Abundance and Characteristics of Microplastics Found in The Gastrointestinal Tract of Commercial Marine Fish from Bitung, North Sulawesi, Indonesia

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Abstract

Microplastic (MP) pollution is an emerging environmental problem that threatens food security, food safety, and human health since it has been reported to be found in commercial fish consumed by humans. Bitung, North Sulawesi, is one of the biggest contributors to capture fishery production in Indonesia. However, there is no data on microplastic pollution in commercial marine fish from Bitung. Therefore, this research aimed to investigate the presence and identify the visual characteristics (color, shape, size) and the polymer type of microplastics found in the gastrointestinal tract of commercial marine fish from Bitung, North Sulawesi. The gastrointestinal tract was extracted using KOH 10%, and the microplastic was observed under a stereo microscope. A total of 753 microplastic particles were found in the gastrointestinal tract of 74 individuals (prevalence 99%), and there was a statistically significant difference in the abundance of microplastics found in the gastrointestinal tract of pelagic and demersal fish. The average number of microplastic particles found in the gastrointestinal tract of pelagic fish (12,24 ± 2,43) is higher than in demersal fish (7,38 ± 3,48). The dominant color and shape of microplastic found in the gastrointestinal tract of the fish were black and fiber, respectively. At the same time, the dominant microplastic size found in the gastrointestinal tract of demersal fish was bigger (1,001-5,000 µm, 39,4%) compared to pelagic fish (150-500 µm, 47%). The Fourier Transform Spectroscopy (FTIR) analysis result shows that microplastics of the same polymer type can be found in the gastrointestinal tract of both pelagic and demersal fish.

Keywords: Bitung, demersal fish, gastrointestinal tract, microplastics, pelagic fish

Introduction

Plastic pollution is one of the most challenging ecological threats nowadays. Recent data on plastic pollution shows there are 4.8 - 12.7 Million Metric Tons (MMT) of plastic waste in the ocean (Veerasingam et al., 2017). Since the 1960s, plastic production has increased by around 8.7% yearly. When plastic waste enters the ocean, the rate of degradation and its persistence varies based on the characteristics of the polymer type, shape, and size (Smith et al., 2018). Plastic can be degraded by the thermal oxidation of UV rays and through a mechanical process to form smaller sizes (Cordova & Wahyudi, 2016). Plastic fragments smaller than 5 mm, either by design or due to a natural fragmentation process, are called microplastics (Gallo et al., 2018; Wu et al., 2018).

Based on several studies conducted from 2017-2022, the microplastic abundance in seawater in Indonesia ranged from 0.000023 to 110737 n/L (Manullang et al., 2023). Microplastics in the ocean can be ingested by marine organisms such as plankton, sea urchins (Echinoidea), shellfish (Exoskeleton-bearing aquatic invertebrates), and fish due to their small size (Chatterjee & Sharma, 2019). Thus, allowing microplastic to enter the digestive tract of an organism and then be translocated to different tissues (Avio et al., 2017). Microplastics ingested can cause damage to the intestinal tissue of the fish (Yin et al., 2019) and accumulate in the gastrointestinal tract in large quantities, thereby blocking the passage of food (Eshun & Pobee, 2022). Another issue caused by fish ingested microplastic was related to its potential to adsorb persistent organic pollutants (POPs), which then accumulated in the adipose tissue of fish (bioaccumulation) and transferred to higher trophic (biomagnification) (Hantoro et al., 2019; Wang et al., 2016; Purba et al., 2019).

Several studies reported the presence of microplastics in commercial marine fish in Indonesia. 169 of 174 (97.13%) fish obtained from Pantai Indah Kapuk coast, Jakarta, were examined had microplastics (Hastuti et al., 2019). Microplastics were found in all sampled fish collected from Pangandaran Bay (Ismail et al., 2019). Ningrum & Patria (2022) stated that based on the total microplastic levels found in the gut of anchovies from fourteen harbors in Indonesia compared to other reports, anchovies (*Stolephorus* spp.) from Indonesia sea were considered the most contaminated fish by the individual. As the global top 10 fish-producing country (Chan et al., 2017) and the second largest contributor of plastic waste to the sea (Organisation for Economic Co-operation and Development [OECD], 2020), microplastic data on commercial marine fish from Indonesia is crucial. Although Bitung is one of the largest fish-producing cities in Indonesia (Ministry of Marine Affairs and Fisheries Republic Of Indonesia, 2018), there was no record of microplastics in fish collected from fish markets in Bitung. Therefore, this research was conducted to investigate the presence and identify the visual characteristics (color, shape, size) and the polymer type of microplastics found in the gastrointestinal tract of commercial marine fish from Bitung, North Sulawesi. This data provides an overview of the levels and possible sources of plastic pollution in Bitung, the requirement for better plastic waste management, and further research.

Materials and Methods

Study Area

A total of 75 individuals from 11 commercial marine fish species were collected from fish markets “Tempat Pelelangan Ikan (TPI) (N 1^{0}26’47.464" – E 125^{0}12’26.994"),” Girian market (N 1^{0}27’25.092" – E 125^{0}11’22.199"), and Winenet Market (N 1^{0}27’1.963" – E 125^{0}12’1.116") in Bitung (Figure 1). Forty-one individuals from 6 pelagic species and 34 from 5 demersal species were obtained from these sampling locations. Each number of species from individuals was diverse and dependent on availability (Table 1).

Preparation of Fish

The total length (cm) and wet weight (g) were measured for each fish before dissection. Fish were dissected using scalpel blade No. 10 (Swann- Morton-England) to remove the gastrointestinal tract from the top of the esophagus to the anus. The gastrointestinal tract was put into a bottle containing 10% KOH solution to digest biological material (Sanchez-Almeida et al., 2022). The gastrointestinal tract bottle was stored in

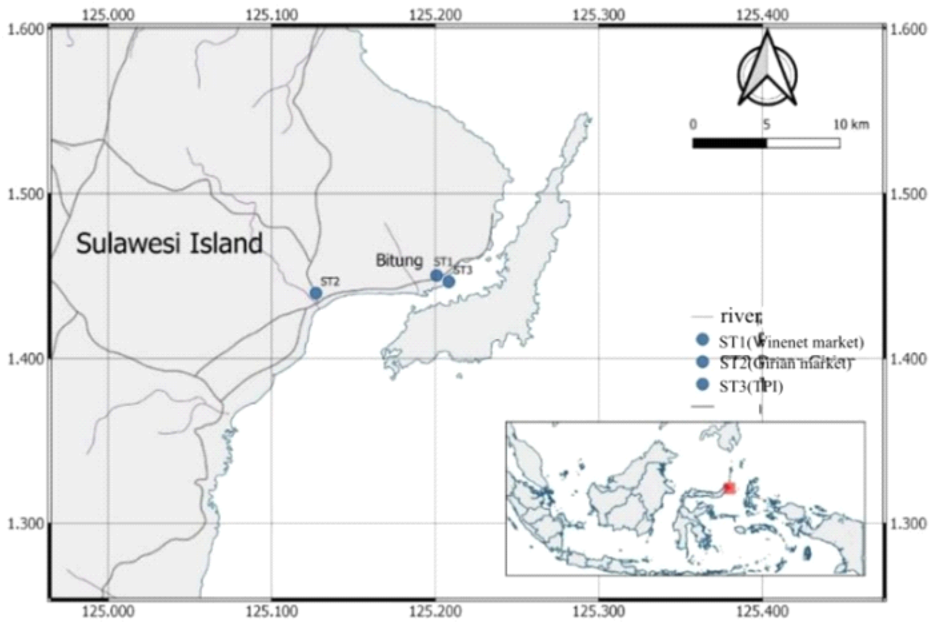

Figure 1. Commercial marine fish sampling locations in Bitung, North Sulawesi; ST1 = Winenet market, ST2 = Girian market, and ST3 = TPI.

Table 1. Sample of commercial marine fish from Bitung, North Sulawesi

Type of Fish	Species	Common Name	Number of Samples
Pelagic Fish	*Decapterus macarellus*	Mackerel Scad	9
	Selar crumenophthalmus	Bigeye Scad	7
	Katsuwonus pelamis	Skipjack Tuna	6
	Rastrelliger spp.	Indian Mackerel	5
	Euthynnus affinis	Mackerel Tuna	5
	Elagatis bipinnulata	Rainbow Yellowtail	9
Demersal Fish	*Scarus* spp.	Parrotfish	9
	Epinephelus spp.	Grouper	10
	Upeneus vittetus	Yellow striped Goatfish	5
	Eubleekeria jonesi	Jones' Pony Fish	5
	Terapon theraps	Large scaled Terapon	5

an oven with a temperature of about 60°C for 24 hours. Treating biological materials with a 10% KOH solution and incubating them in an oven was both time and cost-effective, efficient in digesting biological materials, and had no impact on the integrity of the plastic polymers (Karami et al., 2016). The digested sample was filtered using Whatman Grade 93 Slow filter paper with a pore size of 10 µm. After filtering, the filter paper was transferred into a clean petri dish (Jin-Feng et al.,2019).

Microplastics Visual Characteristics Observation

Visual characteristics of microplastic were observed and referred to as morphological characters, i.e., shape, size, and color. Microplastics filtered on the filter paper were visually observed using the USB Digital Microscope 1600X Zoom Magnifier Monocular Lens. The digital microscope was connected to a computer with Optilab Viewer v2.1 software installed on it to observe the presence and visual characteristics of microplastic. Particle size was measured using Optilab ImageRaster v2.1 software. Microplastic forms were classified into five types: fiber, film, fragments, pellets, and foam (Crawford & Quinn, 2017). The hot needle method was conducted to distinguish microplastics and organic or non-plastic materials. The heated needle is directed to the end of the object. Once the object melts, it indicates that it is microplastic; otherwise, it is organic matter if it does not melt (Cutroneo et al., 2020).

Analysis of the Chemical Composition of Microplastics

The type of microplastic polymer was identified by analyzing the functional groups of microplastic samples using Fourier Transform Spectroscopy (FTIR). The most representative microplastic sample (the most diverse in color and form) from each species was selected for analysis of polymer type. The type of microplastic polymer was identified by analyzing the functional groups of microplastic samples using FTIR Thermo Scientific Nicolet IS10. The analysis results were compared with the data recorded in the instrument library to verify the polymer types.

Data Analysis

Microsoft Excel calculated the microplastic percentage and standard deviation for each character (color, shape, and size) per type of fish (demersal and pelagic). Kolmogorov-Smirnov Test was conducted to test the normality of the data, and Two-Tailed Fisher's Exact test was used to evaluate whether there is a difference between the number of microplastic particles that are found in the gastrointestinal tract of pelagic and demersal fish. The level of significance was set to $p < 0.05$. All statistical analyses were performed in SPSS v.16.

Results and Discussion

Microplastic Content in The Gastrointestinal Tract of Commercial Marine Fish from Bitung, North Sulawesi

A total of 41 individuals from 6 pelagic species (*Decapterus macarellus, Selar crumenophthalmus, Katsuwonus pelamis, Rastrelliger canagurta, Euthynnus affinis,* and *Elagatis bipinnulata*) and 34 individuals from 5 demersal species (*Scarus* spp., *Epinephelus* spp., *Upeneus vittetus, Eubleekeria jonesi*, and

Therapon theraps) were examined (Table 2). Seven hundred fifty-three microplastic particles were found in 74 examined fish (prevalence 99%). This result showed the contamination of microplastics in the fishing grounds in Bitung. The fishing grounds in Bitung include the waters of Tolo Bay, Banda Sea, Tomini Bay, Maluku Sea, Halmahera Sea, Seram Sea, Berau Bay waters, Sulawesi Sea, northern waters of Halmahera Island, and also parts of the Pacific Ocean (Asia et al., 2019).

According to Fisher's Exact Test, there was a statistically significant difference in the abundance of microplastics found in the gastrointestinal tract of pelagic and demersal fish (Asymp. Sig. (2-sided) < 0,05). The average number of microplastic particles found in the gastrointestinal tract of pelagic fish (12,24 ± 2,43) is higher than in demersal fish (7,38 ± 3,48) (Figure 2). This is expected due to microplastics on the water surface, which have a lower density than seawater. Therefore, the microplastics will float in the area for a long time, and the sea surface is the area directly affected by waste originating from the land (Wu et al., 2018). Therefore pelagic fish that live on the surface to the middle layer of water are more susceptible to ingested microplastic (Surwatiningsih et al., 2020; Lopes et al., 2020).

Microplastic polymer, which has a lower density than seawater, such as Polyethylene (PE) and Polypropylene (PP), will remain buoyant on the surface of seawater. These polymers are prevalent in surface, column, and seafloor water. Apart from density, the vertical movement of microplastics can also be influenced by their particle size and shape (Eo et al., 2021). Microplastic movement affects the microplastic content ingested by fish because habitats more polluted by microplastics will increase the possibility of microplastics being ingested (Jabeen et al., 2016; Surwatiningsih et al., 2020). Similar research was conducted by Surwatiningsih et al. (2020) at Baron Beach, Yogyakarta, which stated that the abundance of microplastics in pelagic fish is more than in demersal fish. Research related to microplastics in the gastrointestinal tract of fish has been widely carried out in Indonesia, which indicates fish have unintentionally ingested microplastics in various shapes, colors, and sizes (Jabeen et al., 2016).

Fish can ingest microplastics in two ways: active uptake, when the fish cannot distinguish between microplastics and food or prey, and passive uptake, when microplastics accidentally enter through the mouth when eating, through the gills, and through the food chain. Factors influencing the number of microplastic particles ingested by fish are the concentration of microplastics in the waters, fish feeding habits influenced by food availability, and fish size (Roch et al., 2020).

Microplastic Color Characteristic

The color of microplastics observed from the gastrointestinal tract of pelagic fish were black (452 particles), red (32 particles), green (8 particles), blue (7 particles), and yellow (3 particles). In comparison, the color of microplastics observed from demersal fish was black (192 particles), Red (33 particles), green (12 particles), blue (11 particles), brown (2 particles), and one white particle. Black is the most dominant color in pelagic and demersal fish (Figure 3). Black as the dominant color of microplastic was also reported

Table 2. The abundance of microplastics (MPs) in the gastrointestinal tract of pelagic and demersal fish from Bitung, North Sulawesi

Type of Fish	Species	Number of Samples	Prevalence of MPs (%)	Body Length (cm)	Digestive Tract Weight (g)	Range of MPs/individual	Number of MPs/ Individual (Mean±S.D)
Pelagic Fish	*Decapterus macarellus*	9	100	23,8 ± 1,5	1,3 ± 0,7	10-20	12,8 ± 2,9
	Selar crumenophthalmus	7	100	21,5 ± 0,9	1,08 ±0,7	6-11	9,8 ± 1,8
	Katsuwonus pelamis	6	100	29,3 ± 1,5	3,7 ± 1,2	9-16	13 ± 2,4
	Rastrelliger spp.	5	100	29 ± 1,4	2,8 ± 1,05	9-23	13,4 ± 5,6
	Euthynnus affinis	5	100	43 ± 2	5,01 ± 0,5	8-23	10,4 ± 1,7
	Elagatis bipinnulata	9	100	24,4 ± 2,9	0,7 ± 0,3	7-18	13,3 ± 3,7
Demersal Fish	*Scarus* spp.	9	100	31 ± 4,2	11,1 ± 5,5	1-16	7 ± 4,3
	Epinephelus spp.	10	100	22,5 ± 0,7	1,6 ± 0,6	1-15	7,4 ± 4,8
	Upeneus vittetus	5	100	21 ± 1,4	0,8 ± 0,1	8-13	10,6 ± 1,9
	Eubleekeria jonesi	5	100	12,1 ± 0,4	0,4 ± 0,1	4-11	8,4 ± 2,7
	Terapon theraps	5	80	19 ± 3,9	2,9 ± 0,5	0-9	3,8 ± 3,9

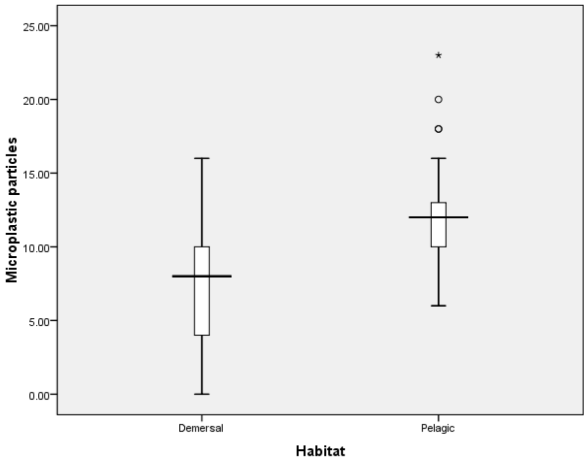

Figure 2. Boxplot of the microplastic found in the gastrointestinal tract of pelagic and demersal fish in Bitung, North Sulawesi.

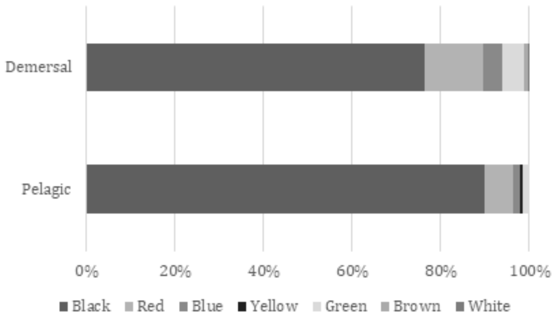

Figure 3. The percentage of microplastic color found in the gastrointestinal tract of pelagic and demersal fish in Bitung, North Sulawesi.

in several studies investigating microplastics from sediment (Naji et al., 2019), seawater (Lenz et al., 2015; Montoto et al., 2020), and marine organisms (Zhang et al., 2021; Duncan et al., 2018). A similar study by Surwatiningsih et al. (2020) reported that black is the most dominant color of microplastics (41,06%) found in pelagic and demersal fish at Baron Beach, Yogyakarta, Indonesia.

Black color can be related to the ability of these microplastics to adsorb pollutants (Tubagus et al., 2020). Black color could indicate high contaminants since microplastic absorbs pollutants once it is released into the environment (Surwatiningsih et al., 2020). The black color also can be the original color of the microplastic itself.

The Color of Microplastics can be used to determine the source of Microplastics (Seijo & Pereira, 2017). Most black microplastic comes from rubber tire production waste (Lenz et al., 2015). The black spectrum of microplastics can indicate that the source of these microplastics comes from domestic wastewater and bottles (Wen et al., 2018).

Fish have a conical eye retina and function to detect the color of their prey (Surwatiningsih et al., 2020). The color of microplastic particles has the same color as the prey or food, so it has the potential to be swallowed by fish (Hastuti et al., 2019). Black microplastics resemble plankton (De Sa et al., 2015); therefore, planktivorous fish, such as *Therapon theraps* and *Epinephelus* spp. (Renjith et al., 2018) will likely mistake black microplastic for its prey.

Microplastic Size Characteristic

Plastics can be divided into macroplastics with a size > 2.5 cm, mesoplastics with a size of 2.5 cm, microplastics < 5 mm, and nano plastics with a size of 1ìm (Chatterjee & Sharma, 2017). In this study, the microplastic sizes were classified into (i) <150, (ii) 150-500, (iii) 501-1,000, and (iv) 1,001-5,000 using ìm units.

The size of the microplastics was measured using the Optilab Viewer software by drawing a line from end to end following the shape so that various microplastics were obtained. Figure 5 shows that microplastic size in pelagic fish tends to be smaller than in demersal fish, with the shortest microplastic observed being 24,1 µm, bigger than the filter paper's pore size used in this study. According to Luo et al. (2022), 10 µm is the detectable minimum size limit of FTIR.

Based on the observations, the size of the microplastics most commonly found in pelagic fish is 150-500 µm (47%), and in demersal fish is 1,001-5,000 µm (39,4%). The dominant microplastic size in demersal fish was bigger (1,001-5,000 µm) compared

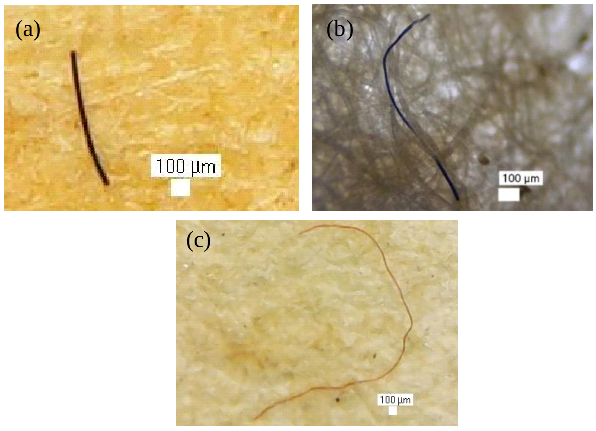

Figure 4. The color of microplastic found in the gastrointestinal tract of pelagic and demersal fish in Bitung, North Sulawesi. (a) Black; (b) Blue; (c) Red.

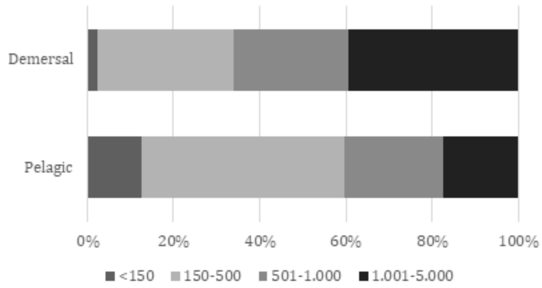

Figure 5. The percentage of the microplastic size found in the gastrointestinal tract of pelagic and demersal fish in Bitung, North Sulawesi.

to pelagic fish (150-500 µm). This is presumably because demersal fish such as Scarus, Epinephelus, and Upenus live mostly in seagrass beds and corals with a sandy substrate (Tebaiy et al., 2014), whereabout the larger microplastic particle size can be easily trapped on a sandy substrate (Silva & Nanny 2020).

The small microplastic size provides a greater surface area for contaminant absorption (Gall & Thompson 2015). Microplastic size can affect fish's ability to enter tissues (Surwatiningsih et al., 2020) and may block the gastrointestinal tract (Eshun & Pobee, 2022).

Microplastic Shape Characteristic

The shape of the microplastic found in this study only consisted of fiber and fragments (Figure 6). As shown in Figure 7, 99% of microplastics found in the gastrointestinal tract of pelagic and demersal fish in Bitung, North Sulawesi, were fiber. A similar study conducted by Surwatiningsih et al. (2020) reported that fiber is the most dominant shape of microplastics (53,14%) found in pelagic and demersal fish at Baron Beach, Yogyakarta, Indonesia.

The high number of fiber microplastic found is assumed due to its lower density than fragments. Fiber is also found on the sea surface and the marine column (Hastuti et al., 2020). In addition, most of the fiber polymers are produced globally, reaching 61 million tons in 2015 (Lusher et al., 2017), and the costs incurred to produce fiber polymers are quite affordable (Maddah, 2016).

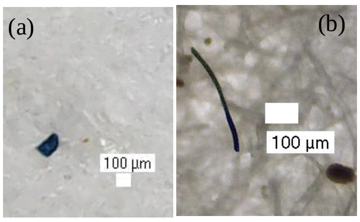

Figure 6. The shape of microplastic found in the gastrointestinal tract of pelagic and demersal fish in Bitung, North Sulawesi. (a) Fragment; (b) Fiber.

The abundance of fiber microplastic is related to its potential sources from several human activities. The source of the fiber comes from the degradation of fishing gear, fish cages, ropes, and waste from laundry clothes (Kasamesiri & Thaimuangphol 2020). Fiber is also found in this study because Bitung is the center of the fishery industry and has an international port. Hence, Bitung waters have high fishing, loading, and unloading activities and domestic and international transportation (Hisna et al., 2020). Also, ship berthing activities can produce waste such as plastic bottle waste, plastic bags, fishing lines, nets, and passenger ropes (Culin & Bielie 2016), so the amount of fiber is higher when compared to fragments. Fiber can also come from raw textile materials (Sait et al., 2021).

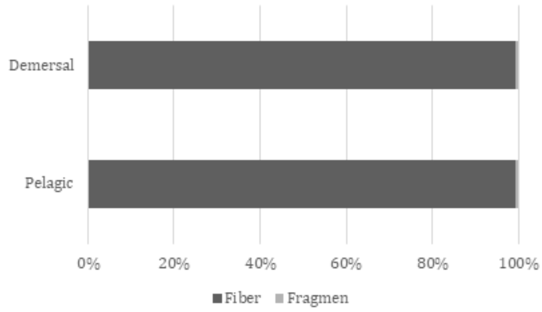

Figure 7. The percentage of microplastic shape found in the gastrointestinal tract of pelagic and demersal fish in Bitung, North Sulawesi.

Fragments have various surface shapes, such as sharp edges, round shapes with smooth surfaces, or rough surfaces. The source of the fragments is quite challenging to identify because it can be a primary microplastic or the result of fragmentation due to their time in the environment (Tanaka & Tanada 2016). Most of the fragments came from plastic bottles, beverage bottles, jars, gallons, and flakes of pipes (Surwatiningsih et al., 2020).

Microplastic Polymer Characteristic

The type of microplastic polymer was identified by analyzing the functional groups of microplastic samples using Fourier Transform Spectroscopy (FTIR). Microplastic samples from each species were selected for analysis of polymer type. The microplastic polymer type analysis results were successful in eight of the eleven samples tested. This is thought to be due to the small size of the particles, which can cause loss when observed using a microscope or FTIR analysis (Kroon et al., 2018).

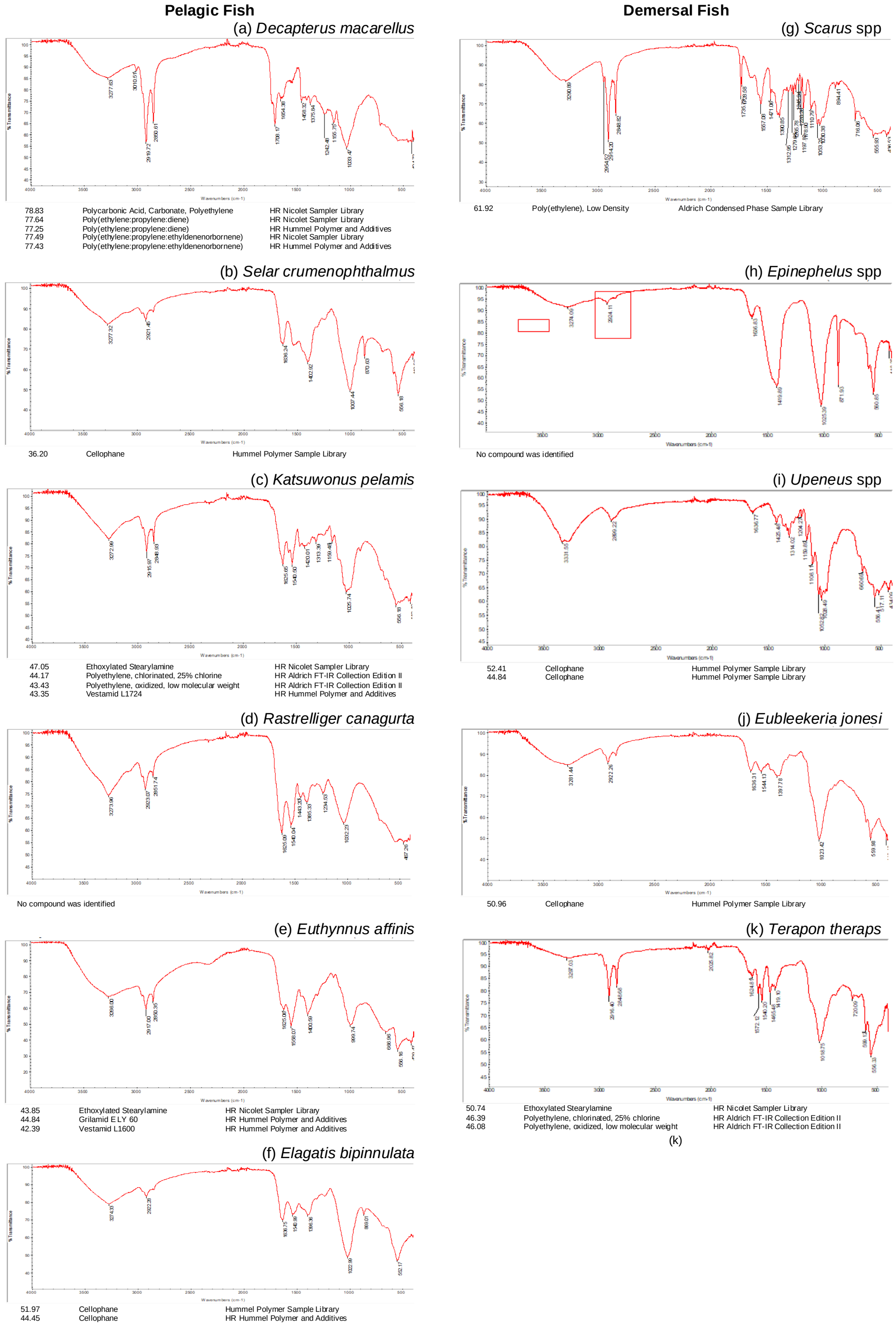

Figure 8. FTIR spectroscopy spectra of the microplastic found in the gastrointestinal tract of commercial fish in Bitung, North Sulawesi.

Based on the FTIR analysis results, it is known that the microplastics found in the gastrointestinal tract of demersal fish have polymer types of Polyethylene (PE), Low-Density Polyethylene (LDPE), and Cellophane. In contrast, the microplastics found in the gastrointestinal tract of pelagic fish have polymer types of PE, Vestamid, Cellophane, and EPDM Rubber. In addition to polymers, FTIR analysis results have identified polymer additives, namely Ethoxylated stearyl amine, used as fabric softeners (Cross & Singer, 2019).

Both PE and Cellophane were found in pelagic and demersal fish. PE and LDPE polymers are the microplastics that are most often found floating on the surface of the water due to the density of PE (0.85-

0.97 g / cm{ ³) being lower than water (Enders et al., 2015). PE and LDPE can also be found in sediments because PE and LDPE can sink if they experience a biofuel process, so the density of the polymer will increase (Erni-Cassola et al., 2019). Meanwhile, The cellophane polymer, derived from regenerated cellulose, is commonly used in food packaging, clothing, fiberglass, and rubber industries (Syafei et al., 2019).

Apart from PE and Cellophane, the type of polymers found in the gastrointestinal tract of pelagic fish were Vestamid and EPDM Rubber. Vestamid is an entire range of polyamides with custom-tailored properties (Evonik, n.d.). Polyamide, commonly called nylon, is widely used for fishing ropes and nets (Lusher et al., 2017). In contrast, EPDM Rubber is currently the fastest-growing general-purpose rubber, especially for outdoor applications. This is because of the advantageous properties of EPDM, such as low density and excellent thermal, moisture resistant, and ozone resistance (Nasrudin & Susanto, 2020).

Microplastics with different polymer types have been found in pelagic and demersal fish. It can indirectly harm human health, especially market fish that humans commonly eat. The long-term negative impact of microplastics on human health is oxidative stress, metabolic disorders, translocation to the circulatory system, disorders of the immune system, and neurotoxicity. Microplastics can discharge organic and inorganic chemicals present in their structure or previously assimilated from their surroundings. Additionally, they can function as vectors for microorganisms (Alberghini et al., 2022). Microplastic also affects the fish; Rochman et al. (2013; 2014) indicated that Japanese medaka *Oryzias laticeps*, which exposure to microplastic (concentration of 0.008 mg L-1 of PE) induced liver toxicity, hepatic stress, and changed endocrine function, as well as gene expression. PE effects are demonstrated at several levels of the sub-organism's biological organization, from oxidative stress at the cellular level to lesions in organs (Lusher et al., 2017). Besides harming human and fish health, Microplastics also harm environmental health. When microplastics enter the waters, contaminants, and additives will associate with microplastics such as bioaccumulative, Persistent Toxic Compounds (PBTs), Potential Toxic Elements (PTEs), and Microbial Pathogens (Alberghini et al., 2022). It can cause water pollution and affect the survival rate of aquatic organisms (Lamichhane et al., 2022).

Conclusions

A total of 753 microplastic particles were found in 74 out of 75 examined fish (prevalence 99%), and there was a significantly different in the abundance of microplastics found in the gastrointestinal tract of pelagic and demersal fish. The average number of microplastic particles found in the gastrointestinal tract of pelagic fish (12,24 ± 2,43) is higher than in demersal fish (7,38 ± 3,48), the dominant color and shape of microplastic found in the gastrointestinal tract of the fish were black and fiber, respectively. At the same time, the dominant microplastic size found in the gastrointestinal tract of demersal fish was bigger (1,001-5,000 µm, 39,4%) compared to pelagic fish (150-500 µm, 47%). The Fourier Transform Spectroscopy (FTIR) analysis result shows that microplastics of the same polymer type can be found in the gastrointestinal tract of both pelagic and demersal fish. The result of this study indicates that microplastic polluted the ocean at every level, with surface water as the most polluted area.

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Supplementary Materials

Supplementary materials is not available for this article.

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