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Identification of Microplastic in the Digestive Tracts of Mackerel Tuna, Lemuru and Sea Water from Pangandaran, Indonesia

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ABSTRACT

Microplastic is a form of marine pollution that accumulates in water and disrupts the food chain in marine ecosystems including fish. If plastic particles accumulate in large quantities in the fish's body, the microplastic can clog the digestive tract of the fish. Pelagic fish have the potential to eat microplastic both intentionally and unintentionally, considering that this type of fish has a very wide variety of properties, and has a level of error-detecting food. The accumulation of microplastic particles in fish intestinal organs can cause inflammation, and physiological disorders (swimming ability, reproductive time). The research was carried out on the waters of Pangandaran East Coast (07°41′31.6″S 108°45′11.9″E), continued at the Biogeochemical Laboratory of the Faculty of Fisheries and Marine Sciences to analyze microplastic abundances in fish intestines and water. The highest microplastic abundance was in mackerel tuna with fragments of 4.61 ± 2.61 particles/g, whereas in the lemuru fish, the highest abundance value was found in the form of fiber as much as 2.45 ± 2.39 particles/g. The highest microplastic abundance value at station two in water samples was recorded at 1.33 particles/m^3 with the dominant fragments. The results of the analysis using the FTIR method found the types of plastic polymers in the three sample forms, namely fragments, fibres and films. The results of FTIR analysis found 2 types of polymers, namely polypropylene (PP) and polyester (PES). In the sample fragments and films found the same type of polymer, namely polypropylene (PP), while the fiber was found in the type of polyester polymer (PES).

INTRODUCTION

Garbage is nowadays the biggest threat in the world, both on land and in the ocean. The volume of waste that enters the waters continues to increase yearly from the remnants of human and natural activities and land waste that is carried to the estuary and ends on the coast and sea. The rubbish that enters the sea is carried by the current and moves in the direction of the ocean currents, becoming what is known as marine debris. Sources of garbage can come from human activities such as household, tourist, and congenital waste from the river **(Yuliadi & Nurruhwati, 2017)**. Marine waste is intentionally discarded or accidentally disposed of or transported from the land through rivers, drainage, or sewage systems which are then carried by water and wind flows **(Hidalgo-Ruz *et al*., 2012)**.

According to the results of the study covering Java, around 68% of plastic waste was found in nine research areas, namely Handeleum Island, Panjang Island, Tunda Island, Pari Island, Biawak Island, Gosong Island, Bali Island, Pangandaran, and Pelabuhan Ratu **(Ismail *et al*., 2018, Ismail *et al*., 2019)**. Plastic materials are commonly used by humans for daily needs. The estimated amount of plastic waste in Indonesia continues to increase from year to year. Indonesia is the second-largest contributor to garbage in the world after China, based on a 2010 study conducted on 192 coastal countries **(Jambeck *et al*., 2016)**. Indonesia, with a coastal population of 187.2 million and an average plastic waste disposal of 0.52kg per person per day, contributes 3.32 million metric tons of plastic waste annually **(Jambeck *et al*., 2016)**.

Plastic waste has recently been found almost everywhere **(Barnes *et al*., 2009)**. It is one type of waste made from polymerized products **(Zheng *et al*., 2005)**. Plastic polymers are very stable, so they will remain intact as polymers for a long period, which means that plastic material that enters the waters takes a long time to decompose **(Hapitasari, 2016)**.

Pangandaran Beach is a beach in Indonesia located in the South of Java Island, Pangandaran Regency. It has the potential to receive garbage in the form of plastic waste. The garbage can interfere with the aesthetics and disrupt the ecological functions of the beach. Plastic bottles and plastic cups are the dominant plastic waste found on Pangandaran Beach **(Yuliadi & Nurruhwati, 2017)**.

Plastics degrades by sunlight (photodegradation), oxidation, and mechanical abrasion form plastic particles **(Thompson *et al*., 2009)**. Small particles that are ≤ 5mm are called microplastic **(Thompson, 2004)**. Microplastics are caused by industrial or domestic activities, or they can be produced by degradation and fragmentation of macroplastics. Microplastics spread across the ocean, and sediments are carried away, mixing with beach sand. Some types of plastic float on the surface of water bodies **(Thompson *et al*., 2009)**.

Microplastics are a form of marine waste, and when they accumulate in aquatic environments, they can disrupt the food chain in fish. If plastic particles build up in large quantities within a fish's body, they can clog the digestive tract **(Browne *et al*., 2013)**, disrupt digestion, or hinder nutrient absorption **(Wright *et al*., 2013)**. Other studies have found microplastics in intertidal zones, tidal areas, and coastal regions of Indonesia **(Uneputty, 1997; Dewi, 2015; Dhamar *et al*., 2017; Ismail *et al*., 2018)**.

Pelagic fish have the potential to eat microplastics both intentionally and unintentionally, considering that this type of fish has an extremely wide variety of properties and has a level of error-detecting food. The accumulation of microplastic particles in fish intestinal organs can cause inflammation and physiological disorders (food response, swimming ability, reproductive time). This factor can cause high mortality before reproducing **(Guilhermino *et al.*, 2018)**. Given the dangers of microplastics, it is essential to conduct research on the identification of microplastic types in the digestive tracts of fish.

MATERIALS AND METHODS

Place and time

The research was carried out on the waters of Pangandaran East Coast (07°41′31.6″S 108°45′11.9″E), continued at the Biogeochemical Laboratory of the Faculty of Fisheries and Marine Sciences to analyze microplastic abundances in fish and water intestines. The study took place from in April - May 2019.

Fig. 1. Sample location

Research materials and methods

Pelagic fish used in this research are mackerel tuna and lemuru fish. These pelagic fish are the catches of coastal trawlers. The water samples used were taken along the fishing trails on the Pangandaran East Coast.

Research procedure

Identification of microplastic in fish intestines

Microplastic identification of fish intestines follows the research method of **Li *et al.* (2015, 2016, 2018)** and **Jabeen *et al.* (2017)**.

a. Sampling

Pelagic fish samples are the catches of Pukat Pantai fishers landed on the East Coast of Pangandaran. The collected samples were inserted into the cool box and given ice (until 0^o Celsius) to maintain its freshness for one day during the transportation process.

b. Preparation of tools for identifying mycorrhea in fish intestine

All tools were rinsed three times using filtered water. This aimed to avoid contamination when conducting research procedures.

Microplastic observation in fish intestine

c. Treatment of hydrogen peroxide (H_2O_2)

Pelagic fish samples were measured for total length and weight. The fish were dissected starting from the anus, along the dorsal side to the lateral line (linea lateralis), then toward the anterior (head), and finally toward the ventral side (towards the stomach) to expose the contents of the digestive tract. The intestines of the fish were removed, weighed, and measured for length. A portion of the fish intestine, weighing approximately 5 grams, was collected from 1-10 fish and placed into a 1-liter glass bottle. All the samples from the same fish type were pooled in one bottle, considered as one replication, with three replications prepared for each fish type. Hydrogen peroxide was used to separate organic matter in the fish intestines, aiding in the extraction and plasticization of the sample. For the process, no more than 5 grams of the fish intestinal sample was added, followed by 200ml of 30% hydrogen peroxide (H_2O_2), which provided the best decomposition results **(Li *et al.*, 2015)**. The bottle was then sealed and placed in an incubator shaker at 60°C with 80rpm for 24 hours to dissolve the fish intestine sample

d. Separation and screening with NaCl

Solution-saturated NaCl solution separates the microplastic from the solution through flotation. 800ml of filtered NaCl solution was added to each bottle. The liquid was mixed and allowed to stand overnight. It was then taken on the surface and filtered directly using the Whatman 1 filter material (125mm) with a vacuum system. After the screening process, the filtration results were stored in a petri dish that had been cleaned and closed to be identified microscopically.

e. Microscopic observations filtration

The results were observed under a microscope, and images were taken with a snap camera on the computer. Visual assessment was applied to identify the type of microplastics according to the physical characteristics of the particles. The microplastics particles were assessed visually using the method of **Hidalgo-Ruz *et al.* (2012)**. Microplastics were classified according to **Jabeen *et al.* (2016)** and categorized by type into fibers (elongated), fragments (small corner pieces), pellets (round, ovoid), and films (thin, soft, transparent).

Water microplastic identification

The method for identifying microplastics in seawater samples followed the procedure outlined by **Kovač Viršek *et al.* (2016)** and **Jabeen *et al.* (2017)**.

a. Water sampling

Method of taking water samples was done by pulling the net plankton that has been installed with a flow meter calibrated. Water sampling was taken horizontally on the water's surface at three points around the traversed fishing area. Plankton net moves in one straight direction for 5 minutes with a boat speed of 2-3 knots. The plankton net was rinsed using seawater from the mouth to the end of the cod to concentrate all the particles attached to the net.

b. Microplastic observations on solid sea water samples

Microplastic observations on solid seawater samples involved transferring the collected samples into a beaker. Then, 20ml of 0.05 M Fe(II) solution and 20ml of 30% H2O2 were added. The mixture was sealed and heated at 75°C for 30 minutes. After boiling, the beaker was removed from the hot plate, and the solution was allowed to cool. If the solution reacted during heating and was at risk of overflowing, distilled water was added to slow down the reaction. Then, 20ml of NaCl was added to help float the microplastic particles, and the mixture was heated at 75°C until the salt dissolved. The beaker was removed from the hot plate and left to cool. The beaker was covered with aluminum foil to prevent contamination and was left overnight for solid objects to settle. Floating solids were collected, and the sample solution was filtered using a 0.3mm filter. The solids that settled and adhered to the filter were rinsed with distilled water, then allowed to dry while covered with aluminum foil for 24 hours. Finally, the sample was examined under a microscope for microplastic identification. During analysis, note that some particles may be easily visible due to their color, shape, and size.

Analysis of microplastic polymer

FT-IR was used to analyze polymers because of their ability to analyze samples directly **(Käppler *et al.*, 2015)**. Microplastic polymer compositions were identified using the Nicolet TM iS5 FT-IR spectrometer equipped with attenuated and corrected total reflection diamond (ATR) accessories in OMNIC ™ software. Polymers were identified based on the vibration band and the comparison of the IR spectrum to the standard in the spectral library. FT-IR was operated using the experimental settings described in **Käppler *et al.* (2015)**, **Löder and Gerdts (2015)** and **Löder *et al.* (2015)**, in single reflection mode with a resolution of 8cm, in the range of 600 and 3800cm^{-1} and 16 scanning times per analysis. FT-IR tested microplastic particles after cleaning the surface using sterile ethanol (96%).

Process of identifying

The identification of microplastic schemes was carried out in the band range of 2780–2980cm^{-1} (vibration stretching CH / CH2 / CH3 group), 1740–1800cm^{-1} (C = O vibration stretching), 1670–1760cm^{-1} (stretching vibration C = O), 1400–1480cm^{-1} (bending vibration CH2), and 1174–1087cm^{-1} (CF2 stretch vibration **(Käppler *et al.*, 2015; Löder *et al.*, 2015)**.

Data processing

Microplastic abundance calculations on fish intestines

According to **Jabeen (2016)** and **Jambeck *et al.* (2016)**, microplastic data processing were calculated using the formula:

$$Microplastic\ abundance\ (particle/g) = \frac{Total\ of\ Microplastic\ (Particle)}{Weight\ of\ intestine\ fish\ (g)}$$

Calculation of microplastic abundance in water

The microplastic abundance in water is calculated based on discharge using the following formula:

$Q = A \times V$

Note :

Q = Water Debit

A = Volume of plankton net

V = Speed up of the boat

Data analysis

The abundance data of microplastics were analyzed descriptively and comparatively. The study focused on identifying microplastics found in mackerel tuna, lemuru, and water.

RESULTS AND DISCUSSION

Research locations

Large amounts of plastic waste, such as food packaging, household waste, and plastic bags, were found along fishing lines. The garbage was still in full form and also in the form of plastic fragments. This proves that the waters in the fishing area have been polluted by garbage originating from the river and carried by the current.

Form-based identification

This research found four microplastic forms: fragments, fibers, films, and pellets (Table 1). Microplastic can affect the possibility of eating microplastic by pelagic organisms **(Boerger *et al.*, 2010)**.

Table 1. Forms of microplastics on samples

Form	Mackerel tuna	Lemuru	Water	Total
Fragment	57	31	60	148
Films	27	2	40	69
Fiber	45	49	11	105
Pellets	2	-	-	2

The abundance value of fragments in the mackerel tuna was 3.92 ± 1.33 particles/g, while the highest abundance value in the lemuru fish was found in the form of 3.41 ± 3.10 particles/g. The results of this study indicated that fiber is the most commonly found form of microplastic in the lemuru fish. This finding aligns with the research conducted by **Lusher *et al.* (2013)**, which showed that fiber (68.3%) is the most prevalent microplastic form found in pelagic fish compared to other types.

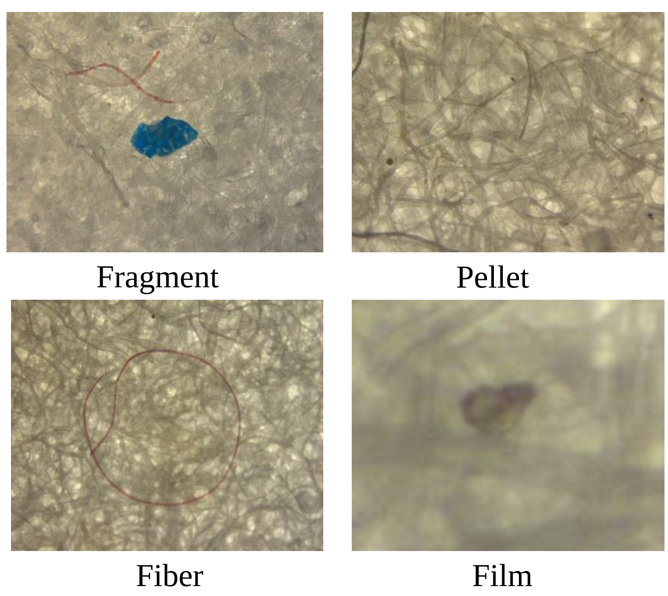

Fig. 2. Microplastic type

Other results found in this research include fragments found in the mackerel tuna and water. As the results of research conducted by Manalu, fragments are more commonly found in the digestive tract of fish **(Manalu *et al.*, 2017)**. Another research conducted by Eriksen found the dominant microplastic form on the surface of the water, and the Great Laurentian Lake is a type of fragment **(Eriksen *et al.*, 2013)**. The form of microplastic fragments come from pieces of plastic bags (macroplastic) found in aquatic environments and are usually associated with residential areas or high population densities. The possibility of microplastic fragments occurs through physical and chemical processes or the help of sunlight, so microplastics are produced in the form of irregular fragments **(Cole *et al.*, 2011, 2015)**. The difference in microplastic forms that are dominantly found in the digestive tract of fish is thought to be influenced by the abundance of each form around the environment of the fish.

The results of the analysis using the FTIR method found the types of plastic polymers in the three sample forms, namely fragments, fibres and films. The results of FTIR analysis found 2 types of polymers, namely polypropylene (PP) and polyester (PES). In the sample fragments and films found the same type of polymer, namely polypropylene (PP), while the fiber was found in the type of polyester polymer (PES).

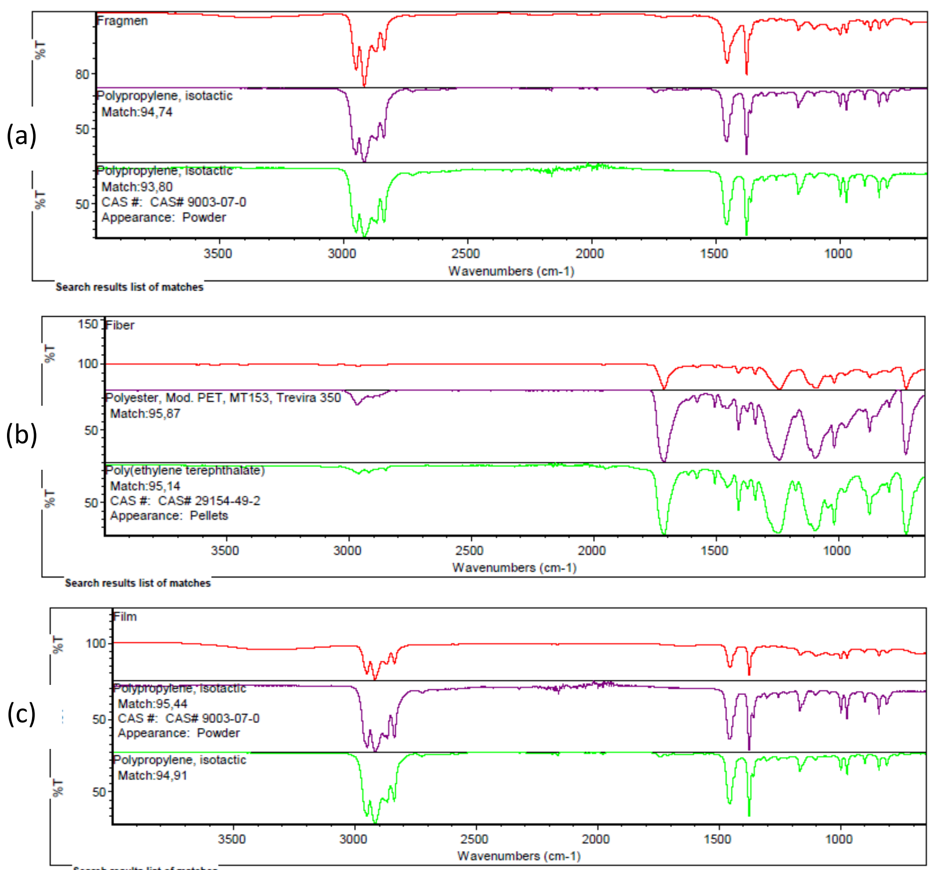

Fig. 3. Microplastic FTIR in samples (a) Fragments; (b) Fiber; (c) Film

Microplastic swallowed by fish or marine organisms will have an impact on the organism of the animal. If ingested, microplastic can pass through the intestine or can survive in the digestive tract **(Browne *et al.*, 2013)**. If plastic particles accumulate in large amounts in the small intestine, they may have the same effect as large plastic waste, clogging the digestive system **(Barnes *et al*., 2009)**. The accumulation of microplastics in an organism's body can lead to the damage of internal organs, as well as carcinogenic effects and endocrine disorders **(Oehlmann *et al*., 2009)**. Accumulation of garbage in the digestive tract can cause a false sense of satiety. This can decrease appetite and cause a decrease in biodiversity **(Ryan, 1988)**. As for concerns if ingested by organisms, small objects from plastic waste may facilitate the transportation of chemical contaminants **(Assuyuti *et al*., 2018)**.

The macarel tuna and lemuru are popular fish for domestic consumption in Indonesia. Therefore, it is worrying that the high levels of microplastic contamination seen in this fish species may have an impact on human health. This species may be used as a bioindicator to determine the level of environmental pollution. The possible harmful consequences of microplastics on people are still up for discussion even though. While photo-oxidative, thermal, chemical, and mechanical factors can break down bigger polymers, the actual process happens very slowly. Additionally, microplastics have been shown to have detrimental effects on the respiratory and digestive systems of humans as well as the reproductive systems of animals **(Fourniel *et al*., 2020)**.

CONCLUSION

The highest microplastic abundance was found in THE mackerel tuna, with fragments measuring 4.61 ± 2.61 particles/g. In lemuru fish, the highest abundance was observed in the form of fibers, with 2.45 ± 2.39 particles/g. In water samples, the highest microplastic abundance was recorded at station two, with 1.33 particles/m^3, dominated by fragments. The presence of microplastics in the water poses a significant threat to both fish and humans.

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