Occurrence of microplastic particles in Milkfish (Chanos chanos) from brackishwater ponds in Bonto Manai Village, Pangkep Regency, South Sulawesi, Indonesia

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Abstract. Pangkep Regency in South Sulawesi, Indonesia, is a milkfish producing region from which milkfish are distributed to fish markets or other regions. The location of the brackishwater ponds which are close to the sea and human settlements could allow microplastics as suspended particles to enter the milkfish gut. This study aimed to observe the number, shape, and color of microplastics in the guts of milkfish (Chanos chanos) from ponds in Bonto Manai Village, Pangkep Regency, and to analyze the relationship between the microplastics found and the hepatosomatic index (HSI). Milkfish samples were collected randomly from one brackishwater pond. Visual identification of the microplastics present in milkfish guts was carried out under a stereomicroscope. The results show that 46 out of 50 (92%) of milkfish sampled had microplastics in their digestive tracts. The average number of microplastics in Chanos chanos milkfish guts was 3.5 items/fish. The microplastic shape was dominated by line (92%) and blue (40%) was the most prevalent color. There was no correlation between the mean number of microplastics in milkfish guts and the mean hepatosomatic index (HSI); however, increasing value of HSI with the increase in number of microplastics found in the gut might indicate a reduction in the health condition of the fish.

1. Introduction

Plastics have become a widespread pollutant and are becoming increasingly prominent in the oceans. In 2010, 275 million metric tonnes (MT) of plastic waste was generated by 192 coastal countries, of which 4.8 to 12.7 million MT entered marine environments [1]. Plastics have advantages, namely that they are multipurpose, lightweight, cheap, do not rust, and can be colored, however, the problems associated with plastics cannot be underestimated. In particular, plastic products are extremely durable and are difficult to break down or melt. In marine waters, a wide variety of rubbish is found on the coast, in shallow coastal water, and even in the middle of the ocean, ranging in size from megaplastic (> 1 mm) down to microscopic nanoplastic (<1 µm).

Small pieces of plastic floating on the ocean surface with a range of less than 5 mm are known as microplastics [2]. Their very small size allows microplastic particles to be mixed with the plankton community, which is the primary food source for several types of fish that inhabit the surface zone, making them highly susceptible to ingesting microplastics. Several studies have found an abundance of microplastics in different marine organism biota, for example in the digestive tract of consumption.
fish and crustacea: pacific saury (Cololabis saira) [3], gudgeons (Gobio gobio) [4], milkfish (Chanos chanos) [5], and brown shrimp (Crangon crangon, Linnaeus 1758) [6].

During the digestion process, nutrients eaten by the fish or shrimp are first digested, then transported through the blood vessels to the liver, where they are used as energy in the metabolic process. The value resulting from a hepatosomatic index (HSI) measurement (derived from the equation between liver weight and fish body weight [7]) is an index that indicates the status of food reserves in animals [8]. The HSI expresses a change in liver size and is widely accepted as a specific bioindicator of contaminant exposure. This also makes it an appropriate index of environmental pollution. Changes in liver size (hypertrophy) and increased hepatocyte count (hyperplasia) are among the defense mechanisms that generally increase the distance between the external environment and the blood within an animal, acting as a barrier that prevents the entry of, and exposure to, contaminants [9].

Bonto Manai Village, Pangkep Regency, is a major milkfish producing area, with the fish living in ponds used by local residents. The proximity of the ponds to both the sea and nearby human settlements is expected to indicate the presence of microplastics as suspended particles in the digestive tract of these milkfish.

Therefore, a study was conducted to examine the number, shape, and color of the microplastics contained in fish intestine samples and to determine the relationship between the microplastics found and the hepatosomatic index (HSI).

2. Materials and Methods
This research was carried out from August to September 2019, at a milkfish brackishwater pond from Bonto Manai Village, Pangkep Regency, South Sulawesi. Further analysis of the samples taken was carried out at the Laboratory of Marine Ecotoxicology, Department of Marine Sciences, Faculty of Marine and Fisheries Sciences, Hasanuddin University.

2.1. Materials
The material used in this study consisted of the milkfish digestive tract, as an object of observation for the identification of microplastics; KOH 10% solution to destroy biological matter, and distilled water to rinse off the residual plastics before analysis. Sampling equipment included GPS and a coolbox for storing fish. Laboratory equipment used consisted of a ruler for measuring fish morphometrics, a cutting board on which fish were prepared, a set of surgical instruments, and a scale for determining fish weight and liver weight.

2.2. Methods
2.2.1. Sampling. A minimum of 50 milkfish [10] were caught at one fish pond location. Samples were stored in a coolbox to preserve them until they could be transported to the laboratory for further analysis.

2.2.2. Sample preparation. Fish morphometric measurements (measurements of fish length and weight) were carried out in the laboratory. Surgery was then performed to remove the intestine as the target organ, and the liver, which was then weighed for the calculation of HSI. After being removed and separated, the intestine was placed in a sample bottle, where alkaline digestion was carried out by adding a 10% KOH solution [11] with a ratio of 1:3 to the sample bottle, which was left to stand for 2 to 3 weeks at room temperature.

2.2.3. Microplastics identification. Once the intestines had degraded and the microplastic particles had been separated, a sample solution of 5-10 ml was placed in a petri dish, where a visual identification of the presence of microplastic particles was conducted with a stereomicroscope. Microplastic observations were carried out until the sample solution ran out. Photographic records of
the microplastics found were then obtained to allow the measurement of microplastic particles to be carried out using ImageJ software.

2.3. Data analysis
The microplastic data obtained from each intestinal sample were presented in the number of microplastic characteristics: namely, shape, color, and size [12]. Furthermore, the number of microplastics ingested was categorized based on their length range and size [13]. The hepatosomatic index was calculated using the HSI formula [14]. All image and data analysis was conducted in Microsoft Excel 2010.

3. Results and Discussion

3.1. Results
Around the brackishwater ponds, in the mangrove ecosystem, and around the jetty, there is a lot of rubbish clearly visible, with plastic being the dominant type of waste. A large amount of plastic waste in and around the brackishwater ponds is a matter of concern for researchers, regarding the safety of food sources for people who daily consume fish in the area, both from the market and from privately-owned brackishwater ponds.

In the sample of milkfish (*Chanos chanos*) studied from one brackishwater pond, it was found that 92% of the 50 randomly collected samples were contaminated by microplastics (MPs), with a total of 175 identified particles at an average abundance of 3.5±2.87 items/fish, while microplastics were not found in the digestive tracts of the remaining 4.

The number of microplastics found was then differentiated based on the size of the microplastics (Figure 1). Based on the identification results after grouping by size category [13], the number of microplastics for each group was tallied. The size category <1 mm had the highest number of microplastics with 90 items (51%). In the other categories, microplastics with the size of 1.01-2 mm totaled 52 items (30%), 2.01-3 mm totaled 17 items (9.7%), 3.01-4 mm totaled 10 items (5.7%), 4.01-5 mm included 4 items (2.3%), while two pieces of plastic (1.1%) sized >5 mm were found and recorded as mesoplastics.

![Figure 1](image.png)

**Figure 1.** Amount of microplastics observed in *C. Chanos*, based on size category.

Observation of microplastic particles in milkfish (*Chanos chanos*) found the presence of different categories of microplastics based on shape and color (Figure 2). Based on the identification results of microplastics in the digestive tract of the milkfish, two shapes of microplastics were found, namely
lines and fragments (Figure 2A). Of these two shapes, the dominant shape was lines, with a total of 162 items (92.6%), while only 13 items (7.4%) were found in the fragments category. Meanwhile, several colors of microplastics were found in the digestive tract of milkfish (Figure 2B), including 3 transparent items (1.71%), 11 red items (6.29%), 7 yellow items (4%), 15 grey items (8.6%), 24 black items (13.7%) and 45 purple items (25.7%), while the predominant color found was blue, with 70 items (40%). Some examples of microplastics found in the milkfish can be seen in Figure 3.

![Figure 2](image-url)

**Figure 2.** Number of microplastics by size category in shape (A) and color (B).

![Figure 3](image-url)

**Figure 3.** Variously colored microplastic shapes: lines (A, B, C), fragments (D, E, F).

The relationship between the number of microplastics ingested and the hepatosomatic index (HSI) of fish is illustrated in Figure 4. There is a positive but insignificant relationship ($R^2= 0.4515; P=0.214$) between the average number of microplastics and the average HSI for milkfish.
3.2. Discussions

Research conducted on 50 milkfish (Chanos chanos) revealed 92% microplastic contamination, where 46 individuals were found to have microplastic particles in the digestive organs totaling 175 microplastic items (MPs) with an average abundance of microplastics in the intestine of 3.5±2.87 items/fish. The abundance of MPs in this study was higher when compared to research conducted on milkfish in Muara Gembong [15] which found an average concentration of microplastics in the intestines and gills of milkfish of 2.33±2.266 items/fish originating from seawater ponds and 2.22±3.768 items/fish originating from brackishwater ponds. The large number of microplastics found in the digestive tract of milkfish in Bontomanai Village, Pangkep Regency is thought to be due to the location of the ponds: as well as being only 800 meters away from the sea, the ponds are also close to land and residential areas, which are one of the main sources of plastic users. This consequently increases the contact between plastic waste and biota.

Based on the results of this study using grouping by size categories, it was found that the highest number of microplastics was in the size category <1 mm (51%) while other microplastic size categories contributed the following: 1.01-2 mm (30%), 2.01-3 mm (9.7%), 3.01-4 mm (5.7%), 4.01-5 mm (2.3%). Mesoplastics with sizes >5 mm were the least abundant size category (1.1%). A study on the fish species Micropogonias furnieri conducted in the estuarine waters of Bahia Blanca in Argentina [13], found 100% microplastic contamination in the digestive tract of fish with a total of 241 items of identified microplastics at an average abundance of 12.1±6.2 items/fish. The range of microplastic sizes found was 0.2-5 mm with the dominant number of microplastic particles (48.3%) coming from the size category <1 mm. The other categories matching those of the present study each contributed 26%, 15%, 6.6%, 2.8%, and 1% respectively. The results of this study are in line with the results of the present study on milkfish found in brackishwater ponds. From the microplastics obtained, it can be concluded that the larger the microplastic size, the fewer items are found in the digestive tract.

The results of microplastic observations on milkfish contained two shapes of microplastic, namely lines and fragments, which were dominated by lines. GESAMP (2019) divides the shape of the lines into filament types (if they come from fishing gear nets) and fiber types (if they come from textile fibers). Due to the large number of strands found at the research location and the fact that pond farmers use nets made from filamentous material and geomembrane/plastic sheeting made of High-Density Polyethylene (HDPE) plastic (which is used as a base in degraded salt ponds), the dominant...
line shape is assumed to be filamentous. The fragments found generally had characteristic irregularly shaped edges. According to GESAMP (2019), this irregular shape indicates fragmentation from larger plastic waste. The same shapes of microplastics were also found in research conducted where there are four shapes of microplastics, namely: fiber, granule, film, and fragments [16]. Of the four types found, the fiber shape has the highest proportion. The results of the present study found variously colored microplastic items. These colors consisted of: blue, purple, black, gray, yellow, red, and transparent, with blue being the dominant color, which supports the findings on microplastics at two different locations[17]. Several other microplastic colors are also often found including white or transparent, black, red, yellow, brown, and gray [18].

The hepatosomatic index (HSI) is a biological response from an organism that is useful for detecting the harmful effects of pollutants or environmental pressure [14]. An increase in liver size is usually seen in fish that have been exposed to contaminants for a long time, while it is suspected that fish exposed to pollutants may also show decreased HSI if food is limited or when exposure is very short. However, chronic exposure to pollutants where food is not limited or at normal eating conditions will increase the value of the HSI [19].

Based on the results of this study, which can be seen in Figure 4, there is a positive but not significant correlation between the average HSI and the average number of microplastics in the fish gut ($R^2=0.4515; P=0.124$). The positive correlation shows an increase in HSI with an increase in the number of MPs present in the digestive tract of milkfish aged 5 months after seeding, although the coefficient of determination shows only about 45% variation in HSI changes. This can be explained by the amount of ingested MPs, but an increase in HSI may indicate there has been exposed for a long time (chronic), following from the findings of Heal'th (2000) that chronic exposure to pollutants with adequate food conditions can increase the value of the HSI. Experimental research conducted found female catla Catla catla [20] exposed to the high concentration of Bisphenol-A at 1000µg/l experienced an increase in HSI even though fish exposed to Bisphenol-A with low concentrations of 10 and 100 µg/l exhibited no significant change in the HSI. In another experimental study conducted there was also an increase in HSI in Sebastes schlegelii fish exposed to polystyrene microplastics when compared to controls [21].

4. Conclusions
The level of microplastic ingestion in the digestive tract of milkfish is high (92%). The majority of microplastics ingested are line-shaped with a dominant blue color and there is no significant relationship between the value of the hepatosomatic index and the number of microplastics that are ingested.

References
[1] Jambeck J R, Geyer R, Wilcox C, Siegler T R, Perryman M, Andrady A, Narayan R and Law K L 2015 Plastic Waste Inputs from Land Into The Ocean Science (80- ). 347 768–71
[2] Masura J, Baker J E, Foster G D, Arthur C and Herring C 2015 Laboratory methods for the analysis of microplastics in the marine environment: recommendations for quantifying synthetic particles in waters and sediments NOAA Tech. Memo. NOS-OR&R 48
[3] Boerger C M, Lattin G L, Moore S L and Moore C J 2010 Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre Mar. Pollut. Bull. 60 2275–8
[4] Sloothaekers B, Catarci Carteny C, Belpaire C, Saverwyns S, Fremout W, Blust R and Bervoets L 2019 Microplastic contamination in gudgeons (Gobio gobio) from Flemish rivers (Belgium) Environ. Pollut. 244 675–84
[5] Agustian Fareza A and Sembiring E 2020 Occurence of Microplastics in Water, Sediment and Milkfish (Chanos chanos) in Citarum River Downstream (Case Study: Muara Gembong) E3S Web Conf. 148 1–5
[6] Devriese L I, van der Meulen M D, Maes T, Bekaert K, Paul-Pont I, Frère L, Robbens J and Vethaak A D 2015 Microplastic contamination in brown shrimp (Crangon crangon, Linnaeus 2021) 012058 doi:10.1088/1755-1315/763/1/012058
1758) from coastal waters of the Southern North Sea and Channel area *Mar. Pollut. Bull.* **98** 179–87

[7] Kiriratnikom S and Kiriratnikom A 2012 Growth, feed utilization, survival and body composition of fingerlings of slender walking catfish, *Clarias nieuhoffii*, fed diets containing different protein levels *Songklanakarin J. Sci. Technol.* **34** 37–43

[8] Tresnati J U M T and S 2019 Changes in Liver relate to Oocyte Growth of Flatfish *J. Pengelolaan Perair.* **2** 1–14

[9] Mohebbi Derakhsh P, Mashinchian Moradi A, Sharifpour I and Jamili S 2020 Toxic effects of diclofenac on gills, liver and kidney of Cyprinus carpio (Linnaeus, 1758) *Iran. J. Fish. Sci.* **19** 735–47

[10] Hermsen E, Mintenig S M, Besseling E and Koelmans A A 2018 Quality Criteria for the Analysis of Microplastic in Biota Samples: A Critical Review *Environ. Sci. Technol.* **52** 10230–40

[11] Rochman C M, Tahir A, Williams S L, Baxa D V, Lam R, Miller J T, Teh F C, Werorilangi S and Teh W J 2015 Anthropogenic debris in seafood plastic debris and fibers from textiles in fish and bivalves sold for human consumption *Sci. Rep.* **5**

[12] Kershaw P, Turra A and Galgani F 2019 Guidelines for the Monitoring and Assessment of Plastic Litter in the Ocean *GESAMP report Stud.*

[13] Arias A H, Ronda A C, Oliva A L and Marcovecchio J E 2019 Evidence of Microplastic Ingestion by Fish from the Bahía Blanca Estuary in Argentina, South America *Bull. Environ. Contam. Toxicol.* **102** 750–6

[14] Parikh P H and Sadekarpawar S 2013 Gonadosomatic and Hepatosomatic Indices of Freshwater Fish *Oreochromis mossambicus* in Response to a Plant Nutrient Gonadosomatic and Hepatosomatic Indices of Freshwater Fish

[15] Sembiring E, Fareza A A, Suendo V and Reza M 2020 The Presence of Microplastics in Water, Sediment, and Milkfish (*Chanos chanos*) at the Downstream Area of Citarum River, Indonesia *Water. Air. Soil Pollut.* **231**

[16] Priscilla V and Patria M P 2020 Comparison of Microplastic Abundance in Aquaculture Ponds Of Milkfish *Chanos chanos* (Forsskål, 1775) at Muara Kamal and Marunda, Jakarta Bay *IOP Conference Series: Earth and Environmental Science* vol 404 (Bogor: IOP Publishing) p 12027

[17] Lie S, Suyoko A, Romadona E E, Armada B, Wira H A, Rimba I S, Putu N A N A, Illiyyen H N, Rahmasari N and Reza A 2018 Measurement of the microplastic density in the Karimun Jawa National Park, Central Java, Indonesia *Ocean Life* **2**

[18] Hidalgo-Ruz V, Gutow L, Thompson R C and Thiel M 2012 Microplastics in The Marine Environment: A Review of The Methods Used for Identification and Quantification *Environ. Sci. Technol.* **46** 3060–75

[19] Heath A G 1987 Behavior and Nervous System Function *Water Pollut. Fish Physiol.* 181–96

[20] Faheem M, Khaliq S and Lone K P 2020 Molecular characterization and phylogenetic analysis of babesia species isolated from domestic cattle *Pak. Vet. J.* **40** 224–8

[21] Yin L, Chen B, Xia B, Shi X and Qu K 2018 Polystyrene Microplastics Alter the Behavior, Energy Reserve and Nutritional Composition of Marine Jacopever (*Sebastes schlegelii*) *J. Hazard. Mater.* **360** 97–105