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Microplastics contamination in commercial fish landed at Lengkong Fish Auction Point, Central Java, Indonesia

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Abstract: Plastic is one of the main pollutant sources that are difficult to decompose and then carried into the ocean and fragmented into smaller parts (microplastics) due to UV radiation and water currents. Their small size means that microplastics are often ingested by aquatic organisms, such as fish. This research aimed to determine the presence, abundance, and types of microplastics in the digestive tract of four dominant fishes landed at Lengkong Fish Auction Point, Cilacap, Central Java, i.e. threadfin (*Eleutheronema tetradactylum*), mackerel (*Rastrelliger* sp.), threadfin bream (*Nemipterus japonicus*), and hairtail (*Trichiurus lepturus*). We found microplastics in the digestive tract of four selected fishes with a frequency of occurrence of 100%. The concentration of microplastics in fish digestive tracts is relatively high, with a value range of 12 ±2.86 to 28.33 ±8.11 particles·ind.$^{-1}$. Microplastics were found in films, fibres, fragments, and granule shape types with various colours: brown, purple, blue, black, green, transparent, and yellow. The polymers found were polystyrene (PS), nylon, acrylonitrile butadiene styrene (ABS), polyurethane (PU), polypropylene (PP), high-density polyethylene (HDPE), and low-density polyethylene (LDPE). The present study provides baseline data for microplastics contamination in commercial fish species landed at Lengkong Fish Auction Point, Cilacap, Central Java, Indonesia. The fact that we discovered PU, the most harmful polymer, piques our attention.

Keywords: digestive tract, fishes, Lengkong Fish Auction Point, microplastics, polymer

INTRODUCTION

The abundance of waste in the waters is caused by domestic waste, industrial waste, and human activities, such as fishing gear made of nets or plastic fibres deliberately discarded in the sea (Lestari, Haeruddin and Jati, 2021). Plastic waste takes hundreds or even thousands of years to decompose through physical, chemical, mechanical, and photolytic processes (Barnes *et al.*, 2009; Eriksen *et al.*, 2014). According to Woodall *et al.* (2014), due to the physical process that is the presence of currents and sunlight, the plastic waste in the water gradually breaks down into microplastics. Therefore, microplastics are defined as a fragment derived from the description of plastic waste measuring <5 mm and cannot dissolve in water (Hiwari *et al.*, 2019). They are found in the environment and the bodies of organisms. Due to their small size, they are often mistaken for food and ingested by various organisms, such as fish, vertebrates, birds, and marine mammals (Cole *et al.*, 2011; Rochman *et al.*, 2015; Terepocki *et al.*, 2017).

Ingested microplastics are very dangerous and can clog the digestive tract of fish. The particles deposited on sediments can also be consumed by benthic fish living on the water's bottom

(Rijal, Annisa and Firda, 2021). Meanwhile, those that contaminate the body of fish consumed by humans will undoubtedly cause harm (Rijal, Annisa and Firda, 2021). The entry of microplastics can be toxic to the body and causes various physical damage to human cells (Barboza *et al.*, 2018).

The current study, which focused on Lengkong Fish Auction Point, Cilacap, examined the occurrence of microplastics in locally consumed fish. Specifically, this research aimed to determine the presence, abundance and categories of microplastic in commercial fish landed at Lengkong Fish Auction Point, Cilacap.

MATERIALS AND METHODS

SAMPLE COLLECTION

Fish sampling was carried out three times every two months, from September 2021 to February 2022 at the Lengkong Fish Auction Point. Four commercially and dominant important fish species were collected during the period, i.e. threadfin (*Eleutheronema tetradactylum*, Shaw, 1804), mackerel (*Rastrelliger* sp., Bleeker, 1851), threadfin bream (*Nemipterus japonicas*, Bloch, 1791) and hairtail (*Trichiurus lepturus*, Linnaeus, 1758). Totally 36 individuals of fish (9 fishes of each species) were used for this study. They were collected in a cool box (4°C), wrapped in aluminium foil, and transported to the laboratory. They were kept at –20°C until further examination.

SAMPLE PREPARATION AND MICROPLASTICS EXTRACTION

Prior to the investigation, the fish samples were thawed at room temperature. Microplastic extraction was performed according to Sarasita, Yunanto and Yona (2019) and Zhang *et al.* (2020) with some modifications. The process begins by dissecting fish samples. The digestive tract (oesophagus, stomach, and intestines) of every fish was removed and placed into a previously cleaned 250 ml beaker glass, and closed using aluminium foil to protect it from external contamination. Subsequently, 20 ml of 0.05 M Fe (II) solution was added, and 20 ml of H_2O_2 30% was poured into a container with a solution for decomposing existing organic matter. After being left for 24 hours, the softened sample was broken down using a stirring glass.

Furthermore, about 20 ml of H_2O_2 30% and 20 ml of Fe (II) 0.05 M were added and left to stand for five minutes. Then, the stir bar was inserted into the sample, and placed on a hot plate with a temperature of 70°C at a rotation speed of 180 rpm within 30 minutes. During the process, it must be ensured that no organic matter sticks to the walls of the container. If found, the attached organic matter can be pushed into the solution using a stirring glass. Likewise, if fats appear on the surface of the sample solution after the organic matter dissolution process, 4–6 ml of n-Hexane solution can be added, depending on the amount of fat in the sample.

A 0.45 μm Whatman filter paper, with a vacuum filter funnel, tube, and filtering device were prepared. The vacuum filter funnel was placed above the filter tube, and the hose was connected between the filter tube and the device. A small amount of distilled water was poured into the vacuum filter funnel before placing a 0.45 μm Whatman filter paper on top. Furthermore, the entire sample solution was poured, and the vacuum filter device was turned on. After the solution has been filtered, the filter paper is slowly pulled out using tweezers and transferred to the Petri dish. Filtered samples were transferred to a Petri dish and dried using an oven at 70°C for one hour. The dried samples were put into vials to prevent contamination from the outside, and then a visual inspection of the microplastics was carried out using a stereo microscope with 40× magnification.

MICROPLASTIC ANALYSIS

The microplastic analysis process is carried out in two ways, visual observation using a stereo microscope to determine the shape, size, and colour. Then, to determine the type of plastic polymer, we employed Fourier transform infrared (FTIR) spectrometer. FTIR analysis was conducted on a Shimadzu QATR10 FTIR spectrometer equipped with a single reflection attenuated total reflectance (ATR) accessory to collect the infrared spectra. For each sample, 32 individual scans were averaged at a resolution of 4.0 cm^{-1} within the spectrum range of 400–4000 cm^{-1}. The spectra were compared to the reference of the Shimadzu library.

DATA ANALYSIS

Abundance of microplastics was calculated as the amount of microplastics in the digestive tract of fish per number of fish, expressing the data with particle·ind^{-1} (Boerger *et al.*, 2010).

The frequency of occurrence (FO, %) of microplastic ingestion was calculated for each fish species by dividing the number of fish with microplastics by the number of fish examined. The abundances were analysed by descriptive analysis, while shapes, sizes, colours of microplastics, and polymer composition retrieved were expressed as a percentage distribution.

RESULTS AND DISCUSSION

THE PRESENCE AND THE FREQUENCY OF OCCURRENCE (FO, %) OF MICROPLASTICS

A total of 36 fishes belonging to 4 different species from the Lengkong Fish Auction Point, Cilacap were investigated. Microplastics were found in the digestive tracts of all four species of commercially important fish examined, i.e. threadfin (*Eleutheronema tetradactylum*), mackerel (*Rastrelliger* sp.), threadfin bream (*Nemipterus japonicas*) and hairtail (*Trichiurus lepturus*). Threadfin is a type of fish that lives in sea and brackish water. It can reach 200 cm in length but is more commonly found in sizes 45–50 cm. These fish live in the bottom waters or are classified as demersal fish. Threadfin is a carnivorous fish and its diet contains small crustaceans and fish (FAO and Fishery and Aquaculture Economics and Policy Division, 1974). Mackerel is a pelagic fish species that can live at depths of up to 200 m in coastal and marine areas (Handani, 2002). The types of mackerel food are phytoplankton and zooplankton, while adult mackerel fish eat shrimp and fish larvae. Therefore, mackerel is included in the omnivorous fish group (Aye, 2020). Threadfin bream is a type of demersal fish that lives in waters with muddy or sandy substrates and does not migrate naturally and lives in groups at depths of 5–80 m (Sen *et al.*, 2014). This fish belongs to the category of carnivorous fish, with its main food being shrimp. Hairtail generally has a body length of 70–80 cm. Hairtail is a benthopelagic fish and tends to live at the water depth of 0–400 m. This fish, with its sharp and strong teeth in both jaws, is able to eat squid, shrimp, small fishes such as anchovies, sardines and juveniles, so it belongs to the carnivorous fish group (Sarasita, Yunanto and Yona, 2019).

We examined the gastrointestinal tracts of four commercial species and found a frequency of occurrence (FO, %) of 100% from the 144 fishes examined. The FO recorded in this study is higher than the commercial species reported in other works from Orontes River, Lebanon (95%) (Kiliç, Yücel and Sahutoglu, 2022), southern New Zealand (75%) (Clere *et al.*, 2022), and commercial fishes from Alexandria, the Mediterranean coast of Egypt (92%) (El-Sayed *et al.*, 2022). The result provides evidence of the ubiquity of microplastics in this region.

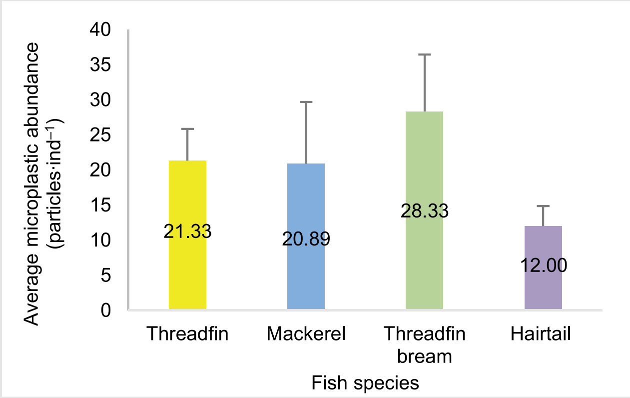


Fig. 1. Average microplastic abundances of commercial fishes collected from Lengkong Fish Auction Point, Central Java, Indonesia (n = 36; 9 samples each species); vertical bars = standard deviation in each case; source: own study

THE ABUNDANCE OF MICROPLASTICS

This study provides a detailed assessment of microplastics for the four most captured commercial fish species. In total, 743 microplastic pieces were retrieved from examined fishes, with an abundance range of 12 ±2.86 particles·ind^{-1} up to 28.33 ±8.11 particles·ind^{-1} and an average of 20.6 ±35.5 individual microplastic particles per fish. The results from this research exhibit higher rates of plastic ingestion in fish when compared to similar studies by Rahmadhani (2019), Sarasita, Yunanto and Yona (2019), and Dalimunthe, Amin and Nasution (2021). Microplastic abundance for every four species is displayed in Figure 1.

Figure 1 shows that the highest average abundance of microplastics found in threadfin bream species is 28.33 ±8.11 particles·ind^{-1}, followed by threadfin – 21.33 ±4.51 particles·ind^{-1} and mackerel with an average of 20.89 ±8.79 particles·ind^{-1}. The lowest abundance was found in hairtail with an average of 12 ±2.86 particles·ind^{-1}. Threadfin, threadfin bream, and hairtail are demersal fishes, while mackerel is a pelagic fish (Nasution, 2009; Foekema *et al.*, 2013; Nurtang *et al.*, 2020; Senduk, Suprijanto and Ridlo, 2021). Generally, demersal species showed higher microplastic abundance compared with the pelagic species as reported by other studies (Jabeen *et al.*, 2017; Wang *et al.*, 2021; Eryaşar, Gedik and Mutlu, 2022; Mistri *et al.*, 2022). Meanwhile, other studies reported that pelagic species ingest more microplastics than demersal ones (Güven *et al.*, 2017).

According to Neves *et al.* (2015), several factors can affect the abundance of microplastics in fish, such as the eating habits and fish species employed in the research. Demersal fish live on the bottom layer of the water (Wahyuni, Hartati and Indarsyah, 2009), while pelagic live on the surface to the middle layer (Susilo, 2010). Foekema *et al.* (2013) and Karthik *et al.* (2018) found that pelagic and demersal fishes can eat microplastics in various ways, such as ingestion on the surface or bottom layer of the water due to size, colour, and shape similar to prey, or eating prey that has previously ingested microplastics.

COMPOSITION OF MICROPLASTIC TYPES

The types of microplastics are divided into four based on shape, i.e. fibre, film, fragment, and granule/pellet (Viršek *et al.*, 2016). According to the overall distribution, the characterisation of microplastics detected in the examined fishes is shown in Figure 2.

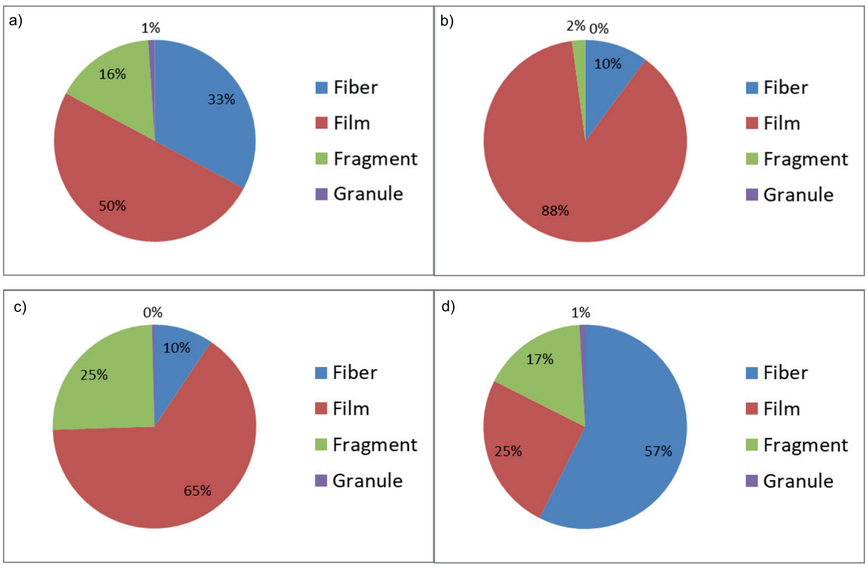


Fig. 2. Composition of microplastic types in: a) threadfin, b) mackerel, c) threadfin bream, d) hairtail; source: own study

The highest type of microplastic found in threadfin, mackerel, and threadfin bream was film, while the highest type of microplastic found in hairtail fish was fibre.

The high number of film types in threadfin, mackerel, and threadfin bream species is suspected because it is in the group of carnivorous aquatic organisms. These organisms can eat plankton, and small fish settle on the bottom layer of the water and resemble prey. The dominant fibre-type microplastics found in hairtail (*Trichiurus lepturus*) are from settlements in coastal areas, with most people working as fishermen who use various fishing gear such as rods and nets. The high density of fibre is also one of the supporting factors, knowing that hairtail is a class of demersal fish on the bottom of the waters (Neves *et al*., 2015). The laundering of synthetic textiles from sewage and river runoff is also considered an essential source of fibres to the marine environment (Browne *et al*., 2011).

The microplastic composition of fragments in threadfin, threadfin bream, and hairtail is quite high at 16, 25, and 17%, but very low in mackerel at 2%. Fragments are derived from plastic pieces with strong synthetic polymers and a higher density. Therefore, the fragment content is quite high compared to mackerel because the three species are demersal fishes. Harikrishnan *et al*. (2023) found that the gills and guts had accumulated more numbers of microplastics (MPs) of which 68% were fibres and fragments.

The microplastic composition study of the four species showed a low number of granule types. This is presumably because the type of microplastic comes directly from the rest of the plastic processing industry or beauty factories. There are no industrial factories close to the Lengkong Fish Auction Site research site, although there are households in the area. However, the percentage of granules in threadfin, threadfin bream, and hairtail is very small, at 1%. Another study on microplastics by Borges-Ramírez *et al*. (2020) also found that granules were the lowest abundance type of microplastics found in 240 individual commercial fish from Campeche Bay, Mexico. Similar results were reported by Eryaşar, Gedik and Mutlu (2022) who found only 3% of the granule type in commercial fish species from the southern Black Sea coast.

AMOUNT OF MICROPLASTICS BY COLOUR

Based on the colour analysis in the digestive tract of 36 individual samples of 4 species of fish, 7 colours of microplastic were obtained, transparent, black, purple, blue, brown, green, and yellow, which can be seen in Figure 3.

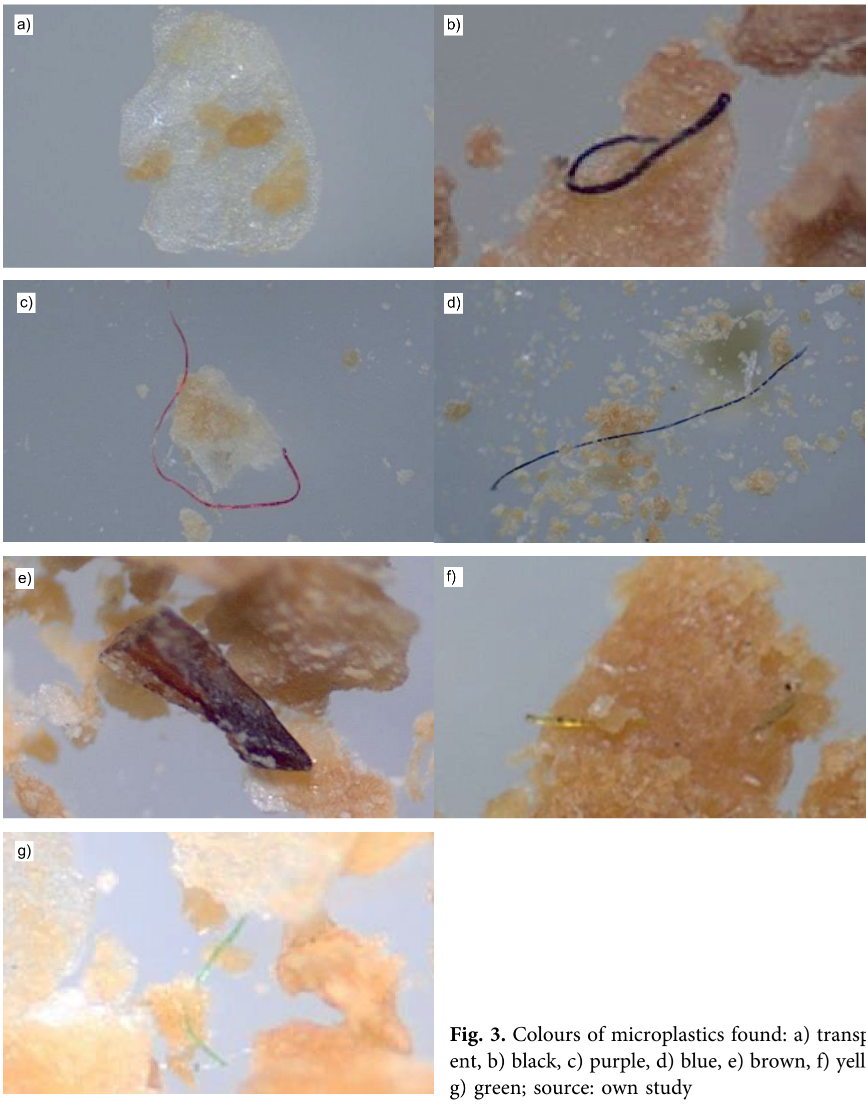


Fig. 3. Colours of microplastics found: a) transparent, b) black, c) purple, d) blue, e) brown, f) yellow, g) green; source: own study

Research on the digestive tract of 36 individual samples consisting of 4 species of fish showed that the colour that dominates is transparent, followed by black, blue, purple, brown, green, and yellow, as shown in Figure 4.

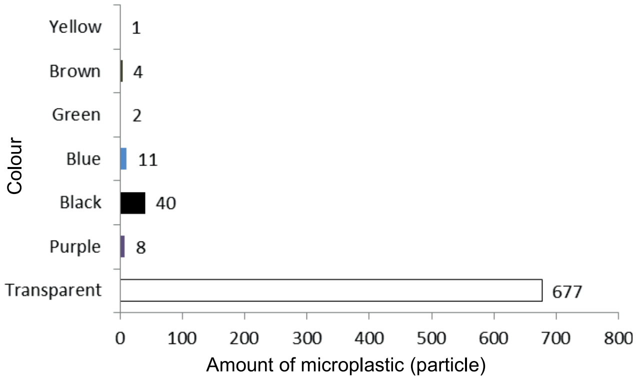
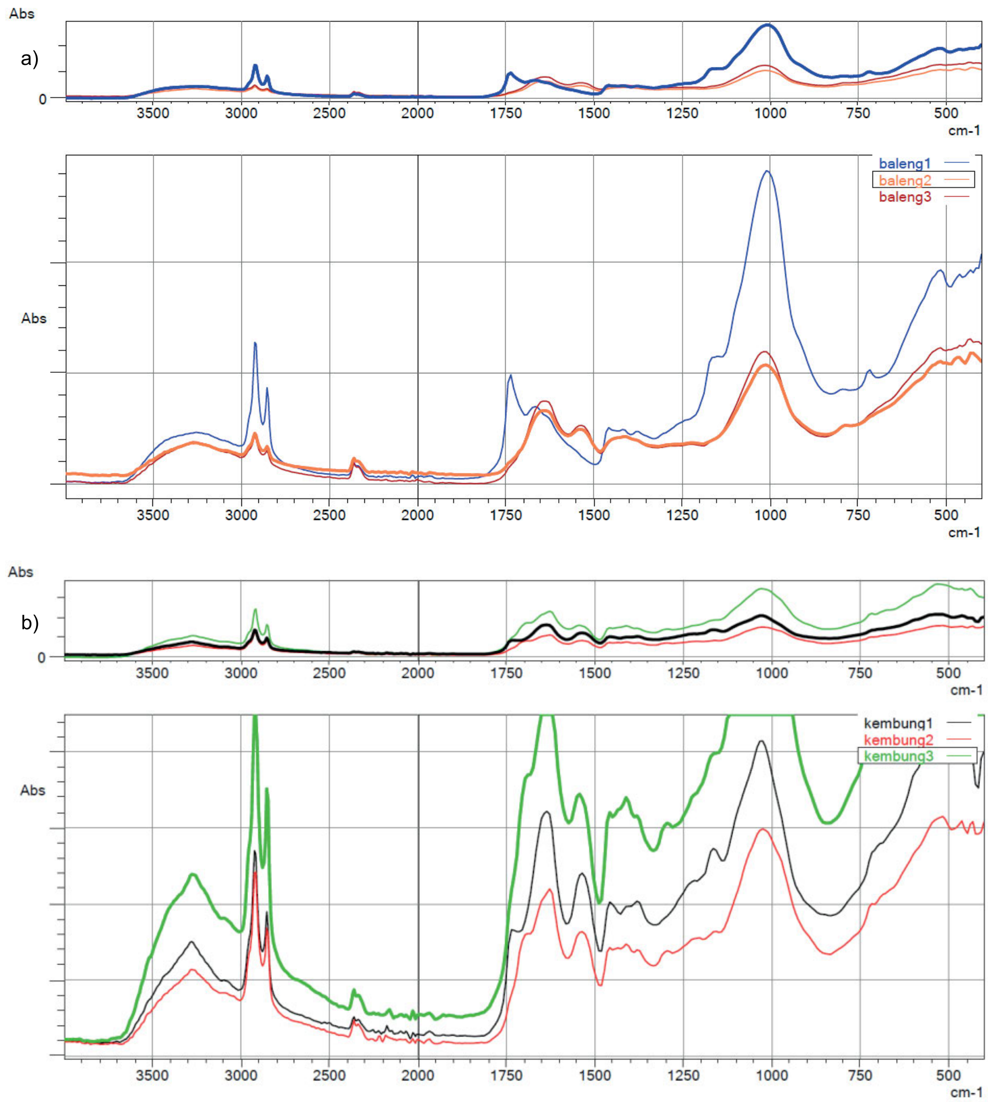


Fig. 4. Amount of microplastics by colour; source: own study

Figure 4 shows that the colour of microplastics with the largest number of observations on 36 individual samples from 4 species of fish is a transparent colour (677 particles). Black is the second most common colour with 40 particles, blue colour is the third with 11 particles, purple, brown, and green are the fourth, fifth, and sixth most common colours with 8, 4, and 2 particles. Yellow is the least common colour of microplastic found (1 particle). Bright or transparent microplastics are influenced by discolouration due to UV rays from the sun (Febriani, Amin and Fauzi, 2020). The transparent colour can also come from newly degraded plastics that have long been degrading (Ridlo *et al.*, 2020). The colour differences in microplastics are not unique, as observed from garbage and waste from multiple sources. This is supported by Kühn *et al.* (2017), which revealed that light-coloured floating microplastics are more abundant.

RESULTS OF FOURIER TRANSFORM INFRARED (FTIR) ANALYSIS

FTIR analysis showed that the tested sample contains seven plastic constituent polymers. These polymers include polystyrene (PS), nylon, acrylonitrile butadiene styrene (ABS), polyurethane (PU), low-density polyethylene (LDPE), high-density polyethylene (HDPE), and polypropylene (PP). Identification of microplastic polymer types in four fish species using FTIR is presented in Figure 5.



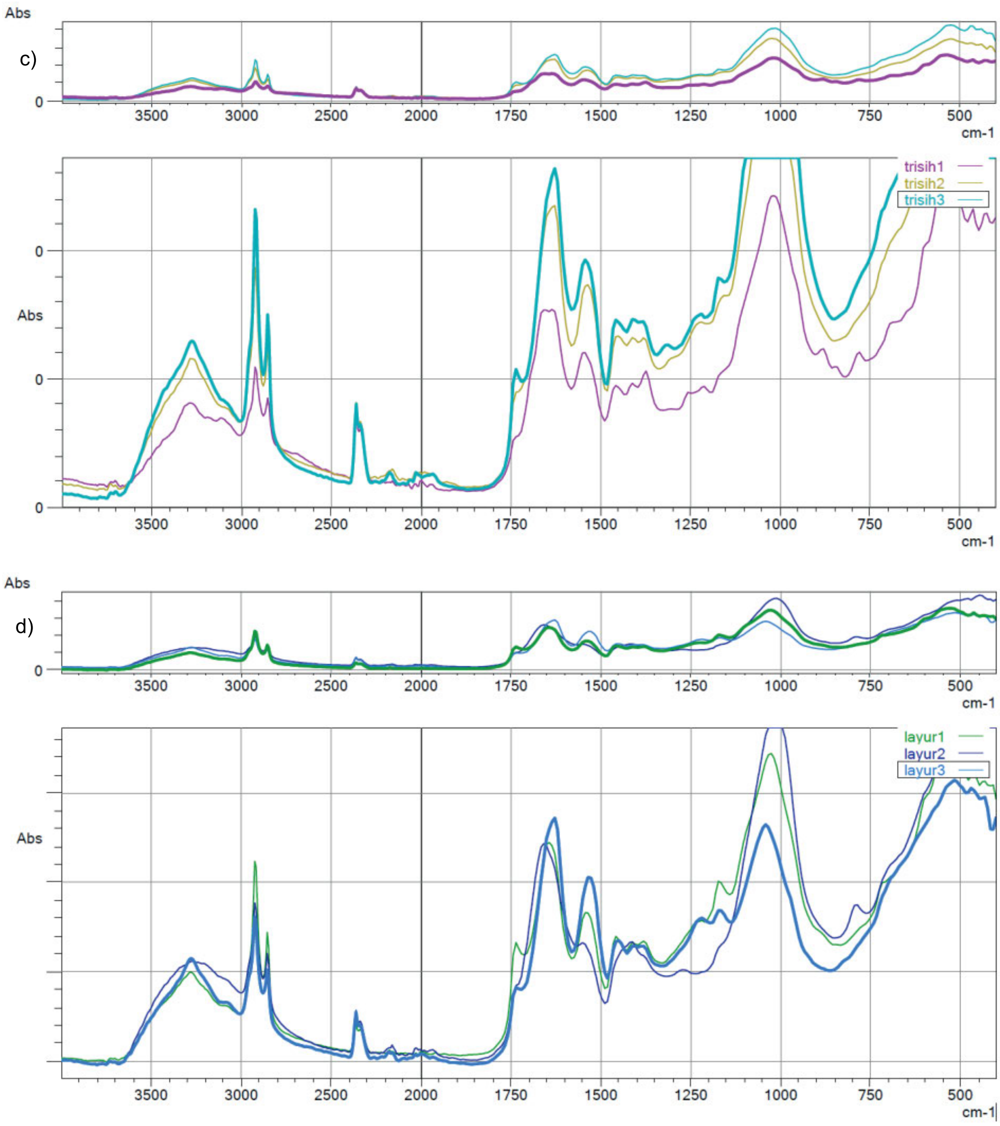


Fig. 5. Identification of microplastic components using Fourier transform infrared (FTIR) in various fish samples collected from Lengkong Fish Auction Point, Central Java, Indonesia: a) threadfin, b) mackerel, c) threadfin bream, d) hairtail; source: own study

The analysis results were later confirmed based on Jung *et al.* (2018) to determine the types of polymers contained in microplastics. PS polymer is indicated by the presence of an aromatic bond CH bend at wave number 1026.13. Nylon polymer is shown by bonding C=O stretch, C–N stretch, and CH stretch at wave numbers 1534.5, 1635.64, and 2854.65, respectively. ABS polymer is shown by the presence of a C–H stretch bond at wave number 2924.09. Meanwhile, PU polymer is demonstrated with a C–N stretch bond at wave number 1535.34. PP, HDPE, and LDPE polymers are shown with a CH_2 bend bond at wave number 1458.18.

According to Jung *et al.* (2018) and Zhao *et al.* (2018), the form of microplastic film is suspected of belonging to the type of PS and PP. Furthermore, the form of microplastic fibre is suspected of belonging to the type of nylon. Microplastic fragments belong to the types of HDPE, LDPE, and ABS. Lithner, Larsson and Dave (2011) revealed that the most dangerous polymer is PU. Granule/pellet is a primary microplastic sourced from microbeads for beauty products or derived from domestic activities in the form of household waste, such as laundry water containing granules (Hiwari *et al.*, 2019; Sugandi *et al.*, 2021).

The plastic constituent polymers can be sourced from various plastic-based wastes. This is supported by Pruter (1987), Andrady (2011), and Karuniastuti (2013), which stated that plastic products are included in the type of polymer. Some plastic products, such as electronics, rearview mirrors, fan holders, and motor vehicle spare parts, use ABS polymer (Puspita and Syamsun, 2011). Fishing tackle, fishing nets, clothing, textiles, and ropes use polyamide (nylon) (Pruter, 1987; Andrady, 2011; Nor and Obbard, 2014). PS is commonly used in tableware such as plastic spoons, plastic forks, cake containers, and food and beverage containers (styrofoam, foam cups) (Pruter, 1987; Andrady, 2011). PP is used in food packaging, beverage packaging, plastic bags, plastic wrapping, straws, household appliances, and fabrics (Andrady, 2011; Nor and Obbard, 2014). LDPE is found in plastic bags, bottles, nets, and straws (Andrady, 2011). Meanwhile, HDPE is widely used for milk bottles, gallons of water, cosmetic bottles, jerry cans, beverage bottles, and medicine bottles (Karuniastuti, 2013).

CONCLUSIONS

Based on the results and discussions, we found microplastics in the digestive tract of the four commercial and dominant fish species used as research objects, namely threadfin (*E. tetradactylum*), threadfin bream (*N. japonicas*), mackerel (*Rastrelliger* sp.), and hairtail (*T. lepturus*) with a frequency of occurrence of 100%. The abundance of microplastics in the digestive tract of fishes is relatively high, with a value range of 12 ±2.86 to 28.33 ±8.11 particles·ind^{-1}. Four main types of microplastics were identified, i.e. film, fibre, fragment, and granule, with film dominating. The seven colours found were transparent, purple, black, blue, green, brown, and yellow, with the most common coloured microplastics ingested being transparent. The polymers found were also varied, PS, nylon, ABS, PU, PP, HDPE, and LDPE. This study shows that fish species differ in their accumulation of microplastics due to differences in diet, feeding strategy and habitat. Generally, pelagic predators collect less plastic than benthic fish species. The confirmation of plastic ingestion by four fish species landed at Lengkong Fish Auction Point contributes significantly to our understanding of the plastic pollution problem in the marine environment. More research is needed to understand the effects of microplastics on the health of these fishes, as well as the possibility of biomagnification.

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