The Impact of Polystyrene Microplastics on Filtration Rate in the Marine Copepod *Acartia tonsa*

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Abstract

Microplastic particles are of increasing environmental concern around the globe and recent studies have shown that these particles are present in the entire water column, thus affecting a range of zooplankton including copepods that have a vital role in the marine food web. This study investigated whether microplastic beads have an effect in the filtration rate in the copepod *Acartia tonsa*, which was collected outside of Ålabodarna located in Oresund. The copepods were exposed to three different solutions during a period of 48h. One group contained solely 10µm of microplastic beads with a concentration of 130 beads per milliliter, the second one contained only a cultured algae mixture with a concentration of 1300 cells per milliliter and the third group contained both plastic beads and algae with the same concentrations as mentioned above. The amount of particles in every container from each group was counted for at t_0, t_{24} and t_{48}. The results showed no significant difference between the groups in the net change in their filtration rate but indicated that their overall survival decreased when being exposed to microplastics. The study concludes that more research is needed to understand what kind of impacts microplastics might have on the copepods filtration rate.

1. Introduction

Since the mid 19th century plastic production has increased dramatically around the world (Statista, 2015) and thus plastic litter has become an increasingly conspicuous within marine ecosystems, which threatens a variety of organisms (A. L. Andrady, 2011; M. Eriksen et al., 2014). While the risks of how larger plastic debris pose to marine life are well documented (C. W. Fowler, 1987; K. Tanaka et al., 2013), we are only just now starting to understand how these microscopic plastic particles, defined as ”microplastics”, may have an impact upon different aquatic organisms (Cunningham & Cunningham, 2013; Morét-Ferguson et al., 2010).

Microplastics are described as plastic fragments <5mm in diameter, with various shapes, and originates either from primary- or secondary sources (J. G. B Deraik, 2002). The first mentioned are those that have been manufactured to be microscopic in size (L. S. Fendall et al., 2009) and the second ones appears due to degradation of larger plastic objects, both by UV-radiation and hydrolysis, that gradually split into microplastic fragments (M. A. Browne et al., 2011). The plastic is made out of polymers, which have proven to be very persistent in the marine environment and are not very easily biodegradable by other organisms (A. L. Andrady, 2003). A particles lifespan in nature does mostly depend on the polymers both chemical- and physical properties, however, the degradation process are many times slower in the ocean compared to land and increases with depth due to the shortage of UV-radiation and low amounts of dissolved oxygen in the water (D. K. A. Barnes et al., 2009).

Because of their change in properties during photodegradation, such as being more hydrophobic than the seawater, adsorption of other small particles from the surrounding environment is of big concern (J. G. B Deraik, 2002). After the fouling, the microplastics

becomes negatively buoyant which enables them to leave the ocean surface, thus entering the water column and in time slowly reaching the sediment (J. Wang et al., 2016; Thompson et al., 2004). While being present in the water column it becomes bioavailable for many different kinds of organisms, particularly filter feeders at the base of the food web, such as zooplankton (S. L. Wright et al., 2013).

As primary consumers in the epipelagic zone and being the most numerous metazoans in the marine ecosystem, copepods have a vital ecological role in the transportation of energy across the whole food chain (K. Lee et al., 2013). They display a range of feeding behaviours, which vary by life-stage, species and prey availability and with the use of different receptors they are able to selectively choose their prey (M. Cole et al., 2013). However this is not always the case, when their external appendages creates feeding currents that indiscriminately draw waterborne particles towards the mouthparts they unfortunately mistake the surrounding particles as a possible food source (M. Cole et al., 2015). It has been shown in previous work that zooplankton is capable of ingesting microplastics, with the particles being found in large amounts in the organisms' intestinal tract, highly concentrated fecal pellets and even being ingested in their pre-adult stage (K. Lee et al., 2013). M. Cole et al. (2013; 2015) found that microplastics were attached in-between the outer extremities which hindered their locomotion and furthermore that the ingested particles led to a decrease in both ingestion rate, fecundity and also reduced growth as a result of energetic deficiencies.

Ålabodarna is located on the west coast of Scania Sweden in the strait called Oresund, which connects the Atlantic Ocean with the heavily polluted Baltic Sea. The concentrations of microplastics in this brackish area and in the Baltic Sea is still unclear, due to insufficient amount of data and also very few surveys with this topic in mind has been conducted (J. Gustavsson – Länsstyrelsen Skåne, 2016). However, in 2013-2014 The Administration Board of Scania carried out a survey on the Swedish West Coast where they found on average 30 particles per litre when using a 10µm filter (F. Norén et al., 2014). In an earlier survey, F. Norén (2008) found as many as 102 000 particles per cubic metre, using an 80µm filter, in one harbor close to a polyethene production plant on the Swedish West Coast.

High concentrations like this could have a negative impact on various marine organisms around the Swedish coasts, not to mention the zooplankton, which are of great ecological importance in the marine food web. The present study focuses on what effect microplastics have on the filtration rate in *Acartia tonsa*, in order to further understand the copepods ability to handle the rising threats of microplastics in our oceans.

2. Materials and methods

2.1. Zooplankton Sampling. Zooplankton sampling was conducted in late October 2015 at two nautical miles west of Ålabodarna in Oresund. A 200µm plankton net was used, at approximately 35m of depth, to collect zooplankton via slow horizontal surface tows and vertical hauls. Collected zooplankton were held in 10L of natural seawater, within an insulated box, and thereafter transported to one of the laboratories at Lund university where they were maintained at ambient sea-surface temperature of 10^0C for 24h to both acclimatize and also allow for a full gut depuration. The following day the adult *Acartia tonsa* were identified and then carefully hand-selected for roughly the same size under a dissecting microscope using a pipette. A number of 12 individuals were then put in each

and every one of the 12 separate 75ml chambers containing artificial seawater with a salinity of 30psu, which were the same as the natural seawater collected at 35m of depth.

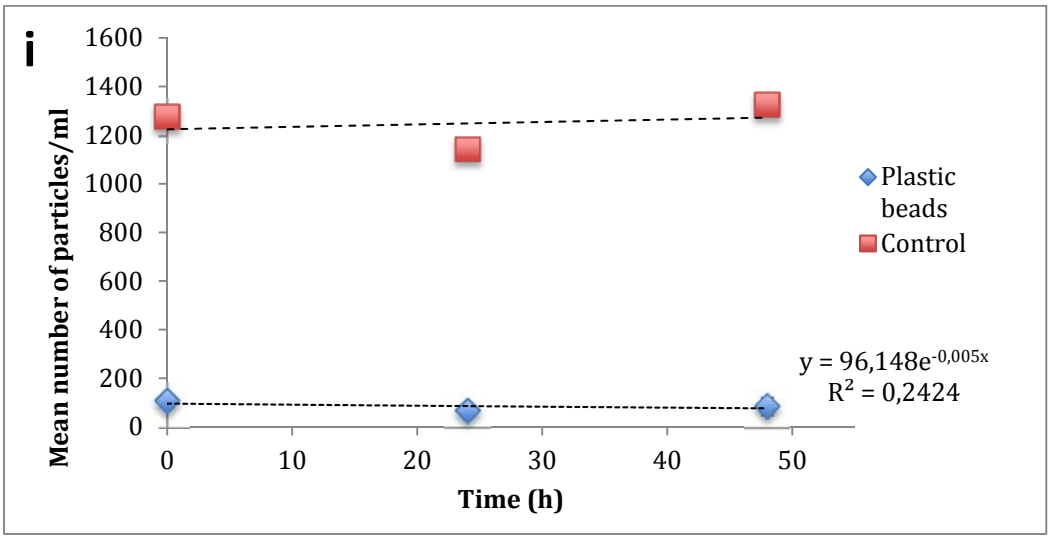
2.2. Treatment. The first group of four chambers (termed "MC_{1-4}") contained both copepods and 130 microplastic beads per ml – the particles were 10µm in diameter. The second group of four chambers (termed "AC_{1-4}") contained both copepods and an algal mixture consisting of *Heterocapsa triquerta*, *Dunaliella tertiolecta* and *Rhodomonas salina* with an approximate amount of 1300 cells per ml – the algae were between 10-15µm in diameter. The third group of four chambers (termed "MAC_{1-4}") contained copepods, microplastics and algae, with the same concentrations per ml as mentioned above. The last group of four chambers (termed "A_{1-4}") were the control group and only contained the same algae mixture – in order to see if the algae concentration would increase or not during the experiment. The choice of 130 particles per milliliter, represented a concentration of approximately 10% of the available food source (1300 algal cells), and were based on the factors of increasing amount of particles at higher depths in the natural environment (D. K. A. Barnes et al., 2009; M. Eriksen et al., 2014) hence >30 particles per litre without reaching extreme concentrations used in other studies (K. Lee et al., 2013; M. Cole et al., 2013). All 16 chambers were strapped to a plankton wheel, inside of an aerated aquarium, which rotated at 3rpm and thus allowed all the particles to be present in the whole water column during the entire experiment. A plankton wheel is a rotating cylinder inside of an aquarium that spins at different velocities depending on how fast one chooses it to turn (W. Kim et al., 2003). The lights were then turned off to inhibit the growth of the algae and the experiment was conducted for 48h.

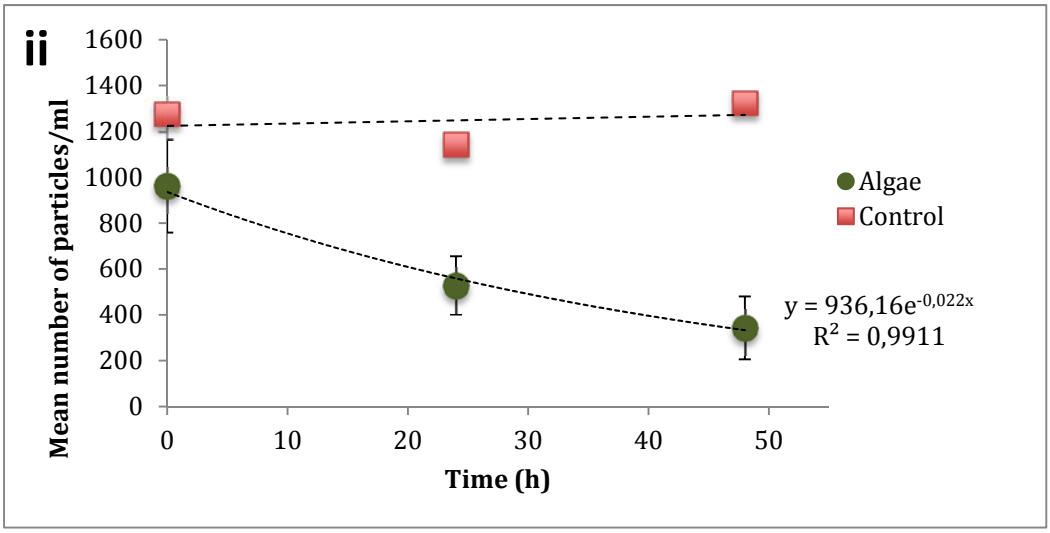
2.3. Measured Ingestion Rate by Using FlowCam. After 24h (t_{24}) all copepods in each chamber were counted for in order to see how many that were still alive, thereafter one ml from each container were extracted and one drop of Lugol´s solution were added in each sample to kill the algae – all dead copepods were removed to prevent contamination. Lugol is an iodine-based solution that is used as a preservative for marine organisms (A. J. Pomroy, 1984). This process was done one more time after another 24h (t_{48}) period. All dead copepods were looked at under a microscope in order to see if the plastic got attached to their extremities. All of the one ml samples taken from each container on t_{24} and t_{48} were then analysed for both algal and plastic content using a FlowCam. The FlowCam is a computer with a built-in microscope and a camera attached to it that takes pictures of all particles in the sample as it passes through a small tube. The device was set to count all particles within the range of 2-30µm, with an efficiency of 17.1% - meaning that 17.1% of the liquid was sampled, if the efficiency is too high then the same particle might be counted for more than one time. The pictures are later analysed by the computer, showing the total amount of particles per ml of samples and in the final step the pictures of every particle were manually checked in order to remove potentially unwanted particles such as fecal pellets.

2.4. Statistics. All data was analyzed in Microsoft excel by making scatterplots for calculating changes in ingestion rate and line charts in order to see the mean change in survival. In order to test for significance, a one-way ANOVA was made on the net-change in all groups.

3. Results

The results from figure 1 shows that the presence of plastic beads had no negative effect on the copepods filtration ability, however, figure 2 illustrates that these plastic particles did in fact have a negative impact on their survival (fig 2 A, C).





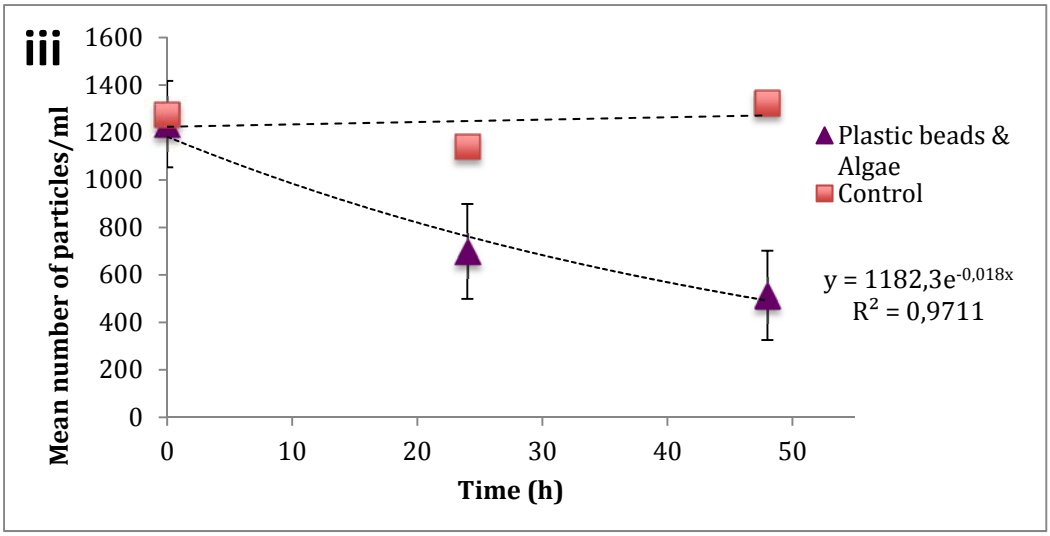


Figure 1. The graphs shows the mean change in number of particles over time (0-48h) in each group compared to the control. The first group "MC_{1-4}" (i) shows the ingestion rate in the copepod *Acartia Tonsa* exposed to only microplastic beads; second group "AC_{1-4}" (ii) shows the ingestion rate of algae; third group "MAC_{1-4}" (iii) shows the ingestion rate of both microplastic beads and algae. There were four number of replicates in each group.

The results also indicate that the copepods that were fed with both plastic beads and algae (fig.1 iii) had the highest ingestion rate followed by the ones that solely had algae as a food source (fig.1 ii). The copepods that were just fed with plastic beads had the slowest ingestion rate of them all (fig.1 i).

The first graph in figure 1 (i) shows the difference in number of plastic particles over time that the copepods ingested, compared to the control. After half of the experimental time the curve started to decrease but later increased towards the end. This increase could be due to errors in the distribution of particles inside the containers when samples were taken. The equation $y = 96.148e^{-0.005x}$ tells us that the group which were solely fed with microplastic beads had the slowest filtration rate of them all. The exponential relationship for the data in the first graph is also much lower, $R^2 = 0.24$, compared to the others, $R^2 = 0.99$ (ii) and 0,97 (iii).

Both the second and third graph (ii, iii) followed a similar pattern with increased filtration rate over time, but unexpectedly the copepods that were fed with nothing more than algae cells had a slower ingestion rate than the ones that were fed with both microplastic beads and algae cells. This can be seen when comparing the both graphs line equations, $y = 936.16e^{-0.022x}$ (ii) and $y = 1182.3e^{-0.018x}$ (iii), where we can see that the third graph has a slightly steeper curve and thus higher filtration rate.

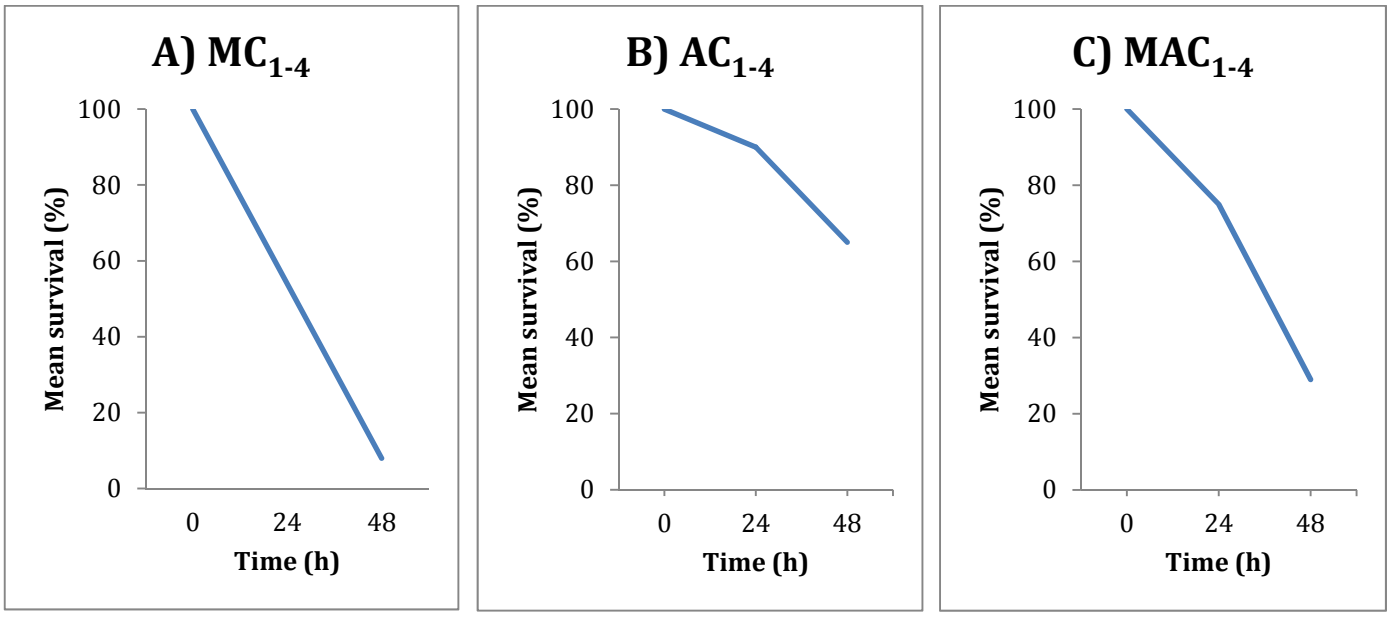


Figure 2. Effect on the survival of the *Acartia Tonsa* exposed to microplastic beads (A), algae (B) and to both microplastic beads and algae (C) over time.

Figure 2 shows the difference between all three groups in the copepods mean survival over time. The ones that only ingested microplastic particles (A) showed a high mortality rate of almost 90% during the 48h period. Group 3 (C), which were fed with a mixture of both microplastic beads and algae cells, did also show for a high mortality rate of approximately 70% whilst the ones that solely fed on algae (B) had the least mortality rate of 35%. P-value = 0,57

Table 1. The net change in mean number of particles per millilitre in all four groups, between t_0 and t_{48}. A one-way ANOVA test was made using the data from the groups beneath, p-value = 0.57.

Mean value / **Time**	*Group 1* Plastic beads	*Group 2* Algae	*Group 3* Plastic beads & algae	*Group 4* Control
t_0	108	963	1235	1277
t_{48}	86	343	514	1325
Net change	-22	-620	-721	48

Table 1 gives us the mean difference in particle uptake per millilitre between t_0 and t_{48} within each group. By comparing the net change between each group it further indicates their differences in filtration rate. Group three (plastic beads and algae) had the highest net change and thus the highest filtration rate, followed by the second group (algae) and then the first (plastic beads). As expected from the control group it had a small increase in algal cells, which indicates that the algae in group two and three might also have grown a bit. The result from the ANOVA-test, which was done on table 1, showed no significant difference ($p = 0.57$) between the groups.

4. Discussion

Microplastics have been found in many parts of the marine food web, especially in the water column with various impacts on the zooplankton (M. Cole et al., 2015). This study investigated what possible affect microplastic beads of 10µm in diameter might have on the filtration ability in the copepod *Acartia tonsa*. The results demonstrated no significant difference in filtration rate between the three groups (table 1), on the other hand, copepods feeding on plastic beads could have had a negative impact on their survival (figure 2 A, C). This in turn could indicate that the copepods living outside of Ålabodarna are able to ingest high concentrations of microplastics of around 130 particles per millilitre without any direct impact on their filtration ability; however, ingesting microplastics of these concentrations might decrease their survival. One possible reason for why the plastic beads did not alter the filtration ability could be due to the short time-scale, a prolonged exposure with a lower plastic concentration might have given negative affect in their filtration rate (K. Lee et al., 2013).

The net change in filtration rate did not show any significant difference between group 2 and 3 (Table 1), suggesting that the presence of microplastics whilst feeding on algae does not change their feeding ability. Group 1, which solely fed on microplastics (table 1), did indicate a slower ingestion rate, in comparison with group 2 and 3. This could mean that the ingestion of microplastics could alter their feeding habit and cause mechanical effects such as a blockage in the feeding appendages or hinder the passage of food through the intestinal tract, leading to a decrease in food intake (J. Wang et al., 2016).

By comparing both figure 1 and 2 one can see how the survival affects the filtration rate. The filtration rate was highest during t_0-t_{24} and later decreased in the last 24h in groups (ii) and (iii), this is most probably due to the fact that many copepods died, even in the containers that had nothing more than algae as a food source. Group (i) had an increase between t_{24}-t_{48} (probably due to errors during the sampling) but the decreasing trend between t_0-t_{24}, which later I presumed stopped, could be because most of them died and the ones that lived might have gotten internal blockages. For future studies it would be interesting to see what kind of impact lower concentrations might have on long-termed exposure to plastics.

The chosen plastic concentration that were used in this study were 4000 times higher per litre than the estimated amount that were found around the Swedish coasts, even though this might seem like a high amount one needs to understand that the concentration at 30m of depth is still unclear. Due to the fact that microplastics do have the ability to accumulate into high amounts at the bottom of the sea floor it is very well plausible for it to get re-suspended into the water column once more but in higher concentrations (D. K. A. Barnes et al., 2009; S. L. Wright et al., 2013). According to M. Eriksen et al. (2014) there are more than 5.25 trillion plastic particles around the world that are currently afloat at the sea, not taking into account for the amount of particles that is suspended in both the water column nor the sediment. One possible source of microplastic leakage into Oresund could be from the Sewage treatment plant in Helsingborg (NSVA). As stated from Jan-Erik Petersson, wastewater treatment specialist in NSVA, the treatment plant does not actively remove microplastics from the wastewater that enters Oresund and as of today they have no data of how many particles that might be present in their wastewater (J. Petersson – NSVA, 2016). Oresund is also located in-between larger cities like Copenhagen, Malmö and Helsingborg; the sediment from these densely populated costal areas can be heavily polluted with plastic particles (S. L. Wright et al., 2013). F. Norén et al. (2010) found a concentration of approximately 100 particles per litre outside a Swedish harbour that were located right next to a polyethylene production factory in Stenungsund.

So when including these factors, the choice of concentration that were used in the present study were set out to be more realistic and accounted for the potential increase in concentrations at increasing depths in Oresund within the scope of a laboratory based study. In similar studies extreme concentrations of around 2.5×10^7 (M. Cole et al., 2013) and 5.2×10^8 beads per litre (K. Lee et al., 2013) were used. Although the information from these experiments is still of great value for future studies, they do not seem to be targeting any realistic concentrations in todays natural environment as the one in this study.

During visual observations under the microscope I noticed that some of the plastic beads got wedged in-between their appendages in figure 2 (A) and (C), which suggests that their locomotion got hindered (M. Cole et al., 2013) and thus making it harder for them to migrate for food. Some of the specimens also had plastics attached to their outer shell, which could have changed their buoyancy (F. M. C. Fazey et al., 2016), thus enable them to spend more energy than is needed when staying at a certain depth. However, this does not entirely explain why more copepods died in figure 2 (A) rather than in (C), because both were fed with equal amounts of plastics. Although this is just a speculation, but maybe the total abundance of distributed algae in (C) were enough for them to survive without having to scavenge for food in the entire container, unlike in (A) where they had less distributed particles in the same amount of volume as in (C). This could furthermore

explain why the survival declined faster after 24h in figure 2 (C) when food became more scarce (figure 1, iii).

Starvation could also be one possible source for why the survival decreased, the copepods that were solely fed on plastics hadn't had any other food source for 72h in total. Nonetheless, this does not explain why the copepods that were fed with both plastics and algae also had quite a high decrease in survival rate. In future studies I recommend having a starvation control, by doing this one can make further conclusions about the impact that microplastics might inflict.

Not only does these particles cause physical harm to the organism, but reports have also shown that persistent organic pollutants (POPs) that occur at low amounts universally in the surrounding water can accumulate into high concentrations on a single plastic particle (U. Mato et al., 2001; A. L. Andrady, 2011). The study made by P. Farrell et al. (2013) indicated that microplastics that are introduced in one trophic level might very well get furthered transferred into another one. However, it is still unknown whether these endocrine disruptors will get released after ingestion and then bioaccumulate in the organisms' tissues, thus furthermore what kind of impact it might have on the biota within each trophic level (A. Bakir et al., 2014; O. Setälä et al., 2014). It would be out of interest to see how animals in the lower thropic level will cope with polluted particles and whether or not they will accumulate it to higher concentrations within them without altering their survival – if they would die from the toxins its not as likely that they will accumulate it and biomagnification might not occur. So with increasing concentrations of microplastics arising all around the globe the potential threat of highly concentrated polluted particles could be of rising interest in the near future.

Another problem with microplastics are their size, D. S. Wilson (1973) found that *Acartia tonsa* are able to ingest particles in various sizes between 7-70µm in diameter. The results from the present study illustrated a trend of decreased survival (figure 2A, C) when ingesting plastic beads in the size of 10µm, suggesting that perhaps larger particles might have another effect on the filtration rate because the increase in size might make the intestinal blockage occur much faster (K. Lee et al., 2013). While the microplastics might not be small enough to enter the tissues of the copepods, recent studies have shown that further degradation of the plastic leads to even smaller particles called nanoplastics which seems to be able to get transported through the cellmembrane or accumulate deeper down in the organisms organs (K. Mattsson et al., 2015; H. Bouwmeester et al., 2015). One important aspect is that the plastic beads that were used in this study were all manufactured in the same size of 10µm and even though this could resemble some of the primary sources of microplastics, it is far more likely that both the size and shape will be different in nature thus have different impacts (K. Lee et al., 2013).

By looking at the net change one can see and compare how many particles that has been ingested by the organism between different timespans. However, as seen in figure 1 (i) the concentration increased at t_{48} and this could be due to error with the FlowCam setup since there shouldn't be a way for the plastics to increase. This fault caused the net change for the plastic group to be less, however the net change between t_0 and t_{24} was the lowest in this group as well and they still cleared the least amount of particles.

One possible source of error in this study could be due to plastic beads not being evenly distributed inside each container, even during the rotation of the plankton wheel, which suggests that the movement of the water was insufficient. Another problem were that increasing the rotation speed might inflict stress or even physical damage that could alter

their survival. Many copepods still died during the experiment (figure 2) and could indicate that maybe even 3rpm were too fast, although this does not explain why a larger amount of copepods died when plastics were present. For future studies I suggest that the speed of the plankton wheel will be more closely focused so that the particles will be more evenly distributed.

Lastly, when making the artificial seawater I used tap water that could have contained traces of copper and because copepods are quite sensitive to waterborne metal particles like copper (B. K. Bielmyer et al., 2006) this might be a possible source for why their survival decreased. Something that speaks against this is that the copepods in figure 2 (B) did not have as high decrease in survival rate as the others, even though the same medium were used in all containers.

5. Conclusion

This study found that the copepod *Acartia tonsa´s* ability to filtrate algal cells of approximately 1300 cells per millilitre did not get altered when being exposed to a microplastic concentration of 130 beads per millilitre. When solely being exposed to microplastics the filtration rate seemed to illustrate a trend of decreased ingestion rate, however, no significant difference in net change between the test groups were found. The individuals that were exposed to microplastics showed a higher decrease in survival in comparison with the ones that only fed on algae. This could mean that even though the filtration rate does not get altered, the overall survival of the organism is threatened at these concentrations. Furthermore the visual observations showed that microplastics could very well get attached to their appendages and thus hinder their locomotion and have a negative impact on their fitness. If being exposed to lower concentrations during a prolonged exposure time the impacts would most probably have been different and their filtration rate could have shown a negative trend without a high decrease in their survival, however this was not studied but would be of interest in future research. Some of the major dangers when it comes to microplastics are their difference in size in nature and the potential damage that persistent organic pollutants might inflict, not only to the targeted organism but also species in other thropic levels. The combination of continued and increasing releases of plastics into the environment all around the globe, and the fact that these objects will remain in the oceans for a long time means that in the future we will probably see further negative impacts on the marine life.

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