Microfiber found on anchovy from Kawal market, Bintan Island and Banyuasin market, South Sumatera, Indonesia

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Abstract. Microplastics (MPs) contamination has become the most central issue in micro-pollutants research due to the possibility of MPs joining toxicity with other marine pollutants. Several studies have recorded that MPs contamination has occurred on the aquatic biota. Nevertheless, the studies on specific small pelagic fish are still few. In this research, we report that MPs in microfiber type are available for commercial fish like anchovy (*Stolephorus* spp.). This research examined the anchovy's digestive tract (n = 15) from both Kawal market, Bintan Island, and Banyuasin market, South Sumatra Province. The samples were destructed with a mixture of 20 mL NaOH (1 M) and 20 mL of dissolved sodium laureth sulfate (SLES) 0.5% and then kept at room temperature for a week. The results show that the total microfiber on the anchovy from the Kawal market is 104±4.12 particles for each individual, followed by the Banyuasin market with 68±0.78 particles for each individual. The size ranges of microfiber are also recorded during the observation. Our findings show that both Kawal and Banyuasin's anchovies ingested 50-500 µm size range of microfiber at most. For the Kawal market, we revealed the novelty of the new group of microfiber size ranges: > 1000 µm that were not detected in anchovies from Banyuasin. Anchovy is always available in all seasons. Hence, the anchovy is listed as one of the highest commercial seafood products in national fishery commodity from Indonesia marine waters. Small pelagic anchovies are critical components on the food web, providing dietary for the higher marine organisms. By consuming anchovy or seafood products regularly, human exposure to MPs contamination is possible.
Key Words: anchovy, Bintan Island, microfiber, microplastics, seafood contamination.

Introduction. Microplastics (MPs) pollution reached the sea by various routes (Frid & Caswell 2017). MPs originate from numerous sources and become marine pollutants. These pollutants enter the ocean directly from maritime activities such as fishing, shipping, offshore industry activities, and coastal recreation activities or indirectly from land-based activities which produce litter and then transferred by the rivers, tidal floods, and atmospheric transfer (Galgani et al 2015). Plastic densities vary between 0.8 and 1.5 g cm^{-3}, while seawater's density is 1.02-1.07 g cm^{-3}. In the aquatic environment, light-density MPs such as polypropylene (PP) and low-density polyethylene (LDPE) tend to float on the surface water. In contrast, the MPs particles with a high density, such as polyester, high-density polyethylene (HDPE), and polyvinylchloride (PVC), are likely to be sinking (Kooi et al 2017). Plastic materials in Sumatera marine water, Indonesia, mainly were found as PP (68%), LDPE (11%), and other types (Syakti et al 2017). The most found MP was fiber in seawater (Yona et al 2019). Microfibers originated from clots, fishing nets and traps, typically in form of foamed polystyrene nylon and LDPE (Tanaka & Takada 2016; Andrady 2017).

MPs prevalence in the aquatic systems makes the MPs particles highly available into fish body (Hantoro et al 2019). Typically, microfiber and microfragment shapes are abundant in the sea water and the most commonly detected in fish (Alomar & Deudero 2017; Savoca et al 2019). Uptake of MPs particles by fish can occur in two ways, directly mistaking MPs as prey and indirectly ingesting another organism contaminated by MPs (Steer et al 2017; Nelms et al 2018; Procter et al 2019). After ingestion, MPs particles remain in the digestive system, including the intestine and stomach (Ismail et al 2019;

Sun et al 2019). Other reports underpin that MPs can be entangled in fish skin and gills or translocated into other tissues such as muscles, livers, circulatory, and lymphatic systems (Feng et al 2019; Su et al 2019). MPs and other pollutants can cause various damage and health problems to the fish after exposure (Hamed et al 2019; Prokić et al 2019).

Similarly, the study on the relationship between the likelihood of MPs uptake and feeding types of intertidal fish shows that the omnivorous fish tend to ingest more MPs particles, rather than carnivorous and herbivorous fish (Mizraji et al 2017). For instance, demersal fish may be more available for HDPE, while pelagic fish are susceptible to LDPE (Lusher et al 2013). New evidence supports this assumption after the analysis of spatial distribution, and temporal plastic variability has been reported. Precisely, 4389 individual fish from 15 species were analysed. As a result, two species are suitable for plastic monitoring, which are demersal cod (*Gadus morhua*) and pelagic sprat (*Sprattus sprattus*) (Kühn et al 2020). Another study reported *Engraulis encrasicolus* and *Mullus barbatus barbatus* as the possible species for bioindicators of marine litter based on their habitat and home ranges (Fossi et al 2018). For this research in both markets, we used anchovies as they are abundant in Indonesia marine waters with commercial importance in the seafood market. This research aims (1) to detect MPs contamination in microfiber type from anchovy and (2) to compare the results between two sites, Kawal Market in Bintan Island, and Banyuasin market in South Sumatera, Indonesia.

Material and Method

Description of the study site. The samples were brought from Kawal, Bintan Island, and Banyuasin markets. Kawal is located on Bintan Island, in the Riau Islands province. This site was selected because it is directly located in the South China Sea. Banyuasin in South Sumatera Province is strategically located because it is open to both the South China Sea and the Java Sea. The samples of the anchovies (*Stolephorus* spp.) were collected in May 2019. Microplastics investigations were then conducted at the Marine Biology Laboratory, Department of Biology, Universitas Indonesia, Depok.

Sample preparation. The anchovies from each market were measured as total length (cm±SD) and wet weight (g±SD). The anchovies of similar length and weight were then selected (n = 15). The measured anchovies were soaked in filtered Milli-Q water for an hour and then rinsed. The anchovies' digestive tracts were isolated and placed in the flask for a destruction step. All dissection materials and glass were cleaned with filtered Milli-Q water (Whatman no. 1 with 11 μm pore size). Subsequently, the MPs particles under 20 μm were not analysed.

Sample destruction protocol. The methodology on organic material destruction was adapted and developed by Budimir et al (2018). The samples were added with 20 mL of NaOH (1 M) and 10 mL of 0.5% dissolved SLES technical grade and then kept at room temperature for a week. The destruction process was finished while no organic materials remained on the bottom of the flask. All chemicals used were filtered through the same filter paper size pore to avoid contamination and stored in a closed place.

Microfiber observation. The samples (1 mL) were pipetted into a Sedgwick rafter and then placed in the Petri dish for microscopic observations. These observations were conducted on triplets. Microfibers were observed with a microscope Leica ICC50 with ten times magnification, then photographed and compared to the references. The size ranges were categorized as 20-50 μm, 50-500 μm, 500-1000 μm, and > 1000 (1-5 mm) (Ningrum & Patria 2019a). The results were displayed in comparison graphics between the two markets. Airborne contamination was avoided by conscientious handling of the samples. The sample was covered with a Petri dish and taken out for microscopic observation only. Visual observation of the MPs can be challenging as training and experience are needed to differentiate the size categories and to distinguish between microfiber and organic contaminants (Lusher et al 2017). Microfibers were photographed and compared to the references (Arias et al 2019; Herrera et al 2019; Hossain et al 2019; Renzi et al 2019; Tajwar et al 2022). The scale was put at 200 μm for easy comparison.

Statistical analyses*.* The Shapiro Wilk test tested the normality assumptions and homoscedasticity on all data. The comparison between microfiber of the two markets at Kawal and Banyuasin was conducted using a t-test. The variation between the size ranges was compared using one-way ANOVA. The significance of the difference was defined at $p < 0.05$. These statistical analyses were conducted with spreadsheets.

Results

Total microfiber on the anchovy*.* Although the size (both for total length and wet weight) of the anchovies from the Kawal market was shorter and lighter compared to those taken from the Banyuasin market (Table 1), the total microfiber on the anchovies from the Kawal market (104±4.12 particle idv^{-1}) was higher than those of the Banyuasin market (68±0.78 particle idv^{-1}). Meanwhile, the t-test results comparing the total microfiber abundance between the two markets showed that both of these markets were similar (0.545 > 0.05) (Figure 1).

Table 1

Summary of length, weight of the anchovy and the total microfiber

Location	*Total length (mm±SD)*	*Weight w/w (g±SD)*	*Total microfiber (particle idv^{-1})*
Kawal market	47.94±0.65	0.52±0.06	104±4.12
Banyuasin market	70.77±0.94	2.08±0.16	68±0.78

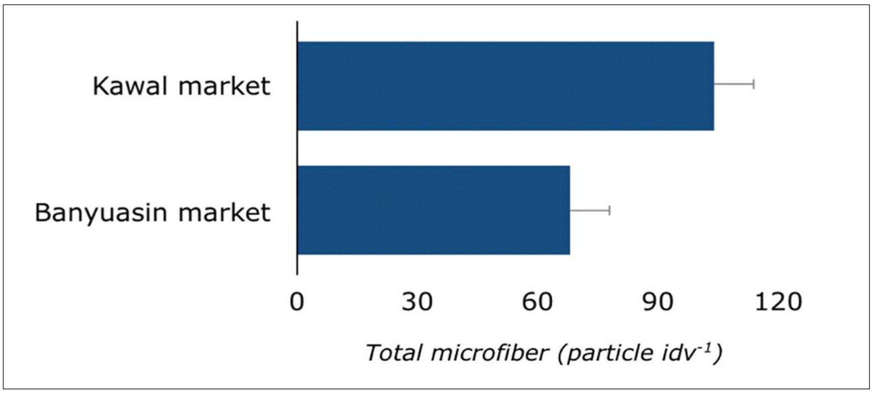

Figure 1. The number of MPs found during microscopic observation.

Microfiber size ranges*.* Microfibers in the 50-500 μm range were mainly found on anchovies from the Kawal and Banyuasin markets, but the difference was statistically insignificant (0.088 > 0.05). The anchovy from the Kawal market had 59% microfiber, while Banyuasin's anchovy had 50% microfiber in the 50-500 μm range (Figure 2). Furthermore, the biggest microfiber size (> 1000 μm) was only detected in anchovy from the Kawal market (Table 2). During the observation, microfibers were typically visible as blue, gray, dark, and clear colors (Figure 3).

Table 2

Microfiber size ranges in anchovy during observation

Location	*20-50 μm (particle idv^{-1})*	*50-500 μm (particle idv^{-1})*	*500-1000 μm (particle idv^{-1})*	*> 1000 μm (particle idv^{-1})*
Kawal market	11±4.04	61±6.66	20±3.46	12±2.31
Banyuasin market	24±0.58	34±1.53	10±1.00	0±0

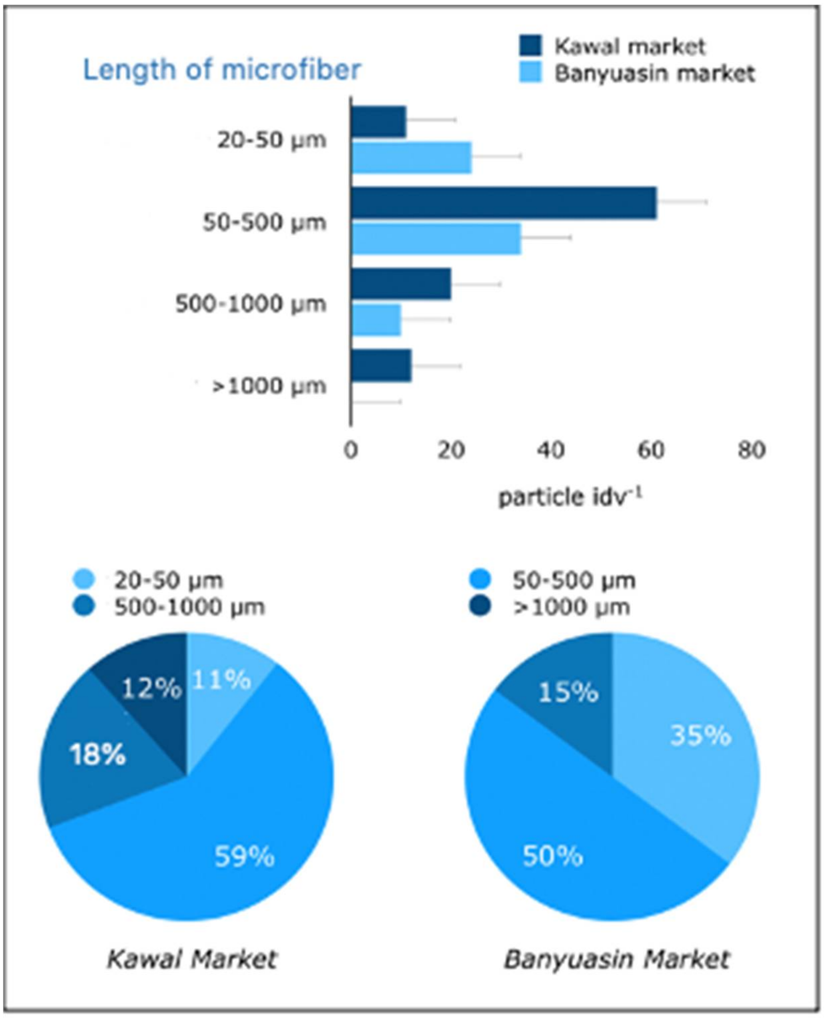

Figure 2. Microfiber size ranges found in anchovy from Kawal and Banyusin market.

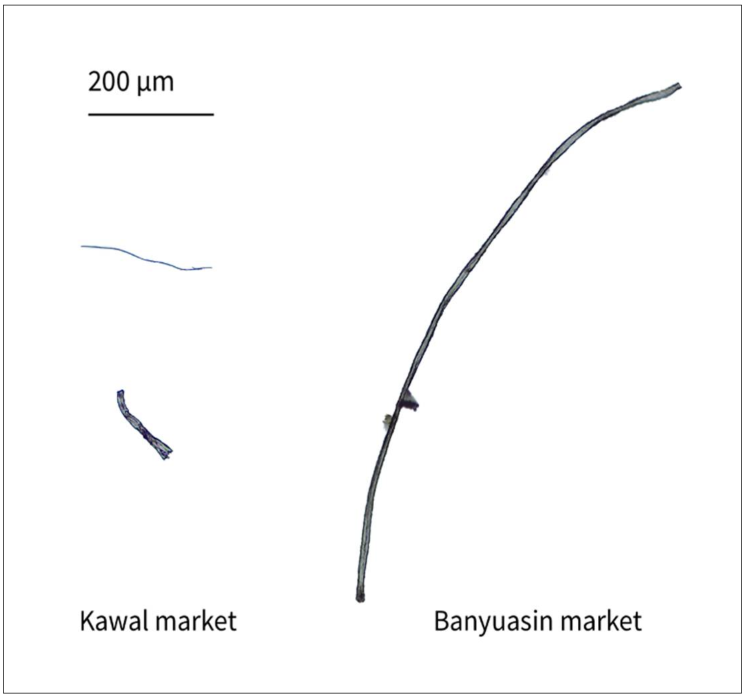

Figure 3. Microscopic images of microfiber carried on the digestive tract of anchovy.

Discussion. Anchovies from the Kawal market have the highest level of microfiber, which is heavier than anchovies from the Banyuasin market. Anchovies from the Kawal market ingested 104±4.12 particle idv^{-1}, weighing only 0.52±0.06 g. Meanwhile, Banyuasin's anchovies only have 68±0.78 particle idv^{-1} microfiber on their body weighing 2.08±0.16 g. This result differs from Ningrum & Patria (2019b), who reported that the heavier anchovies had more microplastic than the lightest. Our result is similar with de Vries et al (2020) report, that found microplastic have no correlation with fish weight.

The external factors include the number of MPs contaminating water and sediment, which could be the most significant factor affecting marine biota. The evidence was confirmed by Al Hamra & Patria (2019) that microplastic was found on the sediment and water of the Kawal coast. As many as 616 particle kg^{-1} microfibers (67.99%) remained in that sediment, followed by 22.74% microfragments and 9.29% microfilms (Al Hamra & Patria 2019). Kawal market is located in Kawal Village, which is closest to the coast and is one of several small islands along the South China Sea border. This area is exposed to tourist attractions and fishing activities. Meanwhile, Almiza & Patria (2021) reported that macroplastics were abundantly apparent, as much as 35 items per m^2 in Musi estuary, Banyuasin. Therefore, microfibers were highly available in anchovy from Kawal and Banyuasin markets in this study.

However, the abundance of microfiber found in anchovies from Kawal and Banyuasin was similar compared to other areas in Indonesia marine waters, such as Alor's anchovies (95% microfiber) and East Lombok's anchovies (51% microfiber) (Ningrum & Patria 2019a). Globally, other reports confirmed that the microfiber was the only MPs shape found on the anchovy collected from the Gulf of Lions (Lefebvre et al 2019). Furthermore, 92.86% microfiber was found on the anchovy collected from the Western Mediterranean Sea (Rios-Fuster et al 2019). The other evidence reported on 87% and 83% of microfibers found in anchovies taken from the Adriatic Sea and the Spanish Western Mediterranean Coast respectively (Compa et al 2018; Renzi et al 2019). Another study supported that microfiber has the most significant percentage of the MPs on seawater and sediment worldwide, up to 100% (Gago et al 2018) (Table 3).

There is only one typically abundant microfiber size in anchovies from both the Kawal and Banyuasin markets which is 50-500 μm (Figure 2). Anchovies from the Kawal market contain 61±6.66 particle idv^{-1} (59%), while Banyuasin's anchovies have 34±1.53 particle idv^{-1} (50%) microfiber in 50-500 μm (Table 2). Likewise, the weight of the anchovies did not follow the model that the biggest ones tend to ingest the bigger particle. The next higher size found in Banyuasin's anchovies was 20-50 μm (35%), while Kawal's anchovies ingested more microfiber in 500-1000 μm (19%). Although there were slightly different microfiber sizes between Kawal and Banyuasin anchovies, these results are statistically insignificant ($p = 0.545$). In other words, both Kawal and Banyuasin anchovies have similar microfiber abundance based on their size categories. These results are compared to other studies. The anchovies from East Lombok, Alor, Balikpapan, and Talisayan harbors significantly ingested 50-500 μm MPs the most (Ningrum & Patria 2019a; Ningrum et al 2019). Although some other global researchers reported that the size ingested by anchovies was wide-ranging, it is still categorized as microfiber. European anchovies (*Engraulis encrasicolus*) from the Adriatic Sea reported by Renzi et al (2019) were ingested 40.1-5000 μm length of microfiber. In addition, Western Mediterranean anchovies (*Engraulis encrasicolus*) ingested 5000 μm length of microfiber, while 200-800 μm length of microfiber were found dominant in the Eastern Mediterranean anchovies (Kazour et al 2019; Rios-Fuster et al 2019).

Handling the samples during the microscopic observation is tricky. For each particle, the identification is made by certain criteria such as gloss surface, homogeneous color and thickness, and the absence of organic and cellular parts. Microfiber photographs were compared with cross-references. They were similar to this study in consistency, gloss surface, homogeneity, and clarity of colors with no organic or cellular compartment (Arias et al 2019; Herrera et al 2019; Hossain et al 2019; Renzi et al 2019; Tajwar et al 2022).

Table 3

Comparison of microplastics found in anchovies

Location	*Organism*	*Total MPs ($MPs\ ind^{-1}$)*	*MPs shape*	*MPs size (μm)*	*References*
Tokyo Bay	Japanese anchovy (*Engraulis japonicus*)	2.3 ±2.5	86.0% fragment, film, 7.3% bead and foam	150-1000	Tanaka & Takada (2016)
Gulf of Lions	European anchovy (*Engraulis encrasicolus*)	0.11±0.31	Fiber	1810-1520	Lefebvre et al (2019)
Gulf of Lions	European anchovy (*Engraulis encrasicolus*)	0.85	Fragment	124-438	Collard et al (2017)
Spanish Western Mediterranean Coast	European anchovy (*Engraulis encrasicolus*)	0.18±0.20	83% microfiber, fragment	not specified	Compa et al (2018)
Eastern Mediterranean Sea (Labanese Coast)	European anchovy (*Engraulis encrasicolus*)	8.3±4.4	Fragment > fiber > film	200-800	Kazour et al (2019)
Adriatic Sea (Italy)	European anchovy (*Engraulis encrasicolus*)	15	87% fiber, 17% film	40.1-2220.6	Renzi et al (2019)
Western Mediterranean Sea	European anchovy (*Engraulis encrasicolus*)	0.03±0.16	92.86% fiber, 7.14% fragment	5000	Rios-Fuster et al (2019)
East Lombok Island	Indonesian anchovy (*Stolephorus* spp.)	88±2.89	51% fiber, 30% film, 17.02% fragment, foam	<20, 20-50, 50-500, 500-1000, >1000	Ningrum & Patria (2019a)
Alor harbor	Indonesian anchovy (*Stolephorus* spp.)	130±1.73	95% microfiber, 2.5% microfilm, 2.5% microfragment	<20, 20-50, 50-500, 500-1000	Ningrum & Patria (2019b)
Balikpapan harbor	Indonesian anchovy (*Stolephorus* spp.)	302±1.00	34.57% microfiber, 43.83% microfilm, 20.99% microfragment, microfoam	<20, 20-50, 50-500, 500-1000, >1000	Ningrum & Patria (2019b)
Talisayan, East Kalimantan	Indonesian anchovy (*Stolephorus* spp.)	366±3.51	29.59% microfiber, 50% microfilm, 18.4% microfragment, microfoam	<20, 20-50, 50-500, 500-1000, >1000	Ningrum et al (2019)
Kawal market, Bintan Island	Indonesian anchovy (*Stolephorus* spp.)	114±9.81	91.3% microfiber, 5.8% microfilm, 2.9% microfragment	20-50, 50-500, 500-1000, >1000	This study
Banyuasin market	Indonesian anchovy (*Stolephorus* spp.)	78±3.06	88.6% microfiber, 10% microfilm, 1.4% microfragment	<20, 20-50, 50-500, 500-1000	This study

The study on MPs in coastal areas reported that seawater and sediment reveal that microfibers were the most abundant MPs types in worldwide seawater (Gago et al 2018). As a result, light-density and low-density MPs materials tend to float on the surface and water column (Kooi et al 2017). Moreover, anchovies are pelagic fish filter-feeders that open their mouths as they swim. They open the mouth while swimming on the surface and water column, which makes the water pass through the gill rakers and esophagus. They could be exposed to MPs particles in this way (Lusher et al 2017).

Similarly, other experts have confirmed that anchovies could mistakenly consume small particles of plastic as part of their diet. Anchovies use their sense of smell to find food and ingest various prey, such as a group of zooplankton, including krills, euphausiids, and copepods (Morote et al 2010). Several studies on their diets supported that anchovies could be exposed to MPs' contaminants throughout their diets. A group of zooplankton ingested 167 μm MPs up to 131.5 pieces m^{-1} while krill ingested 31.5 μm MPs particles, and euphausiids could ingest 556±149 μm MPs particles (Sun et al 2017; Dawson et al 2018). Based on anchovy's habitat and determining their prey, an anchovy was more likely to be a bioindicator for MPs controlling. This evidence is supported by studies on the spatial distribution of various fish and the variability of MPs in the North Sea, which also recommend anchovies as bioindicators for MPs' control of the marine ecosystem (Kühn et al 2020).

Anchovies are the leading capture fisheries commodity in Indonesia. The Indonesian Ministry of Fisheries reported that 247,708 tonnes or 3.7% of the 6,603,631 tonnes caught by fishermen in 2017 were anchovies (KKP RI 2017). Thus, anchovy is always available throughout the year, and it is easy to find anchovy at the fish market, including Kawal and Banyuasin. Moreover, anchovies as small pelagic fish are essential components of the marine ecosystem and fisheries worldwide (Queiros et al 2019).

The fish is available as diets for other taxa, such as whale sharks and salmon (Boldrocchi & Bettinetti 2019; Litz et al 2019). For these reasons, MPs are possibly transferred into the next organism on the food web (Prokić et al 2019). Because MP can bind with heavy metal pollutants such as copper, during transfer to a higher food trophic it will increase the concentration of these heavy metals in the body of the predator (Qiao et al 2019). In addition to support this evidence, another report reveals that MPs could also possibly be combined with other heavy metals and thus increase their toxicity (Jinhui et al 2019). Therefore, humans can be exposed to microplastics and heavy metals if they eat anchovies. However, the safe number of MPs is still unstandardized according to the Food and Drug Administration of Republic of Indonesia (Badan Pengawas Obat dan Pangan, BPOM), National Standardization Agency of Republic of Indonesia (Badan Standarisasi Nasional, BSN), and Food Agriculture Organisation (FAO) due to a lack of studies on this specific issue.

Conclusions. This study has successfully provided evidence of microfiber pollution in anchovy from both Kawal and Banyuasin markets. Total microfibers were similar in anchovies from these locations ($p = 0.545$). Kawal's anchovies contain 104±4.12 particle idv^{-1}, while Banyuasin's anchovies have 68±0.78 particle idv^{-1} in their digestive tracts. Microfiber in 50-500 μm was the highest microfiber compared to the other sizes. Although the findings were slightly different, the size ranges were statistically insignificant. In conclusion, microfibers were similarly found in Kawal and Banyuasin areas.

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Conflict of interest. The authors declare that there is no conflict of interest.

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