Ingestion of microplastics by anchovies from Talisayan harbor, East Kalimantan, Indonesia

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Abstract. Microplastics (MPs) is the most contamination problem on the marine environment now, therefore a small pelagic fish like anchovies, can feed accidentally the microplastic. This research focused on the amount of abundance of microplastics in the anchovies. We measured the length and dry weight of the anchovies and isolated their digestive tracts. The digestive tracts were then digested with NaOH and sodium lauryl sulphate (SLS) technical grade. The microplastics had observed with the using microscope and confirmed with Fourier-transform infrared spectroscopy (FTIR). Here, the investigation result from anchovies digestive tract (Stolephorus spp.) sampled (n = 15). The total microplastics contamination from Talisayan harbor, East Kalimantan is 366 ± 3.51 particles/individual. Kind of microplastics size range detected as categories: < 20 µm, 20–50 µm, 50–500 µm, 500–1000 µm, and >1000 µm. Most of microplastics shapes from Talisayan harbor are microfilm (50%) and microfiber (29.59%). The type of polymers are confirmed by Fourier transform infrared (FTIR) spectroscopy as polypropylene, high-density polyethylene and foamed polystyrene nylon. Our findings reported that specific size range (50–500 µm) is the most majority of microplastics size range which are ingested by anchovies from Talisayan harbor. The microplastics are more dangerous if another contamination found together on it. On this investigation, the mercury contamination also tested and the result showed as not detected mercury contaminant. The anchovies are had the economic value and also small pelagic fish which are play as prey for the other big fish. The exposure are possible to the human by contaminated seafood diet.

1. Introduction
At the oceanic waters and beaches, microplastics (1–5 mm) are one of the most persistent ubiquitous pollutants [1,2]. The recent reporting at marine biota, microplastics had been contamination of biota. Furthermore, Indonesia becomes one of the risked country by produce 0.48–1.29 million matrices tons per year (MMT/year) plastic marine debris on the last 2010 [3].

Microplastics are come from textiles product, personal care products, industrials and home debris which are travelled from rivers, carried by winds, tidal and storm then end up at marine ecosystems. Another causes are from natural phenomenon’s like floods, highly rainfall, tsunami and cyclone which brought microplastics sources into the ocean [4,5]. On the oceanic environment, degradation started with water temperature, ultraviolet radiation, currents and activities of marine degradable organism [6].
Microplastics with its light on the surface of oceanic water are possibly transferred into food chain when it was ingested by zooplankton or small pelagic fishes like anchovies with subsurface foraging behavior [7].

Indonesia marine had widely abundance of anchovies (Stolephorus spp.) during the year. Anchovies are small commercial fish with the high demand on the market. Furthermore, anchovies play as food supply for the higher fish taxa which ingested as prey. Hence, this research focused on the amount of abundance of microplastics in the anchovies. From Talisayan harbor, East Kalimantan, Indonesia as displayed in the Figure 1.

![Figure 1. Sampling locations: Talisayan harbor, East Kalimantan, Indonesia. Source: d-maps.com [8].](image)

2. Materials and methods

2.1. Materials

The used materials were the digestive tract of anchovies (Stolephorus spp.) from Talisayan harbor (near 0.5 g). The used chemicals were Milli-Q water, 10 mL (1M NaOH) and 5 mL Sodium lauryl sulphate (SLS) 0.5% technical grade. Used tools were digital scales, micrometer scrub instrument, filter paper (2.5 m mesh size, we used Whatman No.42), petri dishes, erlenmeyer jar (250 mL), Sedgwick rafter, standard work safety (google, gloves, lab coat, and conducted in the fume hood), Sedgwick rafter for microplastics observation, microscope Leica dm750, Atomic Absorption Spectroscopy (AAS) and Fourier-transform infrared (FTIR) spectroscopy (Thermo Scientific Nicolet iS5IR).

2.2. Methods

Samples were collected from fisherman in Talisayan harbor. Samples were prepared at Marine Biology Laboratory-Faculty Mathematic and Natural Science, presence of microplastics were tested at Research Laboratory of Basic Chemistry Engineering-Faculty of Engineering, University of Indonesia. Samples were measured dry weight (nearest to 0.5 g) by digital scales and length (nearest to 6 cm). Samples soaked in filtered Milli-Q water and rinsed in flow water to avoid contamination from fish body. Fish digestive tract was isolated as a whole up to 0.5 g on total and placed on the erlenmeyer jar with 5 mL 0.5% dissolve SLS and 10 mL (1M NaOH at fume hood, then kept it on the room temperature for 24 hours. After 24 hours, the jar was gently shaken and let to incubate for another 24 hours until all contents dissolved [9]. After the incubation fully dissolved, 1 mL of sample was placed into Sedgwick rafter
(triplet) and observed with magnification 10x on the microscope Leica. Kind of microplastics shapes (microfiber, microfilm, microfragment and microfoam) and sizes range were measured then classified into < 20 μm, 20-50 μm, 50-1000 μm and > 1000 μm. The type of microplastics were confirmed by FTIR instruments. To avoid the contamination, we kept samples in the sealed Erlenmeyer and picked only for microplastics observations. All chemicals were filtered through 2.5 mm mesh size filter paper before ready for microplastics examination.

2.3. Microplastics analysis
Presence of microplastics as its shape and size range were counted with spreadsheets (Ms. Excel) and compared with pictures references [10]. The graphics results of FTIR were compared with the standard to identify the polymer types [2]. The type of microplastics were analysed as Polypropylene (PP), High-density polyethylene (HDPE), Low-density polyethylene (LDPE), Polystyrene (PS), Poly(vinyl chloride) (PVC), Foamed Polystyrene Nylon (PA), Thermoplastic Polyester (PET) and Cellulose Acetate (CA). The statistical analysis were done by SPSS 22.

3. Results
Microplastics were possible to observe by microscope in the 10x magnification after applying modified method to digest the organic material. The retrieval was conduct in triplets in every each of 1 mL sample/Sedgwick rafter and total microplastics/individual as presented in table 1 were (average ± SD) 366 ± 3.51 particles/individual. Considering the anchovies as seafood diet, the results were displayed in total microplastics/g (dry weight) and the microplastics particles were 538 particles/g dry weight. The mercury tested result was not detected on samples from Talisayan harbor.

Table 1. The length, weight and total microplastics found on anchovies collected from Talisayan harbor in May 2018 and analysed for microplastics.

| Location          | Length (cm ± SD) | Weight (g ± SD) | Total MPs/individual | Total MPs/g |
|-------------------|------------------|-----------------|----------------------|------------|
| Talisayan harbor  | 59.96±0.63       | 0.68±0.06       | 366±3.51             | 538        |

Microplastics were observed as shape and range size categories. The percentage of the most microplastics (p < 0.05) found in Talisayan harbor were 50% microfilm and 23.59% microfiber as presented in Figure 2. The number of microplastics size range presented in table 2 and supported by Figure 3 and Figure 4 with scale bars (50 μm) for easy comparison. The most range size found on 50-500 μm (p < 0.05) for each shape of microplastics. The FTIR results were confirmed the type of microplastics. The type of polymers were found as polypropylene (PP), high-density polyethylene (HDPE) and foamed polystyrene nylon (PA).

Table 2. Size range of microplastics and FTIR confirmation.

| Location    | microfiber | microfilm | microfragment | microfoam | FTIR     |
|-------------|------------|-----------|---------------|-----------|----------|
| Talisayan harbor |            |           |               |           |          |
| <20 m       | 1          | 8         | 0             | 0         | PP,      |
| 20-50 m     | 1          | 10        | 2             | 1         | HDPE     |
| 50-500 m    | 44         | 68        | 33            | 3         | and PA   |
| 500-1000 m  | 6          | 8         | 0             | 0         |          |
| >1000 m     | 5          | 5         | 1             | 0         |          |
4. Discussion

This investigation at first is to show the empirical evidence for small pelagic fishes like anchovies which are contaminated by microplastics. Total microplastics/individual anchovy from this harbor is surpassingly high. The anchovies from Talisayan harbor are ingest 366 ± 3.51 particles of microplastics/individual. For comparison, anchovies (*Engraulis encrasicolus*) from Adriatic Sea ingested up to 1.25 particle/individual [11], anchovies (*Engraulis encrasicolus*) from the Gulf of Lion ingested up to 0.11 particle/individual [12] and anchovies (*Engraulis encrasicolus*) from Mediterranean Sea ingested up to 4 particle/individual [13]. Kind of microplastics shape found is 50% microfilm as the most type of ingested microplastics and the HDPE, PP and PA as polymers types. For comparison, at the same small pelagic fish both on *Sardina pilchardus* and *Engraulis encrasicolus* from Mediterranean coast, the most 83% microplastics ingested was microfiber and kind of polymer found as PET, PA and PE [14].
Figure 4. Photographic examples of microplastics found in anchovies from 1-r a) microfiber, b) microfilm, c) microfragment and d) microfoam. Scale bars represent at 50 μm.

The primary source of microfiber has been attributed to washing matching whiles the microfilm come from plastics bag or food wrapper [15,16]. PET are common type of microplastics. Both of PA and PE which found on S. pilcardus and E. encrasicolus are on original synthetic with the other textile contamination such as cotton and wool fiber. Whiles the HDPE are originally form milk and juice jugs which is contributed to film and segment shape. PP comes from rope, netting and bottle cap [17].

Small pelagic fishes like anchovies are foraging by filter feeder which swimming and opening their mouth. The water will pass the mouth to the gills, food particles trapped by gill rakers and transfer into the oesophagus. Microplastics contamination exposures their body through directly exposure and indirectly by ingested the contaminated preys. Several study conducted to gaining the evidence at how anchovies could directly ingested microplastics by mistake. Study on anchovies chemoreception shows the anchovies are using odour to approached their preys. While anchovies responded to their preys, the mechanism of rheotaxis are reduced and the aggregation increased. This mechanism will causing plastic debris smelled like food. Hence, the anchovies mistaken plastic debris as their food [18]. The other theory is indirectly microplastics exposure by ingested the contaminated preys. Study on zooplankton as anchovies preys shows that zooplankton ingested 131.5 pieces/ m³ microplastics in average 167 μm size range. The other prey, euphausiids on small size (1-2 mm) found with 556 ± 149 μm particles size microplastics on its body while the krill ingested 31.5 μm microplastics [19,20]. By the chance, microplastics contamination on anchovies from Talisayan harbor are in the range of 50-500 μm size range. This evidence supported the possibilities of microplastics contamination both by mistaken microplastics as foods or ingested the contaminated preys.

Talisayan harbor is in small village with mostly fishermen workers. With small population area (11,333 in total), kind of microplastics shapes (significant 50% film and 29.59% fiber) are possibly come from domestic litter and fishing activity [21]. The harbor also directly open and connect to the pacific ocean exposure.

5. Conclusion
We present the empirical evidence that microplastics particles are presence on small pelagic fish like anchovies. Our findings report that microfilm within 50-500 μm in size range are the most microplastics size range found from sample of Talisayan harbor. The polymers type are PP, HDPE and PA. The mercury contamination is not detected on sample from Talisayan harbor.

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