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MICROPLASTIC CONCENTRATIONS IN GREEN MUSSEL EPIBIONT FROM LAE-LAE ISLAND MAKASSAR

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Abstract: Microplastic pollution is a serious problem and harms aquatic ecosystems, especially for organisms that are filter feeders. This research aimed to analyse the concentration of microplastics contained in epibionts attached to green mussels (*Perna viridis)* in the water of Lae-Lae Island, Makassar. Sampling was conducted using the *purposive random sampling method,* to obtain 110 green mussels which were divided into three groups of shell length: small (2.42-4.40 cm), medium (4.41-8.01 cm), large (8.02-14.62 cm) groups. In addition, based on the microplastic content, the epibionts associated with green mussels were also divided into four groups, namely (1) microplastics in green mussels with epibiont attached, (2) microplastic in green mussels without epibiont attached, (3) microplastics in epibiont attached to green mussels, (4) microplastics in green mussels and epibiont. Microplastic particles were observed using a stereo microscope with Image J Software. The result depicted that the microplastics were fibres, fragments, and films with blue as the dominant colour. The size of microplastics ranged from 0.03-4.72 mm. The results showed no significant differences in the concentration of microplastics of epibionts at different green mussel shell lengths (p > 0,05). However, there was a statistically significant difference in the concentration of microplastics in epibionts with their host, green mussels, in which the concentration of microplastics in epibionts was greater than that in green mussels ($p > 0.05$). This study suggested that the epibionts and the green mussels played a role in reducing microplastic in waters.

Keywords: Biofouling, *Perna viridis*, microplastics, Lae-Lae Island, Makassar.

Introduction

Plastic pollution has become a global concern and is detrimental to nature, especially marine ecosystems due to the long degradation process (Espiritu *et al.*, 2019). Plastic waste comes from anthropogenic activities that accumulate and spread worldwide in coastal waters and deep seas. Ocean plastic waste almost reaches 60-80% of marine debris (Moore, 2008; Lebreton *et al.*, 2012).

Indonesia is ranked second in the world as the largest contributor of plastic waste to the sea after China (Hoegh-Guldber *et al.*, 2015). The amount of plastic waste produced by Indonesia was more than 5.4 million tons and around 0.48-1.29 million tons each year ended up in the sea (Guldberg *et al.*, 2015). The threat of plastic waste in Indonesia's oceans is increasing significantly every year (Syakti *et al.,* 2017; Purba *et al.,* 2019). Plastic is lightweight, elastic, durable, and cheap, so it is very popular and has become a part of modern life. Besides making a big contribution to human activities, plastic waste seriously threatens ecological sustainability. Plastic waste takes centuries to degrade in nature (Klein *et al.*, 2018; Mierzwa-Hersztek *et al*., 2019; Chamas *et al.*, 2020). Plastics that have undergone a degradation process are known as microplastics if the size is less than 5 mm (Ivleva *et al.*, 2017; Frias & Nash, 2019).

Microplastics can accumulate in huge quantities in seawater and sediments (Hidalgo *et al*., 2012). The presence of microplastics in the environment is a serious problem due to their size and persistent properties which often contain chemicals and are potentially toxic when consumed by organisms (Beaumont *et al.,* 2019). Marine microplastics are also found in marine animals, such as fish, bivalves, and epibionts (Li *et al*., 2016; Baalkhuyur *et al.*, 2020; Davies *et al.*, 2021)

Epibionts are an individual or group of organisms that attach to and grows on either a living organism or an inanimate object. An epibiont may be attached to a wet substrate temporarily or permanently (Wahl, 2020). One of the places where epibiont attaches is green mussels shells (Raman *et al.*, 2013; Taib *et al.*, 2016; Nagabhushanam, 2020). In the waters of the east coast of Thailand, microplastic concentrations in *Balalanus amphitrite* range from 0.23 to 0.43 items/g (Nadeeka *et al.*, 2017). Studies on microplastic content in green mussels have been carried out in global waters. In China, microplastics were found in green mussels with 1.52-5.36 particles/gram (Qu *et al.*, 2018). In waters of Lae-Lae Island, Makassar showed that green mussels contaminated by microplastics in the form of fiber with a relatively low average concentration of 0.04 ± 0.02 - 0.13 ± 0.08 fiber/g wet weight (Fachruddin *et al*., 2020). However, there have been no studies on the concentration of microplastics in epibionts associate with green mussels that live in Indonesian waters, especially in Makassar. Several studies have demonstrated the ability of uptake, ingestion and engestion of bivalves and barnacles (Goldstein & Goodwin, 2013; Woods *et al.,* 2018; Ward *et al.*, 2019; Piarulli and Airoldi, 2020). Indeed, the studies revealed deductively the ecological role of epibionts together with green mussels, as their host, in reducing microplastic pollution. This study aims to analyse the concentration of microplastics in green mussels and their epibionts that live in the waters around Lae-lae Island, Makassar.

Materials and Methods

This research was conducted from September to November 2020. Sampling was carried out in the waters of Lae-Lae Island, Makassar (Figure 1). Furthermore, sample analysis was conducted at Aquatic Animal Physiology, Productivity and Water Quality Laboratories, Department of Fisheries, Faculty of Marine and Fisheries, Hasanuddin University, Makassar. The tools and materials used in this study were: A cool box, a global positioning system, a vacuum pump, a calliper, a digital scale, a stereo microscope, petri dishes, a camera, an identification book of Barnacles Thoracica Excluding The Pyrgomatidae and Acastine (Chan *et al*., 2009) and a Photographic Atlas of Marine Biology (Wisehart *et al.,* 2012). The materials used were potassium hydroxide (KOH) solution (10%), distilled water, and Whatman cellulose nitrate filter 47 mm with a pore size of 0.45 µm.

Sampling and Sample Preparation

Mussel sampling was performed using the purposive random sampling method. Using a knife, the green mussels were collected from the docks and hulls of boats on Lae-Lae Island. Afterwards, the samples were transferred to the laboratory using a cool box. The green mussels were separated from epibionts in the laboratory using a knife. The green mussels' morphometry parameters, length, width, and height were measured using a calliper. The wet tissue weight of green mussels and epibionts attached to green mussels were measured using a digital scale. The types of epibionts found were *Balanus sp* and *Euchema spinosum*. *Balanus sp* was the dominant genus. The samples were divided into three groups of shell length: Small (2.42-4.40 cm), medium (4.41-8.01 cm), and large (8.02-14.62 cm) groups. Based on the microplastic content, the epibionts associated with green mussels were also divided into four groups, namely (1) microplastics in green mussels with epibiont attached, (2) microplastic in green mussels without epibiont attached, (3) microplastics in epibiont attached to green mussels, (4) microplastics both in green mussels and epibiont. Figure 2 depicts the morphological parameters of green mussels, *Perna viridis* (Yaqin *et al.,* 2015).

The green mussels and epibiont tissue were put into sample bottles containing 10% KOH in a volume three times the weight of the samples. The choice of KOH as a solvent was due to its nature which was more effective at removing organic matter than other solvents such as H_2O_2 and maintaining the condition of microplastic particles. After that, the samples were left for approximately 2-3 weeks at room temperature until the organic matter of the green mussels and epibiont tissue was completely dissolved (Foekema *et al*., 2013)**.**

Microplastic Identification

Particles were identified using visual observation under a stereo microscope. Before conducting microplastic observations, the dissolved samples were filtered using Whatman cellulose nitrate filter 47 mm with a pore size of 0.45 μm. Afterwards, the microplastics on the surface of the filter were identified using a stereo microscope with 40x magnification.

Figure 1: Map of sampling locations
Source: Google Satellite 2020

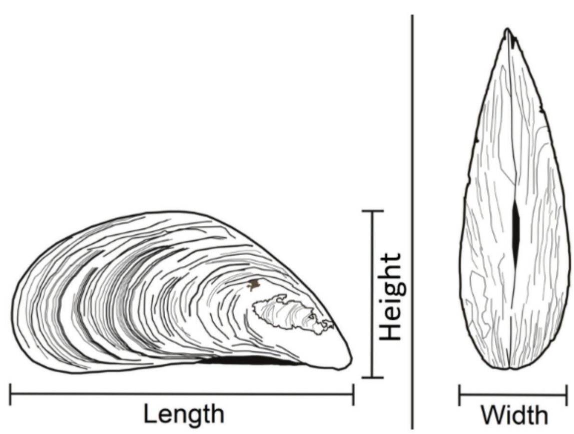

Figure 2: Morphological parameters of green mussels, *Perna viridis* (Yaqin *et al*., 2015)

Subsequently, microplastic images recorded with the camera were documented and then measured using Image J Software.

Calculation of Occurrence Frequency

The frequency of microplastics in the epibiont and green mussel samples was the percentage of the number of shells identified as containing microplastics. The frequency of occurrence was calculated by dividing the number of organisms containing microplastics by the number of organisms observed multiplied by 100%.

Concentration of Microplastic

The concentration of microplastics in the samples was the microplastics that were expressed in units of item/g (wet weight). Microplastic concentrations can be calculated by dividing the number of microplastic in organisms by the wet weight of organisms (Khoironi *et al*., 2018).

Data Analysis

To determine differences in microplastic concentrations in epibionts and green mussels, the concentration data were tested using the one-way ANOVA test. Before the ANOVA test, the data were tested for normality distribution and homogeneity. If the data were normally distributed and homogeneous, then the parametric ANOVA test was used, whereas if the data were not normally distributed, the data were transformed with the log transformation, and the normality and homogeneity were tested again. After being transformed, the data were normally distributed and homogeneous, then continued with the parametric ANOVA test. However, the data were not normally distributed and homogeneous, so non-parametric ANOVA tested the data. Data that did not meet the requirements for statistical analysis were analysed descriptively.

Results and Discussion

Concentration of Microplastics

One hundred and ten mussels were collected and divided into three groups based on shell lengths: small (2.42-4.40 cm), medium (4.41-8.01 cm), and large (8.02-14.62 cm). The number of samples in the small, medium, and large groups was 32, 41, and 37 animals. There were two types of epibiont which were *Balanus* sp. and *Eucheuma spinosum*. Because the presence of *Balanus sp* dominated the epibionts attached to green mussels, the use of epibiont terminology in the results and discussion section is more directed to *Balanus* sp. which has feeding behaviour as a filter feeder.

The current study found that the microplastic concentrations in epibionts were 5.592-16.040 items/g (wet weight). The concentrations were high when compared to the study of Nadeeka *et al*. (2017) which found that the concentration of microplastics in the *Balanus* sp. from the waters of the east coast of Thailand at 0.23-0.43 items/g. The study by Xu *et al*. (2020) showed that the concentration of microplastics in barnacles, *Fistulobalanus albicostatus* in Hong Kong waters ranged from 2.03 to 2.66 items/g (wet weight). The different concentrations of microplastics found in Lae-Lae Island, Makassar, Indonesia and those found in Thailand and Hong Kong are thought to be caused by differences in the concentrations of microplastics in the waters of the three places. The comparison of the microplastic concentrations of each size group of mussels can be seen in Figure 3 and Table 1.

Table 1: The concentration of microplastics in epibiont in various groups of mussel shell lengths. Small (2.42-4.40 cm), medium (4.41-8.01 cm) and large (8.02-14.62 cm)

Size	Average (item/g)	Standard Error
Small	12.208	3.970
Medium	16.040	4.078
Large	5.592	2.881

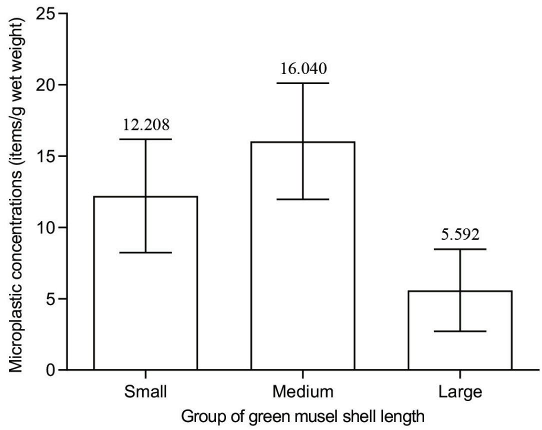

Figure 3: The concentration of microplastics in epibiont in various groups of mussel shell lengths. Small (2.42-4.40 cm), medium (4.41-8.01 cm) and large (8.02-14.62 cm). There were no significant differences in microplastic concentration between the shell length groups statistically (p <0.05). The bars are the standard error

There were no significant differences ($p > 0.05$) in the concentration of microplastics in the epibionts associated with different lengths of green mussel shells. This showed that the epibionts associated with green mussels with different shell lengths had the same opportunity to filter microplastics. The greater the length of the green mussel shell as substrates of the epibionts did not reduce the ability of the epibiont to filter microplastics. The presence of microplastics in the waters is responded to by filter feeder organisms as other organic and inorganic particles in the waters so that epibionts and green mussels take up the microplastics. The presence of associated epibionts in blue mussel shells did not indicate spatial competition between species in any form, so it did not cause negative effects for the two organisms (host and epibionts) (Laihonen & Furman 1986; Gutiérrez & Palomo, 2016). Byssogenesis of blue mussels is reduced by the presence of epibiont (Garner & Litvaitis, 2017) which can have implications for the mussel's ability to withstand water movements such as waves and currents.

Puccinelli and McQuaid, (2021) observed that the relationship between mussels and barnacles was amensalistic. This means that the relationship does not impact the mussel and harms the barnacles. However, very complex relationships that do not reflect the relationship defined by the classical ecological theory are demonstrated by Zuykov *et al*. (2021) that occur between the blue mussel and the epibionts (Zuykov *et al*., 2014; Zuykov *et al*., 2018a; Zuykov *et al*., 2018b; Zhao *et al*., 2019; Zuykov *et al*., 2020).

Microplastic Form

Three forms of microplastics were found in epibionts, namely fibre, fragment, and film (Figure 4).

The concentration of microplastics in epibionts based on shape can be seen in Figure 5 and Table 2. The microplastics found in epibionts in Lae-Lae Island waters were in the form of fibres, fragments, and films. There are no significant differences between fibre, fragment, and film concentration. Xu *et al*. (2020) also found that fibres, fragments, and films were the microplastics contaminated in epibiont in Hong Kong waters. Descriptively, fibre was found to be the most abundant compared to other forms. Barrows *et al*., (2018) found that fibre microplastics were the dominant type in global marine waters, with a percentage reaching 91% compared to other microplastics.

The number of microplastics in the form of fiber at the study site was expected to come from boat ropes and fishing gear used by a fisherman on Lae-Lae Island and its surroundings. Browne *et al*. (2011)suggested that fibre microplastics

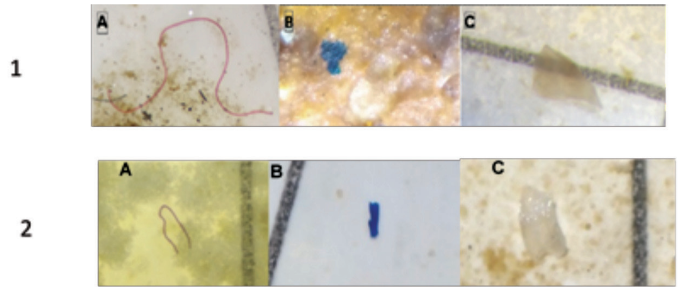

Figure 4: The form of microplastics found in epibionts and green mussels. (1) Microplastic of epibionts, (2) microplastic of green mussel. (A) Fibre, (B) Fragments, and (C) Film

Table 2: The concentration of microplastic shapes found in epibiont in various groups of mussel shell lengths from Lae-Lae waters

Shape	Average (item/g)	Standard Error
Fibre	9.579	2.201
Fragment	4.713	1.345
Film	5.468	1.235

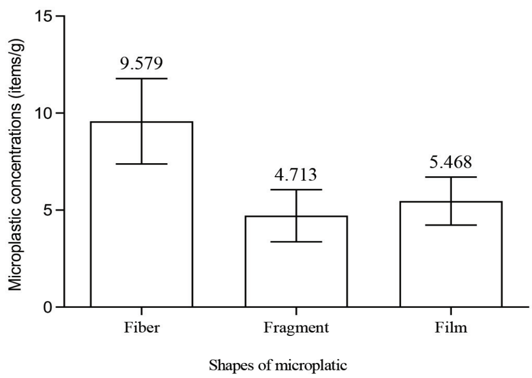

Figure 5: The concentration of microplastic shapes found in epibiont in Lae-Lae waters. There were no significant differences in microplastic concentration between the shapes statistically ($P < 0.05$). The bars were the standard error

originated from cloth, ropes widely used by the community for fishing gear, and fishing boat ropes. In addition to fibres and fragments, microplastics in the form of films were also found. Film-shaped microplastics have an irregular shape, such as thin sheets, are flexible in texture, and have a transparent or clear character. The sources of these microplastics were the use of plastic bags and plastic packaging (Kovač *et al*., 2016). Wu *et al*. (2019) suggested that film-shaped microplastics had lower density properties compared to fibre, causing the presence of film-form microplastics to be very dynamic in water.

Microplastic Concentration Based on Colour

Four colours of microplastics were observed which were red, blue, white, and black (Figure 6). The concentration of microplastics based on colour in epibionts can be seen in Figure 7 and Table 3.

The results of statistical tests using one-way ANOVA analysis revealed that the concentrations of microplastics in epibionts based on colour were not significantly different ($P > 0.05$). Hidalgo-Ruz *et al.* (2012) also observed various microplastic colours, i.e. white, blue, green, red, grey, yellow, brown, and pigmented colours. The colour variations of microplastics observed in the study were influenced by the sources of plastic waste around the sampling location. The blue microplastics were predicted to have originated from the degradation of fishing gear used by fishermen. The colour variation of the microplastics was also caused by the length of time the microplastics were exposed to sunlight so that the microplastics experience oxidation which caused discolouration of the microplastics (Browne *et al.*, 2011). The colour of the microplastic particles observed in the study could be the original colour of the microplastic, or it could be caused by the colour degradation that occurs due to the release of the original colour due to photochemical processes and other processes that occur in the water (Zhu *et al.,* 2020; Zhang *et al.*, 2021).

Frequency of Microplastic Occurrence

The frequency of microparticle occurrence indicated the number of green mussels and epibiont contaminated with microplastic particles. Figure 8 depicted that the highest

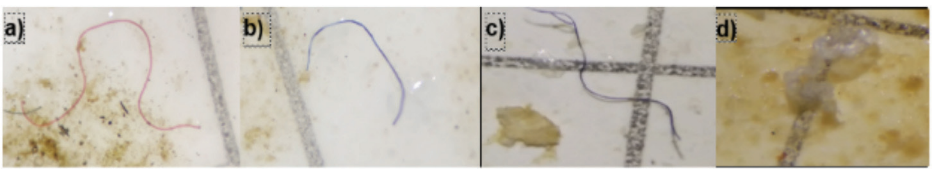

Figure 6: Color microplastics found in epibionts in Lae-Lae waters.
(a) Red, (b) Blue, (c) Black, (d) White

Table 3: Microplastic concentration based on colour in epibiont

Colour	Average (item/g)	Standard Error
Blue	8.828	2.177
Red	7.780	2.968
White	5.107	1.487
Black	4,751	2.839

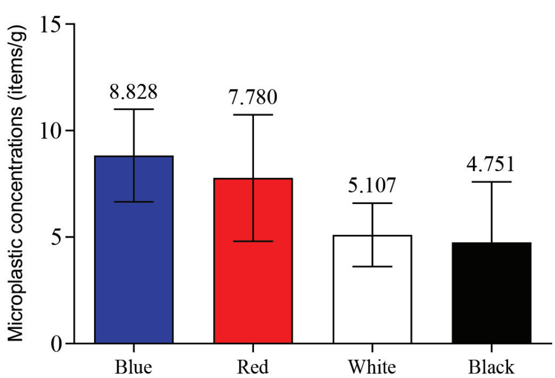

Figure 7: Microplastic concentration based on colour in epibiont. There were no significant differences in microplastic concentration between the colour statistically (P < 0.05). The bars were the standard error

percentage of the frequency of the occurrence of microplastics was 2 groups at 57%, followed by 3, 1, and 4 groups at 33.71%, 15.73%, and 12.36%, respectively. The results revealed that the non-epibiont green mussels were more contaminated by microplastic than the epibiont-associated green mussels. These suggested that epibionts associated with green mussels could reduce microplastic contamination in green mussels. The data also illustrated a commensalism or parasitism relationship between green mussels and epibionts ecologically. Laihonen and Furman (1986) described that by attaching to blue mussels, epibionts obtained more food, therefore they grew better when attached to blue mussels.

On the contrary, Buschbaum and Saier (2001) conducted a field experiment that showed the growth of mussels reduced by epibiont. Laihonen and Furman (1986) depicted a commensalism relationship, while Buschbaum and Saier (2001) emphasized the parasitism relationship between blue mussels and epibionts. However, considering that the non-epibiont green mussel contains more microplastics than the other groups, especially the epibiont-associated green mussel, we could conclude that the relationship between a green mussel and epibiont was parasitic. However, if we considered that microplastic was something that endangered green mussels, then the existence of epibionts for green mussels in terms of microplastic were ecologically beneficial. So, the relationship between green mussel and epibiont could be mutualistic. It is due to the epibiont can reduce the chance for green mussel to be exposed to microplastics.

Goldstein and Goodwin (2013) observed that 33.5% of the gooseneck barnacles (*Lepas* spp.) were contaminated by microplastic in their gastrointestinal tract, ranging from one plastic particle to a maximum of 30 particles. Indeed, microplastic contaminated the barnacle since the nauplii stage and caused an inter-generational effect on the barnacle (Yu & Chan, 2020b).

The Concentration of Microplastics in Green Mussels and Epibiont

The concentrations of microplastics in the green mussel and epibiont tissues are the ratios between the number of microplastic particles obtained with the epibiont total weight and the wet weight of green muscle tissue. The comparison of microplastic concentrations based on the relationship between a green mussel and epibiont can be seen in Figure 9 and Table 4.

In contrast to the frequency of microplastics, the concentration of microplastics describes the number of microplastics per organism weight. The results showed that the associated epibiont green mussels (1) and non-epibiont green mussels (2) contained no different microplastic concentrations ($p > 0.05$). These two groups significantly differed in their microplastic content compared to those in epibiont (3) and

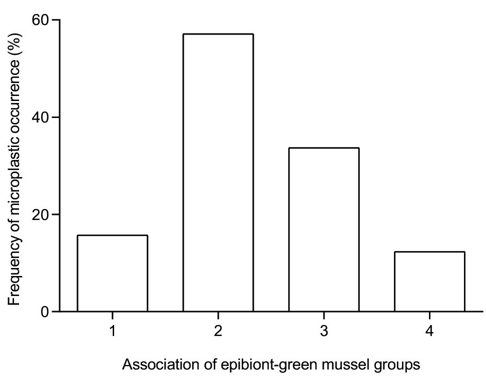

Figure 8: Frequency of occurrence of microplastics. (1) microplastics in green mussels with epibiont attached, (2) Microplastic in green mussels without epibiont attached, (3) microplastics in epibiont attached to green mussels, (4) microplastics in green mussels and epibiont

Table 4: Microplastic concentrations in green mussels' tissue and epibiont

Group	Average (item/g)	Standard Error
1	0.918	0.116
2	0.947	0.138
3	11.915	2.296
4	11.001	4.002

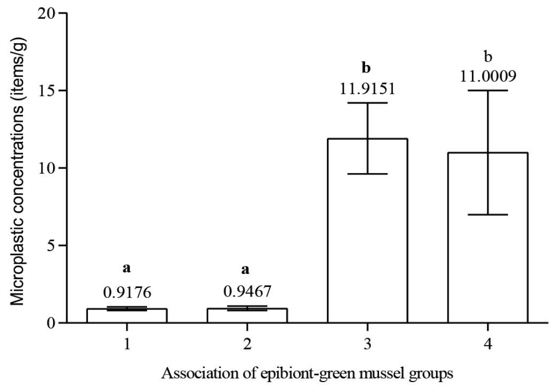

Figure 9*:* Comparison of microplastic concentrations in green mussels' tissue and epibiont: (1) microplastics in green mussels with epibiont attached, (2) Microplastic in green mussels without epibiont attached, (3) microplastics in epibiont attached to green mussels, (4) microplastics in green mussels and epibiont. The bars were the standard error

green mussel and epibiont (4). The microplastic content of groups 1, 2, 3, and 4 were 0.9176, 0.9466, 11.6578, and 11.0009 items/g wet weight. In Hong Kong, Xu *et al*. (2019) observed that microplastic-contaminated barnacles ranged from 0.26 to 10.26 items g^{-1}.

In Thailand, Nadeeka *et al.* (2017) found that the microplastic concentration in barnacles was 0.23–0.43 item/g. Microplastic-contaminated green mussels (*Perna canlicultaus*) contain 0.48 particles/g tissue (wet wt) (Webb *et al.,* 2019). In Jakarta Bay, green mussels with length shells 3, 6, and 9 cm contained microplastic 5.35, 24.99, and 39 particles/g, respectively (Fathoniah & Patria, 2021). Microplastic concentrations in green mussels that inhabit Pangkajene Kepulauan South Sulawesi which were found in small (2-3.9 cm), medium (4-5.9 cm), large (6-7.9 cm) shells were 0.60, 0.45, and 0.32 items/g respectively (Ramli *et al*., 2021). In *Mytilus coruscus*, micro/nano plastic accumulation in the digestive tract was negatively size-dependent (Wang *et al.,* 2021).

In mussels, microplastic not only reduced byssus production (Green *et al*., 2019; Rahmat *et al*., 2020) and the attachment strength of byssus but also altered the hemolymph proteome which had implications for vital biological processes such as immune regulation, detoxification, metabolism and structural development (Green *et al.*, 2019), abrasion of the ciliated structure of the gills, shrinkage of the nucleus of the digestive gland (Vasanthi *et al*., 2021) which eventually lead to death in green mussels (Rist *et al*., 2016; Rahim *et al*., 2020).

Microplastic per se may not harm barnacles, as a study shown by Yu and Chan (2020a) exposing naupliar and cypris larvae of barnacles to polystyrene. In that study, there were no microplastic effects on mortality, settlement, and metamorphosis, feeding rate, and development of barnacle larvae. However, when the barnacle larvae were exposed to plastic leachates, the larvae experienced death and if they did not die, their ability to settle down was impaired (Li *et al*., 2016). In the barnacle, *Amphibalanus amphitrite*, microplastics did not affect larvae or adult animals. However, the offspring of barnacles exposed to microplastics experienced delayed development and death (Yu & Chan, 2020b).

Microplastic Size

The sizes of microplastics found in the body of green and barnacles were different. The maximum length of the microplastic was 4.72 mm, while the minimum length was 0.03 mm. The microplastic length ranges for 1, 2, 3, and 4 classes were 0.03-4.56, 0.12-3.82, 0.03-4.72, and 0.03-4.56 mm, respectively.

The lengths of the microplastics in epibionts were different for each size of the green mussels. The microplastic lengths in epibiont found during the study had a different size range for each shell length. The minimum size of microplastics was 0.03 mm, while the maximum length was 4.72 mm. The ranges of the microplastic length of epibiont in each group of green mussel shell length, small, medium, and large, were 0.15-4.61, 0.03-4.72, and 0.16-4.49 cm, respectively. The difference in the microplastics' length indicated that the epibiont in green mussels could ingest microplastics at various microplastic lengths. This is supported by a filtration strategy carried out by the epibiont, especially the barnacle, in filtering micro-organisms in the waters including microplastics. Barnacles use denticles and setae to capture food and support the muscular system responsible for movement control to carry out the filtration process properly (Hindenberg *et al*., 2022). Zhang *et al*. (2022) found microplastics in *Balanus albicostatus* ecotype A and ecotype B that live in Yellow Sea China with a length size range of 69.83 to 3,743.20 μm and 194.66 to 2,885.65 μm respectively.

Finally, it can be said that this research describes the association between epibionts and green mussels as hosts in dealing with microplastic pollutants in waters. However, several things have not been part of this research: The type of microplastic polymer found in epibiont and green mussels. Second, this study did not examine the correlation between the microplastic content in the column and the microplastics in the green mussel and epibiont. Research on the types of microplastic polymers can provide information on the certainty of the microplastics found. Research on the correlation between microplastics in waters and green mussels and epibionts will illustrate the ability of green mussels and their epibionts to filter microplastics in waters. This latter information can be the basis for using green mussels and epibionts apart from being sentinel organisms in ecotoxicological studies, they can also be used as microplastic bioremediation agents in situ.

In conclusion, the epibionts from Lae-Lae Island, Makassar, were identified to contain microplastic particles. Various forms of microplastic particles were found in epibiont including fibres, fragments and films. The microplastic colours found were blue, red, black and white. The identified microplastic sizes ranged from 0.03 to 4.72 mm. The highest frequency of microplastic presence was in the non-epibiont green mussel, with the percentage of occurrence frequency at 57.4%. The lowest percentage of occurrence frequency was in green mussels and epibiont at 12.36%. The microplastic concentrations in epibionts, which were analysed based on differences in the length of the green mussel shells attached to the epibiont, were 5.592-16.040 items/g wet weight.

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