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Microplastic contamination in Indonesian anchovies from fourteen locations

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Abstract. *Ningrum EW, Patria MP. 2021. Microplastic contamination in Indonesian anchovies from fourteen locations. Biodiversitas 23: 125-134.* Microplastics in seawater can enter into the food chain of pelagic fish with subsurface foraging behavior such as anchovies (*Stolephorus* spp.). Anchovies are valuable commercial fish with high market demand. Hence, measuring potential marine pollutants in these fish is needed. Microplastics as marine contaminants are reportedly more dangerous if they occur together with other contaminants such as trace metals. This research aims to detect microplastic contaminants in anchovies caught in the Indonesia Sea. This research compared between the microplastic contamination on anchovies from 14 harbors: 6 in Western Indonesia (Meulaboh, Krui, Pangkalpinang, Muara Angke, Karimunjawa, and Sidoarjo) and 8 in Eastern Indonesia (Manado, Mamuju, Makassar, Kendari, Ambon, Sorong, Fakfak, and Waingapu). We isolated the digestive tracts of anchovies and measured their length and dry weight. The organic materials were digested with NaOH and a technical-grade sodium Laureth sulfate (SLES) solution approximately one week after collection. Microplastics were observed using a microscope and confirmed with Fourier transform infrared spectroscopy (FTIR). We found that most microplastic contaminants in anchovies were in fiber and film shapes. The majority of the sizes ranged from 50-500 μm, followed by a range of 20-50 μm. Microplastics were surprisingly high in samples from Mamuju (688±1.15 MPs idv^{-1}) and Krui (645±7.02 MPs idv^{-1}), higher than any contaminated biota ever reported for anchovies. As reported, anchovies from the Indonesia Sea are contaminated by microplastics. Moreover, human exposure to microplastic contaminants is possible and may affect consumer health in long-term exposure.

Keywords: Microfiber, microfilm, micro fragment, MPs, *Stolephorus*

INTRODUCTION

Globally, synthetic polymer (plastic) is one of the most ubiquitous persistent pollutants and is easily found in oceans and on beaches (Syakti et al. 2017). Several studies have reported that microplastics (1 μm-5 mm) in surface water and water columns may be transferred into marine food chains. Microplastics are found in zooplankton (Lo and Chan 2018) and fish juveniles (Ory et al. 2018). The marine biota had ingested microplastics directly as their food or indirectly by ingesting contaminated prey (Savoca et al. 2017; Patria et al. 2020). At higher trophic levels, the contamination of microplastics was reported by Karami et al. (2018) in canned fish and other commercial fish (Pozo et al. 2019).

Several methods, including Fourier Transform Infrared Spectroscopy (FTIR) analysis, Pyr-GC/MS, and Raman spectroscopy, have been adapted to monitor marine ecosystems' microplastics. Jung et al. (2018) reported that microplastics were found as Polyethylene (PE), Polypropylene (PP) mixture, unknown PE, Low-density polyethylene (LDPE), High-density polyethylene (HDPE), nylon, polypropylene (PP), polyvinyl chloride, and polystyrene. The most abundant plastic materials reported by Syakti et al. (2017) were PP (68%) and LDPP (11%), with the predominant colors were blue, white, and green. These kinds of microplastic shapes come from different sources. Fibrous microplastics originate from textile fibers or fishing lines, while film-shaped microplastics come from plastic bags or food wrappers. The fragment-shaped microplastics originate from more extensive fragmented materials. Foam shape or microbead possibly comes from daily products (Tanaka and Takada 2016).

The original microplastics are divided into five types: polyethylene (PE), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polypropylene (PP), and polystyrene (PS) (Andrady 2017). According to the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP), microplastics are classified into three groups: 1-5 mm as the large microplastics, >5-25 mm as mesoplastics, and >25 mm as macroplastics (GESAMP 2019). Meanwhile, the National Oceanic and Atmospheric Administration (NOAA) classify <5 mm as microplastics. NOAA describes constituting plastics with kinds of PET from soda bottles, PES from polyester clothing, PE from a plastic bag, HDPE from a detergent bottle, PVC from plumbing pipes, PP from drinking straw, PA from toothbrush and PS from take-out food containers (NOAA 2020).

In the ocean, microplastics represent recalcitrant substances for microorganisms (Oberbeckmann and Labrenz 2020). In the aquatic habitat, surface microplastics serve as colonization ground for diverse microbial communities (Dussud et al. 2018), and Camacho et al. (2019) also reported that microplastics could associate with persistent organic pollutants (POPs). Plastic has semicrystalline, showing as ordered segments of the polymer chain. The thick lines (polymer chain) have a crystal-like property embedded in thin lines (amorphous) polymer matrix. These plastic properties can dissolve POPs compounds (Andrady 2017).

Microplastic impacts on marine biota are recorded widely around the world. The presence of microplastic in aquatic habitats will potentially threaten their biota (Setälä et al. 2014). The European hake as a top predator, *Merluccius merluccius*, consumed by people, ingested 31 black fibers in only one fish (Mancuso et al. 2019). A laboratory experiment was conducted in zebrafish (*Danio rerio*) to gain an understanding of the potential damage if microplastics were combined with chemicals such as derivatives of polychlorinated biphenyls (PCBs) and methylmercury. Rainieri et al. (2018) reported that the zebrafish reached hemostasis after three weeks of exposure to the contaminant mixtures. Evidence was reported by Germanov et al. (2018) that the presence of microplastics in the gut could block nutrients and cause damage to the gut.

Microplastic particles occupy the same size range as some planktonic organisms and sediment, pervasive in almost all aquatic habitats types (Kumar et al. 2018). Fish frequently ingested other digestible material such as wood, shells, sand, and other indigestible items. However, microplastics may impact fish health. Additional materials such as plasticizers, lubricants, flame-retardants, colorants, ultraviolet absorbers, fillers, stabilizers, coupling agents, antioxidants, and natural preservatives may include deleterious effects on fish (Jovanović 2017). Mizraji et al. (2017) investigated microplastics' bioavailability compared to herbivorous, carnivorous, and omnivorous feeding types and the results showed that omnivorous fish tend to consume a higher amount of microplastics.

The anchovy is categorized as a small pelagic fish that feeds on zooplankton. In the recent decade, worldwide, annually caught has increased by 1.2 million tons (Garibaldi and Funge-Smith 2018). Anchovies are small pelagic fish widely abundant in the Indo-Pacific Ocean (Andamari et al. 2013). Anchovies are easily found in the coastal ocean areas (0-50 m deep) and sometimes seen in brackish water. The characteristics of anchovies are a body length of ±12 cm, a length at maturity of ± 6.5-7 cm, a weight of ±14.40 g with a maximal weight of 17.8 g, and 18-19 dorsal fins with or without an anal or dorsal fin (Whitehead 1988). Anchovies are carnivorous fish that forage in a large group. Their preys are zooplankton and crustaceans both in the juvenile and adult phases. Anchovies can ingest up to 22 times their body mass. Their predators are birds (*Laridae* spp.) and Osteichthyes fish from the Scombridae and Synodontidae families (Whitehead 1988).

Microplastics are lightweight and float on the subsurface, in water columns, near the bottom, and in the thermocline layer, all areas where anchovies forage for prey (Zobkov et al. 2019). With these areas of contamination, the chances of human exposure to microplastics by consuming anchovies are elevated. Anchovy in Indonesia has become one of the top commodities (3.7%) on catch fishery category based on national production (KKP RI 2017). In this research, anchovy was chosen as it plays commercial importance on the fish market and is abundant on the Indonesian sea. This research aims to (i) detect contamination in anchovy and (ii) compare the results between the two locations as defined by the western and eastern locations.

MATERIALS AND METHODS

Sample collection

Fourteen (14) harbors in Indonesia were chosen based on their location. The Meulaboh and Krui harbors in Sumatra are located in the Indian Ocean, Sorong harbor in Papua, and Manado harbor in Sulawesi are located in the Pacific Ocean. The Makassar and Mamuju harbors in Sulawesi were chosen because they are strategically located in the Makassar Strait, while Kendari harbor in Sulawesi, Ambon harbor in Maluku, and Fakfak harbor in Papua are in the Banda Sea current. Two harbors were chosen because they were subjected to both the Makassar Strait and the time Banda Sea currents (the Sidoarjo and Waingapu harbors). The rest of the harbors were chosen because they were subjected to the Java Sea current (Karimunjawa and Pangkalpinang), especially Muara Angke harbor, which had overlapping effects from the Java Sea and the Indian Ocean. With these fourteen harbors, microplastic contamination in Indonesian marine anchovies located in the Western harbors was compared to those in the Eastern harbors of the Indonesia Sea. The Western harbors were Meulaboh, Krui, Pangkalpinang, Muara Angke, Karimunjawa, and Sidoarjo, while the Eastern harbors were Manado, Mamuju, Makassar, Kendari, Ambon, Sorong, Fakfak, and Waingapu.

The fifteen (15) anchovies (*Stolephorus* spp.) were randomly picked from each of the 14 marine harbors and a fish market in these harbors during May-August 2018 (Figure 1). Samples were dried naturally within the sunlight then stored in a jar to be shipped. We avoided using alcoholic preparation to avoid destroying the small size range microplastics (Budimir et al. 2018, personal communication). Samples were rinsed by filtered Milli Q water in the laboratory three times and soaked then rinsed. Only the digestive system is isolated after measurement to continue for microplastic observation.

Procedural blank

The method for sample preparation was adapted from Budimir et al. (2018). Validation was carried out with anchovies (*Stolephorus* spp.). The anchovy length (mm±SD) and dry weight (g±SD) were measured by digital scales and photographed (Figure 2). Measured anchovies were soaked in filtered Milli-Q water and rinsed three times by flowing water to avoid contamination from external contaminants on the fish body. Anchovies' digestive tracts were isolated as a whole (± 1 g total for individual tracts) and placed in an Erlenmeyer flask for the digestive process.

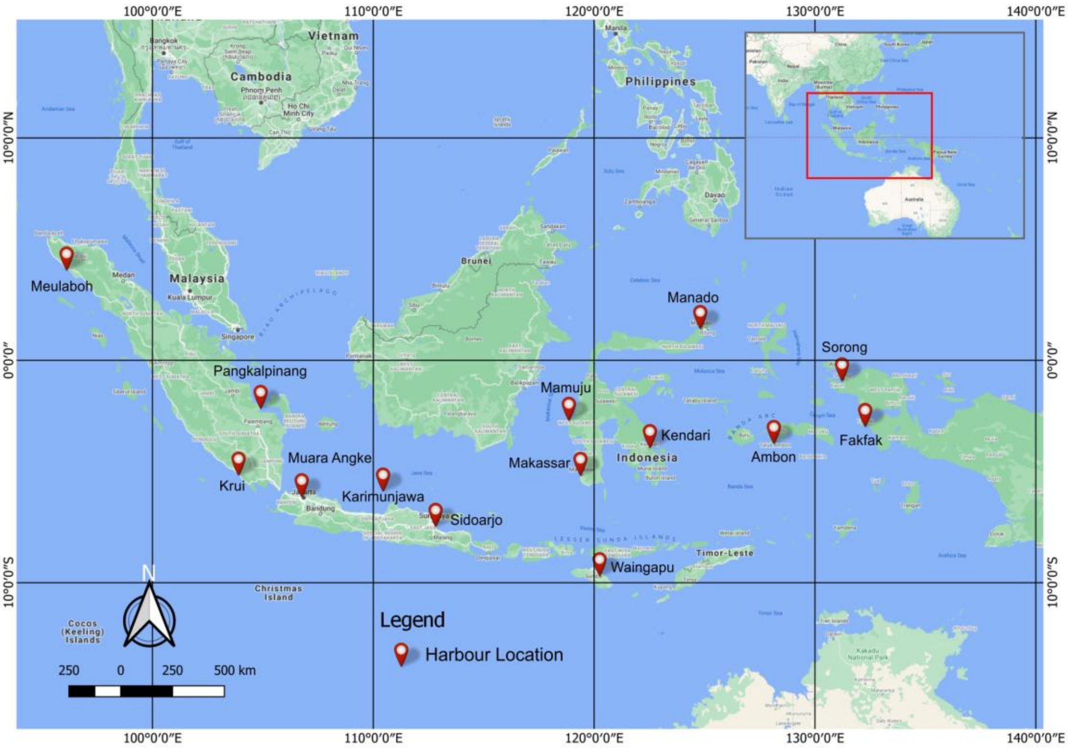

Figure 1. Study sites in the fourteen harbors in Indonesia

Figure 2. The dried anchovy (*Stolephorus* spp.). Bar = 2 cm

During this pre-testing phase, the different volume of the chemicals (Sodium laureth sulfate SLES, NaOH) were applied and the effect of incubation time (2-7 days), and sample processing steps (filtering vs. without filtering) were examined. The digestion time took seven days. Another sample needs more time to digest (up to 14 days), then the chemicals at the same concentration and volume were added (Budimir 2018, personal communication). The digestion process worked like the developed protocol. Sodium dodecyl sulfate (SDS) was changed by sodium Laureth sulfate (SLES) as the SLES was available in the laboratory. The SLES is a common commercial detergent recently been used for denaturing proteins and destructing the organic material in another study on marine microplastics (Ningrum and Patria 2019a).

The Milli-Q water was tested for microplastics contamination, and the results show that only microfiber was found. Most microfiber found on 20-50 μm (246 particles). For this, the Milli-Q water and all chemicals used were filtered with filter paper<10 μm pore size (Whatman No. 24/21, No. 2, or if the stock was run-out the Whatman No.1 was used). To assess the effectiveness of the filtration process, 1 mL of filtered Milli-Q water was placed in the slide for microscope validation. All filtered chemicals were stored in a safe place and only taken as needed.

Sample digestive processing

The digestive tract (n: 210) was isolated using a scalpel blade No. 42 in the 150x15mm pyrex petri dish at none airflow table. Anchovies' digestive tracts were placed in a rinsed Erlenmeyer (50 mL) flask by sterile Milli-Q water. Chemicals of 10 mL (1 M) NaOH, together with 5 mL 0.5% dissolved SLES, were added under a fume hood with a volumetric pipette 10 mL for each chemical. The chemical and sample flasks were covered by aluminum foil and only opened when it is needed. The SLES was categorized as a salts group of sulfated ethoxylated alcohols (Robinson et al. 2010). All glassware was rinsed with filtered Milli-Q water. The Erlenmeyer flask was kept at room temperature for 24 hours. The next day, samples were gently shaken and incubated for another 24 hours. Some samples needed more time to digest completely. Most of the samples were wholly digested within a week. If any remaining gut tissues persisted in the Erlenmeyer flask at the end of the week, we added another 10 mL and 5 mL of NaOH and SLES. Excessive solvent usage will damage the smaller microplastics; however, conservative solvent usage will prolong the sample digestive process. We use only two solutions to prevent damage to microplastics and leaching. The leaching was marked as particle with its transparent color. After complete digestion, samples were filtered through the filter paper. In this phase, the organic materials were completely digested. No hydrochloric acid

(HCl) is needed to remove the calciferous structures. Following the filtration, filters should be thoroughly rinsed with Milli-Q water. The samples were then homogeneous and taken as triplicate in the top, middle, and bottom of the flask. The error bar was indicated (Figure 3) the microplastics were distributed homogeneously. The samples were placed in the Sedgwick rafter for microplastic observation, then stored in a petri dish covered with lids and ready for microscope observation (Budimir et al. 2018).

Observation of microplastics

Total microplastics per individual were calculated to estimate dietary intake (in wet weight) even the maximum microplastic intake still not standardized for both national and international regulation. An aliquot of the sample (1 mL) was placed with a clean pipette in a Sedgwick rafter for microscopic observation. Microplastics were manually counted and measured under the light microscope, Leica dm750 (10 × and 40 ×), and photographed using a Nikon Microscope. Sedgwick rafter has transects across its cells and is easy to measure microplastics' size range (Fendall and Sewell 2009). Microplastic size was validated by scale bar on Leica. Triplicate observations were conducted for each anchovy sample. The average of the three samples for each anchovies samples was taken as the abundance (MPs idv^{-1}) for that harbor.

The microplastics were characterized based on their shape and size range. Each shape and size were photographed and manipulated as one picture for easy comparison using Adobe comp version 3.0.5.1.6 and Adobe Photoshop mix version 2.8.1. After being photographed, the shapes were prepared for an FTIR test, and the rest of the samples were kept in the sealed Erlenmeyer flask with aluminum foil seal to avoid contamination and only obtained for microplastics examination (Budimir et al. 2018).

The kinds of microplastic shapes were characterized as fibers, films, fragments, or foam. The fibers were mainly thin as fishing lines; films were transparent, thin, and soft while the fragment was hard, flat and jagged (Lares et al. 2018; Zhou et al. 2018). Its appearance categorized the foam compared to another reference (Chen et al. 2018). The observed sizes were classified as <20 μm, 20-50 μm, 50-500 μm, 500-1000 μm, and >1000 μm. The shape and size of each harbor were displayed as graphics. Microplastic types were confirmed by FTIR analysis by cross-referencing the spectra with the spectral instrument library.

Contamination prevention

The contamination protocol was adapted from before (Budimir et al. 2018). All the anchovy samples were placed in a Petri dish during the preparation sample. HDPE materials were avoided during this process, and only glassware was used.

Procedural blanks were conducted as controls for laboratory contamination. The blank procedure was conducted exactly like the field samples were treated without the fish digestive tract. Two prepared Milli-Q water were placed in the Erlenmeyer flasks without filtering, and after filtered using Whatman No. 1, No. 2, and No. 24/21 with < 10 μm pore size. There were no microplastics found from Milli-Q water after filtering. Meanwhile, we found only microplastics contamination from Milli-Q water without filtered indicated that Milli-Q water needs to be filtered. Milli-Q water and all chemicals used were prepared by filtering through these filter papers. All the glassware was then rinsed with filtered Milli-Q water.

During the observation process, we placed the blank Sedgwick rafter with Milli-Q water to observe the airborne contamination. No microplastics were found in the controls, but still, the samples were placed in the Sedwick rafter, and covered by a Petri dish during the observation process. Great care was taken not to contaminate the sample by working quickly in the observation process, since the contamination was not possible to prevent entirely.

FTIR analysis

FTIR analysis was conducted using an FTIR diamond Thermo Nicolet™ iS™ 5 instrument (Syakti et al. 2017). Medium-sized (>500 μm) microplastics were prepared for diamond crystal ATR (attenuated total reflection). The microplastics size was chosen because of difficulties in observation and to avoid the smallest one from loss. Fourteenth samples (n: 14) from each harbor were tested for FTIR. The particles were chosen randomized to identify the polymer type. Samples were placed on filter paper in a Petri dish to avoid air contamination. The attenuated total reflection (ATR) was recorded and corrected by ATR correction. The spectra between 4000 and 450 cm^{-1} were acquired. The kinds of microplastics analyzed were PP, PS, LDPE, HDPE, PVC, PA, PET, and CA (Jung et al. 2018). Microplastics found were validated by cross-referencing after being confirmed on the spectral instrument library.

Statistical analysis

Statistical analyses were conducted utilizing a normality test and homoscedasticity analysis with the Kolmogorov-Smirnoff test for the data (n: 210). The comparison between total MPs of the Western and the Eastern harbors was conducted by using a t-test. The variation between the shapes and the size ranges of the Western and the Eastern harbors was compared using a two-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Non-parametric Kruskal Wallis was applied to any of the data without normal distribution followed by post hoc test Mann Whitney. The differences were determined with a significant value of $p<0.05$. This statistical analysis was performed using the statistical software IBM SPSS 25.

RESULTS AND DISCUSSION

Measured the total microplastic contaminants on the anchovy

The anchovies from Krui were heavier (3.56±0.33 g) and longer (103.08±3.51 mm) compared to all collected samples from 14 locations on the Indonesian sea (Table 1). The lighter anchovies (0.52±0.07 g) were from Waingapu and the shorter (47.43±1.20 mm) were from Makassar compared to all collected samples. The highest level of microplastic contamination is found in Mamuju harbor (688±1.15 MPs idv^{-1}), followed by contamination in anchovies from Krui harbor (645±7.02 MPs idv^{-1}), which also had the most extended bodies. Those from the Karimunjawa and Waingapu harbors have the shortest and lightest bodies and the lowest microplastic contamination from all contaminated anchovies. From Table 1, we can expect that microplastic contamination is directly proportional to increasing biomass (length and weight). The highest number of fibers were found from Waingapu (65.45%) and Karimunjawa (64.71%). Film types were mostly found from Mamuju (68.32%) then followed by Ambon (58.90%), while fragment type was highly found from Fakfak (39.39%) and Sorong (30.69%). The lowest percentage of microplastic type was foam. Foam was commonly found from Muara Angke (8.57%) followed by Krui, Lampung (6.36%). Fiber was the most microplastic type found from these locations. The other report from Ningrum and Patria (2021) stated that fiber was the most microplastics from Indonesia Anchovies compared to the four harbors.

The t-test result comparing the total MPs abundance between the Western and the Eastern harbors showed these both harbors were similar (p: 0.18). Total microplastics per individual for each harbor are displayed with standard deviation in (Figure 3).

Possibilities of microplastics in marine anchovies

The total microplastic levels detected in the gut of anchovies from the fourteen harbors were surprisingly high. Compared to other reported biota results, anchovies from the Indonesia Sea were considered the most contaminated fish by individual (Mamuju has 688±1.15 MPs idv^{-1} and Krui, has 645±7.02 MPs idv^{-1}). As a comparison, sardines (*Sardina pilchardus*) and (*Engraulis encrasicolus*) contaminated with microplastics were reported by Renzi et al. (2019) with contamination levels up to 4.63 pieces and 1.25 pieces per individual. The other report from Japanese anchovies reported levels of contamination of 2.3 pieces per individual (Tanaka and Takada 2016). Concerning European anchovies, it was reported that 8 of 10 fish contained nine pieces of microplastics (Collard et al. 2017). Hence, these reports make it more challenging for comparisons to be drawn between our results.

Although there is limited study using anchovy in the laboratory, Avio et al. (2015) used European hake (*M. merluccius*) as a fish model to draw understanding in mechanism of microplastics contamination. The European hake was exposed to 2500 microplastics per liter during a laboratory experiment. This microplastic exposure is much higher than microplastics that have been found in the marine environment. This past study provided evidence of the possibility of a marine biota investigation to determine microplastic contaminants.

Considering the lack of microplastic contamination data in Indonesia marine anchovies, the comparison is drawn between the Eastern harbors and the Western harbors compared to another report. Our findings indicated nothing different between the total microplastic contamination of anchovies in Eastern harbors and Western harbors. However, the difference in microplastics found in anchovies can be seen between those from the outer harbors and those located inside Indonesia marine zones. The outer harbors are Meulaboh, Krui, Sorong, and Manado, which exhibited high microplastic contamination of anchovies. The harbors subjected to the effect of the Pacific current, such as Makassar, Mamuju, and Kendari, are also highly contaminated with microplastics, as indicated by the microplastic contents in anchovies caught from these harbors. Compared to another report, anchovies from Talisayan (366±3. 51 MPs idv^{-1}), and Balikpapan (130±1.73 MPs idv^{-1}) which are located near to Makassar strait, also have higher microplastics (Ningrum and Patria 2019b; Ningrum et al. 2019).

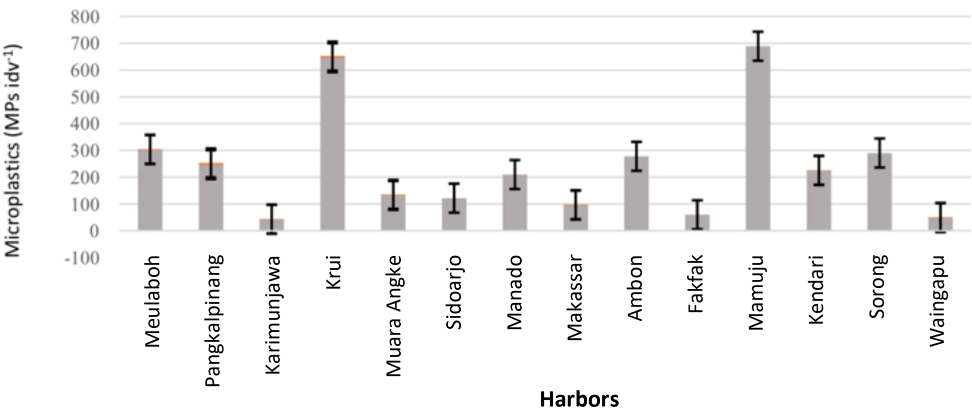

Figure 3. Total microplastics on 1 mL sample from each harbor with standard deviation

Table 1. The number of samples, fish weight, fish length, total microplastics (MPs), and percentage of microplastic type (fiber, film, fragment, and foam) identified on sampled Anchovies (*Stolephorus* spp.) from 14 harbors

Harbors	n	Weight (g±SD)	Length (mm±SD)	Total particle (MPs idv^{-1})	Total MPs (wet weight)	Fiber (%)	Film (%)	Fragment (%)	Foam (%)
The western harbors									
Meulaboh	15	0.58±0.05	56.29±0.99	302±2.65	520	33.33	39.74	26.92	0
Pangkalpinang	15	1.00±0.18	59.11±3.57	246±7.57	246	61.97	24.65	12.68	0.70
Karimunjawa	15	0.64±0.05	48.63±0.37	42±2.08	66	64.71	25.49	9.80	0
Krui	15	3.56±0.33	103.08±3.51	645±7.02	181	33.64	52.73	7.27	6.36
Muara Angke	15	1.77±0.28	71.28±1.58	131±5.29	74	55.71	12.86	22.86	8.57
Sidoarjo	15	0.78±0.08	65.66±1.00	121±1.15	156	52.31	26.15	18.46	3.08
Average	15	1.39	248	248	207	50.28	30.27	16.33	3.12
The eastern harbors									
Manado	15	0.81±0.08	64.76±2.46	209±1.15	258	27.78	37.04	35.19	0
Makassar	15	0.35±0.06	47.43±1.20	95±2.65	272	50.98	33.33	13.73	1.96
Ambon	15	1.36±0.22	74.19±3.67	277±1.15	204	16.44	58.90	24.66	0
Fakfak	15	0.82±0.08	65.09±0.68	59±1.00	72	33.33	27.27	39.39	0
Mamuju	15	0.58±0.11	58.30±2.36	688±1.15	1186	10.21	68.32	19.63	1.83
Kendari	15	1.31±0.08	76.08±1.40	224±2.31	171	41.67	39.17	17.50	1.67
Sorong	15	0.94±0.12	71.14±1.51	290±0.00	308	41.58	26.73	30.69	0.99
Waingapu	15	0.52±0.07	55.73±0.66	48±2.31	92	65.45	10.91	23.64	0
Average	15	0.84	64.09	236	320	35.93	37.71	25.55	0.81

The harbors located in the Java Sea, such as Karimunjawa, Sidoarjo, Muara Angke, and Pangkalpinang, have lower microplastic contaminants than those mentioned above. Waingapu harbor is far from the Java Sea, the Makassar Strait, or the Banda Sea, and its microplastic contaminants are also at lower levels than those detected in the other harbors. Compared to another report, anchovy from East Lombok harbor, which is included in this category, also has lower microplastic contamination (88±2.89 MPs idv^{-1}) (Ningrum and Patria 2019a). Total microplastic contaminants in anchovies from both the outer harbors and the harbors subjected to the Pacific current are highest. The results indicate that microplastic contamination in anchovies is displayed in total microplastic MPs g^{-1} (dry weight), considering the impact of microplastics on human health. The increasing amount of seafood intake by consumers will increase the amount of human exposure to microplastics.

The majority of microplastic shapes are found to be fiber-shaped, followed by the film- and fragment-shaped microplastics (Figure 4 and Figure 5). The harbors with the most fiber-shaped microplastic contaminants found in anchovies were Karimunjawa, Kendari, Makassar, Muara Angke, Pangkalpinang, Sidoarjo, Sorong, and Waingapu. The harbors with the most film-shaped microplastics found in anchovies were Ambon, Krui, Mamuju, Manado, and Meulaboh. Meanwhile, the Mann-Whitney test showed that fiber and film found in the Western and the Eastern harbors were similar (p: 0.98). Fakfak is the only harbor with anchovies contaminated mainly by fragmented microplastics ($p<0.05$). Conversely, foam microplastics are rarely found in anchovies (p: 1.00). Classified by their shape, the fibrous and film microplastics found in this research are also similar to the reported anchovy from East Lombok and Alor, which has microplastics in the most fiber shape (Ningrum and Patria 2019a; Ningrum and Patria 2019b).

Microplastics ranged in size 50-500 μm, followed by a 20-50 μm size range at most ($p<0.05$). All harbors reflected 50-500 μm as the most common size range except Fakfak harbor (20-50 μm) and Manado harbor (<20 μm) (Figures 4 and 5). Unique microplastic shape and size ranges are found in anchovies from the Fakfak (micro fragments in the 20-50 μm range) and Manado (micro fragments in the <20 μm range) harbors. Photographed microplastics are displayed in (Figure 6), and the scale bars represent 200 μm.

Anchovies ingest krill, zooplankton, and euphausiids as their prey. A study on anchovies' prey showed that zooplankton ingested 131.5 microplastic pieces per m^3 water while krill ingested 31.5 μm microplastics and broke them down into nano plastics, and Euphausiids ingested 816 μm sized microplastics (Sun et al. 2017; Dawson et al. 2018). Furthermore, the study on anchovy larvae (9-15 mm) showed that anchovy larvae ingested zooplankton >150 μm in size, which had ingested microplastics (Morote et al. 2010). Additional research has supported the evidence that juvenile anchovies have a mouth width ranging from 2.5 mm up to 8 mm (Viñas and Santos 2000). Adult anchovies possibly have wider mouths. These studies provide evidence that anchovies' prey and anchovies in the larval stage will eat microplastics. Moreover, the results of the observation of Indonesia anchovies show that most microplastics found ranged from 50-500 μm ($p<0.05$) compared to another microplastics size range reported from Japanese anchovy (150-1000 μm), European anchovy from the Gulf of Lions (1810-1520 μm), European anchovy from Lebanese cost (200-800 μm) and European anchovy from Adriatic sea (40.1-2220.6 μm) (Tanaka and Takada 2016; Kazour et al. 2019; Lefebvre et al. 2019; Renzi et al. 2019).

FTIR confirmed microplastics' presence, and they are identified as PP, PS, LDPE, HDPE, PET, PA, and CA (Table 2). The most abundant polymer detected was PA, followed by HDPE. Cross-references validated the FTIR spectrum values. For example, in most dominant PA and HDPE. The PA was showed the N-H stretch (a), CH stretch (b), CH stretch (c), C=O stretch (d), NH bend and C-N stretch (e), CH_2 bend (f), CH_2 bend (g), NH bend and C-N stretch (h), CH_2 bend (i), NH bend and C=O bend (j). The HDPE was showed the C-H stretch (a), C-H stretch (b), CH_2 bend (c), CH_2 bend (d), CH_2rock (e) and CH_2rock (f) (Rotter and Ishida 1992; Verleye et al. 2001; Nishikida and Coates 2003; Noda et al. 2007; Asensio et al. 2009).

The PA microplastic type was primarily found in this investigation, followed by HDPE. The PA was likely sourced from netting and traps, which matched the fibrous microplastics mainly found in anchovies. The HDPE originated from milk and juice jugs, which contributed to the film and fragment microplastic types. Furthermore, LDPE comes from plastic bags, bottles, fishing nets, and drinking straws. The PP comes from rope, netting, and bottle caps. The PS is primarily used in the production of food containers and plastic utensils. The PET is from plastic beverage bottles. The CA comes from cigarette filters (Andrady 2017).

Microplastic contamination in marine anchovies

Anchovies are pelagic fish with filter-feeder foraging behavior. Anchovies use odors to determine their prey (Savoca et al. 2017). Like their prey, zooplankton passes through the mouth or is sieved by gill rakers and transferred into the esophagus. We isolated the gut of anchovies based on this exposure mechanism. Therefore, water contaminated with microplastics will cause direct microplastic exposure to anchovies (Desforges et al. 2015). According to a chemoreception study, sometimes their chemoreceptors decode plastic debris as prey, which results in microplastics being mistaken for food (Savoca et al. 2017). A recent study on the North Sea has categorized the anchovy as one of the microplastics bioindicators on the marine ecosystem (Kühn et al. 2020). As they play a part of the marine food web, anchovies are possibly accumulating microplastics and transporting them to the higher taxa such as a shark (Boldrocchi and Bettinetti 2019). The anchovies' capacity to ingest up to 22 times their body mass (Whitehead 1988) made the contamination worse. Consequently, microplastic contamination is open to the broader community throughout the food web. We suggested counting this kind of possible contamination way when conservation and management are developed.

Table 2. Presence of microplastics from each harbor confirmed by FTIR

Harbour	Type of polymer
Ambon	PA
Fakfak	HDPE, PA
Karimunjawa	HDPE, PS
Kendari	PA, PET
Krui	HDPE, LDPE, PA, CA
Makassar	HDPE, PS, PA
Mamuju	PS
Manado	PA
Meulaboh	PS, PA
Muara Angke	PP, PA
Pangkalpinang	HDPE, PA
Sidoarjo	HDPE, PP, PET
Sorong	HDPE, PA
Waingapu	PS

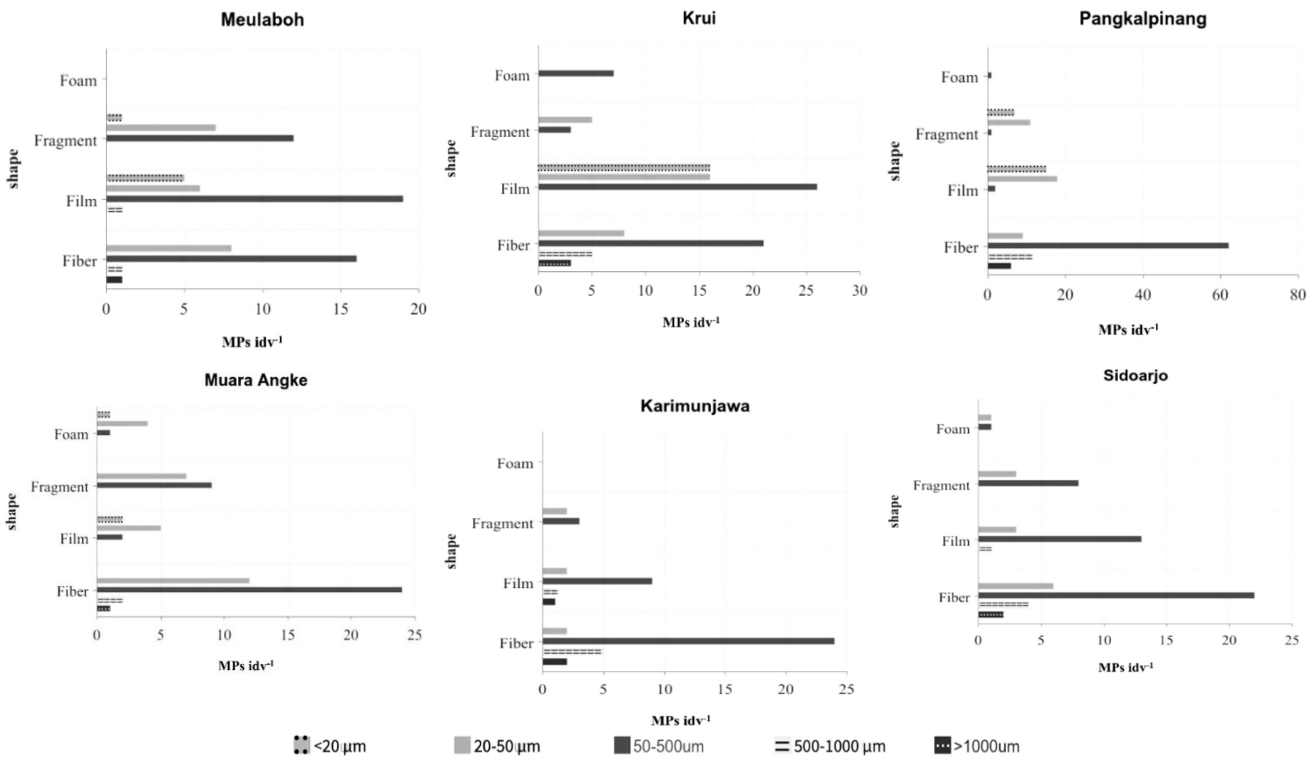

Figure 4. Microplastic shape and size range from each harbour in the Western area, Indonesia

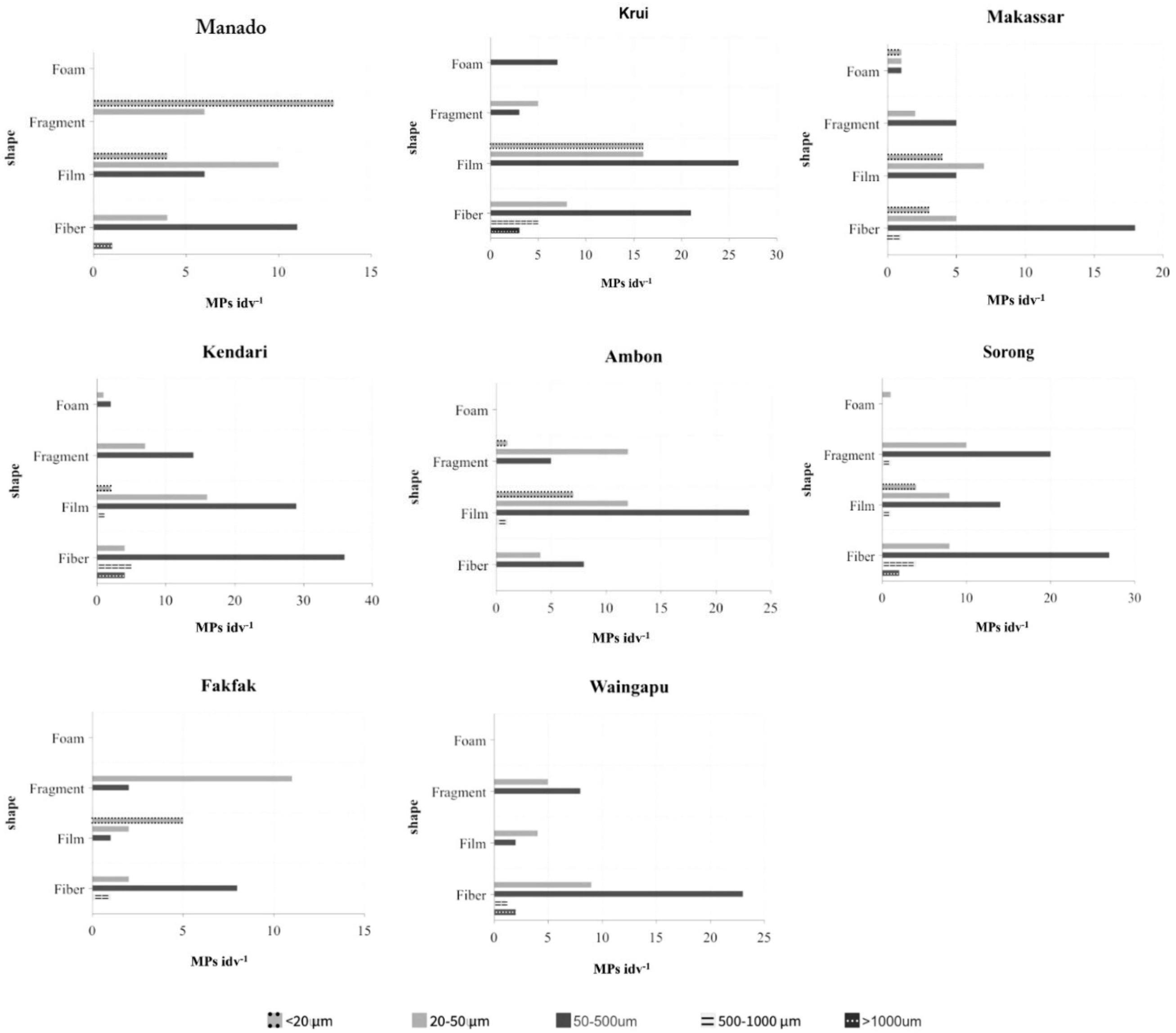

Figure 5. Microplastics shape and size range from each harbour in the Eastern area, Indonesia

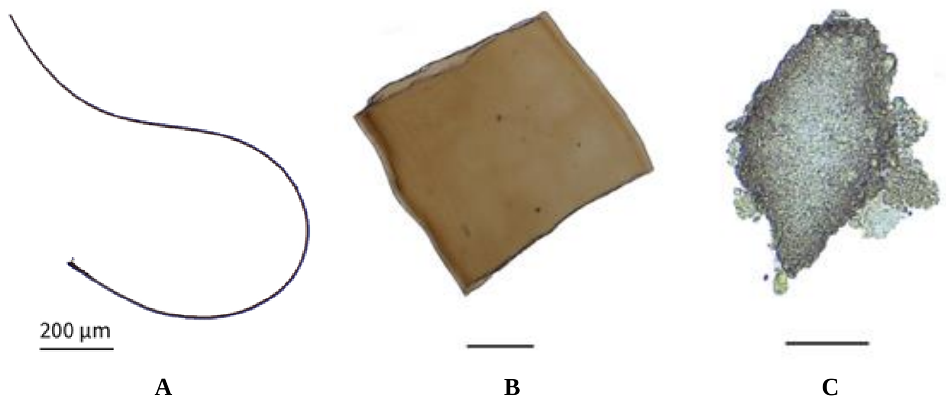

Figure 6. Photographic examples of microplastics found in anchovies from 1-r A) fiber, B) film, and C) fragment. Scale bars represent at 200 µm

In conclusion, the anchovies (*Stolephorus* spp.) from 14 locations of Indonesia sea, proven to be contaminated by microplastics. The most microplastic contamination was found in anchovies from Mamuju (688±1.15 MPs idv^{-1}) followed by Krui (645±7.02 MPs idv^{-1}). Fiber, film, fragment are the types of microplastics found in anchovies. Fiber is the most type of microplastics found in which the majority of the sizes ranged from 50-500 μm, followed by a range of 20-50 μm. Many fish showed a high level of microplastics in their digestive systems. We found microfiber and microfilm in 50-500 μm size categories at most. Meanwhile, our findings indicated nothing different between the total microplastic contamination of anchovies in Eastern harbors and Western harbors.

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