Ingestion of microplastics by anchovies of the Madura Strait, Indonesia

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Abstract. The accumulation of microplastics in tissues has been documented in many marine organisms. The objective of this study was to investigate the abundance of microplastics in some species of anchovies of the Madura Strait. The surrounding areas are highly populated, which elevate microplastics pollution to the waters and therefore may aggravate the ingestion of microplastics by fish. The ingested microplastics were examined from three anchovy species: Indian anchovy (*Stolephorus indicus*), Hardenberg's anchovy (*Stolephorus insularis*) and Commerson's anchovy (*Stolephorus commersonnii*). The anchovies were captured from the Madura Strait, Indonesia. The degradation of organic matter was conducted using 30% H_2O_2. Results showed that microplastics were found in all anchovies. *S. indicus* had the highest abundance (13.66±2.41 items ind^{-1}), followed by *S. insularis* and *S. commersonnii* (6.8±1.62 items ind^{-1} and 1.87±0.64 items ind^{-1}, respectively). Fibers had the highest abundance in all anchovy species with a concentration of 8±1.77 items ind^{-1} and 4.67±0.98 items ind^{-1} for *S. indicus* and *S. insularis*, respectively. In total, there were 335 plastic particles extracted from 45 anchovy samples, out of which fibers and fragments represented 62.98% and 34.03%, respectively. Meanwhile, film was the least abundanct plastic, with a total average of 0.22±0.47 item ind^{-1}, or only 2.98% of total microplastics found in all anchovy samples. These results highlight that microplastics are ubiquitous in the anchovies of the Madura Strait. Given the high level of microplastic ingestion in the anchovies, there is a concern and necessity to understand the impact of microplastics in the food webs and on human health.

Key Words: fibers, films, H_2O_2, *Stolephorus indicus*, *Stolephorus insularis*.

Introduction. Microplastics are small plastics particles <5 mm originated from the degradation of larger plastic debris or from direct environmental emission (Anderson et al 2016; Yona et al 2019). Microplastics are widespread and now considered a global environmental threat (de Souza Machado et al 2018; Asadi et al 2019a; Peixoto et al 2019). The contaminant has been reported in every part of the marine environment from the poles to the tropic waters (Waller et al 2017; Lacerda et al 2019), and even from the most secluded beaches to the deepest oceanic trenches (Barnes et al 2009; Lavers et al 2019).

Microplastics can persist in marine environments for many years (Barnes et al 2009; Mao et al 2020), leading to the availability of the contaminants to a wide range of marine organisms in different habitats (Waller et al 2017; Barboza et al 2020). Benthic organisms such as molluscs, amphipods and polychaete worms are prone to encounter microplastics that are denser than water, while pelagic organisms like zooplankton are more likely to interact with floating microplastics (de Sá et al 2018; Sfriso et al 2020). Both pelagic and demersal fish may ingest microplastics directly or indirectly through the food chain (Murphy et al 2017; de Sá et al 2018). Planktivorous pelagic fish like anchovies are prone to accumulate microplastics, as the feeding mechanisms may not distinguish between prey and microplastics, which may have the same shape, color, and size as their prey (Gong & Xie 2020; Lopes et al 2020).

Stolephorus indicus, *S. insularis* and *S. commersonnii* are commonly caught around the Madura Strait and are widely distributed along its coastal areas, but also in many other parts of Indonesia (Savitri et al 2018). Fresh or dried anchovies are typically consumed without the removal of the digestive tract (Tanaka & Takada 2016). Therefore, the consumption of anchovies presents a potential risk for human health (Barboza et al

2020). In this study, we investigated the microplastics in some species of anchovies sold by fishermen in Lekok beach, Pasuruan, Indonesia, where fish were caught from the surrounding waters (the Madura Strait).

Material and Method

Sampling and processing. Indian anchovy (*S. indicus*), Hardenberg's anchovy (*S. insularis*) and Commerson's anchovy (*S. commersonnii*) were bought from local fishermen in Lekok, Pasuruan, Indonesia. The samples were caught around Pasuruan waters of the Madura Strait on 21 February 2020 (Figure 1). There were 15 fish of each species, totaling 45 anchovies. The fish were placed in iced water and transported immediately to the laboratory. The whole body of each sample was subjected to the microplastics extraction, as the average total length of the samples was small (5.49±2.19 cm), and the fish would have been eaten without the removal of the digestive tract.

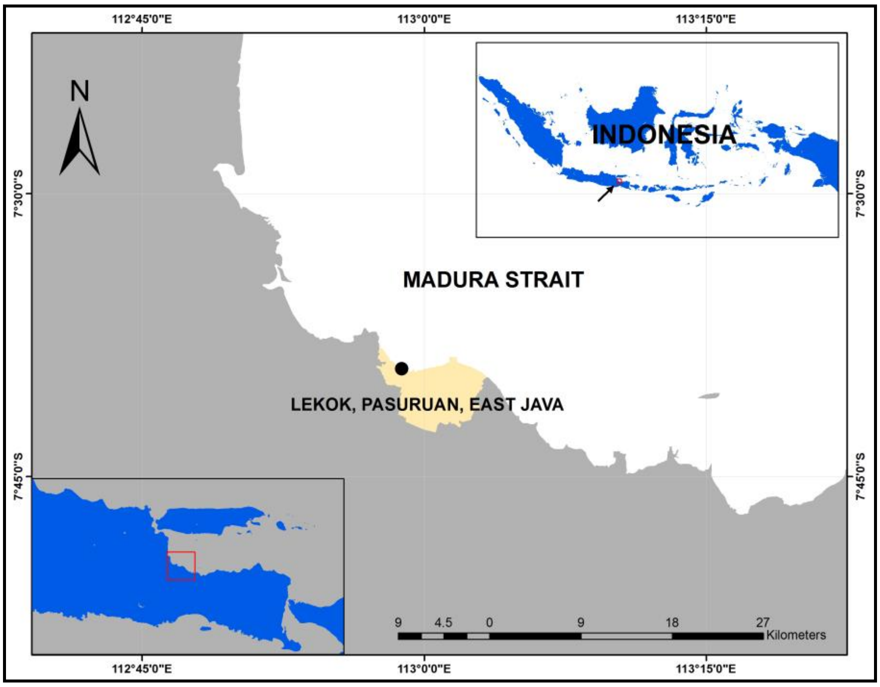

Figure 1. Sampling location of microplastics in the anchovies of the Madura Strait.

Before microplastics extraction, the total length and the dry weight of each fish were measured by a meter scale to the nearest millimeter and by an electronic balance (0.1 mg accuracy), respectively. As the weight of fresh fish is different in each species and individual, it should be standardized, and, therefore, the microplastics were also expressed as item per 1 g of dry weight of each species, in addition to items per individual. The drying took place in an oven, at 60°C, for 8 hours. The degradation of organic matter was performed according to Digka et al (2018), with minor modifications. Each fish was placed into a glass beaker with 10 mL of hydrogen peroxide (30% H_2O_2). The degradation of organic matter was observed to be completed in 1 to 5 days. The samples were then heated on a hot plate at 60°C to evaporate H_2O_2. If organic matter was not fully degraded, 2 mL of H_2O_2 was added to digest the remnants. Furthermore, each sample was diluted with 100 mL of distilled water, stirred and filtered with glass microfiber filters (1.6 μm pore size, Whatmann). The filters were then settled in Petri dishes and dried overnight at room temperature.

Classification and identification of microplastics. Filters were observed under a microscope (Olympus, CX41) for the physical identification of microplastics. Items were counted and classified according to microplastic types (films, fibers, fragments). The guideline of microplastic characteristics was based on Hidalgo-Ruz et al (2012). When microplastics could not be distinguished from organic matter, the hot needle test, based on De Witte et al (2014), was applied. In each species, the abundance of microplastics was expressed as the average number of microplastic items per individual and average number of microplastic items per gram of dry weight of anchovy tissue.

Statistical analysis. Statistical analyses were performed using GraphPad Prism 9.0. The abundance and types of microplastics ingested by individuals from each species were analyzed using Two-way ANOVA. Post hoc analysis was performed using Tukey's multiple comparisons test for comparing microplastics abundance between each anchovy species and between each type of microplastics.

Results and Discussion

The occurrence and abundance of microplastics in the anchovies. The Madura Strait is situated between the northeastern part of Java Island and the southern part of Madura Island. Both areas have high population density and are highly influenced by anthropogenic activities. Therefore, the strait may have a high microplastics concentration as population density and human activities have a direct effect on microplastics abundance (Mbedzi et al 2020). There were not many documentations of microplastics in the Madura Strait. However, high concentration of microplastics had been documented in the sediment of the Bama Beach, Baluran Nature Reserve, which is a protected area situated in the easternmost of Madura Strait. The microplastics penetrated the sediment as deep as 30 cm, with an overall concentration of 116.41±80.78 items kg^{-1} dry weight (Asadi et al 2019b).

The abundance and occurrence of microplastics in anchovies caught from the Madura Strait were higher than those from the Mediterranean Sea, Tokyo Bay and Adriatic Sea (Tanaka & Takada 2016; Collard et al 2017; Renzi et al 2019). All anchovies in this study ingested microplastics in the range of 1 to 18 items ind^{-1}, with the overall average concentration of 7.44±5.16 items ind^{-1}. Meanwhile, other studies showed an ingestion rate between 30% and 95.8% and microplastics abundance between 0.3 and 2.3 items ind^{-1} (Table 1).

Table 1

The abundance and occurrence of microplastics in anchovies reported in this and other studies

Species	*Abundance items ind^{-1}*	*Occurrence (%)*	*Location*	*Reference*
Engraulis encrasicolus	1.74	60%	Western Mediterranean Sea	(Pennino et al 2020)
Engraulis japonicus	2.3	77%	Tokyo Bay	(Tanaka & Takada 2016)
Engraulis encrasicolus	-	80%	Mediterranean Sea	(Collard et al 2017)
Engraulis mordax	0.3	30%	Fish market in California, USA	(Rochman et al 2015)
Engraulis encrasicolus	1.23	>90%	Adriatic Sea	(Renzi et al 2019)
Engraulis encrasicolus	-	95.8%	NW Mediterranean Sea	(Capone et al 2020)
S. commersonnii, S. insularis, S. indicus	1-18 (7.44)	100%	Madura Strait, Indonesia	The current study

Statistical analysis using Two-way ANOVA showed significant differences in the microplastics abundance among anchovy species ($p<0.001$). Tukey's multiple comparisons test showed significant differences in the microplastics ingestion between *S. commersonnii* and *S. insularis* ($p<0.001$), *S. commersonnii* and *S. indicus* ($p<0.001$), and between *S. insularis* and *S. indicus* ($p<0.001$). The microplastics content in each species were 1.87±0.64 items ind^{-1}, 6.8±1.62 items ind^{-1}, 13.66±2.41 items ind^{-1} for *S. commersonnii, S. insularis*, and *S. indicus*, respectively (Figure 2). Weight measurement of the samples also revealed that *S. indicus* significantly accumulated higher microplastics than other species ($p<0.001$). The species held on average 14.99±1.57 items g^{-1}, while *S. commersonnii* and *S. insularis* accumulated 5.22±1.69 items g^{-1} and 12.14±1.95 items g^{-1}, respectively (Figure 2). The samples of *S. indicus* had a higher body length and weight, and, therefore, the species may have a higher tropic position in the food chain. Thus, the species had a higher rate of plastic particles ingestion, as transfer pathways of microplastics in organisms occur through the food web (Barnes et al 2009; Tanaka & Takada 2016; Roch et al 2020; Sfriso et al 2020).

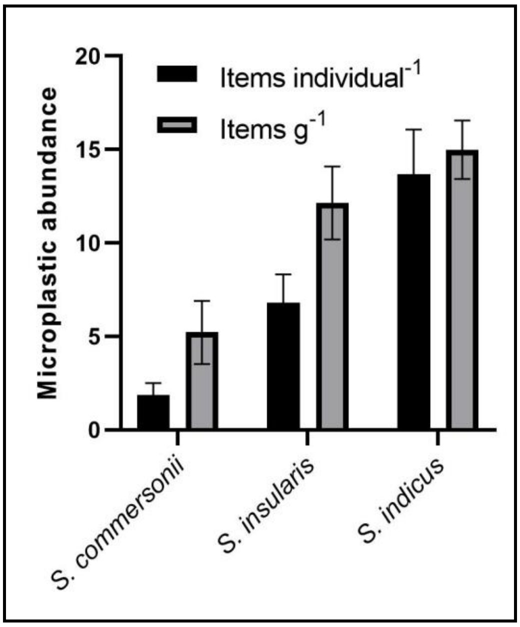

Figure 2. The average concentration of microplastics in some anchovy species of the Madura Strait, Indonesia.

The ubiquity of these plastic particles could damage the anchovy communities, as the uptake of microplastics has adverse effects on marine biota (Murphy et al 2017; Waller et al 2017; Barboza et al 2020). Hydrophobic nature and high surface/volume ratio of microplastic particles can efficiently adsorb toxic compounds like metals or aromatic hydrocarbons (de Sá et al 2018; Pittura et al 2018). These ingested microplastics along with the compounds may further be taken up and absorbed by cells and tissues (Murphy et al 2017; de Sá et al 2018), deteriorating physiological functions such as respiration, growth, maturity and reproduction (Pittura et al 2018; Prinz & Korez 2020).

The toxic compounds attached to microplastics adversely affect not only the organisms that consume them, but also the species higher up in the food web through biomagnification processes (Frid & Caswell 2017). Therefore, as their tropic position in the pelagic food web is low, microplastics ingestion in planktivorous fish like anchovies can have knock-on effects on the trophic structure and ecosystem functioning (Tanaka & Takada 2016; Critchell & Hoogenboom 2018; Pennino et al 2020).

Types of microplastics found in the anchovies. Two-way ANOVA and Tukey's multiple comparisons test of fiber vs. fragment, fiber vs. film, and fragment vs. film showed significant differences ($p<0.001$). The abundance of fibers was 1.4±0.51 fibers ind^{-1}, 4.67±0.98 fibers ind^{-1}, 8±1.77 fibers ind^{-1} for *S. commersonnii, S. insularis*, and *S. indicus*, respectively. Meanwhile, films were the least common microplastics found in the anchovies. *S. indicus* had the highest film concentration with the average of 0.47±0.64 film ind^{-1} (Figure 3). A study of microplastics in the sediment of coastal areas of Lamongan, East Java, showed that the estuary, where microplastics from hinterland urban areas could enter the marine environment, has a much higher number of fibers compared to other coastal areas (Asadi et al 2019a). Therefore, the abundance of plastic fibers in the anchovies and other marine organisms may occur due to the high anthropogenic input of fibers from surrounding terrestrial areas. Fibers originate from a variety of sources, including textiles, fishing nets and ropes (Lusher et al 2013; Tanaka & Takada 2016 Bessa et al 2018).

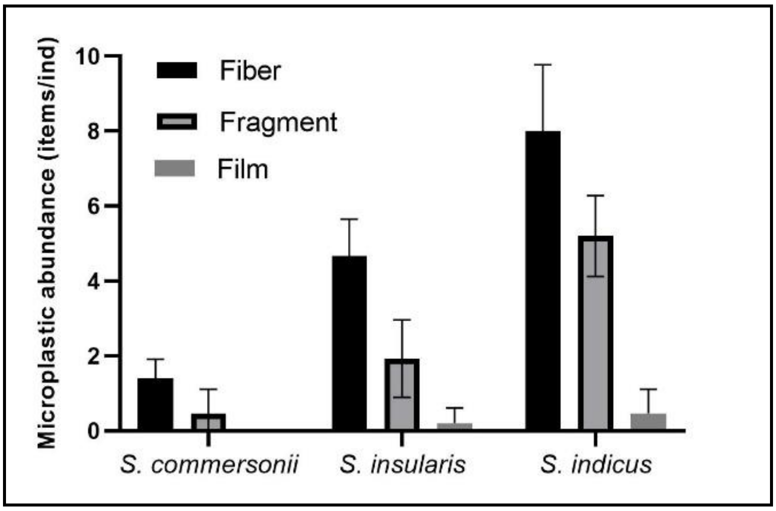

Figure 3. The average abundance (n=15) of the type microplastics in *S. commersonnii, S. insularis*, and *S. indicus.*

Furthermore, in total, there were 211 and 114 items of microfibers and fragments extracted from all anchovy samples, which represented 62.98% and 34% of total microplastics, respectively. Meanwhile, there were only 10 items (2.98%) of plastic films (Figure 4). The predominance of fibers has been also observed in fish from the English Channel (Lusher et al 2013), and from the Mondego estuary (Portugal), which constituted 96% of microplastics found in all fish samples from the estuary (Bessa et al 2018). In wild fish from North East Atlantic Ocean, fibers constituted 54% of the total microplastics (Barboza et al 2020). On the other hand, microplastics in Japanese anchovy (*Engraulis japonicus*) of Tokyo Bay were 86% fragments, with only 5.3% microfibers found in the samples (Tanaka & Takada 2016). This difference may be due to tropic structure, feeding habits and strategy of fish species (Walkinshaw et al 2020), or regional source differences (Tanaka & Takada 2016).

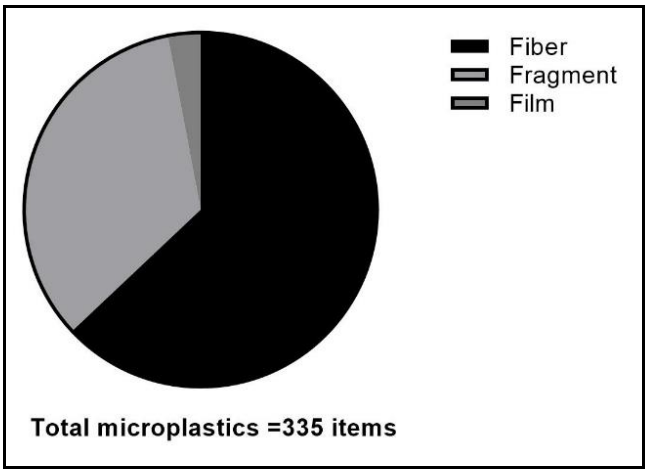

Figure 4. Microplastic types composition in anchovies (*S. commersonnii, S. insularis*, and *S. indicus*).

Anchovies are among important foods for local communities and are often used in Indonesian cuisine. As their size are relatively small, the fish is commonly consumed without removing their gills and digestive tracts. As the concentration of microplastics in the anchovies is relatively high, ingestion of microplastics presents concerns, being a substantial risk to marine biota, food webs and the health human consumers.

Conclusions. The results of this study indicate that anchovies from the Madura Strait are highly contaminated with microplastics, as the pollutant was observed in all individuals. *S. indicus* appears to be more prone to microplastic ingestion than other anchovy species. Fibers not only dominated the type of microplastics, but were also found in all anchovies. Bengawan Solo and Brantas rivers are the main road for all fiber and other microplastics pollution. Therefore, household and industrial wastewater management in the coastal areas and hinterland regions should be implemented to protect anchovies and marine food webs from microplastic pollution.

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

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