The Effect of Zooplankton Grazing on Phytoplankton Biomass

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An ex situ study investigating the effects of microzooplankton grazing on phytoplankton biomass was conducted at the Estuarine and Freshwater laboratory at the Nelson Mandela University (33°S, 25°E), from samples taken at North End Lake, in Port Elizabeth using the dilution technique. The study aimed at exploring the potential use of microzooplankton as a form of biocontrol of heavily polluted algal systems in relation to chemical controls. The North End Lake is a prime example of a hypereutrophic system that is prone to algal blooms consequently from industrial processes that increase the nutrient load in the system. Water samples were collected from the North-End Lake, in Port Elizabeth (33°S, 25°E), where three treatment groups were setup – a control treatment consisting of no grazers, a low-density grazer treatment and a high-density grazer treatment, at ratios of 1:4 and 1:1 of concentrated grazers against filtered water, respectively. A proportional relationship was predicted between grazer density and phytoplankton biomass; where increased grazing consequently of a higher density of microzooplankton will cause a significant decrease in the phytoplankton biomass. The results of the study found that at higher microzooplankton densities, phytoplankton biomass reduced significantly as compared to lower microzooplankton densities. Phytoplankton biomass decreased non-sequentially throughout the study. Initially, all three treatment groups had an average of 65µg/L, where phytoplankton biomass significantly dropped, to 10µg/L in the last week of the study for the control and low-density grazer treatments; the high-density grazer treatment recorded no biomass during the same period. Further statistical analyses outline that physicochemical parameters played a non-significant role in controlling phytoplankton biomass (p=0.0785 for temperature and, p=0.0652 dissolved oxygen). The microzooplankton community largely comprised of Copepoda and Rotifera species with a relatively lower number of Cladocera species.

Keywords: Zooplankton grazing, phytoplankton biomass, herbivory, top-down control, eutrophic systems

1 Introduction

Phytoplankton are microscopic autotrophic organisms, also referred to as microalgae, that inhabit the photic zone of the water column (Vanni, 1987; Rautio & Vincent, 2006). These organisms range between 5-200µm in body size and can be found in freshwater and marine ecosystems (Vanni, 1987; Levine et al., 1999). Phytoplankton convert light energy to organic compounds, namely carbohydrates in the form of sugars, that heterotrophic organisms utilize as a food source (Behrenfeld et al., 2001; Rautio & Vincent, 2006). Consequently, the occurrence of phytoplankton in aquatic ecosystems is critically important in the formation and sustenance of complex food-webs that enable the proliferation of biodiversity as we see it – where the long-term sustenance of these food webs is through carbon fixation – keeping the energy flow in aquatic ecosystems balanced (Levine et al., 1999; Behrenfeld et al., 2001).

Studies, (*e.g.,* Behrenfeld et al., 2001; Rautio & Vincent, 2006; Henson et al., 2010), have found that phytoplankton contribute to about 50% of the world's photosynthetic activity, which clearly showcases the significant role of phytoplankton in aquatic ecosystems.

Microzooplankton are heterotrophic organisms that range between 20-200µm in size (Vanni, 1987; Kim et al., 2006). Like phytoplankton, these organisms are important in aquatic food webs, as predators that control phytoplankton abundance/biomass (Kim et al., 2006). Since they feed on the phytoplankton (primary producers), they create a link between larger predators and smaller prey through a trophic cascade that is important in nutrient cycling and dissolved and particulate organic matter (Levine et al., 1999; Kim et al., 2006). Kim et al. (2006) outlined that grazing in the ocean accounts for 67-75% of the daily growth of phytoplankton, of which holds true in freshwater ecosystems as well**.** This influence of grazing, however, could be attributed to the trophic levels above the zooplankton, mainly planktivorous fish, which feed on the zooplankton, reducing the grazing pressure of microzooplankton on phytoplankton (Vanni, 1987; Kim et al., 2006).

Understanding the factors that influence the abundance of phytoplankton is important ecologically, considering the intensification of environmental stressors related to global warming. Changes in climate conditions may affect the behaviour and physiology alike of these microorganisms where temperature differences may affect different metabolic reactions that depend on stable environmental temperature conditions (Vanni, 1987; Levine et al., 1999). These changes may also cause vertical thermal stratification of the water column affecting the mixing of surface and deep waters, which in turn would have a negative impact on the supply of nutrients to surface phytoplankton communities leading to massive die-offs of species in higher trophic levels that depend on phytoplankton for energy generation (Henson et al., 2010). Another important factor that can be altered is the feeding strategies of microzooplankton on phytoplankton biomass, which consequently, may contribute to the formation of harmful algal blooms (HABs).

Lair & Ali (1989) found that the grazing of *Rotifera* species on phytoplankton is an important factor that influences the seasonal succession of phytoplankton communities. This is ecologically important because it limits the development of algal blooms, which negatively impact the health of ecosystems (Lair & Ali, 1989). Algal blooms occur when there is a higher growth rate relative to the removal rate of phytoplankton (Turner & Anderson, 1983; Vanni,1987). Although algal blooms are naturally occurring, these can be influenced by anthropogenic impacts such as eutrophication, where high concentrations of nutrients, such as phosphorous and nitrogen, are introduced into a low-nutrient system causing an imbalance (Vanni, 1987). Algal blooms are harmful in that they create anoxic conditions that negatively affect other species such as shellfish, which as a result, become highly toxic – posing threats to higher trophic levels (Turner & Anderson, 1983; Henson et al., 2010).

Physicochemical factors such as light and temperature also affect phytoplankton biomass (Levine et al., 1999; Liu et al., 2002; Kim et al., 2006). These factors directly affect phytoplankton through the alteration of key physical mechanisms such as the vertical mixing and sinking wherein, causing changes in nutrient availability (Kim et al., 2006). Seasonal changes also influence grazing (Liu et al., 2002; Liu et al., 2006). In different seasons, the amount of nutrients available in the water column varies because of mixing processes. Winter and summer have lower nutrient levels because of surface water

temperatures that results in the thermal stratification of the water column (Liu et al., 2002; Liu et al., 2006), which in turn limits productivity and subsequent grazing rates. The amount of nutrients available for phytoplankton is limited in winter and summer; limiting the amount of food available for the microzooplankton to feed on.

This study aims at investigating how herbivory from varying microzooplankton grazing densities influence the phytoplankton biomass, wherein a conclusion can be drawn as to whether this can be used as an alternative form of biocontrol for the formation of HABs in eutrophic freshwater systems. It was hypothesized that microzooplankton are effective grazers that control the phytoplankton's biomass, directly via herbivory and indirectly via the introduction of interspecific competition. A proportional relationship was predicted between microzooplankton grazing and phytoplankton biomass, where higher microzooplankton densities will result in greater herbivory of phytoplankton and subsequent reduction in phytoplankton biomass.

2 Methods & Materials

2.1 Study site

The North-End lake is a highly polluted system; the surrounding industrial companies release nutrient-rich runoffs in the form of effluent into the system, which as a result, elevates the nutrient load of the system causing it to be higher than its natural state (Kampire et al., 2015). Because of these runoffs, the North-End Lake has a high abundance of *Escherichia coli* and is highly contaminated with heavy metals, decreasing the aesthetic value of the lake (Kampire et al., 2015). The rehabilitation of this system has focused much on the use of biosynthetic materials which, over time, present further ecological problems such as ecosystem degradation. The *ex situ* study was conducted at the Estuarine and Freshwater laboratory at the Nelson Mandela University (33°S, 25°E) using the dilution technique (Hasset & Landry, 1982).

2.2 Sampling

The water samples were collected at the North-End Lake in Port Elizabeth. Water samples from this system were filtered through an 80µm, to remove debris and macrozooplankton which could alter the study. The water was further filtered through a 30µm net to culture the microzooplankton grazers, wherein the final filtrate was free of grazers. The filtered water samples, collected into 20L carboys, were then transported back to the laboratory for analysis. The carboys were sterilized using 70% alcohol, prior to site collection. Upon arrival on site, these carboys were first rinsed with the lake's water before being filled, to reduce any cross contamination.

2.3 Laboratory work

The collected water sample was filtered through a 30µm to culture the microzooplankton grazers desired for the study (*i.e.* 30-80µm). Afterwards, nine Erlenmeyer flasks (500ml) were setup in a randomised block design as tanks to mimic natural systems – where a set

of three flasks were set out for each treatment group – namely the control, low-density grazer, and high-density grazer treatments. The maximum volume of each flask was set to 400ml for each replicate of the three treatments. The control flasks were filled with 400ml of the filtered sample water, which consisted of no grazers.

Afterwards, the two other treatment groups (*i.e.* low and high-density grazer treatments), had each flask filled with 350ml of the filtered water with no grazers. The remaining 50ml was to be diluted as per the dilution technique (Hasset & Landry, 1982). The 50ml for the low-density grazing treatment was diluted in the ratio of 1:4, where 1 part consisted of microzooplankton culture and 4 parts were the filtered water; a total of 275 grazers introduced. The high-density grazing treatment was diluted in a 1:1 ratio, with a total of 653 grazers introduced.

Over four weeks, on a weekly basis, the physicochemical parameters of each individual flask were measured and recorded, under the same time of day. Thereafter, 50ml in each of the replicates of each treatment were extracted into marked plastic vials and filtered. The extracted 50ml was then replaced by stored filtered water from a designated reservoir, holding the initial 400ml volume. The filter paper obtained from filtering each of the 50ml in the vials was then placed inside a marked plastic vial with 10ml of 70% ethanol, which was then kept in the freezer over 24 hours.

After 24 hours had elapsed, the plastic vial was removed from the freezer where the contents were filtered once again. The filtrate was then analysed using the spectrophotometer to measure absorbance. The filtrate from each vial was transferred into a glass cuvette, which was then placed inside the spectrophotometer to measure the absorbance. After the first absorbance reading was taken, ~1ml of acid (1N HCl) was then added to the cuvette and a second absorbance reading was then taken. The monochromatic equation (Hasset & Landry, 1982) was used to calculate the chlorophyll *a* concentration (herein Chl. *a*).

The microzooplankton community structure was determined using a light microscope. 50ml of both treatment groups – low and high-density, were analysed after preservation in 70% ethanol. The different phyla were distinguished, where after, individuals of each family were counted. Using a pipette, the 50ml was spread along a raceway slide. This was then placed under a light microscope. The frequency of individuals from the different families were recorded. This was done at the initial stage of the study and at the end of the study.

3 Results

3.1 Experimental data

Figure 1 shows the average biomass (Chl. *a*) for the different treatments over four weeks. Initially, all treatments showed high levels of biomass that ranged between 60-70µg/L. After a week of the study, the biomass remained relatively constant as it was in the initial week, with the low treatment recording 82µg/L, the highest biomass amongst all treatments (65µg/L and 50µg/L for the control and high-density treatments). From week 2 onwards, there was a significant drop in biomass across all treatments with a ~70µg/L change for the low-density treatment between week1 and 2 . In the last two weeks, the high grazing

treatment recorded no biomass whilst the biomass remained constant for the other two treatments (*i.e.* recording 10µg/L).

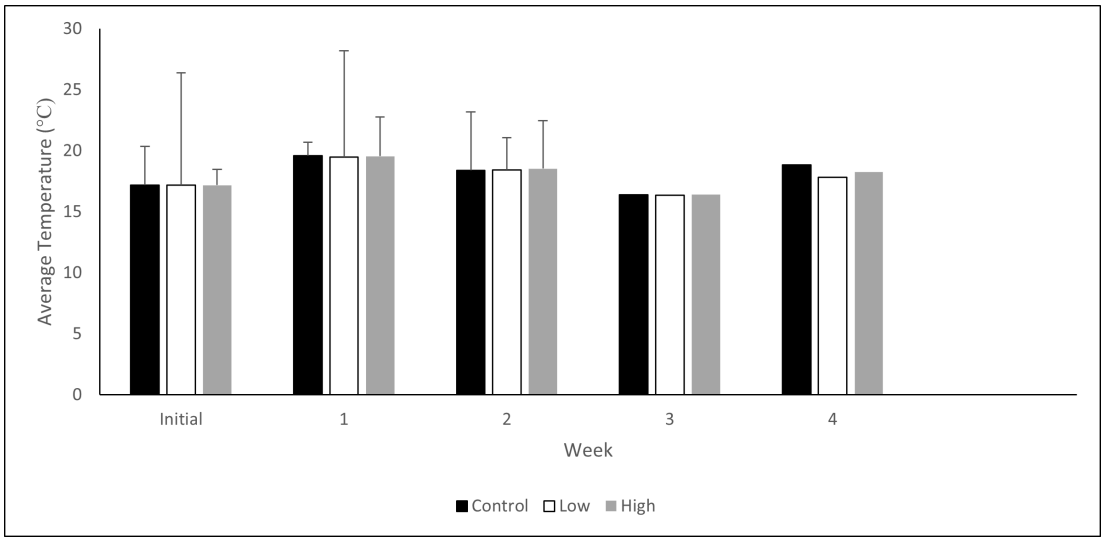

Figure 1: the average phytobiomass (Chl. a) for the different treatments over four weeks

Figure 2 shows the average temperature for the different treatments over four weeks of the study. The temperature recorded over the four weeks remained relatively constant between 15 - 20°C with no significant differences overall across the four weeks. Week 1 had the highest temperature recorded at 18°C, whereas week 3 had the lowest temperature recording at 15°C.

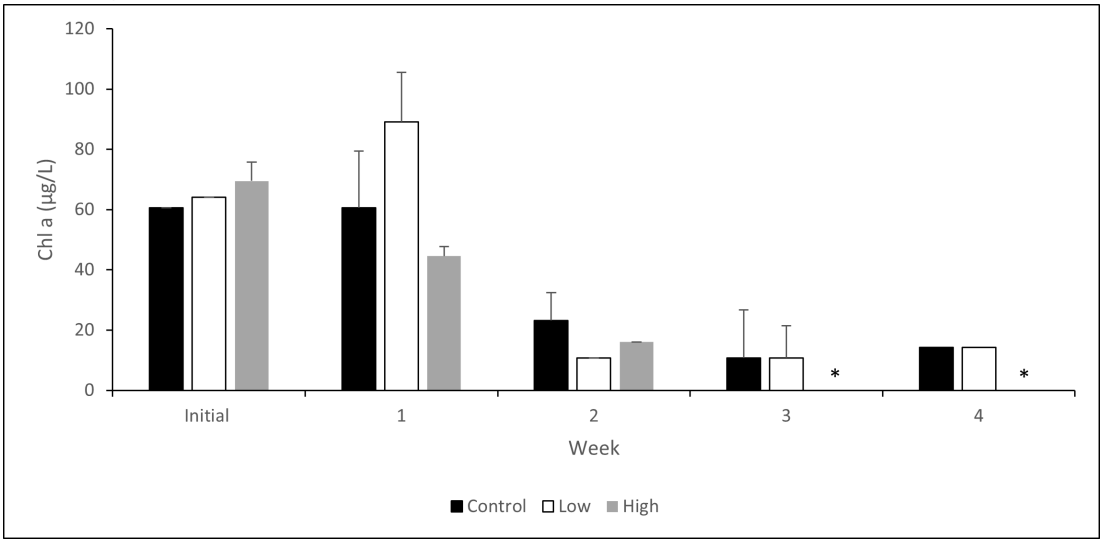

Figure 2: the average temperature for the different treatments over four weeks

Figure 3 shows the average dissolved oxygen for the different treatments over four weeks. Initially, the dissolved oxygen was relatively high, at 100% for all treatments. In week 1, the levels dropped significantly across all treatments (*ca.* 60% across all three treatment groups). In week 2, the dissolved oxygen across all treatments increased significantly

ranging between 100-110%. In the last two weeks, the dissolved oxygen maintained a relatively constant rate at *ca.* 80%.

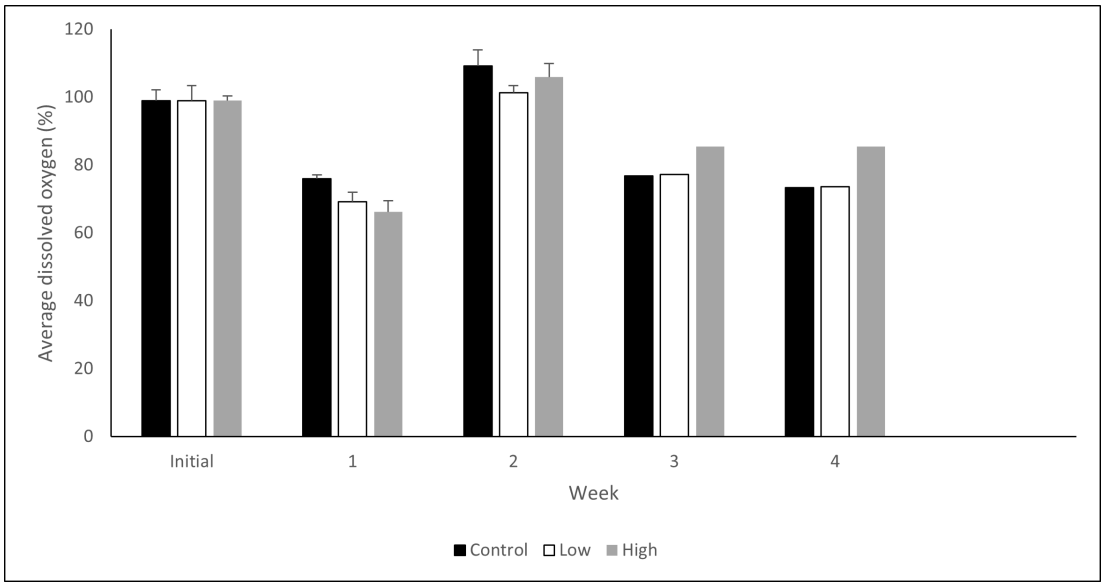

Figure 3: the average dissolved oxygen for the three different treatments over four weeks

Table 1 shows the physicochemical parameters for the different treatments over four weeks. The physicochemical parameters recorded include pH, electrical conductivity (µs/cm), salinity and total dissolved solids. These parameters showed relatively little change over the four weeks where salinity ranged between 0.71-0.79. The total dissolved solids also ranged between 435 – 776. The pH also remained constant between 6.75 – 7.24. * week 1 however had the highest pH measurements across all weeks because of instrumental error.

Date	Treatment	pH	Electrical Conductivity (µs/cm)	Salinity	Total Dissolved Solids
Initial	Control	7,2 ± 0.0	1552 ± 0.0	0.8 ± 0.0	776 ± 0.0
	High	7,2 ± 0.0	1552 ± 0.0	0.8 ± 0.0	776 ± 0.0
	Low	7,2 ± 0.0	1552 ± 0.0	0.8 ± 0.0	776 ± 0.0
Week 1	Control	*10,2 ± 0.1	836 ± 40.5	0,4 ± 0.0	435 ± 48.7
	Low	*10,2 ± 0.1	1530 ± 16.0	0.8 ± 0.0	766 ± 7.2
	High	*10,1 ± 0.1	1417 ± 11.9	0.7 ± 0.0	729 ± 38.3
Week 2	Control	6,9 ± 0.0	1498 ± 39.5	0.8 ± 0.0	749 ± 19.5
	Low	6,8 ± 0.0	1449 ± 9.9	0.8 ± 0.0	725 ± 4.4
	High	6,8 ± 0.0	1332 ± 92	0.7 ± 0.0	666 ± 46.6

Week 3	Control	8.0 ± 0.1	1552 ± 24.7	0.8 ± 0.0	744 ± 24.6
	Low	7.9 ± 0.1	1532 ± 11.0	0.8 ± 0.0	768 ± 5.5
	High	8.1 ± 0.1	1448 ± 21.0	0.7 ± 0.0	726 ± 4.9
Week 4	Control	7.4 ± 0.4	1543 ± 12.9	0.8 ± 0.0	760 ± 25.6
	Low	7.4 ± 0.5	1521 ± 9.5	0.8 ± 0.0	763 ± 5.7
	High	6.9 ± 0.3	1434 ± 42.0	0.7 ± 0.0	726 ± 5.7

Table 1: the physicochemical parameters for the different treatments over four weeks (mean±SE).

Table 2 shows the community structure of the microzooplankton. The number of grazers increased with concentration, where the low concentration was diluted (in the ratio 1:4) and the high concentration undiluted. Overall, the number of grazers was relatively high initially than in week 4. *Rotifera* and *Copepoda* individuals were more abundant, 510 and 410 for both treatments, than *Cladocera* species which were less abundant, 13 in total over both treatments. After week 4, the trend observed was the same as that seen initially. High concentrations had a higher number of Copepoda individuals, 334, than *Rotifera*, 309 and *Cladocera*, 10. The reverse was observed in low concentrations, where there was a higher number of *Rotifera* individuals than *Copepoda* and *Cladocera*.

	Rotifera	***Cladocera***	***Copepoda***
Initial			
Low grazing.	201	3	71
High grazing.	309	10	334
Total	**510**	**13**	**405**
Week 4			
Low grazing.	24	3	8
High grazing.	37	5	58
Total	**61**	**8**	**66**

Table 2: the zooplankton community structure for low and high grazing treatments at the start and at the end of the experiment

Statistical analyses using a multilinear regression (MLR) showed that temperature and dissolved oxygen influenced the change in phytoplankton biomass in relation to other environmental variables. A two-way ANOVA showed that the relationship between grazing density and the phytoplankton biomass over the four weeks was significant, p=0.0371 (p<0.05). Change in phytobiomass (Chl. *a*) was not significantly influenced by temperature, p=0.0785 nor dissolved oxygen, p=0.0652 (p>0.05).

4 Discussion

The results obtained from the study supported the hypothesis tested, microzooplankton are effective grazers that control phytoplankton biomass, directly via herbivory and indirectly via the introduction of interspecific competition. As predicted, microzooplankton grazing and phytoplankton biomass were proportional, where higher microzooplankton densities resulted in greater herbivory of phytoplankton and subsequent reduction in phytoplankton biomass (herein phytobiomass). Furthermore, the study found that phytobiomass was not significantly influenced by environmental factors, as is by grazing, although the temperature and dissolved oxygen had an influence in grazing activity, with cooler days resulting in greater activity (*c.f.* figure 1 and figure 2).

Phytobiomass over the study period declined significantly in the last two weeks of the experiment – more so for the high-density treatment – with the control maintaining higher phytobiomass throughout the duration of the study. This trend (*c.f.* figure 1) was expected since the more densely populated a given treatment was with grazers, the higher the grazing pressure on the phytoplankton biomass, subsequently resulting in a greater removal of the phytobiomass (Anderson & Harvey,2019).

4.1 Environmental effects on phytobiomass

In the first week, the low-density treatment had the highest phytobiomass recorded against the other two treatments. Two explanations could be used to explain this observation – the first of those being the increase of phytoplankton productivity consequently due to environmental factors, with the second being the selectivity of the microzooplankton grazers (Vanni, 1987; Kim et al., 2018). Firstly, since the tanks were randomly assorted using the randomised block setup, some of these were shielded, and others exposed to direct sunlight, wherein the intensity of light reaching the different tanks might have influenced phytoplankton productivity. In our experiment, week 1 had the highest temperature (*c.f.* figure 2) and minimal cloud cover (*field observation*), relative to the other three weeks where temperatures were relatively lower with frequent cloud covers (*field observation*).

Phytoplankton are primary producers that require sunlight for production (Rautio & Vince, 2006; Cottiene et al., 2001) where these species tend to occur in the photic zone of the water column where maximum sunlight is available for photosynthesis (Levine et al.,1999; Liu et al., 2002). A limitation of sunlight would have reduced the productivity during those periods, negating an increase in biomass. Thus, the placement of control tanks in an area that was exposed to high sunlight favoured for production wherein increasing the overall phytobiomass relative to the other treatment groups whose tanks may have been less exposed to direct sunlight. Notably also, during this period, the dissolved oxygen in the control treatment (*c.f.* figure 3) in the control treatment decreased, relative to the other treatment groups, as a consequence of increased phytoplankton productivity (Kim et al., 2018; Anderson & Harvey, 2019). This further reiterates that the presence of grazers reduced the possibility of an algal bloom by maintaining high levels of oxygen in the treatments consisting of grazers.

4.2 Selective feeding by microzooplankton

Secondly, microzooplankton are selective feeders (Schoenberg & Carlson, 1984). These grazers tend to select for palatable over unpalatable phytoplankton, where the selection depends primarily on body size, because of an energy trade-off where the energy gained by the grazer is greater than the energy used in predation (Vanni, 1987; Liu et al., 2006). Therefore, larger-bodied grazers tend to feed extensively on smaller phytoplankton relative to large phytoplankton (Vanni, 1987), as the greater the prey the greater the energy used in its predation. Consequently, this selectivity tends to shape the phytoplankton community structure, where larger species tend to be more prominent than their smaller-sized conspecifics, wherein these produce more and thus retain high phytobiomass.

The community structure of the microzooplankton in our study was primarily made up of *Rotifera* and Copepoda species – these species are generally selective in their feeding as compared to other *grazers* such as Daphnia which are less selective (Cottiene et al., 2001). Since copepods and rotifers prefer frequent feeding, the cost of predating on larger-bodied phytoplankton is greater than smaller-bodied phytoplankton. The phytoplankton species present in the tanks of the low-density treatment (in week 1, *c.f.* figure 1) might have been selected against by the lower number of grazers, leading to a reluctance in the grazing of these by the microzooplankton grazers, herein allowing these to produce and retain a high phytobiomass.

However, with the intensified removal of the smaller phytoplankton and the prominence of the larger phytoplankton, the selectivity of these may reduce, as a means of survival (Pomati et al., 2020). This is evident in week 2 onwards, with the phytobiomass greatly reducing in the grazer treatments, with the high-density treatment recording no phytobiomass in the last weeks of the study. Greater reduction in the high-density treatment was expected as various factors influenced grazing in this treatment group, which includes interspecific competition amongst the grazers consequently due to higher grazer numbers (Kim et al., 2019). Another interesting observation from this study is how the control treatment responds over the duration of the study – with a frequent decline of phytobiomass regardless of not having active grazers, as in the other two treatments.

4.3 Interspecific competition on phytobiomass

Theoretically, this treatment should have retained high phytobiomass throughout the four weeks – which surprisingly does not hold in a practical setup. Various factors may be at play, with interspecific competition being a key reason. The absence of prey, which acts as a controlling factor, introduced an increased need for the competition for essential resources (*e.g.* nutrients and adequate light) amongst phytoplankton species, wherein individuals that were outcompeted could not reproduce and subsequently died (Vanni, 1987; Kim et al., 2018). Those that survived, reproduced further increasing competition for resources. With the random assortment of the tanks, where these could have been placed in areas of reduced sunlight and reduced nutrient cycling in the water, the phytobiomass of this treatment rapidly declined over time. The combination of high interspecific competition and low nutrient cycling in the tanks, intensified selection, regardless of not having any grazing pressure.

5 Conclusion

It is evident from this study that grazing acts as a control for phytoplankton biomass. This outlines that intertrophic relationships can be used to maintain healthy ecosystems when these are used as biocontrol agents, in relation to chemical methods. It also outlined how various factors influence grazing activity and phytoplankton productivity, where in the event of an algal bloom, these can be manipulated to control and limit the intensity and persistence of such.

Understanding that nutrient enrichment influences phytoplankton growth (Behrenfeld et al., 2001), introducing measures to divert such nutrient offloading into water bodies is only half the problem solved; the next would be to create a healthy ecosystem by manipulating intertrophic relationships. In the case of the North End Lake, the introduction of grazers may increase herbivory and herein maintain high phytoplankton diversity (McCauley & Briand, 1979, Schoenberg & Carlson, 1984), limiting the abundance of persistent algal bloom-causing species. This, in turn, promotes a healthier ecosystem in relation to chemical treatments that further persist in the water column which in future may result in heavy metal overloads in the water.

Areas for further research includes the use of an improved technique to estimate phytobiomass. The dilution technique used in the study, if done incorrectly, might introduce bias, since there is a large room for error that can stem from the cross-contamination of samples. Another factor to consider is the replenishment of the water that acts as a reservoir for the extracted water. The use of this might influence the growth rates of the phytoplankton mainly as a consequence of the difference in physicochemical properties – the water used to replenish these tanks might be less oxygenated or have elevated levels of nutrients, which will ultimately affect the growth of the phytoplankton. Kimmance et al., (2007) discusses how changes in the physiological conditions of the phytoplankton due to experimental conditions differing from natural conditions may affect both grazing efficiency and rate.

Secondly, instead of focusing solely on microzooplankton grazer density, these can be fractioned by body size. This may allow for a better understanding of which grazers are most efficient in controlling phytobiomass. This in turn, will allow for better planning around algal blooms and their subsequent control, with the most effective grazer fraction sizes being introduced first as a means to effectively control the phytoplankton population.

Lastly, season may also have an impact altogether since at different seasons, thermoclines influence nutrient availability which, affect the abundance of phytoplankton and microzooplankton communities in natural systems (Zheng et al.,2006; Anderson & Harvey, 2019). Winter has a less persistent thermocline, but mixing is prohibited by light availability; summer unlike winter, has a persistent thermocline where surface and deep waters are prohibited from mixing resulting in low nutrient availability whereas in spring and autumn the opposite occurs (Zheng et al., 2015; Anderson & Harvey, 2019). Therefore, ensuring that the study is designed around such can reduce the bias. Essentially, for a more accurate understanding, one should run the experiment using samples from two different seasons as these will account for nutrient differences in the water and subsequently provide

a better argument for the use of microzooplankton grazing as a control for phytoplankton blooms, more importantly, HABs.

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