Effect of microplastics and natural microparticles on green Mussel (Perna viridis)

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Abstract. There are so many studies of microplastics on marine animals, but almost all of them do not compare the effect of microplastics with natural microparticles which are also can be a stressor for the animals. This can lead us to overestimate the effect of microplastics on marine animals. Therefore we conducted a study to see whether the effect of microplastics was the same as the effect of natural microparticles on green mussels (Perna viridis). This research was conducted experimentally in the laboratory for 21 days. Green mussels were exposed to PVC as microplastics and red clay as natural microparticles with 3 different concentrations (1.5 mg / l, 15 mg /l and 150 mg /l). We observed mortality, clearance rates, and BCI index. Clearance rates and mortality values were not statistically significant, while the BCI results showed that the effects of PVC and red clay at each concentration were only less than 10%. This suggests that the effect of microplastics and natural microparticles is similar, and it is necessary to include natural microparticles as a comparison in research related to microplastics.

1. Introduction

Mussels are widely used for microplastics research, as they are filter feeders and they have important economic values in society as well. Filter feeders get their food by filtering the water. An estuary is one of the habitats of filter feeders in the coastal zone. High levels of mixing in estuary waters lead to high numbers of suspended particles. This can cause the suspended particles in the water column to get ingested by them.

The source of suspended particles can be natural as in resuspended sediment (e.g., sand, clay, silt, diatom shell) or artificial (e.g., microplastics). Microplastics are plastic particles with the size of 1µm-5mm [1,2] The sources of microplastics are divided into primary source and secondary source. The primary source means that the microplastics are produced in very small size and the secondary source means that the microplastics are the result of degradation of larger plastic products [3]. These small sizes cause filter feeders to ingest the microplastics with their food [4-7].

Microplastics research has provided a lot of information about its impact on the marine organism. It can decrease animal reproduction and growth, change their feeding behavior, and also can be lethal for fish, crustaceans, and algae [8,9]. Filter feeders have the ability to emit microplastics from their body as pseudofeces [10] but still be affected. One consequence is that the animal can have low body conditions or changes in physiological function due to diversion of growth and develop energy to emit microplastics [11-15].

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Most of the studies only reported the impact of microplastics on animals by referring to the absence of other non-digestible particles. It is important to include natural microparticles in the study, because we may overlook the impact of microplastics on animals. As we know both plastics and natural microparticles can be a stressor for filter feeders. To date, only two studies directly compared the effect of plastics and natural microparticles on marine animals [16,17]. Therefore we conduct a study to see whether the effect of microplastics was the same as the effect of natural microparticles on green mussels (*Perna viridis*).

2. Methods

2.1. Time and location

This study was conducted in Marine Habitat Laboratory FPIK, IPB University. We conducted the experiment for 21 days from 15th July 2019 to 5th August 2019. Green mussels (*Perna viridis*) that were used for this study were obtained from Muara Kamal, Jakarta Bay, Special Region of Jakarta.

2.2. Sample

Green mussels were collected from Muara Kamal, Jakarta Bay, Capital Region of Jakarta. The transport used dry methods using coolbox with sufficient air supply. After arrived at the Laboratory mussels got sorted, cleaned, and being measured their initial conditions (wet weight, width, and length). Mussels that have size 1-3 cm were acclimated to an aquarium. Each aquarium contained 25 mussels in 20 L of seawater with a consistent air supply. After 10 days of acclimation, mussels were well adapted to laboratory conditions, seen by byssus production, active movement, and absence of mucus.

The experiment was conducted in single individual containers. Each observation container contains single mussel that is chosen randomly from the acclimation aquarium and filled with 1L seawater. The air supply in the observation container was supplied for 24 hours, used straw all the way to the bottom to ensure sufficient oxygen and constant re-suspension particles. 50% of seawater in observation containers was renewed daily. Every day we also cleaned the container from feces and were feeding the mussels with *Spirulina* sp.

2.3. Treatment

The experiment had six treatment combinations, consisted of two particle types (microplastics and natural microparticles) across three concentrations (1.5 mg/l, 15 mg/l, and 150 mg/l). The particles compared are Polyvinyl chloride (PVC) and red clay. The particle was produced in Germany by PyroPowders (PVC) and now® solutions (Moroccan red clay powder). This particle is chosen because it is in the natural size range of particles commonly found in the field (0.1-100 μm).

2.4. Response variables

2.4.1. Survival rates. Survival rates were observed by visual. Dead mussels usually open their shells widely and did not respond when stimulated by disturbing or touching.

2.4.2. Clearance rates. Clearance rates were measured by adding microalgae (*Botryococcus* sp) with a density ±100,000 ind/ml into observation containers. The first measurement was taken initially after adding the microalgae, then after 40 minutes, the second measurement was taken. Microalgae abundance was calculated using the Sedgewick rafter counting cell under a light microscope. Then clearance rates were calculated with the formula:

\[
\text{Clearance rates} = \frac{\ln \left( \frac{\text{Algae (start)}}{\text{Algae (end)}} \right) \times \left( \frac{\text{Volume}}{\text{Time}} \right)}{\text{Wet Weight}}
\]
2.4.3. Body condition index (BCI). Body condition index was measured by separating soft body tissue with the shell of green mussel, then the mussel was placed into an oven with a temperature of 60 °C until the weight is stable. BCI was calculated with the formula [19].

\[
BCI = \frac{\text{Dry weight of the soft tissue}}{\text{Dry weight of the shell}}
\]

2.5. Statistical analysis
Data analyses were performed in the R software. Survival was analyzed with coxph regression. Clearance rates were analyzed with GLM negative binomial and BCI were analyzed with GLM quasipoisson. We used type III sums of squares for the statistical models using \(\alpha = 0.05\) to determine statistical significance.

3. Result and discussion
3.1. Survival rates
Green mussels had high mortality in the 3rd - 4th days of exposure and after the 18th day of exposure (fig 1.). Green mussels exposed to plastics and natural microparticles did not show a significant difference in survival rates (p>0.05) (table 1).

![Figure 1](#)

**Figure 1.** Survival of *Perna viridis* by the days over three weeks of exposure to different particle types and concentrations.

3.2. Clearance rates
Green mussel exposed to microplastics has lower clearance rates compared to green mussel exposed to natural microparticles (figure 2). In statistical analysis did not show the significant difference between particle types, concentration, and interaction between particle types and concentration (p>0.05) (table 1).

3.3. Body condition index (BCI)
Green mussels exposed to microplastics show a lower BCI at the lowest and middle concentrations than mussels exposed to natural microparticles, on the other hand at the highest concentration a green mussel exposed to microplastics has a higher BCI than a green mussel exposed to natural microparticles (figure 2.) This is also reflected in statistical results that show the significance of interactions between particles and concentrations in experiments (p<0.05) (table 1).
Figure 2. Clearance rates (a) Body condition index (b) of *Perna viridis* after three weeks of exposure to different particle types and concentrations.

Table 1. Statistical analysis of survival, clearance rates, and BCI. ** means statistically significant.

|                | LR Chisq | df | Pr(>Chisq) |
|----------------|----------|----|------------|
| **Survival**   |          |    |            |
| **Particle**   | 3.3206   | 5  | 0.6507     |
| **Concentration** | 0.16084  | 2  | 0.9227     |
| **Interaction** | 1.14975  | 2  | 0.5628     |
| **Clearance rates** |          |    |            |
| **Particle**   | 2.33565  | 1  | 0.1264     |
| **Concentration** | 0.0022   | 2  | 0.9967     |
| **Interaction** | 12.2497  | 2  | 0.0022     ** |

After green mussels were exposed to plastics and natural microparticles (PVC and red clay), the results of survival rates and clearance rates showed that the difference between the two particles across three concentrations is not significant. Only the results of BCI showed a significant difference in the interaction between particles and concentration.

This result is in contrast to the results by [16] which stated that mussels exposed to microplastic at their highest concentrations (> 1250 particles/ml) had lower clearance rates by 62% than mussels exposed to natural microparticles at the same concentration. These different results can be caused by very different concentrations that being used and the different exposure times. Their mussel exposed within an hour with the highest concentration >1250 particles/ml while we did for three weeks with the highest concentration 150 mg/l [16]. They also measured the clearance rates with particles in the environments, while we measured the absences of particles in the environment. It can certainly cause differences in the result of the observations.

Compared to [17] that did their experiment for 5 weeks, we interestingly found a similar result to the study that we did. The results showed mussels which are exposed to microplastics had 14% BCI than mussel exposed to natural microparticles, while other response variable observed did not show any significant differences between the effects of the two particles.

The length of observation seems to have a profound effect on the results obtained. This also can be seen in the survival rates. At the beginning of the experiment, survival rates dropped the most. This can be caused by the high stress level, which is caused by separating the mussels from each other in an acclimation tank and placing them in individual containers.

Mussels that can adapt well to plastic and natural micro particles exposure become robust so that the variable response (ex. clearance rates) shows there is no difference between plastic and microparticles. However, prolonged exposure can have a carry-over effect for mussel, further affecting the health status of mussel which can be seen from the mussel’s body condition index (BCI) [6].
BCI in general can be used as an indicator of the nutritional status and health of mussel in the facing of environmental stress [20]. The significant difference in BCI can indicate that mussels are able to sort the particles on their gills and labial palps [21,22], but still need the proper method to support this hypothesis.

4. Conclusion
This experiment suggests that the effect of plastics and natural microparticles are comparable, and it is necessary to include natural microparticles as a comparison in research related to microplastics.

References
[1] Anbumani S and Kakkar P 2018 Ecotoxicological effects of microplastics on biota: a review Environ. Sci. Pollut. 25 14373–14396
[2] Gigault J, Halle A T, Baudrimont M, Pascal P Y, Gauffre F, Phi T L, El Hadri H, Grassl B, Reynaud S 2018 Current opinion: what is a nanoplastic? Environ. Pollut. 235 1030–1034
[3] Andrady A L 2011 Microplastics in the marine environment Mar. Pollut. Bull. 62 1596–1605
[4] Von Moos N 2012 Uptake and effects of microplastics on cells and tissue of the blue mussel Mytilus edulis J. Environ. Sci. Technol. 46 11327 – 1133
[5] Avio C G, Gorbi S, Milan M., Benedetti M, Fattorini D, d’Errico G, Pauletto M, Bargelloni L, Regoli F 2015 Pollutants bioavailability and toxicological risk from microplastics to marine mussels J. Environ. Pollut. 198 211-222
[6] Rist S E, Assidqi K, Zamani N P, Appel D, Perschke M, Huhn M, Lenz M 2016 Suspended micro-sized PVC particles impair the performance and decrease survival in the Asian green mussel Perna viridis Mar. Pollut. Bull. 111 213–220
[7] Sussarellu R, Suquet M, Thomas Y, Lambert C, Fabioux C, Eve M, Pernet J 2016 Oyster reproduction is affected by exposure to polystyrene microplastics. Proc. Nat. Acad. Sci. 113(9) 2430–2435
[8] Chae Y, An Y J 2017 Effects of micro- and nanoplastics on aquatic ecosystems: Current research trends and perspectives Mar. Pollut. Bull. 124 624–632. doi: 10.1016/j.marpolbul.2017.01.070.
[9] Haegerbaeumer A, Mueller M T, Fueser H, Traunspurger W 2019 Impacts of micro- and nano-sized plastic particles on benthic invertebrates: a literature review and gap analysis Front. Environ. Sci. 7 17
[10] Garrido M V, Chaparro O R, Thompson R J, Garrido O, Navarro J M 2012 Particles sorting and formation and elimination of pseudofaeces in the bivalve Mulina edulis (siphonate) and Mytilus chilensis (asiphonate) J. Mar Biol 159 987-1000
[11] Brillant M G S, MacDonald B A 2000 Postingestive selection in the sea scallop, Placopecten magellanicus (Gmelin): the role of particle size and density J. Exp. Mar. Biol. Ecol. 253 211-227
[12] Shumway S E, Cucci T L, Newell R C, Yentsch C M 1985 Particle selection, ingestion and absorption in filter-feeding bivalves J. Exp. Mar. Biol. Ecol. 91 77-92
[13] Sprung M, Rose U 1988 Influence of food size and food quantity on the feeding of the mussel Dreissena polymorpha J. Oceologia 77 526-532
[14] Rosa M, Ward J E, Shumway S E, Wikfors G H, Pales-Espinosa E, Allam B 2013 Effect of particle surface properties on feeding selectivity in the eastern oyster Crassostrea virginica and the blue mussel Mytilus edulis J. Exp. Mar. Biol. Ecol. 446 320-327
[15] Widdows J, Fietch P, Worrall C M 1979. Relationship between seston, available food and feeding activity in the common mussel Mytilus edulis J. Mar. Biol. 50 195-207
[16] Harris L S T, Carrington E 2019 Impacts of microplastic vs. natural abiotic particles on the clearance rate of a marine mussel Limnol. Oceanogr. Lett. 5 66–73
[17] Yap V H S, Chase Z, Wright J T, Hurd C L, Lavers J L, Lenz M 2020 A comparison with natural particles reveals a small specific effect of PVC microplastics on mussel performance Mar. Pollut. Bull. 160 111703
[18] Riisgard H U, Pleissner D, Larsen P 2014 Allometric equations for maximum filtration rate in blue mussel *Mytilus edulis* and importance of condition index *Helgol Mar. Res.* **68**(1) DOI: 10.1007/s10152-013-0377-9

[19] Huhn M *et al.* 2016 Tolerance to stress differs between Asian green mussels *Perna viridis* from the impacted Jakarta Bay and from natural habitats along the coast of West Java *Mar. Pollut. Bull.* **110**(2) 757-766 https://doi.org/10.1016/j.marpolbul.2016.02.020

[20] Lucas A, Beninger P G 1985 The use of physiological condition indices in marine bivalve aquaculture *J. Aquaculture* **44** 187-200

[21] Kiørboe T, Møhlenberg F, Nøhr O 1981 Effect of suspended bottom material on growth and energetics in *Mytilus edulis* *Mar. Biol.* **61** 283–288

[22] Riisgård H U, Pleissner D, Lundgreen K, Larsen P S 2013 Growth of mussels *Mytilus edulis* at algal (*Rhodomonas salina*) concentrations below and above saturation levels for reduced filtration rate *Mar. Biol. Res.* **9** 1005–1017

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