Analysis location of microplastics in green mussel's *Perna viridis* Gills (Linnaeus, 1758)

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Abstract. This study describes the location, type, and range of microplastic sizes found in the gills of green mussel *Perna viridis*. This study continues the results of Fathoniah's research on 2017 about microplastic abundance in green mussels at Kamal Muara, North Jakarta. Sample of 3 green mussels with a size of about 7 cm was taken from Kamal Muara. Gills from both the outer and inner demi branch were isolated and made as preparations. Every part of the gills marked into posterior, anterior, proximal, and distal. After that, the gills were observed under the light optical microscope. Position and type of microplastic in the gills was recorded and measured using the LAZ EZ application. The results showed that the distal area of the gill contains more microplastic than proximal. The dominant microplastic group found in the gills of green mussels was fiber. The range of microplastic sizes found in green mussel’s gills was 20-4500 μm.

1. Introduction
Plastic waste is the most common waste found in the oceans. Plastic waste in the ocean will eventually be degraded due to several factors, such as radiation from the sun, temperature, hydrolysis of water, and biodegradation. Plastics that degraded to <5mm (5000 μm) are called microplastics [1]. Microplastic is very small, so marine organisms can inadvertently have ingested and eventually accumulate microplastics in their body. Microplastics in marine organisms can move to higher trophic levels organism through the food chain [2]. Microplastics also can cause internal injury in organ of organism, obstruction of the digestive tract, and can disrupt the physiological functions of the organism's body [3].

Green mussel (*Perna viridis*) is filter feeder organism. Green mussel filtering the water that contains particles with gills. The water pumped through the inhalant chiffon, then filtered with gills [4]. According to Manalu et al [5], Jakarta Bay waters are polluted by plastic waste that can enter the body of marine organisms such as green shells. This was proven by Fatoniah and Patria [6] which stated that green mussels in the area were contaminated with microplastics. Most microplastics are found in the breathing shells of green shells, but the location of accumulation, type of microplastic, and microplastic size contained in that section is unknown.

Microplastics can enter the body of green mussels. Gills accumulated microplastic the most because it filtered suspended particles from the water before entering the mantle cavity. The presence of microplastic in the gills of green mussels can disrupt its physiological functions. It is important to know...
the location and size of the microplastic in the gills and the type of microplastic groups that predominate in the gills of green shells in Kamal Muara. This study aims to: (i) determine the location and position of most microplastic accumulations in the gills of Perna viridis green shells, (ii) analyze the dominant microplastic groups found in the gills of green shells in Kamal Muara, and (iii) determine the range of microplastic sizes lodged in the gills of green mussels in Kamal Muara.

2. Materials and methods

2.1. Materials
The material used in this research is monocular microscope [Nikon], light microscope [Leica], LAZ EZ imaging software, mobile camera [iPhone], ruler, tweezers, needles, surgical scissors, pipettes, paraffin surgical boards, and 100 mL beaker glass [IWAKI]. 3 individu of green mussels with approximantly size 7cm from 3 different points. Other materials are aquades, filter paper with a pore size of 0.42 μm [Whatman], tissue, gloves, disposable mask [Sensi], 70% alcohol, glass jars, glass preparations, and cover glass.

2.2. Method

2.2.1. Sampling. Sampling was carried out from Kamal Muara. Sampling was carried out at relatively the same depth of around 5m to 6m. The green mussels that have been taken are then preserved in glass jars containing 70% alcohol and taken to the Marine Biological Laboratory.

2.2.2. Isolation and preparation of Perna viridis green mussels. Green shells are opened and the gills are dissected using surgical scissors and transferred to preparations glass. Samples were dropped with filtered aquades and then carefully closed by object glass [7]. Gills are relatively thick if directly used as a preparation, so the sample must be pressed slowly until translucent. The preparations are marked on the posterior, anterior gills, food grooves (proximal), and the axis (distal) so that they are not confused.

2.2.3. Analysis of microplastic location of Perna viridis green mussels. Samples of the green mussels were observed under the light microscope to see the microplastic location in each part of the gill. Samples were then observed under a Leica camera microscope to find out the type of microplastic (fiber, fragment, particles, or pellet particles) that accumulated in the gills of green mussels. Microplastic particles were observed with magnifications of 4x10, 10x10 and 100x10. Each microplastic in the gill portion of the green shell was observed for its type and recorded. The microplastic contained in the gills was measured using the LAZ EZ imaging software application. Measurements are made by opening the menu section then clicking "line", after that drag the line according to the microplastic width observed. Microplastic size will be visible on the screen. The observed particles were counted and photographed [7].

2.2.4. Data processing. The size of the gills and the number of microplastics found in each part of the gill are recorded. Each microplastic that is found from observations will be grouped according to its type and analyzed which type is frequently encountered and least encountered then represented in the form of a pie chart. Microplastics from each group encountered were measured and compared with the size of the gills and made a mapping. The data that has been processed is then described and adjusted to the literature.

3. Results and discussion

3.1. The most frequent type of microplastic found in the gills of Perna viridis Green Mussels
Microplastic types found in Perna viridis green mussels are fiber, film, and fragment. Pellet were not found in the observation using a light microscope. Total microplastics found in the three green shell
samples are shown in Table 1. The most common microplastics are fiber types as much as 44% of the total microplastics of all parts of the green shell gills, followed by 30% film types and fragment types as much as 26%.

Table 1. Total microplastic of each individual based on microplastic type group.

| Microplastic type | Sample I | Sample II | Sample III | Total | Percentage |
|------------------|----------|-----------|------------|-------|------------|
| Film             | 31       | 32        | 33         | 96    | 30%        |
| Fragment         | 28       | 26        | 27         | 81    | 26%        |
| Fiber            | 39       | 43        | 42         | 124   | 44%        |
| Total            | 301      |           |            | 100%  |            |

Percentage of Microplastic type Composition in Green Mussles Gills were 44% fibers, 30% film, and 26% fragments. Pellet type were not found because it has a really small size (0.05—6 μm) and difficult to see just by using a light microscope. Fiber was the most common type that found in green mussel gills because in the Kamal Muara area many fisheries activities carried out by the majority of the community [8]. The fishing activity uses source of fiber microplastics such as fishing lines, fish nets, and ship mines. In addition, the substrate used for the cultivation of green mussels is a rope, which is also the source of fiber microplastics.

Least fragment of microplastics types found in the gills of green shells. Fragment microplastics have a density that tends to be heavier than film and fiber microplastics so most of them sink into the bottom of the water and are rarely found suspended in the water column [9,10].

3.2. The microplastic size range found in Perna viridis green mussel

Fragment microplastic types found in the size range 20-100 μm (Figure 1), fiber microplastics types found in the size range of 600 - 4500 μm (Figure 2) and 130-2000 μm for film microplastics types (Figure 3). Fiber and film type microplastics that were seen when observations have a relatively larger size than the type of fragment microplastics.

According to von Moos [11], only microplastics size under 100 μm can enter the digestive tract of bivalves are. Microplastics sizes found in green mussel gills are about 20-4500 μm. Previous studies have not found the ability of gills to grind food particles into smaller sizes. This support the statement that green mussles sort their food particles based on size, quality, and other factors such as the nutrients contained by the particles [12-14].

Figure 1. Fragment microplastic found in green mussel’s gills. The red arrow shows fragment microplastic with scale = 50 μm.

Figure 2. Fiber microplastic found in green mussel’s gills. The red arrow shows fiber microplastic with scale = 500 μm and blue arrow shows the filament of the gills.
Figure 3. Film microplastic found in green mussel’s. The red arrow shows film microplastic with scale bar = 50 μm.

3.3. Microplastic locations in the gills of *Perna Viridis* green mussels

Based on observations, microplastics tend to be more in the axis (distal) than the food groove (proximal) (figure 4b). Microplastic abundance was also compared in the posterior-anterior portion and the outer-dembbranch demibranch. Microplastic abundance in the posterior is more than in the anterior portion (figure 4c). The distribution of plastics in the outer demibranchs is more than in demibranches (figure 4a.). The data graphic is served in Figure 4.

Figure 4. Comparison of plastic abundance location in green mussel’s gills; a) comparation between axis and food groove; b) comparation between inner demibranch and outer demibranch; c) comparation between anterior and posterior.

Microplastic particles are more commonly found on the axis. This is because the mussels are able to choose food particles to be digested based on nutritional quality so that most food grooves are food particles. Nonnutritive particles like microplastics are rejected as pseudofeces. Water flow containing pseudofescs is carried to the exhalent siphon that flowing through the axis and some of microplastics attach there and hard to remove [15].
Microplastic is more abundance in the posterior part than anterior. This is because the main pathway of particles transport begins through inhalant siphon near the posterior part. The pseudopheses component will pass through the axis region (distal) and exit through the exhalation siphon which is also near the posterior part [15,16].

The distribution of microplastics in the inner demibranchs tends to be less than on the outside. But, particles that has been selected by the shell will be retained on the outerdemibranch, while the pseudofeses are moved to the inner demibranch and removed. This might be happened because microplastics in the outer demibranch have the sizes according to the preference of shells [3]. Final mapping of microplastic position in green mussel’s gills can be seen in figure 5.

![Figure 5](image.png)

Figure 5. Mapping of microplastic location in green mussel gills; black arrow shows the water flow; white arrow shows the food flow; red dots are presenting microplastic and green dots are presenting food particles.

Plastic that attached to the gills can block the transfer of food particles carried by cilia to food groove. This situation can make the green mussels experiencing nutrient deficiencies and disrupted its growth. In addition, microplastics particles that accumulate in green mussels can also consumed by humans and settle in the human body. Plastic accumulation in the body of living organism can be harmful for the physiological functions [3].

4. Conclusion
The most abundance microplastics in the gills of green mussels is located in the axis (distal) posterior to the outer demibranch. The dominant microplastic type found is fiber, and the size range of the microplastics found in the gills were 20 - 4500 μm.

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