

The Effect of Exposure to Microplastic Particles on Baltic Sea Blue Mussel (*Mytilus edulis*) Filtration Rate.

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Abstract

This study sought to investigate the effect of microplastics on *Mytilus edulis*'s ability to filtrate water. The mussels were sampled at Hanö bay located in the Baltic Sea, and were transported to a controlled environment. The mussels were exposed to three different solutions, one containing a concentration of 1 000 Microbeads (diameter 10 µm) per liter, one containing the same amount of plastic beads in addition to algae in a concentration of 3 000-5 000 cells per ml, and the third group containing only algae. The mussels were kept in these different conditions for six days. After this acclimatization period the mussels were given filtrated salt water, and were all fed the same amount of algae during four hours. The number of algal cells per liter was counted every half hour, by removing a one ml sample and analyzing it using a FlowCam in order to test for any differences in filtration rate between the different groups. The study found that there was no significant difference in the net change in algal content between the groups, thus drawing the conclusion that microplastics in a concentration of 1000 beads per liter does not have a short-term effect on the filtration rate of the mussels. These results are discussed here to suggest that that the mussels are able to separate the microplastic into non-food particles before ingesting them. The results indicate that this process requires some amount of energy, which in turn make the mussels hungrier. This could have negative effects on the fitness of the mussels in the long-term, however the long term effects were not tested in this study. The study concludes that more research is needed.

Introduction

The worldwide demand and production of plastics is today at an all-time high, and is ever increasing (Andrady, 2011). Plastics in the ocean were first noticed in the 1970s (Fowler, 1987). This spawned heavy research on its effect on the marine ecosystem. Early on, entanglement was the main research purpose (Laist, 1997) as well as the ingestion of plastics by marine birds (Mallory, 2008), and while today the public is, mostly, very much aware of the immediate harmful effects of marine littering, a new threat termed microplastics, is gaining more and more attention from researchers around the world.

With an increased use of plastics in today's society, almost 300 million tons being produced annually (Statista, 2015), together with growing cities around coastlines, it is likely that marine littering is something that will increase in the future (Ribic et al., 2010). Plastic are not easily biodegradable, meaning bacteria have a hard time to break down the materials. However, photodegradation, through UV radiation, have the capability of degrading plastic into smaller and smaller pieces. This process is however considerably slower in the ocean compared to land surfaces, meaning the plastic have a longer lifespan in the ocean compared to on land (Andrady et al., 1993). UV radiation breaks down the plastic into smaller and smaller particles, which eventually results in microplastics. These are usually defined as < 5mm in diameter (Arthur et al., 2009). Microplastics have been shown to be harmful to marine organisms with the particles accumulating in tissues. In addition, microplastics have the ability to bind certain toxins. In a sense microplastics clean the water of toxins as toxic substances accumulate around the plastic beads. However once these beads enter an organism the toxins might be released and cause considerable harm (GESAMP, 2010).

Mussels are benthic filter feeders, who can filter a considerable amount of water per day. They are important organisms in marine systems, since they are able to recycle nutrients into the system (Nielsen & Maar, 2007). It has been shown in previous work that the blue mussel (*Mytilus edulis*) is capable of taking up microplastics, with plastics being found in the animal tissue as well as the feces of the mussels (Wegner et al., 2012). Browne et al. (2008) found that micro plastic beads were ingested by the blue mussel and translocated from the digestive system to the circulatory system and that smaller particles are more easily accumulated in the mussels. Furthermore it has been shown that the microplastics decrease the filtering ability of the mussels, (Wegner et al., 2012). These mussels, however, were exposed to an extremely high concentration of microplastics. Therefore there is a need to investigate the response of these organisms to more environmentally relevant concentrations.

The Baltic Sea is today considered to be one of the world's most polluted seas, with frequent algal blooms and low oxygen levels. Hanö bay is located on the East Coast of Scania Sweden. It contains brackish water of salinity 9 ppm. The County Administration Board of Scania is currently surveying the area for marine litter, including microplastics (County Administration Board Scania, 2015). Although the data from this survey is not yet available, a similar survey, however, was carried out on the West Coast of Sweden in 2013/2014. The study found on average 30 particles per liter using a 10 µm filter. Around *Galterön*, an island west of Sandviken the survey found as many as 534 particles per liter, most of which was believed to be microplastics (Norén Fredrik et al., 2014).

The blue mussel serves an incredibly important role in the marine systems around the Swedish coast by recycling nutrients in an ever more polluted sea. This study will investigate what effect microplastics have on the *Mytilus edulis*'s ability to filtrate water, in order to further understand the blue mussel's ability to cope with the threat of rising levels of microplastics.

Materials methods

Sampling

The mussels used in the experiment were taken from a shallow and calm bay at Landön, located in the Hanö Bay of the Baltic Sea (fig 1). 60 mussels were sampled from a depth of roughly 0.5 m. The mussels were 5 – 20 mm in diameter, all of which were attached to rocks. We also brought 100 L of water from the sampling area, which was later filtered and used in the experiment.

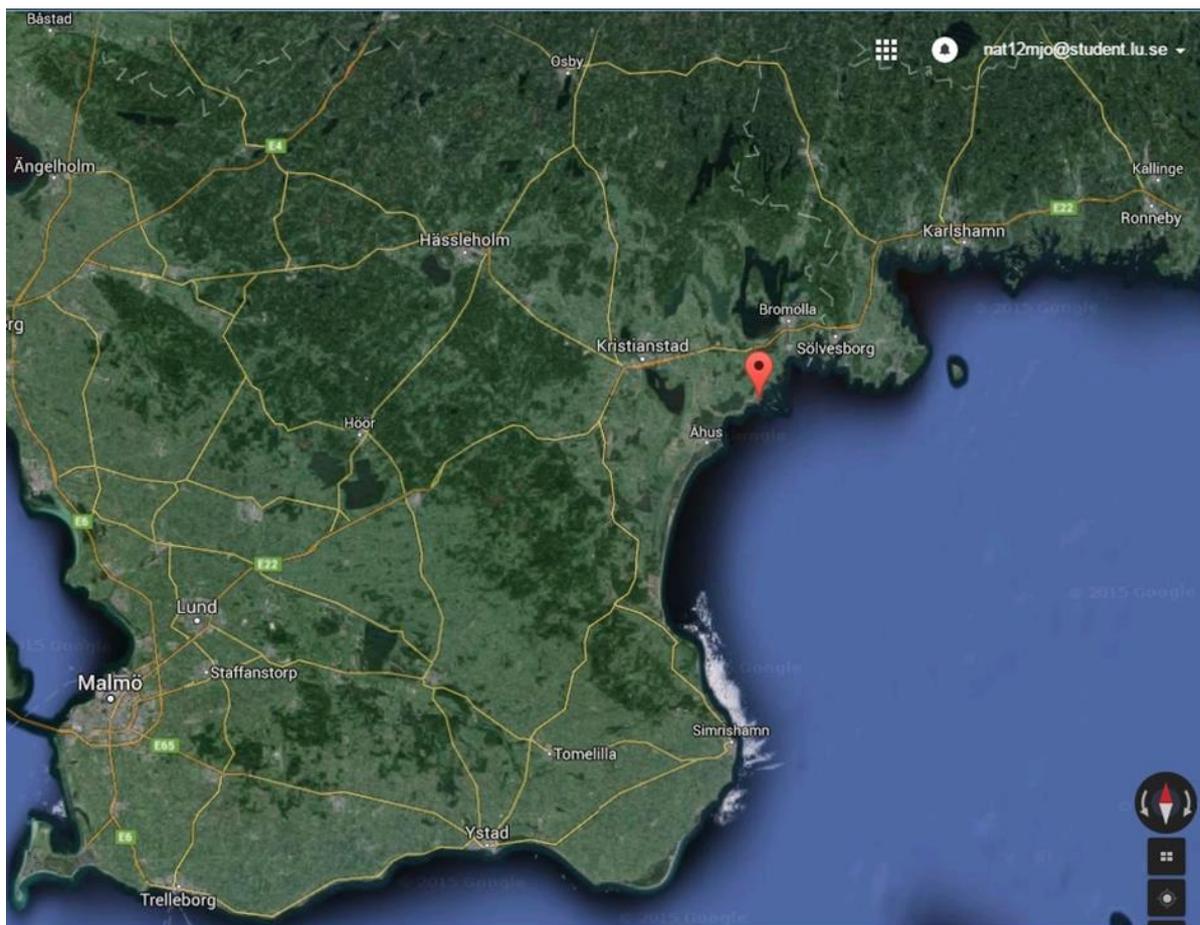


Figure 1. The sample area at Landön in the north eastern part of Scania.

Acclimatization

Once the mussels were back at the laboratory, they were kept in a cooling room set to 10°C, which was the same temperature as in the water where we collected them. The water was aerated and kept in motion by a pump. After 5 days the mussels were separated into 3 groups of 20 and placed in 12 separate containers, meaning 5 mussels in each 1 L container. The mussels were divided in respect to size, so that all containers contained mussels of roughly the same amount of biomass. Once the mussels were separated, the containers were filled with 1 l of filtered sea water (9 PSU salinity). Next, one group of mussels were fed plastic beads (termed “plastic”), with size 10 µm in diameter, in a concentration of 1000 beads per liter and no algae. The second group of mussels (termed “plastic + algae”) were fed the same amount of plastics as well as an algae mixture consisting of *Dunaliella tertiolecta* (≈ 10 µm diameter), *Rhodomonas salina* (≈ 6 x 10 µm) and *Heterocapsa triquetra* (≈10 x 20 µm) This mixture was added in a concentration of 3000 - 5000 cells per ml. The proportion of these was 1:2:1 respectively. Lastly, the third group of mussels (termed “algae”) was fed the same amount of algae, but no plastics. All mixtures and water were replaced twice a day for 6 days.

Testing filtration

After the six day acclimatization period, where the mussels were fed either plastics, algae + plastics, or only algae, the containers with the mussels were rinsed out thoroughly and filled with one liter of filtered seawater. The mussels were then fed an algal mixture, same as the one used during the acclimatization period, of a concentration of roughly 1000 – 2000 cells/ml. The control group was a container with the same concentration of algae, but no mussels, in order to account for any errors that could affect the number of algal cells. As soon as the algal mixture had been added to the mussels a one ml sample was removed for analysis. This was repeated every half hour for two hours. Once the samples had been extracted they were fixed using one drop of lugol's solution.

Once the samples were fixed, they were analyzed for algal content using a FlowCam. The FlowCam is essentially a microscope with a camera attached, which takes pictures of all particles in the sample, as it is passed through a small tube. The pictures are later analyzed to find the total number of algal cells per ml. The FlowCam was set to count all particles within the size range 2 μm – 30 μm , with an efficiency of 17.1%, meaning 17.1% of the liquid was sampled, the machine is not able to have 100% efficiency, and too high of an efficiency might cause the same particle to be counted several times. Afterwards the pictures from the FlowCam were manually checked to remove any potential non algal particles, in order to get as precise an estimate of the number of cells as possible, this gives a better accuracy in finding the concentration of algal cells in the sample, compared to using a spectrophotometer for instance, which would take into account fragments, and other particles.

Statistics

The data was analyzed using Microsoft excel 2010 by constructing scatter plots and line equations, as well as running a one way ANOVA on the net change in algal cell concentration to test for significance.

Results

The results in fig 2, 3 and 4 illustrates that the mussel's ability to filtrate was not negatively affected by the presence of plastic particles, at least in a short-term timescale. The results indicate a trend where the mussels in the group "plastic", were the ones that filtrated the fastest (fig 2), followed by the "plastic + algae" group (fig 3), while the "algae" group filtrated the slowest (fig 4).

Figure 2 shows the difference in number of algal cells over time for mussels that were fed only plastic beads during the acclimatization period, compared to the control. The curve follows an expected curve, where the number of algal cells decreases exponentially. The line equation $y = 1264.8e^{-1.015x}$ indicates that the algae was filtrated the fastest by this group.

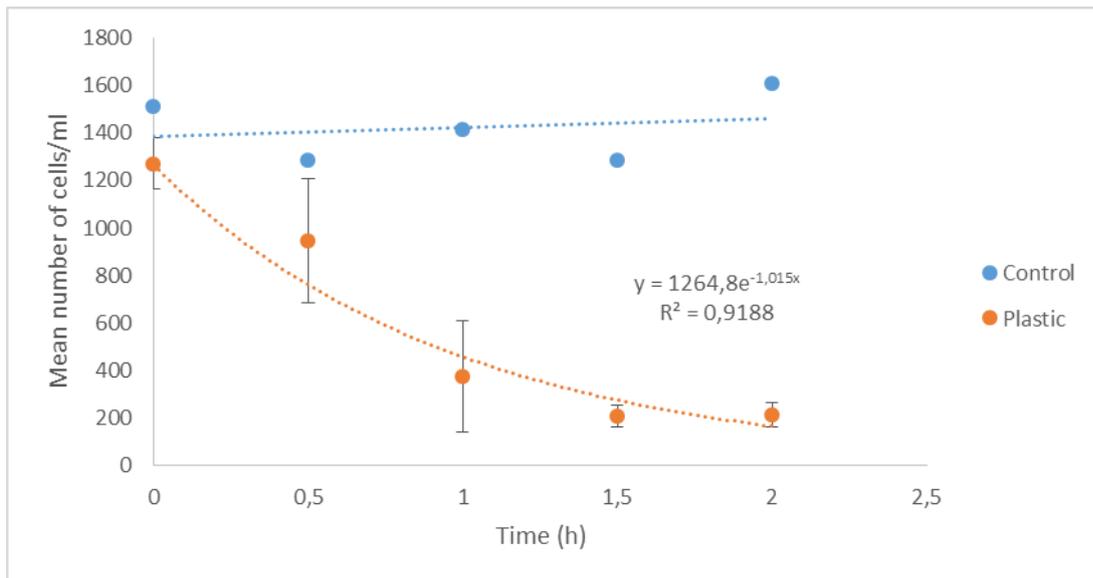


Figure 2. Change in number of cells over time by mussels who were only exposed to microplastics during the acclimatization period. Number of replicates n=4.

Figure 3 shows that the mussels that were fed both microplastics and algae, followed a similar curve as the mussels that were only fed microplastics (fig 2). However the line equation $y = 1136,1e^{-0,431x}$ indicates a less steep curve compared to fig 2. This shows that the “plastic + algae” group were filtrating slightly slower than the “plastic” group. In addition the net change in number of algal cells was lower for the “plastic + algae” group, compared to the “plastic” group, as seen in table 1.

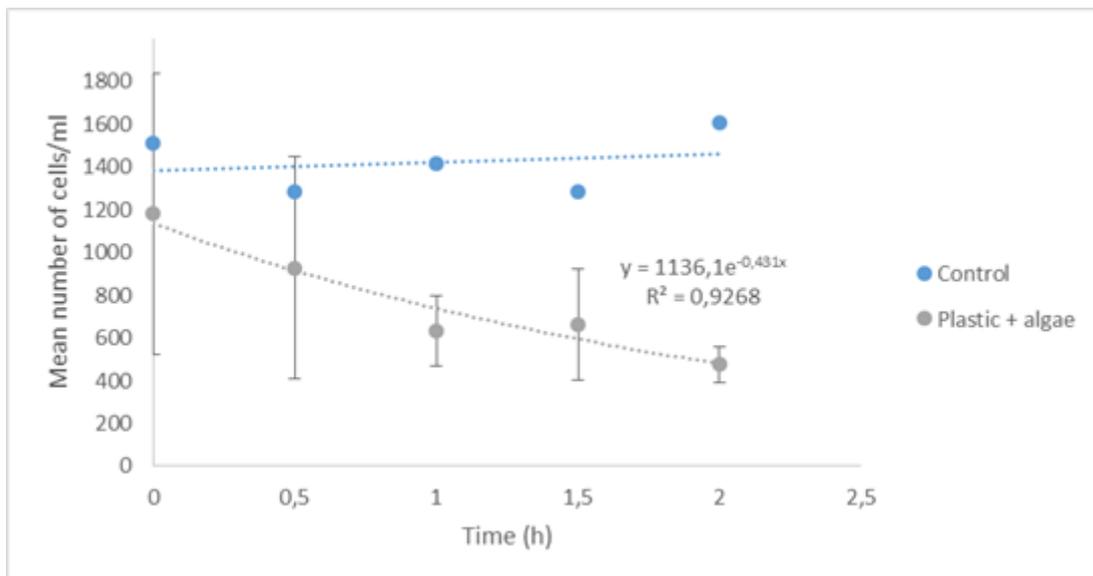


Figure 3. Change in number of algal cells over time for the “plastic + algae” group. Number of replicates n=4.

Figure 4 differs somewhat from figs 2 and 3 in that there is a very poor exponential relationship for the data, $R^2 = 0,13$ compared to 0,92 (fig 2) and 0,93 (fig 3). The data in fig 4

almost shows a linear relationship. The number of algal cells was decreasing until the first hour, then the concentration started to increase. This should obviously not happen and could be the result of some unknown errors.

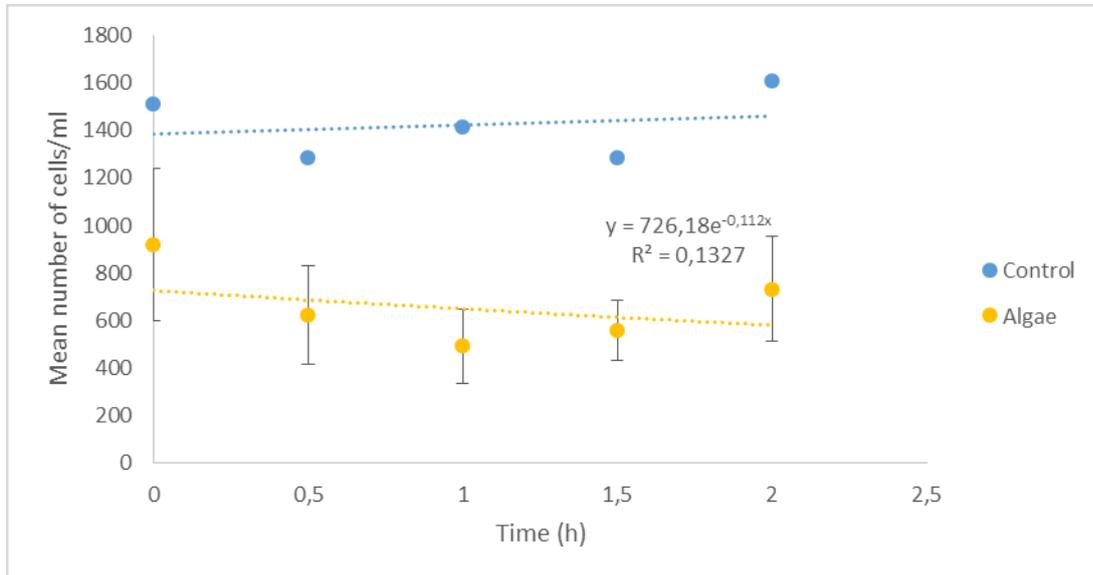


Figure 4. Change in number of algal cells over time for the “algae” group. Number of replicates n=4.

To further illustrate the differences in algal uptake between the four groups (“control”, “plastic”, “plastic + algae”, and “algae”) the net change in mean number of algal cells between t0 and t2 was calculated (table 1). The results showed that the control had increased, the “plastic” group had been able to take up the most algae and the mussels in the “algae” group took up the least amount of algae. A one way ANOVA was performed on the data in table 1 in order to test whether there was any significant difference between the three groups. No significance was found ($p = 0.06$).

Table 1. Net change in mean number of algal cells between t0 and t2.

Mean number of algal cells				
	Control	Plastic	Plastic + algae	Algae
t0	1512	1270	1182	920
t2	1608	214	476	733
net change	96	-1057	-706	-187

Discussion

This experiment sought to investigate how microplastics would affect the ability for the blue mussel to filtrate algae. The results showed no significant difference between the three different mussel groups (table 1). Thus the mussels in Hanö bay seem to be able to cope with microplastic concentrations of up to 1 000 beads per liter. There are numerous micro and nanoparticles already existing in the world's ocean, average being 30 particles per liter around the Swedish west coast (this is just counting particles larger than 10 μm . (Norén Fredrik et al., 2014)). These micro and nanoparticles do not seem to cause any harm to filter feeding animals, whether it is regarding a mussel or a baleen whale, which all come into contact with these particles every day. Perhaps then, the microplastic particles, on their own, are not as dangerous as one might think. While some studies reports negative effects due to microplastics, one should bear in mind that extremely high concentrations of particles were used in these experiments. For instance Wegner et al. (2012) used a concentration of 0.2 g/l, which by my calculations would result in a concentration of roughly 18.1×10^6 beads per liter. Van Cauwenberghe et al. (2015) reasons around this issue and estimates that the concentrations of microplastics in most of the experiments in this field are up to 5000 times greater than realistic environmental concentrations. While there is certainly value in these types of experiments, conducting studies with more realistic concentrations certainly has its value too. The experiment conducted in this project, set out to have a more realistic concentration while at the same time account for the potential increase in concentrations. The net change in algal cells showed no significant difference between the different groups (table 1), suggesting that micro beads have to be present in a very large concentration, for them to negatively impact the filtration mechanism for the mussel, in the short-term at least. While this certainly could be seen as a positive result, there are still many unknown effects of microplastics that need to be studied. Going forward, an important way of studying the effects of microplastics would be to study the animals in their natural environment. In the ocean the microplastic bind toxins that float around in the water column, essentially becoming very effective transportation mechanisms of delivering toxins to organisms (GESAMP, 2010). Shifting the focus from studying the physical harm, caused by a very large concentration of microplastics to studying the effects of the toxins, and the microplastic beads ability to bind these, I believe is an important step forward in this field. However, it is one that is not easily replicable in a laboratory environment.

While the results does not indicate any reduction in the filtrating abilities of the mussels (table 1). The results seem to indicate that the mussels that were fed plastic were on average hungrier than the ones that were just fed algae (table 1). This could be an indication that the mussels were spending more energy separating or excreting the microplastic through pseudofeces (material taken up by filtration but rejected before ingestion (Gosling, 2003)), thus making them hungrier. This would then potentially have a negative long-term impact on the health and fitness of the mussel. Recently a study was conducted testing whether there was a change in cellular energy allocation for mussels who had been exposed to microplastic. The results showed, however, that the energy allocation was not affected by the microplastics. The authors noticed, however, that the mussels exposed to plastics had an increase in respiration (Van Cauwenberghe et al., 2015). Furthermore Wegner et al. (2012), found an increase in pseudofeces production by mussels exposed to microplastics. This could suggest a reduction in energy acquisition. In addition, Browne et al. (2008), found that as plastic is broken down

into smaller and smaller particles, they are more easily accumulated in the mussel's tissues. They did not, however, find any significant short term biological effects. Nonetheless, since plastics are very long lived they will most likely have some long-term effects, should they accumulate in the tissues. The uptake of plastic particles by the mussels have been found both in laboratory experiments and in wild specimens, though the retention efficiency of the plastic particles is estimated to be quite low $\approx 0.003\%$ (Van Cauwenberghe et al., 2015). The long-term effects of microplastics is poorly understood. It is likely that the microplastics that are being accumulated in the tissues will have long-term effects, and due to the low degradability of plastic it could have biomagnifying effects reaching further up the food web. Nevertheless, due its low retention rate, and its ability to cope with environmentally realistic concentrations, as suggested by table 1, the blue mussel will likely be able to cope with the current microplastic concentration of the ocean, rather well.

While the presence of microplastics certainly could have caused an increase in respiration for the mussels, another explanation for the large net-change in algal cells, for the "plastic" group specifically, could be that the mussels were starved for food. The mussels in this group were only fed plastic for the duration of the acclimatization period, which naturally would cause them to be hungry. The "plastic + algae" group, however, did have a larger net change in algal cells compared to the "algae" group (table 1), which could suggest that the plastic caused the mussels to spend more energy. In future studies I recommend having a starvation control, by keeping the mussels in clear salt water with no food, in order to account for starvation. By doing this you can draw more conclusions about the effect of microplastics. In addition, I would recommend performing the filtration rate test for several consecutive days. This would remove the effect of starvation and furthermore it could give an insight to any potential long-term effects of the microplastic.

The use of net change should be a good indicator of how much algae has been taken up by the mussel. However, as seen in fig 4, the data for the algae group shows that the algal concentration increased at t1.5 and t2. This could indicate an error with the FlowCam setup, since there shouldn't be a way for the number of algal cells to increase. Nevertheless this caused the net change for the Algae group to be less, compared to if it had followed an exponential curve. However, even comparing the net change at t1 the Algae group still cleared the least cells (fig 2, 3 & 4).

The experiment might have gotten different results if the mussels had been exposed to the higher levels of microplastics for a longer period of time. It is possible that the mussels have a threshold value for the number of junk particles they can handle in any given time, so it is possible that the mussels are quite fine with seemingly high levels of plastic, at least when only considering the short-term effects. But should the plastic concentration rise too high, it might have severe effects on the mussels (Wegner, 2012). Due to time restraints this was not possible to test, however it should be considered in future research. In addition, Wegner et al. (2012) found that the mussels, that were fed plastics, had an increased defecation of pseudofaeces, which suggests that the mussels can excrete the plastic beads. In future studies this defecation should be examined, as well as investigate the mussels interior to find where the plastic ends up.

In future research it is important to investigate the effect of microplastic on the earlier parts of the lifecycle of the mussel. The larvae (although lecithotrophic) and juveniles might be differently affected by microplastics than the adult life stage.

An important aspect to consider, regarding this study, is that the plastic beads used in this experiment were all factory manufactured with the same diameter of 10 μm . In the ocean the mussels would be exposed to different sizes, and more importantly, different chemicals that can be bound to the plastic. It is possible the biggest threat with microplastics is not the beads themselves, but the toxins that accumulate on them, making the bead a transfer device for toxins into the organism (GESAMP, 2010).

One possible source of error in this experiment is that the plastic beads might not have been kept suspended in the containers. The experiment was designed in such a way that movement of air into the containers would create enough of a current to keep the dense plastic beads suspended. It is, however, quite difficult to know for sure. It is possible that the plastic simply got shot away to the other side of the container where the water was not turned around as much, thus allowing the plastic beads to settle. It was noted during the experiment that algae had settled on the bottom, thus indicating that the movement of the water might have been insufficient. Throughout the duration of the experiment, however, all the mussels had opened shells, indicating that they were at least filtering the water. I suggest for future research to use something like a magnetic stirrer in order to keep all the particles suspended in the medium, and thus obtainable for the mussels.

Conclusion

In conclusion, this study found that the mussel's ability, to filtrate algal cells of a concentration of 1000 – 2000 cells/ml was not negatively affected by being exposed to a microplastic concentration of 1000 beads/L. The results did show, however, a trend where the mussels that had been exposed to microplastics for six days, were hungrier compared to the ones that were not exposed to microplastics. This could mean that mussels need to expend more energy in the presence of microplastics in order to separate them from other food particles. In the long term this could have negative effects for the overall fitness of the animal. However this was not tested in this study, instead it shows a need for further research. Although no immediate negative effects were noticed in the experiment, the report emphasizes that one of the major dangers with microplastics is its ability to bind and transfer toxins to the organisms, the effect of this ability of the plastic beads require further research. It is clear that we need to stop polluting our oceans with plastic materials, however this study show some optimistic results for the blue mussel. Its selective feeding might make it to cope with an increased concentration of microplastics in the world's oceans.

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