Microplastic abundance in the water, seagrass, and sea hare
*Dolabella auricularia* in Pramuka Island, Seribu Islands, Jakarta Bay, Indonesia

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**Abstract.** This research focused on the amount of abundance of microplastics in the sea hare *Dolabella auricularia* as well as the seagrass fields along the southern coast of Pramuka Island. Sampling of 8 individuals of *Dolabella auricularia* along with seagrass *Cymodocea rotundata* leafblades was done at the southern coast of Pramuka Island, after which the samples were preserved and brought to a laboratory in Depok for microplastic analysis. The sea hares’ digestive tracts were extracted and dissolved in strong nitric acid. A 1 cm² portion of a seagrass leaf blade was cut for observation. Prepared samples were observed under a monocular microscope and further analysis was done. Microplastic fibers and film particles were found in highest abundance within the digestive tracts of each sample of *Dolabella auricularia* as well as on the seagrass surface where the sea hare obtains its algae from, with fragment particles found in much lower amounts. Overall, number of microplastics was found between 40.1 to 73.7 particles/g weight of sea hare digestive tract and the estimated amount of microplastic found at seagrass leafblade was 185 particles/cm². Results provide evidence that microplastics in the ocean brought by water currents could adsorb on to algae through which it enters the food chain as it is consumed by marine biota.

1. **Introduction**

Microplastics are solid micro-particles made out of synthetic polymer [1]. The National Oceanic and Atmospheric Administration decided upon the designated size of 5 mm for a plastic to be classified as a microplastic [1]. The small size and light weight of microplastic allow it to be easily distributed throughout the oceans that it could be found at every depth throughout the water column and in every ocean around the globe, making it a considerably large threat to global marine ecosystems [2]. Microplastics are so small that they could easily be swallowed along with food or through respiration by various marine organisms as small as zooplankton and as large as baleen whales. Accumulation of microplastic particles in an organism’s body may affect it in multiple ways such as abrasion against internal organs or clogging of ducts or vessels of the body’s organ systems. Several components of microplastic such as plastic monomers could also become toxins that inhibit the body’s physiological processes [3,4].
Research has shown that other than the accidental consuming by marine biota, microplastics could also adhere to surfaces of macroalgae. Presence of microplastic on such primary producers contributes to the entering of microplastic into the marine food chain [5]. Analytical research of microplastic has been done towards various marine animals such as fish and mussels with varying feeding behaviours (filter feeders, sediment feeders, or detritus feeders) with microplastic particles found in nearly all samples such as the case in Karlsson et al.’s research in 2017 [6].

This research focuses on a mollusk, the wedge sea hare Dolabella auricularia. It is neither a filter feeder, a sediment feeder, nor a detritus feeder, as it feeds on nearly all types of algae that can be found around its habitat [7]. The research is done in Pramuka Island, Seribu Islands, Jakarta where the sea hare is commonly found among the seagrass along the southern coast of the island. It is believed that microplastics could be found in high abundance in the sea hares found on the island’s coast as Pramuka Island has one of the most polluted shores among the islands of the Seribu Islands district. The reason for it is likely because the island is geographically located in the southern area of the district, not too far from the Bay of Jakarta, an estuary at which 13 rivers conjoin and flow out unto the sea, carrying all the pollutants from the mega city to the ocean [8].

The wedge sea hare local to Pramuka Island is known to feed on microalgae on the surface of seagrass leaves among which the sea hares live. Such information was given by the local people of the island who have often observed the behavior of these mollusks. Hence, sampling was done in the shallow waters of the seagrass fields, taking the sea hares and its primary source of food, the seagrass leaves. Presence of microplastic in the water brought by the currents could carry it to the seagrass leaves where it could adhere and therefore it is very much possible that as the sea hares feed on the microalgae by sucking on the surface of the leaves, they also consume the microplastic that was present there.

The research aims to: 1) analyze the types and abundance of microplastic eaten by Dolabella auricularia by means of extracting its digestive tract and, 2) analyze the presence and abundance of microplastic particles on the leaf surface of the seagrass Cymodocea rotundata upon which the algal feed of sea hares are found. The qualitative aspect of this research would be the types of microplastic found in each sample, as microplastics can be classified into various groups based on its size and shape. The common types are the round granules, circular pellets, long and thin fibers, thin and clear films, or large and irregular fragments [9]. The quantitative aspect would be the number of microplastics found in each sample, per gram of each sea hare sample, per mL of the water sample, and per cm² of the seagrass sample. The quantitative and qualitative results may hopefully provide more information on an alternative path taken by microplastic to enter the marine food web that is through adhesion onto the surface of primary producers and then consumed by marine biota. The theory, if proved, could be additional information in the environmental fight against plastic pollution and the importance of reducing it.

2. Materials and methods

2.1. Sample origins and sampling process
Sampling was done on the 14th of April 2018 on the southern coast of Pramuka Island on the coordinates S 06.33690° E 106.68021° at around 6 to 7 am when the sea hares are out feeding. The sampling station of about a 15 meter radius around the mentioned coordinate was where 8 samples of Dolabella auricularia were taken along with 3 leaf blades of the seagrass Cymodocea rotundata. The 8 sea hares were placed into a plastic container box filled with alcohol 70% for preservation and sealed shut. The water sample was taken in a glass jar which afterwards was closed tightly with a lid. Another glass jar with a lid was used for storage of the seagrass leaves. All the samples were brought to the laboratorium in the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia in Depok for extraction and analysis.
2.2. Materials and equipment

Materials used for the research include distilled water, analytical nitric acid for use in extraction and analysis of the sea hare samples, and alcohol 70%. The analytical 65% nitric acid was obtained from Mallinckrodt chemicals. The remaining products were obtained from local chemical material stores. Equipment used included a Gold Cross dissecting set, Pyrex beakers with various sizes (250 mL, 500 mL, 1000 mL, and 2000 mL), object glass counting chambers, regular object glass, a glass funnel, plastic tray, a dissecting tray, Garmin GPS for coordinate tagging on the field, a kitchen sieve, counter, Nikon 891451 monocular light microscope, and glass pipettes.

2.3. Extraction, observation, and analysis of microplastic

Each sample of *Dolabella auricularia* was weighed and then dissected to isolate its digestive tract from the esophagus to the anus. The digestive tracts were put into different containers containing alcohol 70% for preservation. Each container was labeled with a sample number and its original weight. Before dissolving of the body tissue, each digestive tract was re-weighed. Strong nitric acid was used to disintegrate each whole digestive tract as it is found to be one of the most effective reagents for the digestion method [10]. The 2000 mL and 1000 mL beakers were used for this process as each digestive tract sample was given acid in the ratio 1:3 of weight of digestive tract against volume of nitric acid. The mixture was left covered for 24 hours to dissolve the body tissue completely, after which distilled water was added in the ratio 1:7 of volume of acid against volume of distilled water. This was done to dilute the acid, allowing it to be safe for observation under the microscope.

For analysis of the seagrass sample, the length of each leaf blade was measured, after which 1 cm² area of one leaf was cut from the *Cymodocea rotundata* leaf blade and its surface was scraped to obtain every particle that could adhere on it. The substance obtained was placed directly on an object glass and given three drops of distilled water from a pipette. This was covered with the counting chamber and directly observed.

All of the samples were observed under a monocular light microscope using a counting chamber on top of the object glass. Each prepared sample solution of dissolved sea hare digestive tract has a surface volume of 15 mL from which 1 mL is taken for observation and counting. The types of microplastic on all samples – 8 sea hare samples and one seagrass sample – are identified based on the appearance and shape, and the abundance of each type of microplastic was counted. Further data processing was done to calculate an estimate of the total amount of microplastics in one sea hare individual and on a whole seagrass leaf blade based on the amount of microplastic found through observation. The final data were tabulated and discussed.

3. Results

3.1. Microplastic in sea hare samples

Three types of microplastic particles were found upon analysis of the solution containing the disintegrated sea hare digestive tracts. Films were found in highest abundance along with fiber particles. Fragments were found as well, though in much lower amounts. The highest number of fibers found in one sample was 140, belonging to the sample with the heaviest gross body weight out of the eight sea hares. That same sample contained the highest number of film particles, as much as 148 particles, and the highest number of fragment particles, as much as 17 particles. However, another sample also reached a number of 17 fragment particles, the sample belonging to the sea hare with the third heaviest gross body weight.

Data processing by calculation showed the estimated amount of particles that could possibly be found within the whole digestive tract of each individual sea hare, with the highest amount being 4575 particles (Table 1). Calculations were also done to show the amount of particles per gross weight of the digestive tract. The highest amount is 73.7 particles/g and the lowest amount is 40.1 particles/g (Table 1). It was found that the average amount of microplastic particles per gross body weight within a sea hare individual is about 54.9 particles/g based on the findings using the 8 samples.
Table 1. Abundance of microplastics found per sample of *Dolabella auricularia*.

| Sea Hare Sample # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|-------------------|---|---|---|---|---|---|---|---|
| Gross Body Weight (g) | 266 | 264 | 242 | 393 | 275 | 363 | 229 | 210 |
| Gross Weight of Isolated Digestive Tract (g) | 61 | 49 | 58 | 65 | 55 | 73 | 60 | 46 |
| Microplastic in 1 ml. of prepared solution | | | | | | | | |
| Fiber | 68 | 65 | 59 | 140 | 78 | 130 | 99 | 107 |
| Film | 99 | 96 | 87 | 148 | 113 | 94 | 105 | 108 |
| Fragment | 13 | 10 | 9 | 17 | 17 | 14 | 7 | 11 |
| Total | 180 | 171 | 155 | 305 | 208 | 238 | 211 | 226 |
| Particles/Individual | 2700 | 2565 | 2325 | 4575 | 3120 | 3570 | 3165 | 3390 |
| Particle/Gross Weight of Digestive Tract (/g) | 44.3 | 52.3 | 40.1 | 70.4 | 56.7 | 48.9 | 52.8 | 73.7 |

3.2. Microplastic in water and seagrass samples
The same types of microplastic particles found in the sea hare samples were also found in both the water sample as well as the surface of the seagrass leaf. Fibers and film particles were found in high abundance in both the water and on the seagrass (Table 2). Fibers were found in highest abundance in the water sample, while film particles were found in highest abundance on the seagrass leaf surface. A total of 185 particles were found on the 1 cm$^2$ surface of the seagrass leaf (Table 2).

The seagrass *Cymodocea rotundata* samples had an average leaf length of 7 cm with a width of 1 cm. It was calculated that if 1 cm$^2$ had 185 particles, a whole leaf blade with both sides taken into account would have 2,590 microplastic particles.

Table 2. Abundance of microplastics found on the seagrass leaf surface.

| Sample | Seagrass Leaf Surface (1 cm$^2$) |
|--------|----------------------------------|
| Fiber  | 57                               |
| Film   | 121                              |
| Fragment | 7                              |
| Total  | 185                              |

4. Discussion
The sample of sea hares had an extremely high abundance of microplastics that can be due to two factors which are the relatively large bodies of wedge sea hares and a considerably high level of pollution in the southern coast of Pramuka Island. The bodies of the wedge sea hare samples were around 15 to 25 cm in length and had gross weights ranging from 210 to 393 grams. Larger bodies mean that they would have to feed more than the average macrozoobenthos living in shallow water ecosystems such as the seagrass fields. The large amount of food and water intake would mean larger amounts of microplastic that are accidentally consumed. It is possible for microplastics to exit the body with feces however many would remain adhered to the walls of the digestive tract. Film particles, which composed most of the microplastics found in the sea hare, are small, thin, and light, originating from plastic bags and wrappings [9], that it is easy for them to adhere to the linings of the intestine. Fibers, which were also found in high abundance, are commonly derived from fishing lines or textile [9]. Pramuka Island as a
hub for fishermen [8], would most likely produce a lot of fishing line remains into the ocean. Textile waste could also reach the island as Jakarta, from which the water currents flow, is home to a number of textile plants [8].

Fiber particles are in abundance most likely because it is derived from textile waste as well as the degradation of fishing lines [11]. However, fiber seems to be more likely to wash away, while film is more likely to adhere to surfaces, hence why there are more film particles than any other microplastic particles on the digestive tracts of the sea hares as well as on the seagrass leaf surface.

Fragments are found in very low amounts in all samples. This could be due to the large nature of fragments, which are results of the breaking down of larger plastic material, yet can still be broken down into smaller particles [9].

There may be slight inaccuracy in the numbers given in the final research data that may be a slight overestimate of microplastics in nature due to a number of reasons such as the possible presence of microplastics in any of the equipment used that could possibly originate from the tap water used to wash them. However, results are the closest estimate. Some of the samples were counted twice or thrice and then averaged to obtain a more accurate number.

Results serve as evidence that the fore-mentioned theory of a microplastic pathway into the marine food web may be true. High amounts of microplastic were found in all samples, and of the same particle types. Fibers and film particles were very abundant in the sea hares and seagrass, while fragments were very few in all samples. The daily intake of water and algae for each sea hare is not known; hence it is not possible to find a correlation between food intake and amount of microplastic found. However, the data shows that primary producers, even in the form of microalgae on the surface of seagrass leaves, may act as a vector to bring microplastic from the marine environment into the marine food chain. The results are according to microplastic research done on seaweed as aquatic primary producers by Gutow et al. in 2015 that also show a pathway through which microplastics affect marine life [5].

5. Conclusion
The research succeeded in finding qualitative and quantitative data of microplastics in the seawater, seagrass, and sea hare samples from the southern coast of Pramuka Island. There is an estimate of 185 particles/cm² on the entire surface of one Cymodocea rotundata leaf blade. Microplastics of between 40.1 to 73.7 particles/g gross weight of digestive tract was found in the samples of seahare Dolabella auricularia with a calculated average of 54.9 particles/g gross weight of Dolabella auricularia. Results act as evidence of how microplastics in seawater may adhere to the surface of a primary producer and be accidentally eaten by a marine biota, entering the marine food chain.

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