

# Microplastic concentration in asiatic hard clam meretrix meretrix.

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## Microplastic concentration in asiatic hard clam *meretrix meretrix* (Linnaeus, 1758) from Lemo Beach, Burau District, Luwu Timur Regency, South Sulawesi

Sarnila Tamrin, Khusnul Yaqin, Sri Wahyuni Rahim, Dwi Fajriyati Inaku and Moh. Tuhid Umar

Department of Fisheries, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar 90245, Indonesia

Email: khusnul@gmail.com

**Abstract.** Microplastic pollution is a problem that is currently attracting the attention of various groups, from scientists to ordinary people. This type of pollution can have a negative impact on aquatic biota, especially organisms that have filter feeder behavior. Asiatic hard clam (*Meretrix meretrix*) is a filter feeder organism that has a considerable risk of being exposed to microplastics. This study aims to analyze the concentration of microplastics in Asiatic hard clam (*M. meretrix*) at Lemo Beach, Burau District, East Luwu Regency, South Sulawesi. Sampling was performed using purposive random sampling method. The number of samples of Asiatic hard clam was 118 which were divided into three groups of shell length, namely class A (2.75 - 3.40 cm), class B (3.41 - 4.21 cm), class C (4.22 - 5.24 cm). Observation of microplastic particles is carried out using a stereo microscope equipped with a camera (Miconos optical lab). The results of the frequency analysis of the presence of microplastics in the clam showed that class A was the class with the highest frequency, namely 95.67%. The observed microplastics were in the form of fibers, fragments, and films with a predominantly black and transparent color. The size of the observed microplastics ranged from 0.033-0.88 mm. The results of the microplastic concentration analysis showed that size class A had a greater concentration than class B and class C.

### 1. Introduction

The number of pollution cases that occur from year to year is increasingly troubling in several countries. Pollution that is very dangerous and is rife, namely pollution caused by plastic waste because it is a major threat to life, both in human life and in organisms that live on land and also in the ocean. Plastics are increasingly being used around the world in a variety of applications in everyday life. The market share for plastics continues to increase to 600 million tonnes in 2025 and exceeds 1 billion tons in 2050 [1]. This increase is motivated by the durable nature of the plastic and its relatively efficient use. However, this property makes plastic a bad impact on the surrounding environment, especially marine waters. Almost all types of plastics float in water bodies, which causes plastics to be degraded by sunlight (photodegradation) in oxidation process occurs and mechanical abrasion occurs which will form plastic particles [2,3]. Microplastic pollution is a global problem and is the focus of research by environmentalists around the world. The presence of microplastics in the environment is a problem because they are persistent, contain chemical base materials that are potentially toxic and carcinogenic [4]. Microplastics have been identified throughout the environment including water bodies, sediments

and aquatic organisms [5,6]. When consumed by organisms, it will affect the aquatic ecosystem [7]. The results of a study conducted by Lusher *et al.*, (2013) showed that microplastics are widespread in the oceans; on the ocean floor, coast, and ocean surface [5].

One area that has the potential to be contaminated by microplastics is Lemo Beach, which is located in East Luwu Regency, South Sulawesi Province. Lemo Beach is one of the most popular tourist destinations in East Luwu Regency, flanked by two estuaries, namely Muara Singgeni and Muara Saloanna. Both estuaries have long rivers which accumulate a lot of garbage eventually enters the ocean by the current. The source of microplastics is thought to come from activities around coastal waters, namely visitor activities and community activities, and from other waters. Community activities at Lemo Beach which are at risk as a source of microplastic waste in marine waters, such as household activities, fishing activities, seaweed cultivation and pond activities. Microplastics that are carried by the currents will accumulate in the waters, settle in the sediment and can be eaten by organisms on the Lemo Beach, especially organisms that have filter feeder properties, one of which is the bivalve class.

Various studies on microplastics in bivalves have been carried out in various regions and have shown that microplastics are present in the bodies of shellfish organisms. Li *et al.*, (2016) found bivalves of the *Mytilus edulis* in coastal areas China, whether collected directly on the beach or cultivated, were shown to contain microplastics with concentrations of 0.9 - 4.6 items/g with the form of fibers and fragments [6]. In addition Naji *et al.*, (2018) found microplastics in the form of fibers, fragments and films in the shellfish *Amiantis umbonella* and *Amiantis purpuratus* of the Veneridae family with a concentration of 12.8 - 20.0 items/g [6]. Wahdani *et al.*, (2020) also found that microplastics in bivalves from the Veneridae family of the *Venerupis philippinarum* found in Maccini Baji Waters, Pangkajene Islands Regency, have been contaminated with microplastics in the form of fibers and fragments.

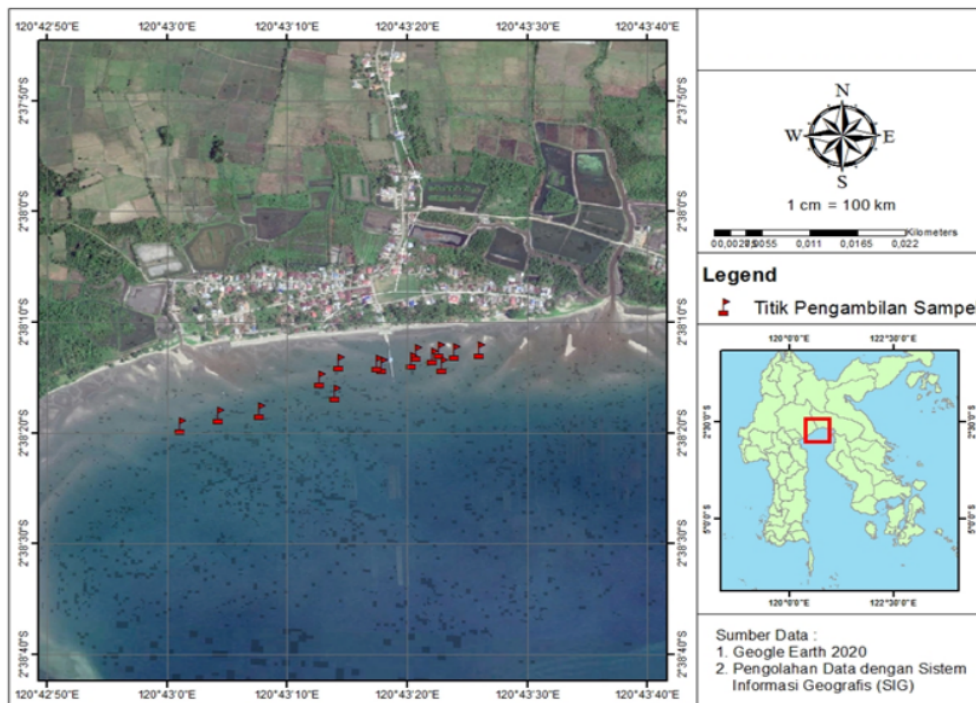
Asiatic hard clam (*Meretrix meretrix*) are a class of bivalves in the Veneridae family which are filter feeders. As a filter feeder organism, asiatic hard clam obtain their food by filtering particles of organic matter and phytoplankton suspended in water and living permanently on a substrate by immersing themselves in coarse and fine sandy substrates [11]. Therefore, asiatic hard clams have the risk of being exposed to various pollutants from sea water and accumulating in their bodies. Asiatic hard clam (*M. meretrix*) is one of the livelihood objects of fishermen in the waters of Lemo Beach and is widely consumed by people in East Luwu Regency. Indeed, there is no information on microplastic contamination in the area of East Luwu Regency, because pollution research has never been carried out in that area.

Based on the description above, it is necessary to conduct research on the microplastic content in *M. meretrix*. Previously, a preliminary test was carried out to prove the presence of microplastic particles in the shellfish tissue in the waters of Lemo Beach. The results obtained were that there were microplastics in the bivalve which were used as preliminary samples. Therefore, a more in-depth study was conducted to determine the concentration of microplastics in Asiatic hard clam by looking at the concentration of microplastics based on the number, shape, size and color of the microplastics.

## 2. Materials and Methods

### 2.1. Study area

This research was conducted in March-June 2020. The sampling location was Lemo Beach, Burau District, East Luwu Regency, South Sulawesi Province (Figure 1). Furthermore, it will be analyzed at the Water Quality Laboratory, Fisheries Department, Faculty Marine of Sciences and Fisheries, Hasanuddin University, Makassar.



**Figure 1.** Map of the location for sampling *M. meretrix* at Lemo Beach, Burau District, East Luwu Regency, South Sulawesi (Google earth, 2020)

2.2. Research Procedure

2.2.1. *Sampling.* Sampling was done using purposive random sampling technique. Sugiyono (2007) states that purposive random sampling is a technique of determining a sample with certain considerations and is based on certain characteristics or adjusted to the criteria applied based on the research objectives. Samples were taken in the morning in low tide condition. The bivalves were taken them up by hand. Afterward, the samples were transported using coolbox to the laboratory. The sample was put in the refrigerator during the preparation process for the analysis Khoironi et al., (2018) To determine the required number of samples, the sample was first divided into three classes of shell lengths based on the class determination method with the largest and smallest shell length logarithms of the shell samples. Then, the bivalves are grouped according to their respective classes. To calculate the required sample size, the formula Yamane (1967) is used

$$n = \frac{N}{1 + N(d^2)} \tag{1}$$

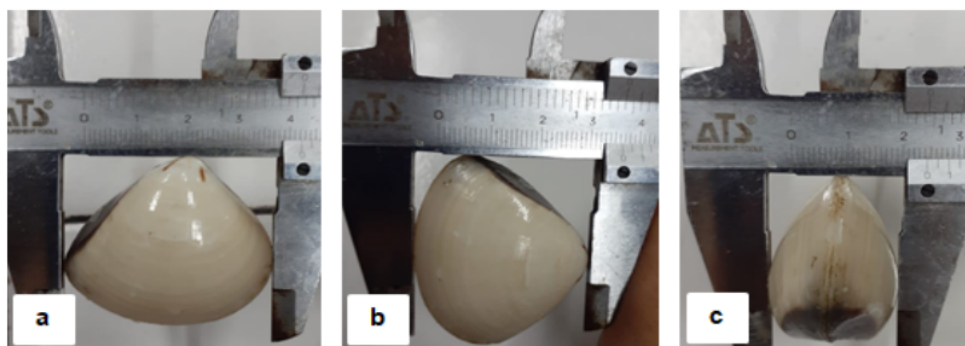
Note: n: samples needed, N: total samples obtained in the field (total number of shells in 3 classes), d: 5% confidence level.

To determine the number of samples for each class using the proportional allocation formula according to Sugiyono (2007):

$$n_i = \frac{N_i}{N} \times n \quad (2)$$

Note:  $n_i$ : samples needed in class  $i$ ,  $n$ : samples needed,  $N$ : total samples obtained in the field (total number of shells in 3 classes),  $N_i$ : samples in class  $i$ .

**2.2.2. Measurement of sample morphometrical characteristics.** Morphometric measurements were carried out to determine the index of shellfish conditions. The biota samples that have been collected are cleaned from the adhered fouling. Measurements of sample morphometrical characteristics including total length, height and shell width were measured using a caliper (Figure 3). Before the sample is dissected, the total weight of the shells is measured using a Fujitsu brand with an accuracy of 0.01 g. Then the clams are dissected to separate the clam meat and shell. The weight of the meat and shell is weighed individually using a scale. The weighed shellfish was put into a sample bottle and then closed. This is done to keep the sample from contaminating the outside air.



**Figure 2.** How to measure the length, height and width of the bivalve shell morfometry; (a) The length of the shells, (b) The height of the shells and (c) the width of the shells

**2.2.3. Digestion of sample.** Before identifying microplastic particles, the organic materials in shellfish must be removed and separated from the microplastics. The method used is by adding an alkaline solvent in the form of KOH 10% in each sample bottle containing shellfish. The addition of 10% KOH was included as much as 3 times the weight of shellfish meat [14,15]. The choice of KOH as a solvent is due to its nature which is more effective at removing organic matter than other solvents such as  $H_2O_2$ , and is better at maintaining microplastic conditions (Ding et al., 2018). After that, the samples were allowed to stand for approximately two to three weeks at room temperature until the bivalve tissue was completely dissolved.

**2.2.4. Microplastic identification.** Identification of microplastics using visual observation methods, namely by looking at the shape, size, and color of the microplastic particles. Observation of the microplastics on shellfish was started by pouring the sample solution into a petri dish using a dropper of 2-3 drops. Then the observation was carried out in a stereo microscope, if the sample observed contained microplastics, then filtering was carried out using the  $0.45 \mu m$  Whatman filter paper. This technique is carried out because the microplastic analysis method with filtering, the results are better than without filtering (Fachruddin et al., 2020). The results of these filters will be observed under a stereo microscope using an optical tool by looking at the shape and color of the microplastic particles. Meanwhile, to determine the size of microplastics using the Image raster application by measuring the largest length of the microplastics by using the line option for fragment and film microplastics and using the path selection in the raster image for fiber microplastics by following the shape of the microplastics.

### 2.3. Research variables

**2.3.1. Microplastic concentration.** The concentration of microplastics in bivalves is the microplastic content found in the tissue of bivalves, and can be expressed in units of item/g. The microplastic concentration can be calculated using the formula of Khoironi *et al.*, (2018):

$$\text{Microplastic Concentration (item/g)} = \frac{\text{Number microplastic in bivalve}}{\text{Weight of wet tissue}} \quad (3)$$

The results of the calculation of the concentration of each shell will then be compared per size class to see the difference, and presented in a graph.

**2.3.2 Frequency of attendance.** The presence frequency (FK) of microplastics in bivalve samples is the percentage of the number of bivalves identified as containing microplastics. The presence frequency is calculated based on the presence frequency formula of Krebs (2014) little modification:

$$\text{FK} = \frac{\text{The number of bivalve cobtaminated by microplastic}}{\text{The total number of bivalve}} \times 100\% \quad (4)$$

### 2.4. Data analysis

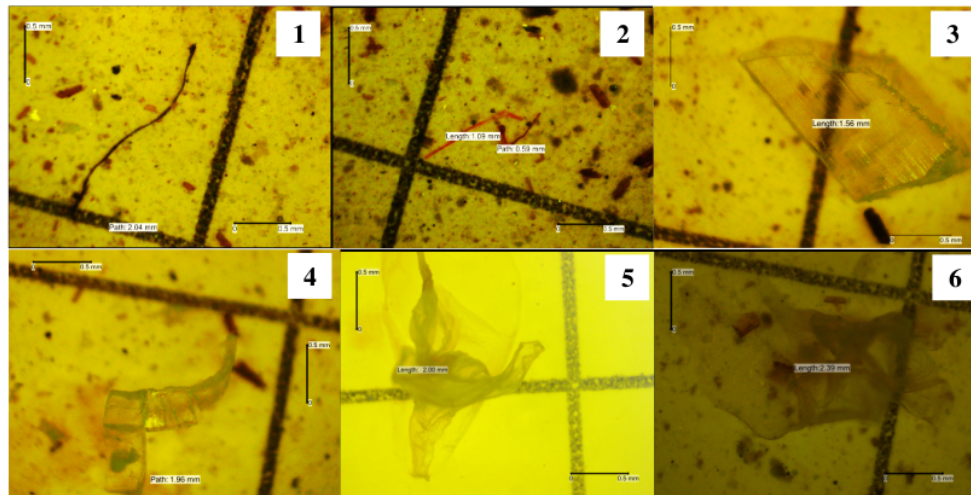
Parametric analysis of variance (ANOVA) was used to determine differences in microplastic concentrations in bivalves with groups of different shell lengths. This was because the data was normally distributed and homogeneous.

## 3. Results and Discussion

The number of samples of *M. meretrix* used in this study were 118 samples, divided into three groups of clam shell length classes. The clam shell length groups were class A (2.75 - 3.40 cm), class B (3.41 - 4.21 cm) and class C (4.22 - 5.24 cm). The results showed that *M. meretrix* contained microplastic particles. This is because bivalves are filter feeder organisms, which feed by filtering whatever is around them, such as water and sediment particles. Therefore, various kinds of waste in the aquatic environment can enter to the clams, including microplastic particles. The release of plastics into the marine environment occurs through various routes, including river and atmospheric transportation, coastal waste which is directly dumped into the sea through aquaculture, shipping, fishing [16] and other land-based plastic sources, such as household activities, tourism and industry (Browne *et al.*, 2011). Microplastics carried by the currents will accumulate in the waters, settle in the sediment and can be eaten by organisms in the waters, especially organisms that have filter feeder behavior [19]. Walkinshaw *et al.*, (2020) suggested that organisms at lower trophic levels, such as clams, are more likely and at risk of being contaminated by microplastics than predators, because microplastic concentrations at different trophic levels tend not to be biomagnified.

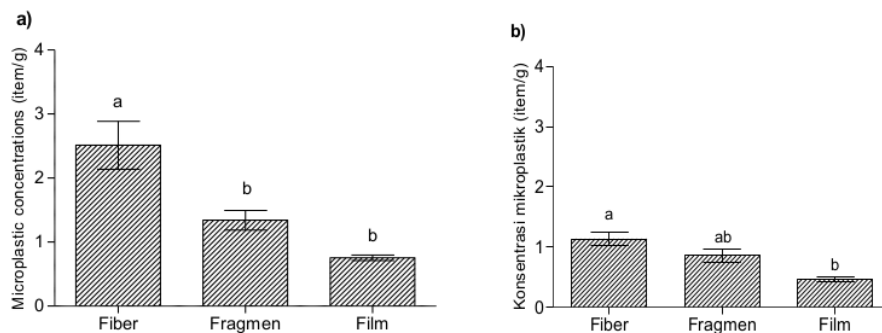
### 3.1. Microplastic concentration based on shape

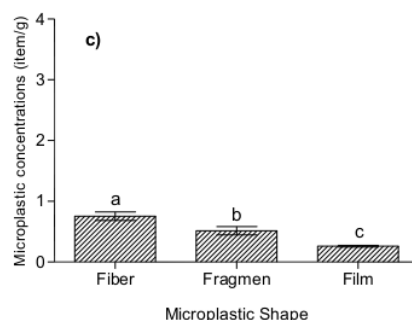
The microplastic particles found consisted of three forms, namely fiber, fragment and film (Figure 3). According to Claessens *et al.*, (2011); Browne *et al.*, (2011) that fiber is a microplastic that has a long and thin shape, can come from clothing fibers, ropes such as ropes used by the community for seaweed cultivation and fishing gears such as fishing rods and fishing nets. used by fishermen. Fragments are microplastics that have a rigid structure, irregular shapes that come from the remains of jars, drink bottles, mica folders, and pieces of small pipes (Tanaka & Takada, 2016; Lushe *et al.*, 2017). Film Has an irregular shape, is thinner and is more flexible than fragments. The source of microplastics comes from food packaging. The film also has a transparent white color.



**Figure 3.** Forms of microplastics found (1-2) Fiber; (3-4) Fragments; (5-6) films

Based on the results of statistical analysis, it can be seen that the microplastic concentrations of class A shellfish are significantly different from the shape of the fragments as well as the form of fiber and film. Meanwhile, the form of film and fragment had no significant difference in concentration. Class B which has a significantly different concentration is the form of fiber and film. Meanwhile, the shape of the fragments and films, the forms of fibers and fragments, had no significant different concentrations. Meanwhile, Class C has a significantly different concentration of each form of microplastic (Figure 4). The average value of microplastic concentrations obtained in each form (fiber, film and film) in class A were 2.51, 1.34 and 0.75 items/g. The average value of the microplastic concentration in each form (fiber, film and film) for class B were 1.13, 0.86 and 0.47 respectively. While class C has a concentration value of 0.75, 0.52 and 0.26 items/g respectively. Based on this value, it can be seen that microplastic with the form of fiber is the dominant form found in each group of shell length and film is the form that is the least found.





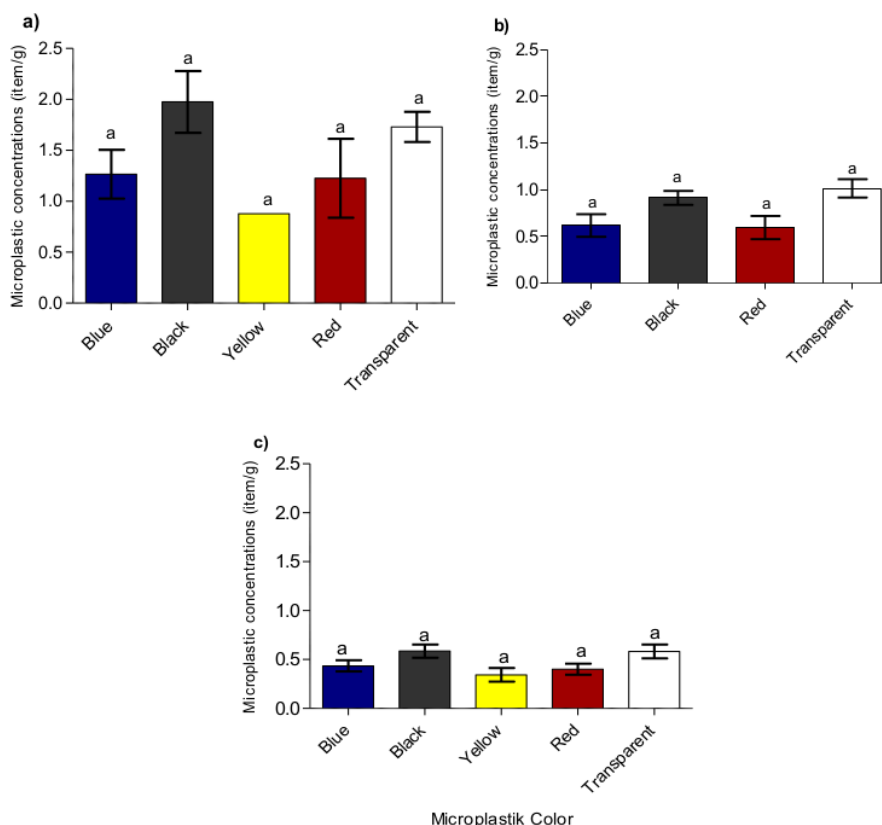
**Figure 4.** Microplastic concentration based on microplastic shape ( $X \pm SE$ ,  $N = 118$ ). Different letter symbols indicated statistically significant differences ( $p < 0.05$ ). a) Class A (2.75-3.40 cm), b) Class B (3.41-4.21 cm), c) Class C (4.22-5.24 cm).

Fiber is the dominant microplastic form found in each group of bivalve shell lengths. Research conducted by Fang *et al.*, (2019) also found microplastics in the form of fibers, fragments and films in Xiami, China and fiber is the dominant form of microplastics found in *M. meretrix* in the region. Barrows *et al.*, (2018) shows that microplastic forms of fiber can contribute up to 91% in global marine waters compared to other forms of microplastic. The number of microplastics in the form of fiber found is thought to have originated from the rope of seaweed cultivation which is almost along the waters of Lemo Beach, fishing gear and boat ropes used by fishermen. Film is the least microplastic found in each group of bivalve shell length. This is because the microplastic film has a lower density so it tends to float in the water column and is easier to transport to another places (Barnes *et al.*, 2009)

Some of the microplastics found in asiatic hard clam in the waters of Lemo Beach are thought to be because the sampling area is where various activities are carried out such as tourism, seaweed cultivation, fishing and there are also two estuaries that can accumulate waste from various activities on land to enter the waters. Lemo Beach. Some of the microplastics found in bivalves in the waters of Lemo Beach are expected due to the sampling area is a place for various activities such as tourism, seaweed cultivation, fishing and there are also two estuaries that can accumulate rubbish from various activities on land where the garbage enters to Lemo Beach. In addition, the form of microplastics is widely used to determine the category of microplastics and determine the source of microplastics [26]. The form of fibers, films and fragments is classified as a source of secondary microplastics. Secondary microplastics come from the larger fragments of plastic goods produced during the use of plastic products (for example textiles, wheels and tires) or after plastic is disposed of into the aquatic environment, for example food packaging plastics secondary microplastics are derived from the larger shards of plastic that are generated during the use of plastic products (e.g. textiles, wheels and tires) or after plastic is dumped into the aquatic environment (Lusher *et al.*, 2017). According to Peng *et al.*, (2020) most of the microplastics in the marine environment come from secondary source microplastics.

### 3.2. Concentration of Microplastics by Color

Apart from observing the shape of the microplastic, the characteristics of the microplastic can also be seen based on the color of the microplastic. The results show that the microplastic colors are black, transparent, red, yellow and blue. The results of microplastic concentration analysis based on microplastic color can be seen in Figure 4.



**Figure 5.** Microplastic concentration based on microplastic color ( $X \pm SE$ ,  $N = 118$ ). Different letter symbols indicated statistically significant differences ( $p < 0.05$ ). a) Class A (2.75-3.40 cm), b) Class B (3.41-4.21 cm), c) Class C (4.22-5.24 cm).

Color might be used to identify the initial composition of microplastics (Ryan *et al.*, 2019) but the color of microplastics does not affect the consumption of organisms, especially organisms that have filter feeder behavior and the potential for microplastics to be consumed by biota is thought to depend on the presence of microplastics in an environment (Ory *et al.*, 2018). The dominant colors found in *M. meretrix* were black and transparent white (Figure 4). The black microplastic is thought to have originated from the degradation of black plastic and the presence of organic materials that stick to the surface of the plastic (Hiwari *et al.*, 2019). Transparent color microplastic particles were found in microplastic fragments and films. Fragments are microplastics that have a rigid structure and are irregular in shape from the remains of jars, straws and drink bottles. The film has an irregular shape, is thinner and is more flexible when compared to fragments which derived from food packaging (Kovač Viršek *et al.*, 2016). According to Hastuti *et al.*, (2019) the transparent colors found are thought to have come from transparent white plastic bags, drink bottles and straws which are the original colors of microplastics and are widely used by people in everyday life. In addition, transparent white microplastic is also suspected due to the change in the color of microplastic particles in the waters to a transparent color caused by UV rays (Hiwari *et al.*, 2019). Light colored microplastics indicate that microplastic particles have not experienced (discoloring) or significant color changes (Solmon *et al.*, 2016). In

In addition, light colored microplastics, namely blue, yellow and red, are found in fiber microplastics, thought to have originated from the degradation of seaweed cultivation ropes and fishing gear thrown into the sea.

3.3. Microplastic concentrations in various groups of shell length

The results of the microplastic concentration were obtained from the ratio of the number of microplastic particles to the weight of the wet shellfish. Based on the results of statistical test analysis, it was found that the microplastic concentration for each group of bivalve shell length had a significant difference, namely the concentration of microplastics in class A was significantly different to class B and class C, as well as class B was significantly different to class C (Figure 5). The average value of the microplastic concentration in each group of shell lengths from class A, B, and C was 3,736, 1,916, 1,182 items/g.

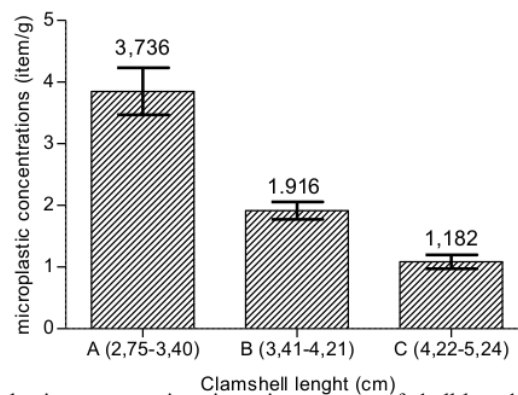


Figure 6. Microplastic concentrations in various groups of shell length (X ± SE, N = 118)

The smaller the size of the shells, the greater the concentration value contained in the shells when compared to the size of large shells. This is in accordance with the research of Fachruddin *et al.*, (2020) who found that the longer the shell size, the less the concentration of microplastics in the shells body and vice versa because small clams have a fast filtration rate and growth so they can filter their food faster. According to Yaqin *et al.*, (2014) the absorption of waste in green mussel is thought to be influenced by the filtration rate. Tantanasarit *et al.*, (2013) suggested that there is a difference in the filtration rate between small and large shells. The filtration rate of small bivalves is faster than the filtration rate of large bivalves. Yaqin *et al.*, (2014) stated that when the growth of bivalve slows down, the filtration rate of the bivalve also slows down and vice versa and the growth rate is constant when the mussels have reached their maximum size limit. Sakila *et al.*, (2018) also found that young *M. meretrix* have rapid growth, when they reach old age, the growth rate will slow down and even tend to be static (fixed) when it reaches its maximum length.

3.4. Microplastic size and frequency of presence

The microplastics found consisted of large microplastics (1 mm - ≤5 mm) and small microplastics (1 μm - ≤1000 μm). The microplastic length ranges in classes A, B, and C were 0.03-4.66, 0.05-4.81, and 0.15-4.88 mm, respectively (Table 1).

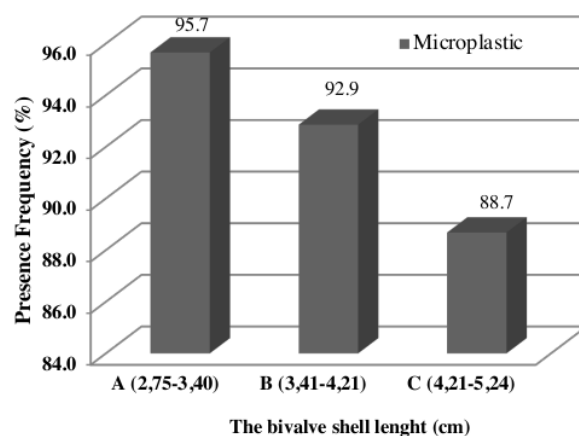
Table 1. Microplastic based on the length range of microplastics

No	Clamshell length (cm)	Microplastic length range (mm)	Average length of microplastics (mm)
1	2.75-4.40	0.03-4.76	1.3640
2	3.41-4.21	0.05-4.81	1.3240

3                      4.22-5.24                      0.15-4.88                      1.3972

The size of the microplastics in each group of the bivalves has a different length range. The minimum size of the microplastic found was 0.030 mm, while the maximum length was 4.880 mm. Fang *et al.*, (2019) found that the size of microplastic particles found in *Meretrix meretrix* was 0.04-4.72 mm. This indicated that the bivalves could ingest microplastics of various lengths. According to Wright *et al.*, (2013) bivalves do not select in ingesting microplastics based on the size, shape and color of the microplastics. Because they tend to ingest all surrounding particles including microplastics..

The presence frequency of microplastics can indicate the number of the bivalves contaminated with microplastic particles. The results indicated that the highest percentage of microplastic presence frequency is in class A, which is 95.65 %. Whereas the class with the lowest percentage of microplastic presence frequency was in class C with a percentage of 86.79 %. Class B has a frequency percentage of the presence of microplastics of 92.86 % (Figure 5). Overall, the percentage of the total frequency of the presence of microplastics in all groups of the bivalve shell length was 91.77 %. This means that almost all of the shellfish populations sampled were found microplastic particles.



**Figure 7.** Percentage of the frequency of presence of microplastics in the shell length group

Wahdani *et al.*, (2020) found that the percentage of the frequency of the presence of microplastics found in manila shellfish (*Venerupis philippinarum*) was 51.69%. Some of the bivalves that were not identified to contain microplastics were thought to be due to the life strategy of the bivalves. Each bivalve has a different life strategy. According to Puspitasari (2007) when a chemical enters the body of an organism and is distributed, the material can be excreted, stored, or metabolized by the body depending on the concentration and potential of the material. This ability depends on the organism's life strategy and also the characteristics of the chemical enter the body of an organism.

#### 4. Conclusion

Asiatic hard clam (*M. meretrix*) located at Lemo Beach, Burau District, East Luwu Regency were identified to contain microplastics. The microplastic particles found were fibers, fragments and films with predominantly black and transparent colors. The microplastics found ranged in size from 0.030 to 4.880 mm. The highest frequency of microplastic presence was in the group of shell length 2.75-3.40 cm (class A), which was 95.67%. While the group with the lowest frequency of microplastic presence was in class C with the size of 4.22-5.24 cm with a percentage of 87.79%. The microplastic concentration with the highest average value was in the bivalve shell length group 2.75 - 3.40 cm (class A), while the

microplastic concentration with the lowest average value was in the bivalve shell length group of class C.

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