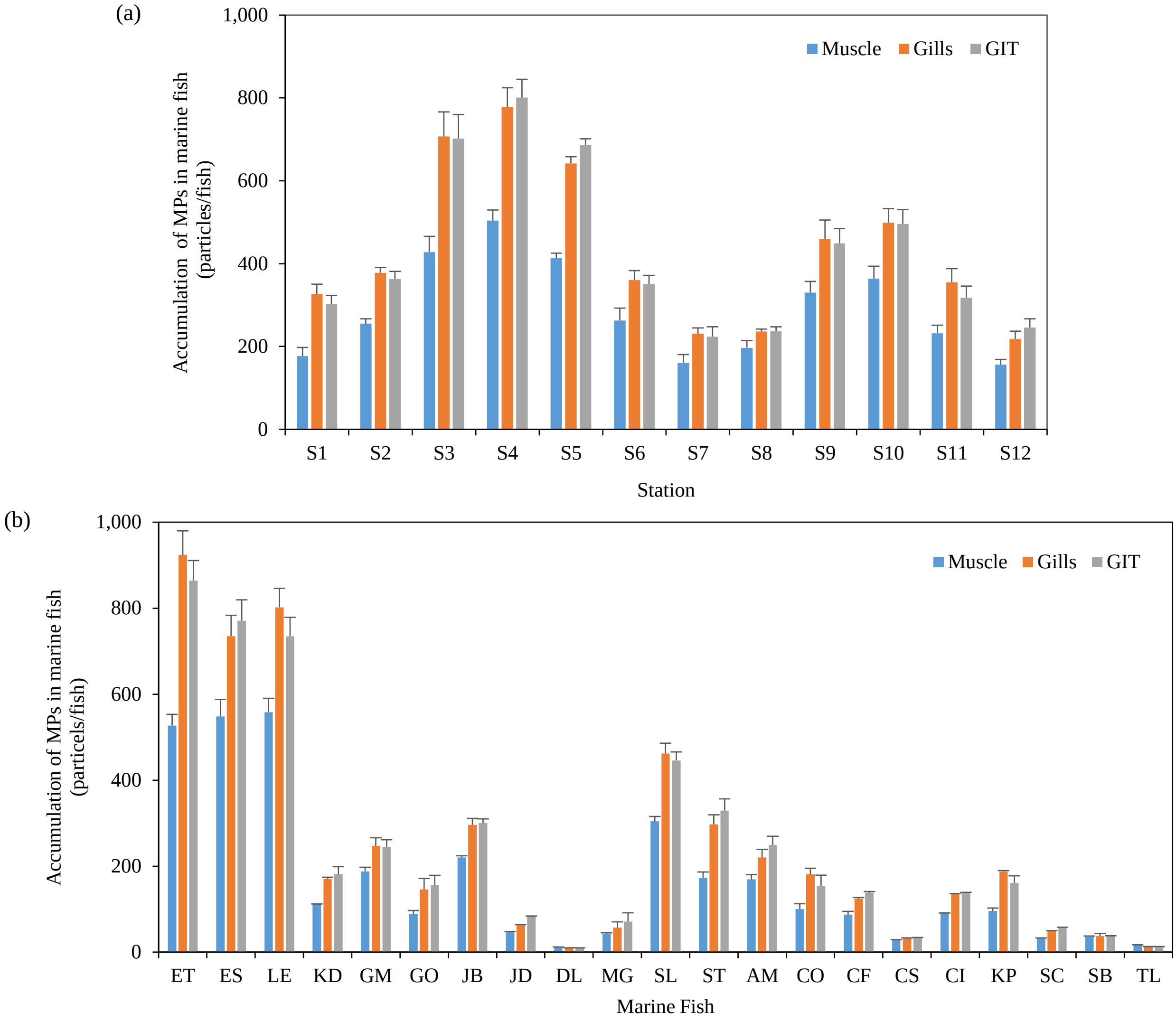

Figure 3. Accumulation of MPs in fish found in each (a) station (a) and (b) fish species

3.3 Microplastics characteristics

In this study, MPs found in the surface seawater were dominated by small particle sizes (<1.5 mm) (Figure 4(a)), similar to the results demonstrated by Guven et al. (2017) and Koongolla et al. (2020). This finding suggested that MPs in marine fish tend to be associated with MPs concentrations in surface seawater. The accumulation of MPs in marine fish may also be affected by their position in the food chains.

The shape of MPs found in surface seawater was dominated by films (73.30%), followed by fibers (13.09%), fragments (12.39%), foams (1.05%), and pellets (0.17%) (Figure 4(b)). Similar results were reported by Pattiaratchi et al. (2022), where film and fiber were the most dominant MPs in the Indian Ocean from various regions. However, in the dorsal muscle of marine fish, the most dominant MP shapes were fragments (70.62%) and fiber (14.29%). Fiber and fragments can be internalized by skin lesions or the blood (Barboza et al., 2020). For the gills and GIT, films were the most dominant MP shape (gills 74.22% and GIT 74.57%) (Figure 4(b)), although differences were found between gills and GIT. Film and fiber accumulate and trigger the perception that MPs are fish food (Galloway et al., 2017).

For MP colors in surface seawater, the most dominant colors were black, brown, and transparent, but they varied in marine fish (Figure 4(c)). Importantly, the color of MPs acts as a visual appearance similar to natural prey in marine ecosystems (Savoca et al., 2017).The most dominant MP polymers in surface seawater included PE (38%), PP (24%), and PET (24%) (Figure 4(d)). It is known that PE and PP have a lower specific gravity, thus they are more commonly found in surface seawater (Amelia et al., 2021). Similar results were reported by Rodrigues et al. (2020).

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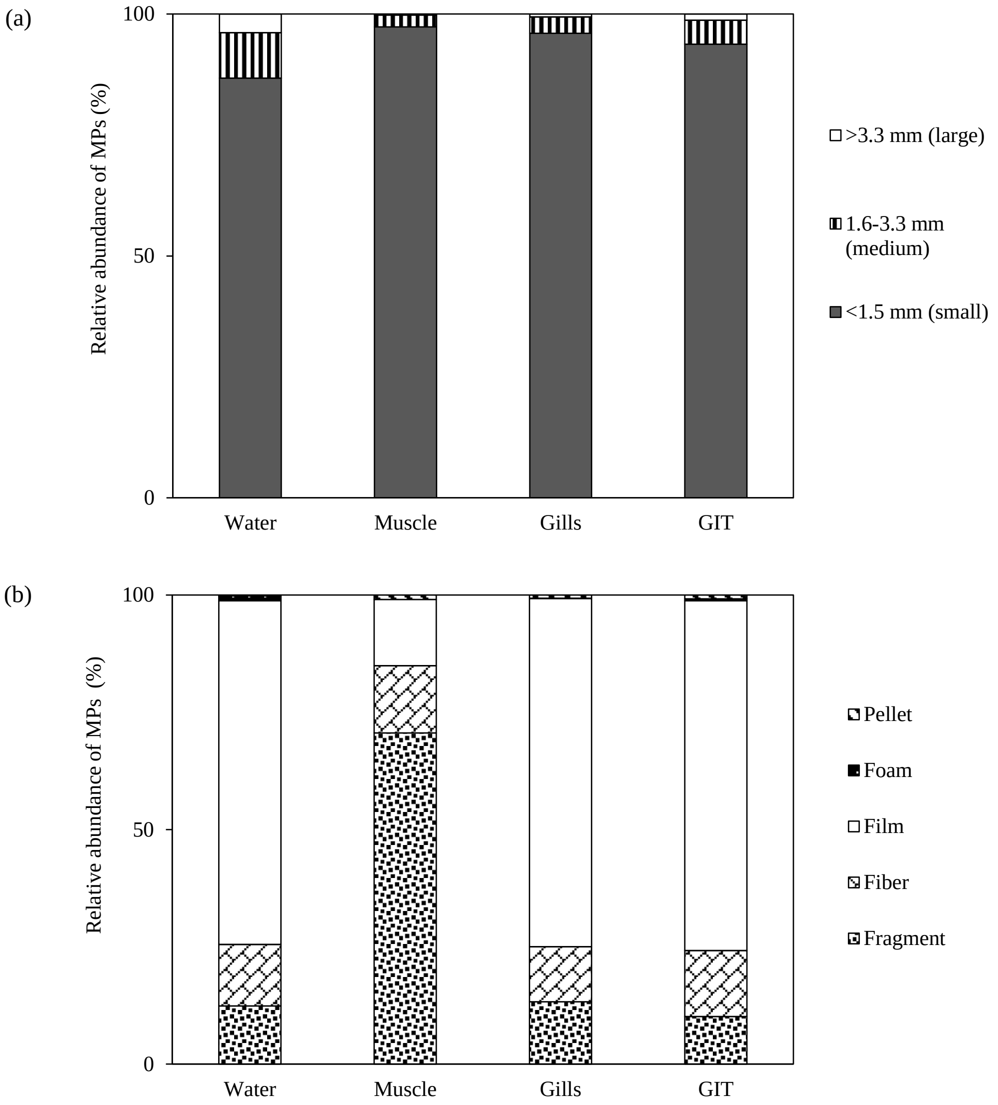

Figure 4. Relative abundance of MPs classified according to their (a) sizes, (b) shapes, (c) colors, and (d) type of polymers in water and fish organs

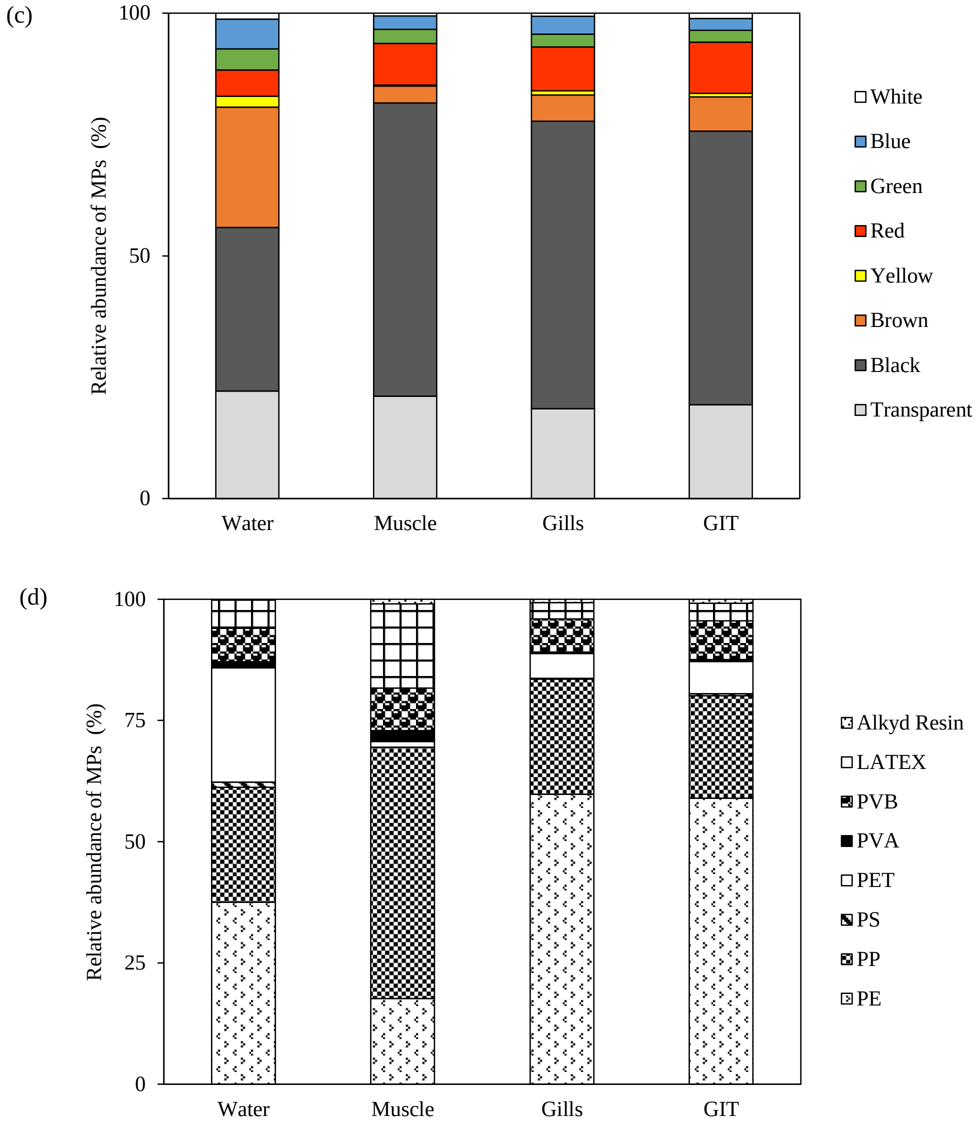

Figure 4. Relative abundance of MPs classified according to their (a) sizes, (b) shapes, (c) colors, and (d) type of polymers in water and fish organs (cont.)

Microplastic polymers that accumulated in marine fish organs were primarily PE and PP (Figure 4(d)). This result was consistent with polymers in surface seawater and other studies by Koongolla et al. (2020), Zhang et al. (2020), and Barboza et al. (2020). These two polymers are the most widely used type of thermoplastics in various human materials, such as packaging and fishery equipment, and are generally found in marine waters and biota (Andrady, 2011). Some of the risks of MPs accumulation in marine fish depend on their physical and chemical characteristics, as previously demonstrated in animal models (Barboza et al., 2020; Limonta et al., 2019). The consumption of marine fish worldwide containing MPs is inevitable, thereby exposing humans to MPs (Smyth and Elliott, 2016).

3.4 Environmental factors and PCA analysis

The results for the environmental variables are shown in Table 2. Sampling was performed from October to November 2021, which is the rainy season. In this study, air and water temperature, salinity, pH, and DO did not show significant differences between stations (p>0.05), as confirmed by Duncan's test analysis (Table 2). These findings highlight which estuarine-marine area had transitional conditions. However, statistically significant differences were detected between (i) low (S3 and S4) and high (S2, S5, S6, S7, and S11) air temperatures, (ii) low (S3) and high (S1, S11, and S12) water temperatures, (iii) low (S7) and high (S10) salinity, (iv) low (S3) and high (S10) pH, and (v) low (S3) and high (S5) water DO. These findings emphasize that environmental factors at the BTB stations varied between the coastal and estuary waters. Pearson correlation analysis found that the MP concentration in surface seawater did not correlate to the average air temperature, water temperature, pH, and DO. However, our findings suggest that the MP concentration in surface waters was correlated to the average salinity.

Our results are similar to those of Defontaine et al. (2020), which demonstrated the turbulent mixing effect of salt-wedge estuaries that can drastically affect the water masses and particles like MPs. Similar features were studied in the Ebro Estuary by Simon-Sánchez et al. (2019), where the estuary acted as a salinity barrier for transporting plastic waste. In this study, stations 3, 4, 9, and 10 had higher salinity levels than the other stations. As expected, the salinity of estuarine-marine waters was influenced by the hydrodynamics in these areas (Smyth and Elliott, 2016). Human activities downstream of the PRE include massive sand mining using heavy equipment, marine tourism activities, Vannamei shrimp aquaculture, and settlements. These conditions most likely contributed to MPs accumulation in the estuary and marine ecosystems (Syafiya and Hadisusanto, 2019; Utami et al., 2022).

Table 2. Environmental factors and concentration of MPs in surface seawater at each station

Stations	Air temperature (°C)	Water temperature (°C)	Salinity (‰)	pH	DO (mg/L)	MPs
S1	29.23^{c}	30.06^{h}	32.31^{e}	7.27^{fg}	6.24^{c}	24.67^{c}
S2	30.19^{e}	29.23^{f}	32.64^{f}	7.23^{de}	6.27^{cd}	14.33^{abc}
S3	28.25^{a}	27.24^{a}	33.43^{i}	6.68^{a}	5.31^{a}	25.00^{c}
S4	28.21^{a}	28.22^{c}	33.36^{i}	6.74^{b}	6.40^{d}	13.67^{abc}
S5	30.24^{e}	29.18^{ef}	32.34^{e}	6.92^{c}	6.70^{e}	21.67^{bc}
S6	30.13^{e}	28.20^{c}	32.13^{d}	7.20^{d}	6.36^{cd}	12.33^{ab}
S7	30.20^{e}	28.08^{b}	30.32^{a}	7.24^{de}	6.35^{cd}	7.00^{a}
S8	29.09^{c}	28.32^{d}	33.14^{g}	7.26^{efg}	6.32^{cd}	10.67^{ab}
S9	28.50^{b}	29.12^{e}	33.22^{h}	7.29^{g}	5.71^{b}	17.67^{abc}
S10	28.35^{ab}	29.61^{g}	33.63^{k}	7.32^{h}	6.36^{cd}	21.00^{bc}
S11	30.10^{e}	30.10^{h}	31.13^{b}	7.23^{de}	6.33^{cd}	12.67^{ab}
S12	29.60^{d}	30.02^{h}	31.29^{c}	7.25^{ef}	6.42^{d}	10.33^{ab}

Numbers followed by the same letter in the same column indicate insignificant differences with Duncan's test with at a significance level of 0.05.

It should be noted that the intensity and frequency of rain events were relatively high during the study period, causing the Progo River to increasingly discharge into the estuary area and bring various wastes to the sea. During the rainy season, starting in August, sea surface runoff transfers MPs to the coastal area, which is also influenced by environmental parameters, including salinity, wind, and other hydrodynamic conditions (Kanhai et al., 2018). In this study, the rainy season was a period of high rainfall, causing high river water discharge that increased the water output to the sea (river flush) via the estuary area. This condition contributed to the fragmentation of plastics into micro or nanoplastics (Lima et al., 2014). The contamination of MPs has been closely related to climate change as plastic production results from the extraction of fossil fuels, although it cannot be determined how much greenhouse gas emissions are produced from plastics, and the distribution of MPs in the sea also influences climate (Ford et al., 2022). This study is consistent with previous results that showed temperature and UV light to greatly contribute to the fragmentation of MPs (Gola et al., 2021).

A PCA analysis was conducted to identify which environmental variables explain the variation in MP concentration at each station. PCA analysis on environmental factors, surface seawater, and marine fish (27 active variables) showed a spatial distribution of the sampling stations in the four quadrants (Figure 5). The biplot revealed an apparent clustering of MP concentrations on the first (F1) and second (F2) axes, explaining >42.95% of the total variance. The first axis (F1) explained 23.76% of the variance, which divided the biplot according to the stations. The highest MP-contaminated seawater was found for S3, ET, MG, ES, ST, KP, SL, and LE samples. Notably, the MP concentration at S3 correlated with the salinity of the seawater. Stations S7, S12, S8, S11, and S6 had lower MP concentrations.

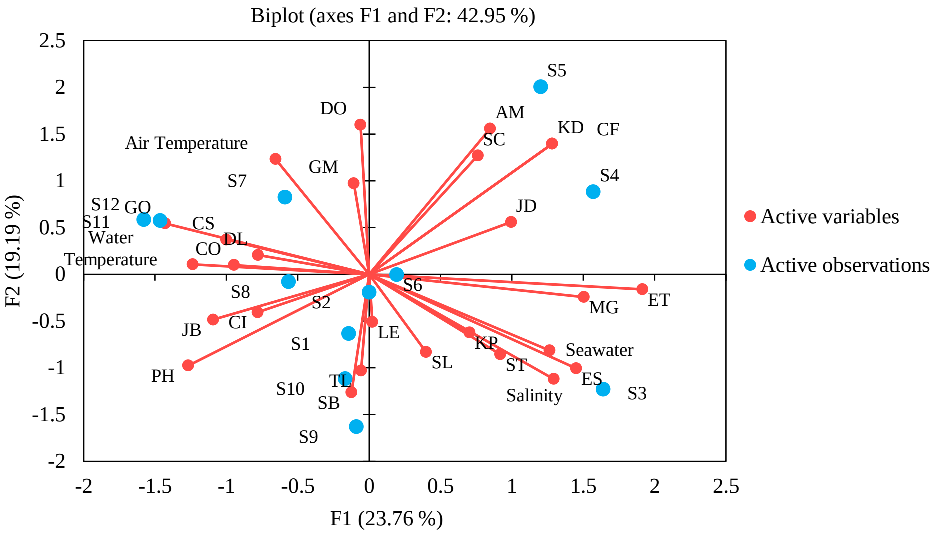

Figure 5. PCA biplot of environmental factors and MP concentration of surface seawater and marine fish

3.5 Histological analysis and MP internalization in the intestine from two demersal marine fish

The histological analysis was conducted to observe potential correlations between structural characteristics with MPs accumulation (Ahrendt et al., 2020). Several findings have revealed that the presence of MPs can affect the histology of the GIT (Limonta et al., 2019; Furukawa et al., 2004; Qiao et al., 2019). In this research, histological analyses were carried out on two demersal fish, *Eleutheronema tetradactylum* (ET) and *Leioghnathus equula* (LE), from three stations using HE and PAS-AB staining (Figure 6) following the methods of Limonta et al. (2019) and Haave et al. (2021) who analyzed the intestinal histology of zebrafish and the gut histology of several wildlife marine biotas, respectively. The two selected fish species are demersal and amphidromous, have a high relative frequency (%), and accumulate MPs. Microplastic internalization of intestinal tissue was analyzed with microscopy and FTIR analysis using MP particles from intestinal coupes. Appropriate steps were taken during the histological process to avoid the presence of artifacts (Batel et al., 2020).

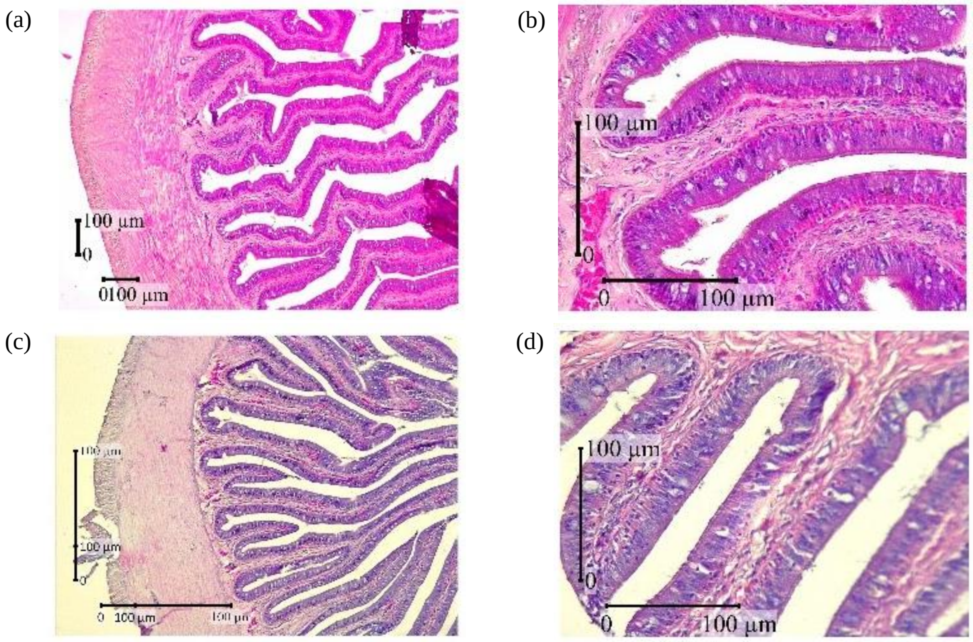

Figure 6. Histology of intestine with HE staining of ET with 100× magnification (a) and 400× magnification (b), LE with 100× magnification (c) and 400× magnification (d), histology of intestine with PAS-AB staining of ET with 100x magnification (e) and 400× magnification (f), LE with 100× magnification (g) and 400× magnification (h)

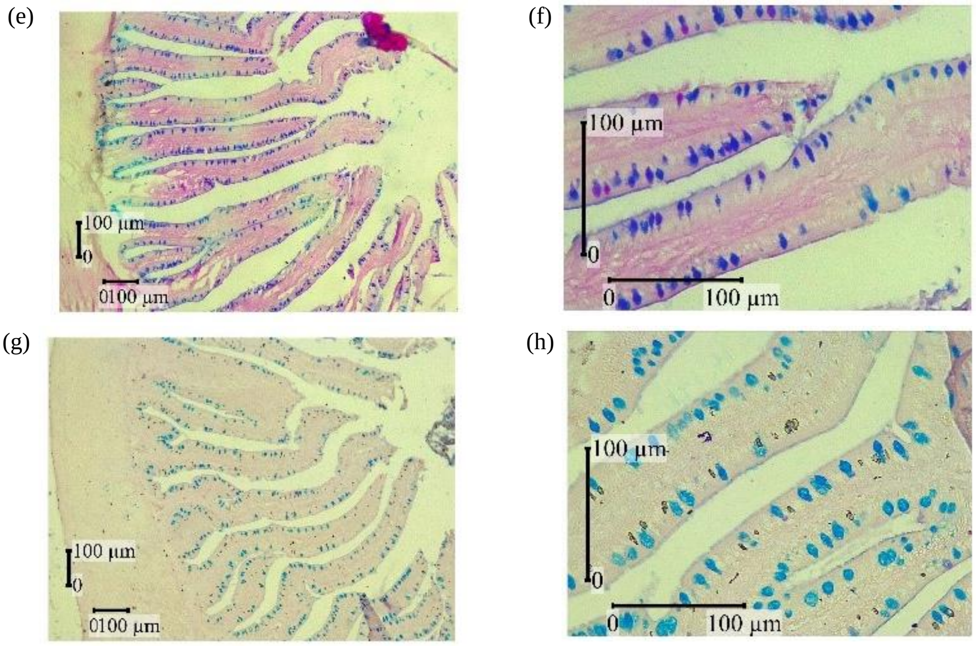

Figure 6. Histology of intestine with HE staining of ET with 100× magnification (a) and 400× magnification (b), LE with 100× magnification (c) and 400× magnification (d), histology of intestine with PAS-AB staining of ET with 100x magnification (e) and 400× magnification (f), LE with 100× magnification (g) and 400× magnification (h) (cont.)

These findings highlight, the MP particles found in the intestine and/or tissue of *E. tetradactylum* and *L. equula* (Figure 7(a-d)). Although our results differ slightly from Haave et al. (2021), who did not find MP particles in the intestinal samples, they are consistent with those of Batel et al. (2020) and Cauwenberghe et al. (2015), who detected MPs in the digestive system of blue mussels (*Mystus edulis* L.) and the zebrafish intestine, respectively. Tissues were positive for MP internalization based on physical characteristics (size, shape, and color) identified by microscopy and FTIR. The Kruskal-Wallis test on MP particles of the *E. tetradactylum* showed a significant ($p<0.05$) difference among the stations. In contrast, the Kruskal-Wallis test on MP particles of the *L. equula* did not differ significantly ($p>0.05$) among stations. This study investigated the internalization of MP particles in intestinal tissue with a focus on polymers (PP, PE, PVA, and LATEX), shapes (fragment and fiber), and colors (transparent, brown, black, red, blue, and green). Microplastics were frequently found in intestinal tissues samples. The presence of MPs in the intestinal tissues was observed by structural histology damage (Figure 7).

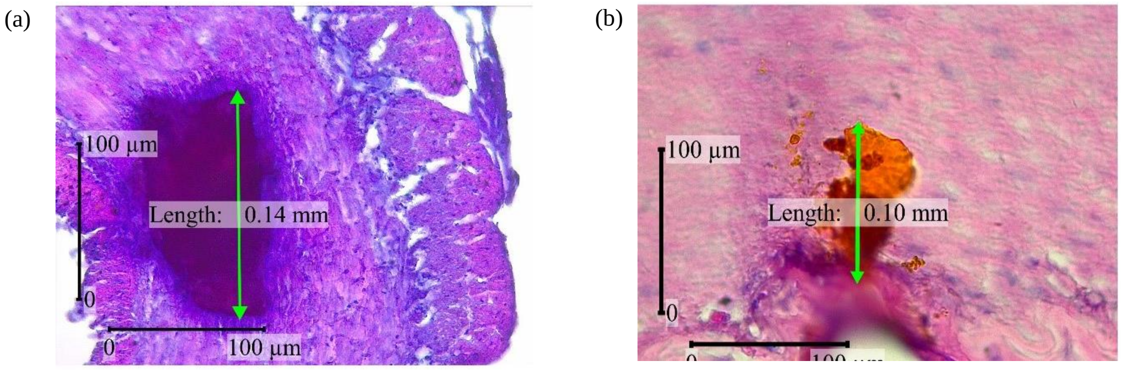

Figure 7. Internalization of MP particles for intestinal histology analysis with HE staining. Microplastics in ET intestinal samples (a-b), MPs in LE intestinal samples (c-d).

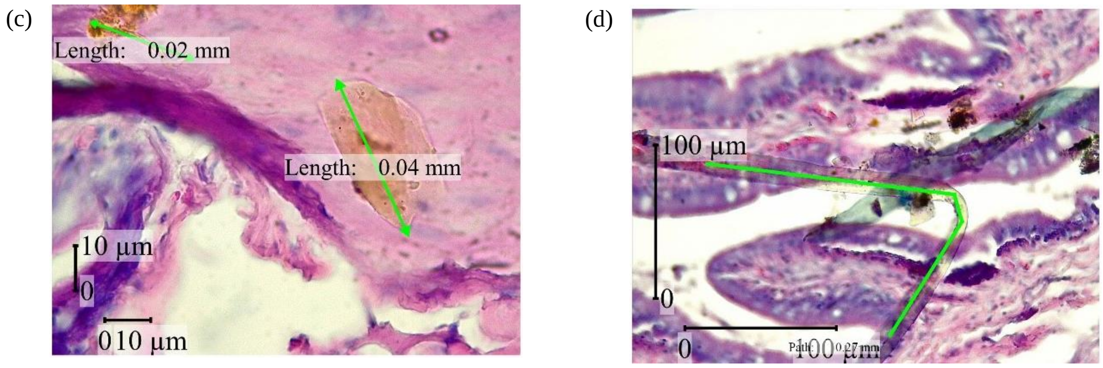

Figure 7. Internalization of MP particles for intestinal histology analysis with HE staining. Microplastics in ET intestinal samples (a-b), MPs in LE intestinal samples (c-d) (cont.).

For research related to the analysis of the impact of MPs accumulation, under natural conditions, on the integrity of the intestinal tissue using histological parameters of the villi cell, crypt cell, goblet cell, and denudation on the epithelial cell under natural exposure, please refer to Ahrendt et al. (2020) and Limonta et al. (2019). Duncan's test analysis (Table 3) confirmed most of the results were not significantly different between stations for *E. tetradactylum* and *L. equula* fish samples. These findings suggest that demersal fish have swimming ranges in both coastal and estuary waters. However, the mean crypt depth of *E. tetradactylum* and the mean villi length of *L. equula* significantly differed among stations. The Pearson correlation found that MP accumulation of MPs in GIT strongly correlated with the average intestinal villi length of *E. tetradactylum* (r=0.728), but it was weakly correlated with the average goblet cells (r=0.389). This result is similar to the study of Ahrendt et al. (2020), which showed that the concentration of MPs in the intestine causes goblet cell loss and a low mean length of the villi. In contrast, *L. equula*, showed that MPs in the GIT were weakly correlated with the average villi length (r=0.314) and were not correlated with the mean crypt depth and the number of goblet cells. This difference might have been influenced by differences in natural prey, and behavior.

Table 3. Average of histology parameters in *E. tetradactylum* (ET) and *L. equula* (LE)

Fish	Stations	Average of villi length (µm)	Average of crypt depth (µm)	Average of goblet's cell	Average of microplastics in GIT
ET	S1	485.37^{a}	75.59^{b}	46.00^{a}	13.33
	S3	498.34^{a}	65.40^{ab}	45.20^{a}	8.33
	S6	485.47^{a}	52.87^{a}	44.40^{a}	11.67
LE	S1	185.05^{a}	19.64^{a}	23.07^{a}	15.67
	S3	157.94^{ab}	15.59^{a}	20.47^{a}	7.67
	S6	269.73^{b}	28.27^{a}	24.80^{a}	6.67

Numbers followed by the same letter in the same column indicate insignificant differences with Duncan's test at a significance level of 0.05.

Accumulation of MPs in the GIT of ET and LE ranged between 8.33-13.33 particles/fish and 6.67-15.67 particles/fish, respectively. In the GIT, the most dominant MPs shape was film, fragment, and fiber. The most dominant MP polymers were PE and PP, similar to the histological sample' physical and chemical results. Therefore, MPs accumulation in the intestine is expected to affect tissue structure and functioning. The effects of MP internalization in the intestine in this study were strongly correlated with the mean villi length. Previous studies have demonstrated that the accumulation of MPs can damage the villus structure and denudation of the epithelium (Ahrendt et al., 2020). The evidence obtained by Zhao et al. (2021) in zebrafish as the animal model also confirms that fish exposed to MPs stimulate intestinal structural damage.

Additionally, this research evaluated the percentage of epithelial denudation in the intestine. Epithelial tissue acts as an important environment-organism interface with several functions, including nutrient transport and osmoregulation, and serves as a barrier against toxicants like MPs (Minghetti et al., 2017). Microplastic accumulation in the intestine causes damage to the epithelial barrier, inflammation, oxidative stress, and microbiota disorder (Jeong et al., 2017). Our study showed that *E. tetradactylum* had higher epithelial denudation than *L. equula*. The percentage of epithelial denudation in *E. tetradactylum* ranged between 70.20% to 89.52%, while *L. equula* ranged between 67.69% to 81.24% (Figure 8). A high percentage of epithelial denudation was found at station 3. This station had a high MP concentration in surface seawater. Nevertheless, the two fish species showed epithelial denudation above 50%, indicating a high level of foreign particles, including internalization of MPs in the intestine (Ahrendt et al., 2020).

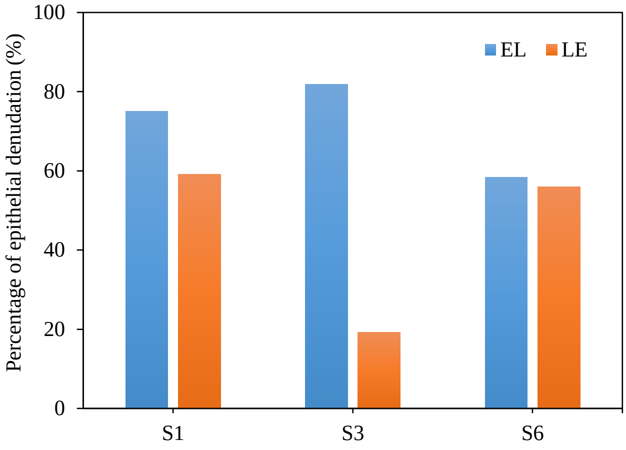

Figure 8. Percentage of epithelial denudation (%) in *E. tetradactylum* (ET) and *L. equula* (LE).

Previous studies by Furukawa et al. (2004) and Qiao et al. (2019) showed that histological analysis of the MP internalized intestine causes significant changes such as damage to epithelial, cracks in the villi, thickening of the walls, and an increase in mucus volume. In this study, the histological structure showed damage to the mean villi's length, goblet cells, and denudation of the villus epithelial cells (Figure 9). These conditions might have been caused by MPs' physical characteristics and polymers that can lead to inflammation.

Previous studies have shown that MPs can destroy the functional barrier of epithelial cells because they increase ion leakage to the lamina propria (Sendra et al., 2021). During MP internalization, the intestine will undergo lysosomal degradation, causing alkalinization and an increase in the reactive oxygen system (Sendra et al., 2021; Jeong et al., 2017). The latter will activate signal transduction mediated by p-ERK, p-38, and Nrf2 (Jeong et al., 2017). Microplastic accumulation has been correlated with the level of mRNA inflammation, such as IL-10, IL-8, IL-IB, SOD, and CAT. Previous studies have confirmed that MPs can cause damage to mucus channels and increase mucus activity, inflammation, and metabolic disorders such as microbiota addiction (Qiao et al., 2019). Microbiota disorders have been changed in gene expression studies, which correlated with changes in the regulation of epithelial integrity in the intestinal cytochrome P450 gene (CYP1A), while dysfunction of the barrier correlated with cortisol stress hormone and inflammatory cytokine release (Furukawa et al., 2004).

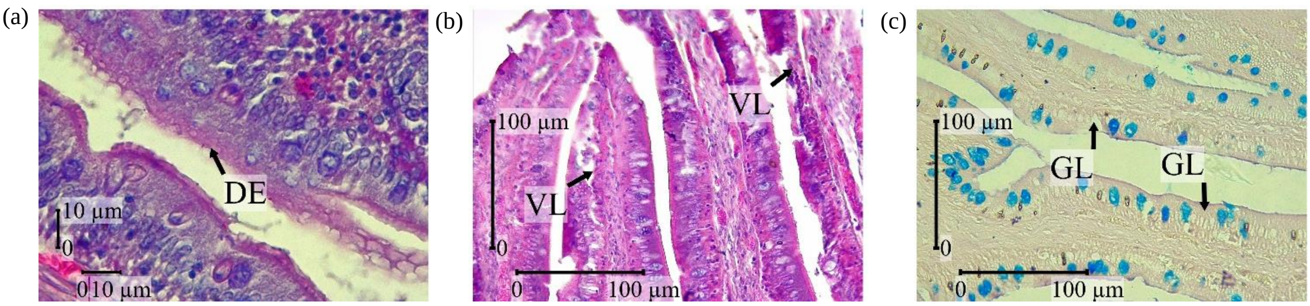

Figure 9. Structural damage to intestinal histology samples with HE and PAS-AB staining. Epithelial denudation with 1,000× magnification (a), villi cell loss (VL) with 400× magnification (b), and goblet cell loss with 400× magnification (c).

The potential of detecting MP accumulation in organs with histological analysis must be expanded. Many researchers have claimed that MPs cause structural damage based on MP particles and polymers (Ahrendt et al., 2020; Limonta et al., 2019), while other studies revealed that MPs do not induce any histological reaction (Batel et al., 2020; De Sales-Ribeiro et al., 2020). Nevertheless, additional research about MPs on the histological structure is important to clarify and understand the transfer of MPs' from the environment to the organs. The discovery of MPs in intestinal histology samples, above the background contamination, suggests the translocation of MPs to tissues of marine fish exposed in their natural habitats. The uptake and release of MPs in animal models have previously been observed (Ahrendt et al., 2020; Limonta et al., 2019; Mbugani et al., 2022), although this is one of the very few studies that have investigated MP accumulation in marine fish under natural exposure.

The structural damage to intestinal samples in this study confirms MP internalization in intestinal tissue with structural damage under naturally exposed conditions, which strongly indicates that MPs accumulation in the GIT of marine fish has a negative effect on epithelial homeostasis, villi structure, crypt structure, and goblet cell condition. Further histology and biomarker studies are needed regarding the internalization of MPs in other tissues in marine fish from different water columns under natural conditions.

4. CONCLUSION

Microplastic pollution was found in surface seawater and marine fish from the coastal waters of BTB, Special Region of Yogyakarta, Indonesia. The MP characteristics, including, size, shape, and color, varied between the different types of MPs. The dominant MP polymers were PP, PE, and PET. Station 3 contained the highest MP concentration in surface seawater Demersal and pelagic were present at the highest relative frequency, and *E. tetradactylum*, *E. splendens*, and *L. equula* (demersal), and *S. lysan* and *S.tala* (pelagic) are recommended for MP biomonitoring. Intestinal histological analysis of *E. tetradactylum* and *L. equula* and FTIR analysis showed MP internalization in tissue with structural damage. This novel study demonstrated the presence of MPs in marine fish tissues from wildlife by histological analysis. This is to our knowledge, the first study to demonstrate MPs in demersal marine fish using histological analysis in relation to the environmental MP concentration in a natural habitat. Further management and method development is needed for MPs mapping and potential accumulation in tissues under natural exposure.

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