How brownification and eutrophication effects a drinking water source – with Lake Bolmen, Sweden, in focus

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Picture first page: Lake Bolmen, picture taken by Stina Stomberg 5/4-2022

Stina Stomberg

MVEM12 Examensarbete för Masterexamen 30 hp, Lunds universitet

Intern handledare: Anna Borgström, Akvatisk Ekologi, Lunds universitet

Extra intern handledare: Johanna Sjöstedt, Akvatisk Ekologi, Lunds universitet

CEC - Centrum för miljö- och klimatvetenskap
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Abstract

The ongoing human impact in form of deforestation, urbanization and intense agriculture is affecting the natural hydrology and our freshwater lakes negatively. Both eutrophication and brownification has seen to increase with the ongoing environmental change which can potentially have large impact on our freshwater ecosystems.

Using a mesocosm experiment, this study investigates the individual and synergistic effects between eutrophication and browning. Specifically with focus on phytoplankton and zooplankton abundance and community structure, as well as toxicity of cyanobacteria. The mesocosm experiment was run over 32 days in Lake Bolmen, Sweden in July-August 2021.

The result from this study shows that zooplankton diversity decreases, and zooplankton abundance increases with brownification. A community shift from Rotifera to Copepoda and then to Cladocera was also noticed independent of treatment. Green algae were the most prominent phytoplankton group in the experiment, followed by diatoms and lastly Cyanobacteria. Both Green algae and Cyanobacteria had highest abundance in the treatment only simulating eutrophication. The highest toxicity per cell, for cyanobacteria, was however found in the treatment simulating brownification and in the Control.

According to results gathered from this study, synergistic effect between eutrophication and brownification may not pose a big threat to zooplankton or phytoplankton abundance and community structure in Lake Bolmen. However, as global warming also is an ongoing problem which both phytoplankton and zooplankton have seen to be affected by, there is still need for further research. Any synergistic affects between brownification, eutrophication and warming need to be investigated as all three environmental changes will continue in the future.

Populärvetenskaplig sammanfattning

Kan förhöjda halter av näring och humusämnen från jordbruksmark och skog påverka kvalitén på vårt dricksvatten?

Försämrad vattenkvalitet, ökade farliga algblomningar och minskad biodiversitet kan vara framtiden för våra vattendrag, på grund av de miljöförändringar vi står inför idag. I detta projekt undersökte jag hur framtiden kan se ut för en av våra sjöar vi får dricksvatten från här i söder, Bolmen. Resultatet visade att kombinationen av mer näring och humusämnen inte verkade ha lika stor påverkan på organismerna som mer näring och humusämnen individuellt.

Sjöar, floder och vattendrag förgyller inte bara vår syn på landskapet utan dom har en väldigt viktig roll i naturen, de skapar balans. Alla djur behöver vatten att dricka och många djur är beroende av dessa sjöar för att överleva. På senaste tiden har dessa vattendrag blivit mer hotade, hotade på grund av oss människor. Att vi människor fortsätter skövla skog, använder market till jordbruk och till städer, påverkar vattnets kretslopp. När vi människor bara blir fler och fler sätter vi också press på vår dricksvattenproduktion, då vi som djuren, är beroende av färskt vatten. Många sjöar används idag till att ge oss vatten att dricka. Tyvärr har det setts en minskande trend i vattenkvalitet på grund av den näring vi låter rinna ut som överskott från jordbruket och den ökade mängden organiskt kol i form av humusämnen från skogsbruket som rinner ut i vattnet.

I detta projekt undersökte jag hur ökad mängd näring och humusämnen kan påverka växt- och djurplankton i en dricksvattentäkt, specifikt Bolmen, i södra Sverige. Jag undersökte hur de två viktiga grupperna av plankton påverkades av olika kombinationer av framtidscenarior, antingen bara med mer näring eller humusämnen separat, eller kombinationer med båda. Organismerna som undersöktes var växtplankton, de som skapar stora slemmiga algblomningar och djurplankton som genom att äta växtplankton och blir ätna av fiskar, kopplar ihop näringskedjan. Djurplankton har ingen direkt påverkan på oss människor. Växtplankton kan dock ha det, specifikt om algblomningen består av giftiga alger (cyanobakterier) som kan orsaka svåra leversjukdomar om människor och djur får

i sig dem. Detta är inget vi vill ha i vårt dricksvatten, därför undersökte projektet om detta verkar vara en risk för Bolmen.

Kombinationen av näring och organiskt kol påverkade kompositionen av både växtplankton och djurplankton vilket kan komma påverka ekosystemet avsevärt, då dessa också påverkar fiskar och andra djur ovanför vattnet. De goda nyheterna är att kombinationen av ökad näring och humusämnen inte gynnade tillväxten av cyanobakterier i projektet. Tyvärr går det inte att pusta ut än för andra aktiva miljöproblem såsom den globala uppvärmningen i kombination med mer humusämnen har visats att gynna tillväxten av de farliga växtplanktonen. Vilket gör det viktigt att fortsätta forskningen om hur olika framtidscenarior kan komma påverka våra vattendrag så vi är beredda med lösningar när det väl händer. Även om vi inte vet exakt hur, kommer en fortsatt påverkan på vattenkvalitén ske. Eftersom förändringen till stor del beror på oss människor har vi också en skyldighet att se till så att vattenkvalitén inte blir för dålig, se till så att både människor och djur kan fortsätta nyttja det livsviktiga vattnet.

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1. Introduction

Many freshwater lakes are experiencing a negative trend in water quality due to human exploitation. As we humans are using more land for urbanization, cultivated land, and engaging in deforestation we are changing the natural hydrology, which is negatively affecting our freshwater lakes (Bakker et al., 2016; Desta et al., 2017).

There are also multiple threats for aquatic ecosystem as a consequence of the ongoing human induced global change (Rasconi et al., 2015). The intensity and frequency of rainfall are expected to change during the next century (Rasconi et al., 2015). As a result of increasing heavy rainfall, the amount of runoff from terrestrial land to lakes increase. This results in increased nutrient runoff from agricultural land which can result in eutrophication (Bláha et al., 2009). It also results in more colored water in the receiving lake as the runoff water contains humic substances and a great amount of dissolved organic carbon (DOC). This coloring of water is called brownification (Rasconi et al., 2015).

Eutrophication is a big problem in both fresh- and saltwaters around the world. It is caused by an oversupply of nutrients, nitrogen (N) and phosphorus (P) usually from agricultural land, into an aquatic system (Fink et al., 2018). This can have big impacts on the whole aquatic ecosystem as it favors toxic algal blooms. Eutrophication is usually also connected to a decrease in water transparency, which often result in a change and reduction of species composition (Smith & Schindler, 2009). There can also be an increase in fish kills which can have big impacts on the ecosystem and results in less harvestable fish for recreational purposes (Smith & Schindler, 2009).

Brownification in freshwater lakes are also of environmental concern as it is affecting both the function and structure of the aquatic system and the water quality (Kritzberg et al., 2020). A change in ecosystem structure is often linked to changes in zooplankton composition as it is a key community that links primary producers, by eating phytoplankton, and predators as they can be eaten by fish (Gutierrez et al., 2020). Zooplankton also play a key role in the nutrient recycling which can be altered if the ecosystem changes. The abundance of zooplankton is highly controlled by the abundance of planktivorous fish (Vanni & Findlay, 2016). An

intense zooplanktivory by fish often results in a zooplankton community dominated by small sized zooplankton (Vanni & Findlay, 2016). Small sized zooplankton have higher mass-specific nutrient excretion rate than larger zooplankton which results in a high nutrient recycle rate to phytoplankton which increases their growth (Vanni & Findlay, 2016). A strong predation on zooplankton by fish can therefore result in large algal blooms (Vanni & Findlay, 2016).

Organisms like cyanobacteria are expected to increase with brownification and eutrophication as the reduction in light penetration favor growth of shade-tolerant species (Smith & Schindler, 2009; Urrutia-Cordero et al., 2017). Not all cyanobacteria produce toxic blooms, but the ones that do can produce toxins with enormous health risks for humans and animals (Bláha et al., 2009). *Microcystis botrys*, is one of the cyanobacterial species that produce the toxin microcystin, that at exposure can cause severe liver damage, heart failure and in worst case death (Bláha et al., 2009).

What now raises further concerns is that brownification has been seen to have synergistic effects with other environmental changes as for example global warming (Hansson et al., 2013). As the air continues to get warmer, the water will also increase in temperature, and an increased water temperature of 4°C is expected in many lakes (Rasconi et al., 2015). Hansson et al. (2013), who looked at a future scenario with 3°C increased water temperature and brownification, found that particularly the cyanobacteria species, *M. botrys* benefited from the combination of brownification and increase in temperature. Ekvall et al. (2013) made a similar study and noticed likewise trends with an increase in abundance of one specific cyanobacterial species, *M. botrys* with the combination of brownification and increase in temperature by 3 °C. In addition, microcystin bloom toxicity increase by 300% in the future scenario, due to the massive overtake of M. *botrys* (Ekvall et al., 2013). If this becomes a fact in our lakes it can have big consequences on lakes biodiversity, ecosystem functioning, and ecosystem services.

When a freshwater lake, especially a lake that is used as a drinking water source as Lake Bolmen, suffers from eutrophication and brownification, it raises mayor concerns. It can have a big impact on several important ecosystem services that freshwater lakes offer us, by altering the fish community and production, the biodiversity and drinking water production (Kritzberg et al., 2020). It can result in health risks and major economic costs for treating the water for it to become safe to drink (Kritzberg et al., 2020; Smith & Schindler, 2009). It is important to see how these factors influence organisms at a species level as some species are key groups and have a big influence on the whole ecosystem (Hansson et al., 2004).

This is why this project focused on both phytoplankton and zooplankton abundance and community structure.

Brownification and eutrophication synergistic response respectively with increasing water temperature, is slightly more researched than the synergistic effect between brownification and eutrophication. This is why the synergistic response between brownification, and eutrophication will be investigated in this research project to increase that understanding. It is crucial to know which exact effects these climate changes have on the ecosystem, down to species level, to be able to implement the proper management method to protect our freshwater lakes.

1.1 Aim

This report aims to give further understanding of what impact increased nutrient and dissolved organic carbon (DOC) concentrations can have on plankton and the ecosystem in a lake. This was done by looking at phytoplankton and zooplankton community composition both independent and dependent of treatments and time, and analyzing the toxicity of the phytoplankton group, cyanobacteria. The samples that were analyzed in this project were taken from a mesocosm experiment preformed in July to August 2021 in Lake Bolmen, and the result therefore provide further understanding of Lake Bolmens future as a drinking water source.

Specially I will investigate:

- How are the zooplankton abundance and community composition in Lake Bolmen affected by an increased concentrations of nutrients and DOC?
- How are phytoplankton abundance and community structure in Lake Bolmen affected by increased concentration of nutrients and DOC?
- How is abundance and toxicity of cyanobacteria in Lake Bolmen affected by an increased concentration of nutrients and DOC?

1.2 Hypotheses

The hypothesis for this project is based on results from previous studies showing that there is a species-specific response by cyanobacteria, where microcystin producing species are favored by an increase in brownification and eutrophication. The hypothesis is that the highest amount of microcystin will be found in treatments with high DOC and nutrient content. Previous studies have indicated that the taxonomic composition of zooplankton changes due to browning and

eutrophication. From a similar Mesocosm study in lake Bolmen, performed in the summer 2018, the result showed an increase of Cladocera and a decrease of Rotifera and Copepods (Gustavsson, 2019). Although the experiment performed 2018 only investigated the effects of brownification, similar pattern should be expected in this present study.

2. Method

This section explains the study site, Lake Bolmen, and the set up for the mesocosm experiment preformed before the start of this project, in summer 2021. It follows with the methods used in this project for laboratory work on samples with zooplankton and cyanobacteria. It also contains a description of which statistical tests were used in this project to analyze the data on zooplankton and cyanobacteria and also data on phytoplankton (which were already gathered on the study site). The section ends with how the Biodiversity index was calculated and some limitations.

2.1 Study site, Lake Bolmen

There are 17 municipalities with close to 1 million citizens connected to the drinking water Sydvatten is producing, with Lake Bolmen as the main water reservoir (Sydvatten 2022). Lake Bolmen is situated in three counties, Kronoberg, Jönköping and Halland in southwestern Sweden (latitude:56.8373◦ N; longitude:13.6738◦ E). The lake has an area of 184 km^2 and is the tenth largest Lake in Sweden (Borgström, 2020). Due to a large island in the middle, the lake is divided into southern, eastern, northern and western sub-lakes with different characteristics. The southern and the northern parts differs in depth as the southern part has a maximum depth of 37 meters (average 8 meters) and the northern part a maximum depth of 13 meters (average 5-6 meters, Borgström, 2020).

Lake Bolmen both receives water from River Storån in the northern part of the lake and from River Murån in the southern part of the lake. The water that comes by River Storån is humic and has a relatively poor nutrient content and the water that comes from River Murån has high levels of humic substances and has therefore high nutrient content in form of phosphorus and nitrogen. The inflow into the northern part of Lake Bolmen is the main inflow. Both River Storån and Murån has a drainage basing consisting of mainly forest, then marshland with some

agricultural lands (Borgström, 2020). Surrounding Lake Bolmen there is mostly forest but also some agricultural land (Borgström, 2020).

2.2 Aquanet in Lake Bolmen, Mesocosm experiment summer 2021

Samples with zooplankton and already gathered data of phytoplankton from a mesocosm experiment in Lake Bolmen performed in July to August 2021 have been studied and analyzed in this project.

The mesocosms were set up in the southern part of lake Bolmen by Sydvatten research station, using a platform previous used for mesocosm experiments in lake Bolmen. Safely attached to the platform 20 mesocosms, 500L each, were put in the water, where they remained during the project. The experiment was set up with four treatments and one control. Each treatment, and the control, had four replicas and all containing water from the lake. The four different treatments consisted of: Treatment 1: DOC and high concentrations of nutrients (Brown + HighNP), treatment 2: DOC and low concentrations of nutrients (Brown + LowNP), treatment 3: high concentration of nutrients (HighNP) and treatment 4: low concentration of nutrients (LowNP).

A Redfield ratio (N:P) of 16:1 was aimed for when nutrients were added as this is the preferable ratio for phytoplankton. For the DOC addition into the treatments symbolizing brownification (Brown + HighNP and Brown + LowNP), a soil extract was used which were extracted from soil taken from the upper soil layer in the catchment area of the lake. The soil extract consisted of dried soil from the catchment area with 1,62kg from coniferous forest, 0,24kg wetland and 0,14kg deciduous forest, summed up to a total of 2kg dried soil. The 2kg soil was mixed with 13,33 L distilled water and 467 g ion exchanger (Diaion CR11).

In Brown + HighNP 4,5 L soil extract and 15 millimole (N) were added to 500 L lake water, creating a N:P ratio of 31 (Table 1). In HighNP 1,7 millimole phosphorus (P) and 18 millimole N were added to 500 L lake water, creating a N:P ratio of 27. In Brown + LowNP 4,5 L soil extract were added to 500 L lake water, which created a N:P ration of 21. In LowNP 1,7 millimole P and 2,86 millimole N were added to 500 L lake water, creating a N:P ratio of 18. No additional nutrients nor DOC were added to Control. The high N:P ratio (268) in Control was due to that the lake is P limited. High values of N and low values of P gives a high N:P

ratio. The low N:P ratio in Brown + LowNP is due to that the soil extract contained nutrients which resulted in addition of nutrients although no individual P nor N was added. The exact amount of nutrients in the soil extract were not determined when produced in the summer 2021.

Table 1. Nutrient addition and calculated Redfield ratio (N:P). Added Phosphorus (P) and Nitrogen (N) to each treatment and the N:P ratio calculated for day 1. Presented in ml 0,1 molar

	P added ml 0,1 molar	N added ml 0,1 molar	Day 1 total N:P
Brown + HighNP	0	150	31
HighNP	17	180	27
Brown + LowNP	0	0	21
LowNP	17	28,6	18
Control	0	0	268

Water samples were collected in 2021 on eight days (5/7, 6/7, 7/7, 8/7 10/7, 14/7, 22/7 and 7/8). Samples was also taken on two set locations outside the mesocosm experiment at the same dates as the sampling in the mesocosms. One in the southern part of the lake (LS) and one in the northern part of the lake (LN) northwest of Bolmsö. Samples from LN and LS was taken to see if and how much the mesocosm, and especially the controls, differed from the actual lake during the project. For this project the samples gathered day 2 (8/7-2021), 16 (22/7- 2021) and 32 (7/8-2021) will be analyzed. Day 2 will symbolize the start of the project, day 16 will show what happens between the different organism groups at a maximum of available resources between and within treatments, and day 32 when the resources decline.

Data on phytoplankton abundance and community structure were on site gathered using Algae lab analyzer (ALA) and were later analyzed in this project. On the eight days of sampling in 2021 10L of water were filtered through a mesh-net of 65µm for gathering of Zooplankton. Zooplankton were fixed and stored in 70% ethanol until analyzed in this project 2022. Samples was also taken for total phosphorus (TP), total nitrogen (TN) and total organic carbon (TOC). Temperature and pH were recorded by sensors in each individual mesocosm. Samples on total microcystin (Mt) were also gathered and later analyzed in this project using Beacon Microcystin Tube Kit.

2.3 Laboratory work on zooplankton

Zooplankton community composition and abundance were analyzed from 66 samples in total in this project, taken day 2 (8/7-2021), 16 (22/7- 2021) and 32 (7/8-2021) of the experiment. For each day there were 20 samples from the mesocosms, and one LS and one LN. Zooplankton was counted for each sample using a counting plate and an Olympus SZX7 microscope. Zooplankton was identified to genus level and species if possible.

2.4 Laboratory work on cyanobacteria

For measuring toxicity of cyanobacteria, Beacon Microcystin Tube Kit were used in this project. Beacon Microcystin Tube Kit is an immunological laboratory test that quantify the amount of microcystin in a sample. The samples were kept in the freezer until the microcystin test started, then thawed and refrozen three times in order to break apart the cells. The Beacon Microcystin Tube Kit manual was followed and a DRLange Cadas 30S spectrometer was used to read the absorbance (whole manual found at Beacon Analytical System Inc, 2021). The Tubes were first measured at 450nm than calculated minus the absorbance at 605nm. The concentration of microcystin in the samples was then calculated using a standard curve of five standards ranging from 0 to 5 microcystin ppb. A trend line was fitted to the graph and the equation was used for calculating the microcystin concentration for the samples.

2.5 Analyzing data with SPSS

The zooplankton species identified were summed up and grouped as Rotifera, Copepoda or Cladocera when doing the statistics. The three zooplankton groups were firstly tested for any significant differences over time of the project, using repeated measure ANOVA. The data of phytoplankton was first analyzed using ALA (Algae Lab Analyzer) where three groups of phytoplankton were identified and used for this project, Green algae, Bluegreen algae (cyanobacteria) and Diatoms. Total biomass concentration was based on chlorophyll a concentration, which was also in the analysis for phytoplankton. Repeated measure ANOVA was

used to test for significant differences over time for the three phytoplankton groups and total biomass concentration. Both the zooplankton and phytoplankton groups were thereafter analyzed for any differences in abundance between treatments for the three chosen sample days separately. This was done by preforming multiple one- way ANOVA with following Post Hoc (Tukey) tests. A significant result corresponds to p <0,05.

2.6 Calculating Biodiversity index

Species richness is the number of species in each sample. An average was calculated for each treatment. Shannon diversity index (H) was used to calculate the diversity of species in each treatment calculated as $H = -\Sigma p_i * \ln(p_i)$, where p_i is the sum of a species divided by the sum of all species in a community. A high H value represent a high diversity of species in a specific community. A low H value correspond to a community with low diversity, with H=0 point to a community with only one species. To measure the evenness of species in a community the Shannon Equitability index, further on referred to as Shannon Evenness, was calculated. A high value (1) on evenness indicates that there is complete evenness of how similar the abundance of different species is in a community and a low value (0) implies the opposite. Shannon Evenness was calculated using $E_H = H/\ln(S)$, where H is Shannon diversity index, and S is the total number of species in the sample.

2.7 Limitations

Zooplankton will only be identified down to genus level and not species, due to limitations of identification tools.

3.Ethical reflection

Personal data from article writers and supervisors will be processed carefully and not used in an unauthorized manner. No discrimination between information will occur due to gender or origin of writers. None of the studied organisms for this project require ethical permits. This study will help in the understanding of how the global environmental problems brownification and eutrophication effects community structure and abundance of phyto- and zooplankton. The result will hopefully help in the discussion how these climate changes will affect the rest of the ecosystem in freshwater lakes, like Bolmen. Sydvatten, Sweden Water Research (SWR) and Lagans vattenråd are continuously monitoring the water quality in Lake Bolmen, in close collaboration with universities, surrounding municipalities and county administration boars. This research will help and add to their understanding of how future scenarios, with increased watercolor and increased amount of nutrients, can affect Lake Bolmen ecosystem as well as its future as a drinking water source. Depending on the results from this report VASYD may have to take further actions to prevent outcomes from future scenarios, which can result in extra costs for VASYD. Although this report aims to highlight eventual problems before they occur which can prevent bigger costs later.

4.Result

4.1 Zooplankton community composition

Across the 60 samples for the three sampling dates, day 2, 16 and 32, the most abundant zooplankton where from the groups of Rotifera, Copepoda and Cladocera.

Four genera of Rotifera were identified, with *Asplancha* sp. (Figure 1A), and *Polyartha* sp. (Figure 1B) being the most abundant (Appendix A Figure A1).

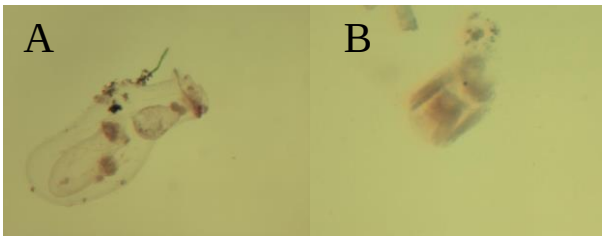

Figure 1. *Asplancha* sp. (A) found day 2 in HighNP**. *Polyartha* sp. (B)** found day 2 in Control

Two genera of Copepoda were identified, *Calanoid Copepod* (Figure 2A) and *Cyclopoid Copepod* (Figure 2B). The nauplii stage of Copepoda was not included in the analysis.

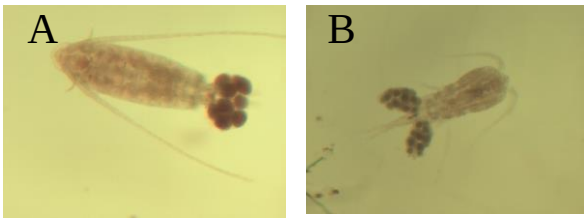

Figure 2. *Calanoid Copepod*. (A). Found day 32 in HighNP. ***Cyclopoid Copepod* (B).** Found day 2 in Brown + LowNP

Withing the group of Cladocera 10 genera was identified, with *Daphnia* sp. (Figure 3A) *Bosmnia* sp. (Figure 3B) and *Chydorus* sp. (Figure 3C) being the most abundant.

Figure 3. *Daphnia* sp. (A). Adult carrying babies. Found day 32 in Brown + HighNP. ***Bosmina* sp. (B).** Adult carrying babies. Found day 16 in Brown + LowNP. ***Chydorus* sp. (C).** Found day 32 in Brown + LowNP

4.1.1 Zooplankton abundance independent of treatment over time

When looking at trends over time independent of treatment, Rotifera were most abundant in samples from day 2 declining drastically until day 16 and being almost completely absent on day 32 (Figure 4). This decrease was significantly different between day 2 and 16 and 2 and 32 (repeated measures anova; p<0,001; p<0,001).

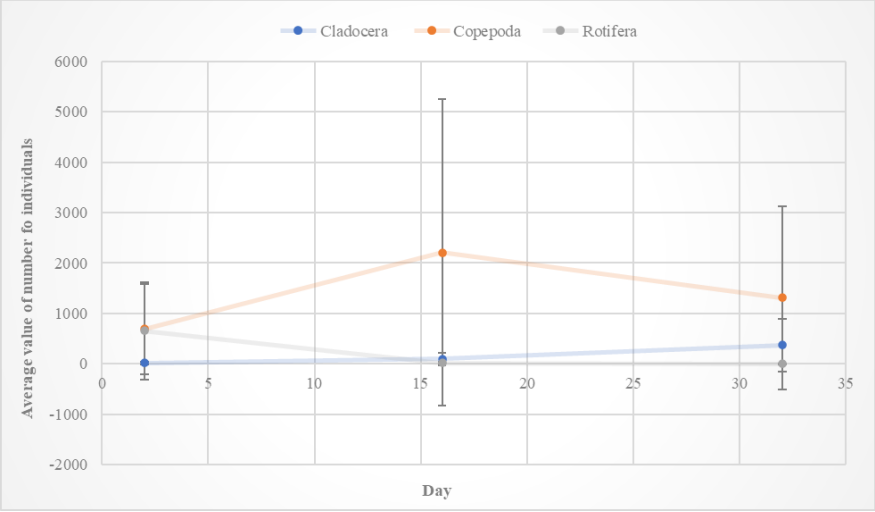

Figure 4. Average value of number of individuals independent on treatments divided into the three identified zooplankton groups, Cladocera (blue), Copepoda oOrange) and Rotifera (gray)

Calanoid Copepod had a far higher abundance than *Cyclopoid Copepod* throughout the experiment and dominated the community on day 16 (Appendix A Figure A2). There was a significantly higher abundance of Copepoda at day 16 compared to day 2 (repeated measures anova; p=0,004, Figure 4). No significant difference between day 2 and 32 nor 16 and 32 was detected for Copepoda due to the big difference in individuals found in the different samples creating large standard deviations.

Cladocera was in low absence day 2 of the experiment, had a slight increase in abundance until day 16 and a bigger increase until day 32 (Figure 4) and the abundance was significantly different between day 2 and 16 and 2 and 32 (repeated measures anova; p=0,048, p=0,014). Throughout the project, Cladocera had the largest amount of different genera with 10 genera on day 2 and 16 (Appendix A Figure A1, A2) and 8 genera on day 32 (Appendix B Figure B1).

In LN (lake north) and LS (lake south), Rotifera was present on all sampling dates and the fraction between individuals of Rotifera, Copepoda and Cladocera remained about the same on all sampling dates. Although a decrease in Rotifera after day 2 and an increase in in Cladocera from day 16 could be seen even here. Due to LN and LS not following the same pattern as in the mesocosms, they were excluded from the following results.

4.1.2 Zooplankton abundance between treatments

Abundance of the three different zooplankton groups between treatments were analyzed for each sampling date. There were significant differences between treatments on day 2 for Rotifera and Copepoda and on day 32 for Cladocera (one-way anova; Table 2).

Table 2. Differences between treatments each day separately for each zooplankton group (one-way anova). Significant differences highlighted in yellow, found for Rotifera day 2, Copepoda day 2 and Cladocera 32.

	2			16			32		
	df	F	p	df	F	p	df	F	p
Rotifiera	4	3,6	0,030	4	3,0	0,054	4	3,8	0,57
Copepoda	4	3,3	0,040	4	2,0	0,14	4	1,4	0,29
Cladocera	4	1,4	0,30	4	1,9	0,16	4	0,75	0,025

The significant differences that could be seen was between Brown + HighNP and other treatments, indicating that the synergistic effects between brownification and eutrophication may have an effect on zooplankton abundance. The high abundance of Rotifera in Brown + HighNP was significantly different from Control (Post-hoc tukey; p = 0,024). There was a significant difference between Brown + HighNP and LowNP on day 2 for Copepoda with also the highest abundance in Brown + HighNP (Post-hoc tukey; p=0,05). Cladocera abundance did not show any significant differences between treatments until day 32, where the high abundance in Brown + HighNP was significantly different from the low abundance in HighNP (Post-hoc tukey; p=0,048), and the low abundance in Control (Post-hoc tukey; p=0,025). The result shows that in many cases Brown + HighNP had a positive effect on abundance of the different zooplankton groups.

4.1.3 Zooplankton abundance over time according to treatment

Trends could be seen over time within the different treatments with Brown + HighNP again showing the most drastic results. Similarities and differences between the separate treatments could also be seen when comparing abundance over time across the treatments.

Cladocera abundance increased over time in Brown + HighNP (Figure 5A), Brown + LowNP (Figure 5C) and in LowNP (Figure 5D). The abundance of Cladocera behaved differently in HighNP then in the other treatments where the abundance increased until day 16 and decreased to day 32 (Figure 5B). The highest abundance of Cladocera was found on day 32 in Brown + HighNP (Figure 5A).

Copepoda increased in abundance from day 2 to day 16 following a decline to day 32 in Brown + HighNP (Figure 5A), HighNP (Figure 5B), and Brown + LowNP (Figure 5C). The highest abundance of Copepoda was found in Brown + HighNP and Brown + LowNP on day 16. Copepoda abundance declined drastically in Brown + LowNP until day 32, while it only leveled off in Brown + HighNP (Figure 5A and 5C). Compared to Cladocera which had an increasing trend over time in most treatments, Copepoda seemed to peek at day 16 and then decline to day 32 in nearly all treatments.

Rotifera declined drastically from day 2 to 16 in all treatments, being almost completely gone to day 32.

Control had similar patterns as the treatments with a slight increase in Cladocera during the whole project, Copepoda declining after day 16 and Rotifera being present day 2 and disappeared to day 32 (Figure 5E). Control, compared to

the other treatments, had a very low abundance of all zooplankton groups throughout the experiment.

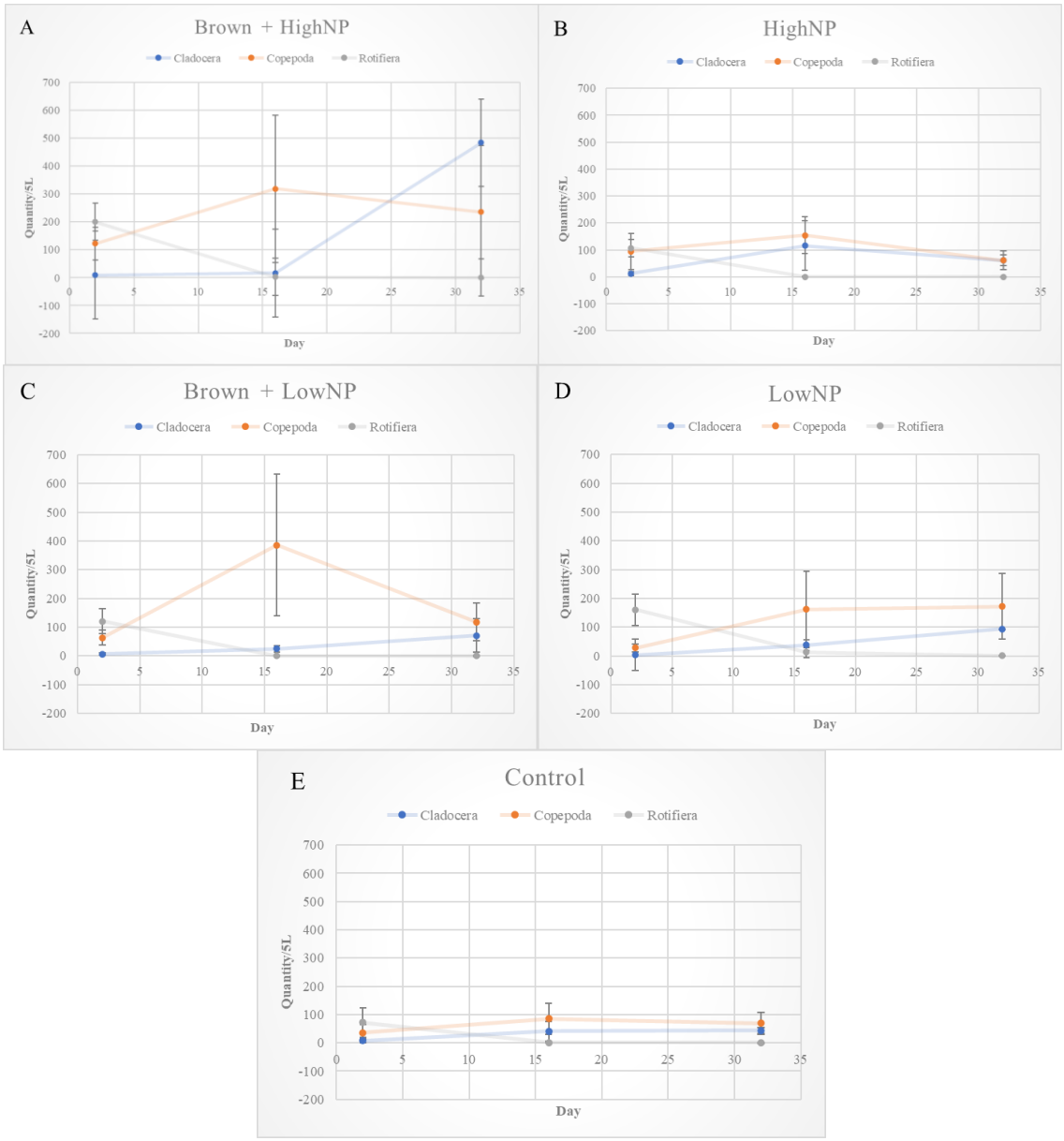

Figure 5. Cladocera (Blue), Copepoda (orange) and Rotifera (grey) abundance over time in each treatment separately. Brown + HighNP (A), HighNP (B), Brown + LowNP (C), LowNP (D) and Control (E).

4.1.4 Zooplankton Biodiversity index

Species richness did not show any significant difference between the different samples on the different sampling days (one-way anova; Table 3). Both diversity and evenness showed significant differences between treatments on day 16 (one-way anova; Table 3).

Table 3. Differences in Richness, Diversity, and evenness between treatments each day separately (one-way anova). Significant results were found on day 16 between treatments for Shannon diversity index and Evenness, which are marked in yellow.

	2			16			32		
	df	F	p	df	F	p	df	F	p
Richness	4	0,075	1,00	4	2,5	0,088	4	0,82	0,53
Diversity	4	1,03	0,42	4	7,2	0,0020	4	0,42	0,79
Evenness	4	0,91	0,48	4	5,4	0,0070	4	0,78	0,56

When analyzing diversity index between the different treatments from day 16 a trend could be seen with the two treatments symbolizing brownification (Brown + HighNP and Brown + LowNP) were significantly different from the treatments containing only nutrients (HighNP and LowNP) and the control (Post-Hoc Tukey; Table 4). The Shannon diversity index (*H*) ranged from low *H* values (0,18 to 0,72) for the brownification treatments and high *H* values (0,69 to 1,48) for the eutrophication treatments and the controls. This correlation could only be seen for day 16.

Table 4. Diversity index between treatments on day 16. Table only presenting the significant results. Significant results could be found between Brown + HighNP against the treatments with nutrients and Control, and Brown + LowNP against the treatments with nutrients and Control.

Treatment	Brown + HighNP	HighNP	Brown + LowNP	LowNP	Control
Brown + HighNP					
HighNP	0,033				
Brown + LowNP		0,016			
LowNP	0,035		0,017		
Control	0,039		0,018		

4.2 Phytoplankton abundance and community structure

4.2.1 Phytoplankton abundance independent of treatment over time

Three groups of phytoplankton were identified and used for the analysis, Green algae, Bluegreen algae (cyanobacteria) and Diatoms. When looking at affect over time independent of treatments, Green algae had the highest abundance on all sampling dates (2, 16 and 32, Figure 6). The abundance of Green algae was significantly different from day 2 to 16 being the highest on day 16 (repeated analysis anova; p=0,006), although not significant, a decrease again until day 32 could be seen. No significant difference could be seen between the three different sampling dates for abundance of Cyanobacteria, which remained low on all analyzed sampling dates. Significant differences in diatom abundance were detected both between day 2 and 16 (repeated analysis anova; p=0,003) and between day 16 and 32 (repeated analysis anova; p <0,001) where the highest abundance was on day 16 in both cases (Figure 6).

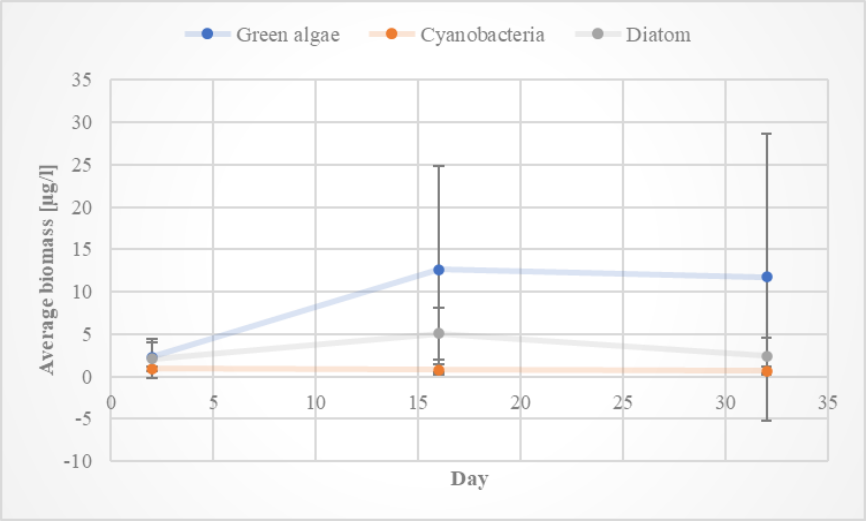

Figure 6. Average biomass of the three different groups of phytoplankton identified independent of treatment. Green algae (blue) having the highest biomass on all three sampling dates (2, 16 and 32), Diatoms (grey) increasing in abundance to day 16 and declined until day 32 and Cyanobacteria (orange) being low in abundance for all samping dates.

4.2.2 Phytoplankton abundance between treatments

Significant difference could be seen between treatments on day 2 for Green algae and on day 16 for Cyanobacteria (Table 5). Diatom showed both significant differences on day 16 and day 32 (Table 5).

Table 5. Differences in Green algae, Cyanobacteria, and Diatoms between treatments each day separately (one-way anova). Significant differences were found between treatments for Green algae on day 2, for cyanobacteria and diatoms on day 16 and for diatoms on day 32. Significant differences marked in yellow.

	2			16			32		
	df	F	p	df	F	p	df	F	p
Green algae	4	7,774	0,001	4	2,757	0,067	4	1,097	0,394
Cyanobacteria	4	0,37	0,826	4	16,59	<0,001	4	1,222	0,343
Diatom	4	2,301	0,106	4	11,69	<0,001	4	35,33	<0,001

Abundance of Green algae on day 2 showed significant differences between the two treatments symbolizing brownification (Brown + HighNP and Brown + LowNP) and the treatments with only nutrients (HighNP and LowNP), and Control (Post-Hoc (Tukey), Appendix C Table C1). The abundance was in all cases higher in the treatments symbolizing brownification.

On day 16, Cyanobacteria abundance was significantly different in HighNP against all other treatments (Post-Hoc (Tukey), Appendix C Table C2) with the highest abundance located in HighNP. This indicates that the addition of nutrients had a bigger influence than browning on cyanobacteria abundance. Brown + HighNP was significantly different from Brown + LowNP, which also indicates that extra addition of nutrients is affecting, as the abundance was higher in Brown + HighNP.

The significant differences in abundance for diatoms on day 16, could be seen between the treatments with high and low nutrient (Post-Hoc (Tukey), Appendix D Table D1), where Brown + HighNP had a higher abundance of diatoms than in both LowNP and Control. The abundance of diatoms was also higher in HighNP against LowNP and Control. Brown + LowNP was also significantly different from Control with the highest abundance in Brown + LowNP (Post-Hoc (Tukey), Appendix D Table D1).

Abundance of Diatoms on day 32 showed the same significant pattern as zooplankton diversity on day 16 and abundance of Cyanobacteria day 2, with the two treatments symbolizing brownification (Brown+NP16 and Brown) being significantly higher from all of the other treatments except each other (Post-hoc (Tukey), Appendix D Table D2).

4.2.3 Phytoplankton abundance over time according to treatment

The total biomass concentration follows the same pattern as Green algae, indicating Green algae was the dominating phytoplankton group throughout the project. The highest abundance of Green algae could be seen in HighNP (Figure 7B) and the second highest in Brown + HighNP (Figure 7A). There was a slightly higher peak in Brown + LowNP (Figure 7C) than LowNP (Figure 7D). LowNP and Control was the only treatments that the total concentration of biomass continued to increase after day 16.

Diatoms increased between day 2 and day 16 and then decreased to day 32 in all treatments including Control. Diatoms had the highest abundance in Brown + HighNP (Figure 7A) and Brown + LowNP (Figure 7C) following HighNP (Figure 7B). Diatom abundance was lowest in LowNP (Figure 7D) and in Control (Figure 7E).

In all treatments Cyanobacteria had much lower biomass throughout the experiment than the other phytoplankton species. The largest biomass of cyanobacteria could be seen in HighNP with the highest value on day 16, however the biomass declined until day 32 again (Figure 7B). In Brown + LowNP and LowNP, a dip on day 16 in biomass for cyanobacteria could be seen with a slight increase again until day 32 (Figure 7C, D). There was a decline of cyanobacteria throughout the experiment in Control and almost no change in Brown + HighNP (Figure 7A).

According to the result the only treatment effect that could be established from the experiment were the high amount of nutrients in HighNP benefiting the growth of Green algae.

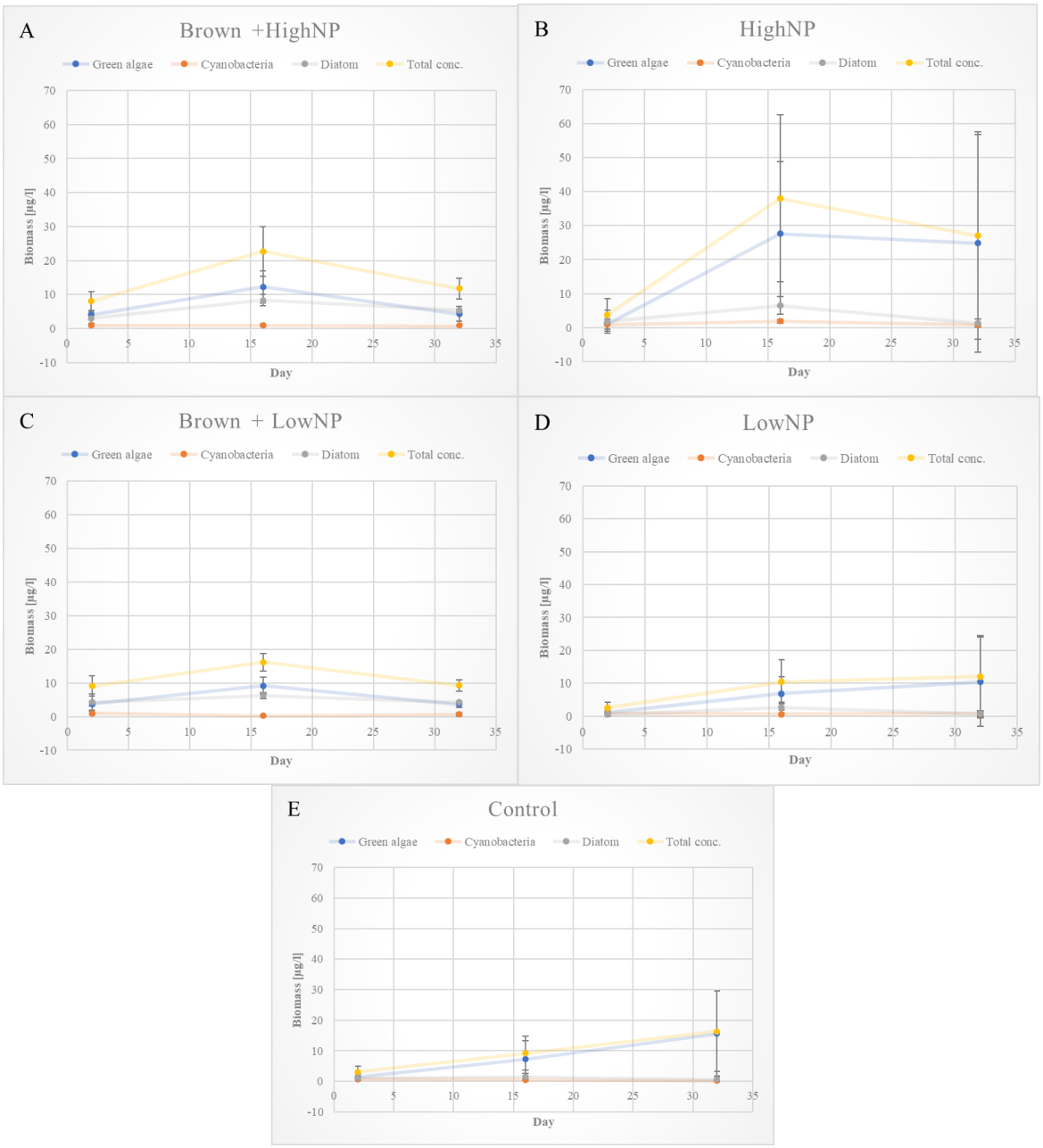

Figure 7. Phytoplankton abundance for each treatment over time. Abundance of Green algae (blue), Cyanobacteria (orange) Diatom (grey) and Total con. (Chl a, yellow), with standard deviation. Brown + HighNP (A), HighNP (B), Brown + LowNP (C), LowNP (D) and Control (E).

4.4 Toxicity of cyanobacteria

The microcystin levels remained low through the whole experiment. There was no detection of microcystin on day 2, on day 16 there were levels of mycrocystin in almost all samples and on day 32 only in a few samples. The amount of microcystin never exceded 0,04 ppb and were around 0,03 ppb when detected.

The highest biomass of Cyanobacteria but also the lowest toxicity per cell, was on day 16 in HighNP (Figure 8A, B). Brown + LowNP had the highest toxicity per cell but the lowest biomass on day 16 (Figure 8B, A). On day 32 all treatments had the similar toxicity per cell values except Control. Control had the highest value of toxicity per cell, and also the lowest cyanobacteria biomass, which occurred on day 32 (Figure 8A, B).

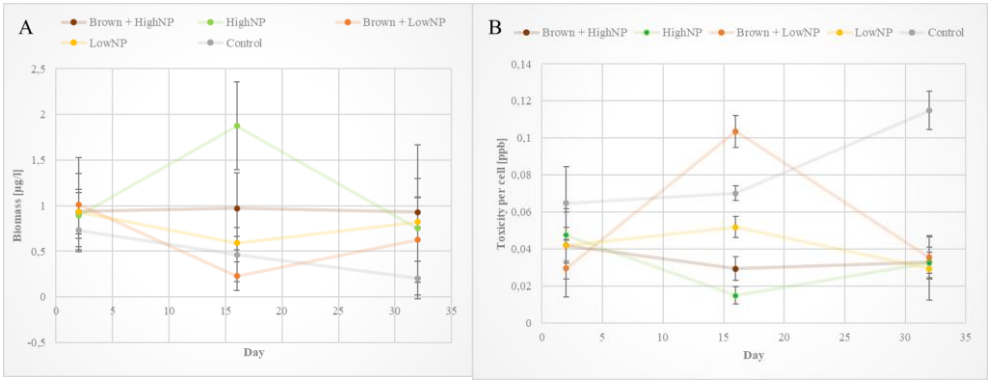

Figure 8. Cyanobacteria biomass (A) and toxicity per cell (B) per treatment. Brown + HighNP (brown), HighNP (green), Brown + LowNP (orange), LowNP (yellow) and Control (grey), with errorbars.

4. 4. 1 Temperature and pH changes

The peak in Cyanobacteria on day 16 in HighNP corresponds to an increase in pH from 6,7 to 9,2 to day 16 whiles the rest of the treatments only had an increase from 6,7 to 7 pH on day 16 (Table 6). The temperature was also the highest on day 16, this was the case for all treatments however, with an increase from 21,5 °C to 22,4 °C to day 16 and a decrease to day 32 to 19 °C (Table 6).

Table 6. Average value of temperature and pH for each treatment each day separately. Temperature presented in ◦C.

	Temp °C				pH		
	Day 2	**Day 16**	**Day 32**		**Day 2**	**Day 16**	**Day 32**
Brown + HighNP	21,5	22,3	18,9	Brown + HighNP	6,2	7,2	7,0
HighNP	21,5	22,4	19,0	HighNP	6,7	9,2	7,9
Brown + LowNP	21,5	22,4	19,0	Brown + LowNP	6,1	7,0	7,0
LowNP	21,4	22,3	18,9	LowNP	6,8	7,8	7,3
Control	21,5	22,3	18,9	Control	6,8	7,2	7,2

5. Discussion

The aim of this project was to gain further understanding of what could happen to a freshwater lake that is exposed to increased nutrients and DOC concentrations, representing eutrophication and brownification, as this is future scenarios many of our lakes are facing. The study investigated the community composition and abundance of zooplankton and phytoplankton across different combinations of high and low increase of nutrients, and addition of DOC.

5.1 Zooplankton community composition shift over time independent of treatment

The result from this study showed that zooplankton community composition shifted over time from being mostly Rotifera on day 2 to a dominance of Copepoda on day 16 and a combination of Copepoda and Cladocera on day 32. As large Copepods feed on Rotifera (Kumar & Rao, 2001) this shift on day 16 could be due to intense feeding by Copepods on Rotifera. The increase of Cladocera to day 32 could be a delayed result of the increase in temperature around day 16 (Table 6), as Cladocera has been noted to increase in abundance with increasing temperature (Ekvall & Hansson, 2012). The same community composition shift in zooplankton was observed in a previous mesocosm experiment in Bolmen 2018, where Rotifera declined over time and Cladocera increased (Gustavsson, 2019). Zooplankton have been shown to have seasonal patterns, with Rotifera having high densities in June and Cladocera and Copepods in July and August (Lehtovaara et al., 2014) the shift in taxa could therefore also be due to natural seasonal patterns.

5.2 Zooplankton community composition differences between treatments

Between treatments on day 2 and on day 32 separately, significant differences could be seen among zooplankton abundance between the treatment Brown + HighNP and the other nutrient treatments. This significant difference, where the abundance

was higher in Brown + HighNP, could already be seen on day 2 for Rotifera and Copepoda and was seen on day 32 for Cladocera. As the samples from day 0 was not investigated any conclusions can not be made if there already was a treatment effect on day 2 or if the significant differences were already there from the beginning. The differences on day 32 however is indicating that the high levels of nutrients in combination with DOC is affecting the abundance of Cladocera, as although not significant, the abundance was higher in Brown + HighNP (Figure 5A) than in all other treatments. That the abundance was low in HighNP is in accordance with Gutierrez et al. (2020) result who also noticed a decline in Cladocera with increasing nutrients. Compared to this study Gutierrez et al. (2020) compared mesotrophic streams against eutrophic streams, where in the mesotrophic streams Cladocera was the dominating group by 58% while in the eutrophic only 4% were Cladocera. Contradictory to this study Gutierrez et al. (2020) observed an increase in Rotifera in the streams with high nutrient concentrations which was not observed in this study.

Although there were no significant differences in abundance of zooplankton between the different treatments on day 16, there was a significant difference in diversity index and evenness (Table 3). The treatments simulating brownification had a significant lower diversity and evenness than the treatments with only nutrients on day 16. This is an indication that browning is affecting competitive interactions and survival among taxa (Arzel et al., 2020). That browning have a negative effect on biodiversity have both been investigated for zooplankton (Arzel et al., 2020) and for phytoplankton (Urrutia-Cordero et al., 2017). There seem to be a threshold were browning and temperature increase stop effecting the biodiversity positively and start to affect it negatively instead (Urrutia-Cordero et al., 2017). The temperature was higher around day 16 (22 °C) than for day 32 (19°C) which could be the reason why the diversity index was higher for the brownification treatments again around day 32, as increase in temperature seem to have a negative effect on biodiversity in combination with browning (Arzel et al., 2020).

5.3 Phytoplankton community composition differences between treatments

When analyzing the statistics Phytoplankton growth seemed to be more affected by the different treatments than zooplankton, as clearer patterns could be seen from treatment effects. Already at day 2 of the experiment Green algae seem to prefer the treatments with high concentrations of nutrients as the growth was highest in HighNP followed by Brown + HighNP. This follows with previous research that an increase in nutrients favors the growth of phytoplankton (Liu et al., 2011;

Prasertphon et al., 2020; Wurtsbaugh et al., 2019). Which phytoplankton group that dominates a system can depend on many factors. Wurtsbaugh et al. (2019) discusses the possibility for different growth stimulation on different algae due to the form of nitrogen and phosphorous that is added. If the nitrogen that is added is in form of nitrate, it can favor growth of diatoms and green algae for example (Wurtsbaugh et al., 2019), which is maybe what happened in this study. Wurtsbaugh et al. (2019) also adds that there are multiple factors that influences the growth of phytoplankton, as each having its own needs and functions. In this experiment the nutrient composition in HighNP seemed to be highly preferable for growth of bluegreen algae, as for in Brown + HighNP something with the addition of DOC made a lower growth of bluegreen algae and a slight higher growth of diatoms.

The highest abundance of Green algae corresponded to the increase in pH to 9 pH in HighNP, this is due to high photosynthetic activity by algae increases pH due to hydrogen use (Axelsson, 1988). The increase in pH did not seem to affect the growth of zooplankton in HighNP and as pH remained around a natural value (~7) in the other treatments no effect of pH could be stated from these results. The increase in temperature on day 16 may have had an effect on the concentration of phytoplankton as the abundance was highest day 16. Phytoplankton have seen to increase in abundance due to increase in temperature (Ekvall et al., 2013; Urrutia-Cordero et al., 2017) but as there are several other factors involved it is difficult to say the magnitude of influence the temperature had in this experiment.

In all treatments, diatoms increased until day 16 and decreased until day 32. On day 16 diatoms seemed to be more affected by the availability of nutrients, and especially phosphorus as the growth was highest in the treatments with high values of nutrients. At day 32 however, diatoms seemed to prefer the treatments symbolizing brownification instead since the abundance was highest in treatments with addition of DOC. There is research of phytoplankton changing what to eat due to grazing pressure (Reynolds, 2009), this is maybe what happened in this study aswell, due to the increase of Green algae uptake of nutrients.

5.4 Cyanobacterial growth and microcystin concentrations

Neither increase in brownification nor eutrophication had a large effect on Cyanobacteria growth in the mesocosms, as the biomass remained low in all treatments during the whole experiment.

The highest biomass of cyanobacteria was in HighNP whereas the cells with most microcystin was in Control followed by Brown + LowNP. That there were

high concentrations per cell in Brown + LowNP are a similar result to other studies (Ekvall et al., 2013; Urrutia-Cordero et al., 2016) where treatment symbolizing brownification has caused a shift towards more toxic cyanobacteria species. This could potentially have happened in this study. However, Cyanobacteria species composition were not analyzed in this study, thus more analysis of the data would be needed.

The control contained lake water, thus had no extra nutrients or DOC added. The low concentrations of nutrients or DOC is likely what resulted in a minimal growth of cyanobacteria. The high toxicity per cell is probably due to the few cyanobacteria that grew was producing microcystin. There are a wide range of different cyanobacteria species, which all prefer different physiological conditions. The maximum replication rate is spread between 20 to 41 degrees between species, (Reynolds, 2009) and the ability to take up and who favors high concentrations of nutrients, differs between species (Carey et al., 2012). This is why a community shift in cyanobacteria is most likely the outcome when lakes are exposed to increased concentrations of DOC, nutrients and higher temperatures.

This study, compared to other studies looking at cyanobacteria, did not investigate the synergetic effects between temperature and browning contra eutrophication. There was though a slight increase in temperature in all mesocosm due to natural causes on day 16, were also the highest abundance of cyanobacteria could be seen.

5.5 DOC:s effect on primary production

With DOC it is yet not determined if it enhances or limits the primary production. As Seekell et al. (2015) explains, this can be due to DOC both stimulate primary production through nutrient availability or inhibit primary production through light extinction. As Bolmen is in a boreal region, where DOC concentrations are usually high, it might have a negative effect on primary production as seen in Seekell et al. (2015), as the high concentrations of DOC effect the light penetration which is important for primary production. This might be the reason why phytoplankton growth in Brown + HighNP, which had DOC addition, did not show the same high growth as in HighNP. Seekell et al. (2015) also claims that lakes in boreal regions in Sweden might face a decrease in primary production if the DOC concentrations continue to increase in the future. This, in combination with DOC having a positive effect on cyanobacterial growth, could be devastating for the primary production (Seekell et al., 2015). DOC can also effect several ecosystem services, that we receive from freshwater lakes, negatively if it continues to increase (Kritzberg et

al., 2020). As it can affect fish abundance, the biodiversity of the lake ecosystem and health risks for humans and animals. It can also affect Lake Bolmens future as a drinking water reservoir if there should be an increase in DOC in the future.

5.6 Limitations

As LN and LS, the samples taken from the lake, did not follow the same pattern as the mesocosm they were excluded from the data analysis. That they did not follow the same pattern as for the mesocosms was expected as they were taken from the lake and not from the limited ecosystems in mesocosms. When constructing mesocosm it is inevitable to exclude multiple important influencing factors.

A factor that was excluded in the mesocosm was the presence of fish. In absence of fish, grazers like several Cladocera species, can rapidly reduce the abundance of phytoplankton (Geller & Müller, 1981; Tessier & Woodruff, 2002). However, in an actual lake the outcome could be different if there is a high abundance of zooplanktivorous fish. The presence of planktivorous fish, keeps the abundance of large-bodied crustacean down (as *Daphnia* sp., Urrutia-Cordero et al., 2016) which even out the food chain and allows smaller plankton to grow. Phytoplankton is common food for several large-bodied zooplankton which are under pressure from the high abundance of zooplanktivorous fish. When zooplankton are heavily grazed on this can lead to increased growth of phytoplankton and massive algal blooms and this effect also increases with increased nutrient concentrations (Urrutia-Cordero et al., 2016).

In the actual lake there is places for both phyto- and zooplankton to seek shelter, a continuous input of nutrients and DOC and repeated mixing which effect the plankton community. In the mesocosm there were no plants or rocks for the plankton to hide under and the only stirring occurred during the 8 sampling dates.

5.7 Lake Bolmens future as a drinking reservoir

Brownification, eutrophication and temperature increase are global problems earths water bodies are facing. Water color has previously increased drastically in Lake Bolmen, however the last 10 years water color has decreased (Borgström, 2020). This means that at the moment browning does not seem to pose an immediate threat to Lake Bolmen. Although watercolor has decreased, browning is still affecting lake Bolmen, posing a potential threat towards the usage as a drinking water rewervoir, commercial fishing and the recreational potential. Therefore it is of great importance to continue monitoring Lake Bolmen in aspect of any shift in the DOC

load, as shown in this project, can influence both phytoplankton and zooplankton abundance and community structure.

Algal bloom in form of green algae is most likely the outcome for Lake Bolmen if nutrient load continues into the Lake. However, it is uncertain what will happen if Lake Bolmen both get higher concentrations of DOC as this does not seem to favor Green algae bloom as addition with only nutrients. None of the future scenarios seemed to favor high growth of microcystin producing cyanobacteria which is a promising result for Lake Bolmens future as a drinking water reservoir.

6. Conclusion

Based on the result from this experiment, a pulse which increases DOC seem to affect the abundance, diversity, and evenness of zooplankton. Throughout the experiment the highest abundance of zooplankton could be seen in the treatments simulating brownification. However, on day 16 the brownification treatments had the lowest diversity index of all treatments. The diversity in the brownification treatments showed a recovery and no difference could be seen on day 32 between treatments.

- These results show that a pulse of DOC can favor and lead to a dominance of certain groups, but it also indicates the possibility for zooplankton species to recover from a high input of DOC. However, if this happens continuously, as is expected with climate change, the outcome could be different. Therefore, future studies need to investigate how repeated input of DOC is affecting the community composition of zooplankton as they are a key species and a change in diversity can have big influences on the ecosystem.

The highest abundance of cyanobacteria, found in HighNP, had also the lowest toxicity per cell. As this project only investigated the toxin microcystin, it left out the possibility to detect if any other toxins were produced.

- There does not seem to be a present threat in Lake Bolmen by toxic cyanobacteria blooms as a result of brownification and eutrophication, as the abundance of cyanobacteria remained low during the experiment. In further studies it could be of interest to include investigation of other toxins apart from microcystin as there are other toxic cyanobacteria that also are of danger to humans and animals.

Green algae as expected had the highest abundance in the treatment simulating eutrophication. As the abundance was not as high in the treatment with both DOC and nutrients, it seems that eutrophication alone can promote Green algae blooms but not eutrophication and brownification together.

- The result from this study indicates that synergistic effects between brownification and eutrophication have an impact on zooplankton abundance and community structure. Phytoplankton does not seem to be as affected by the synergistic effects between brownification and eutrophication as zooplankton. However future similar studies are need as brownification and eutrophication will probably be accompanied with other climate changes as temperature increase, which have shown to have a large effect on phytoplankton.

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Appendix A.

Relative species abundance for day 2 and 16, for all treatments, with all genera found.

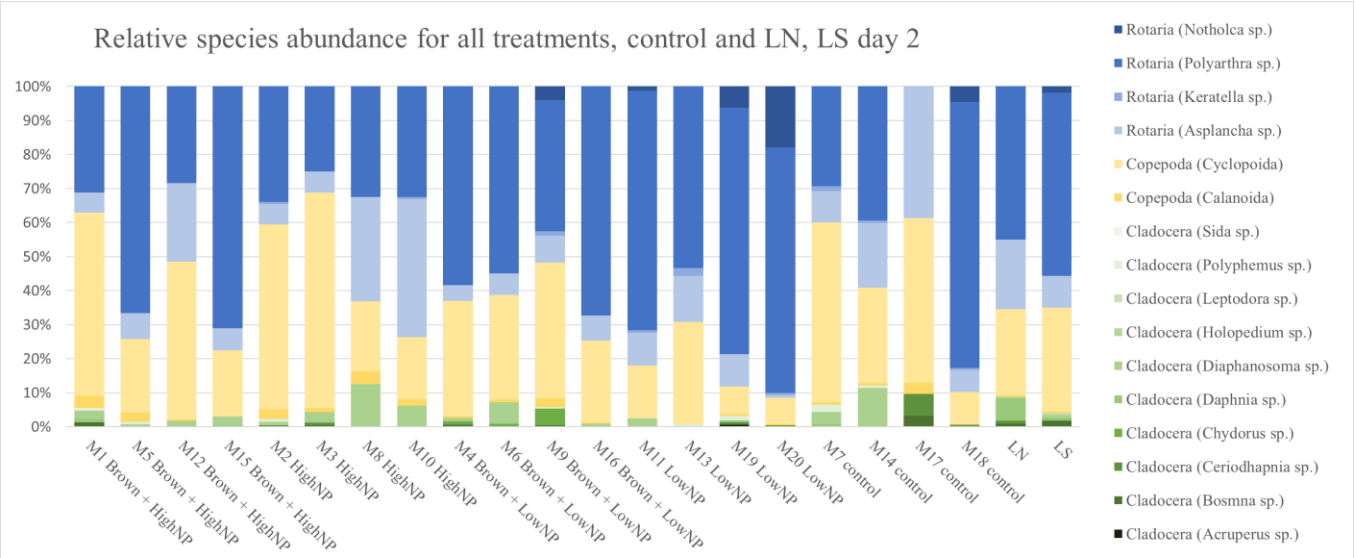

Figure A1. Relative abundance day 2 for all treatments and LN & LS. Rotaria are different shades of blue, Copepoda shades of yellow and Cladocera are different shades green.

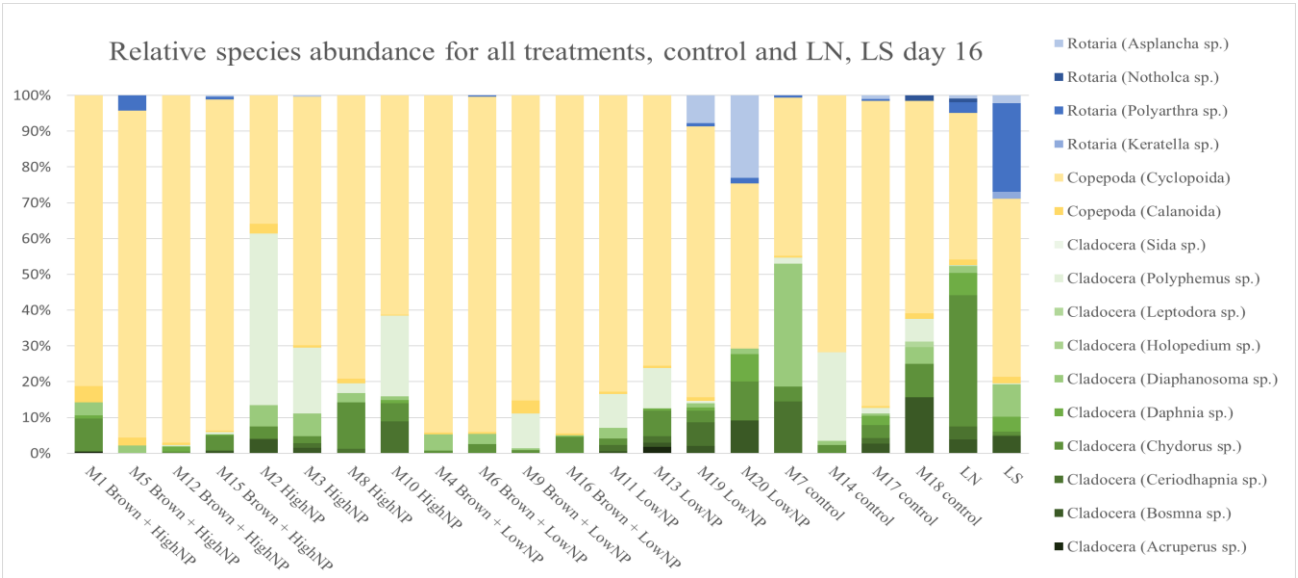

Figure A2. Relative abundance day 16 for all treatments and LN & LS. Rotaria are different shades of blue, Copepoda shades of yellow and Cladocera are different shades green

Appendix B.

Relative species abundance for day 32 for all treatments, with all genera found.

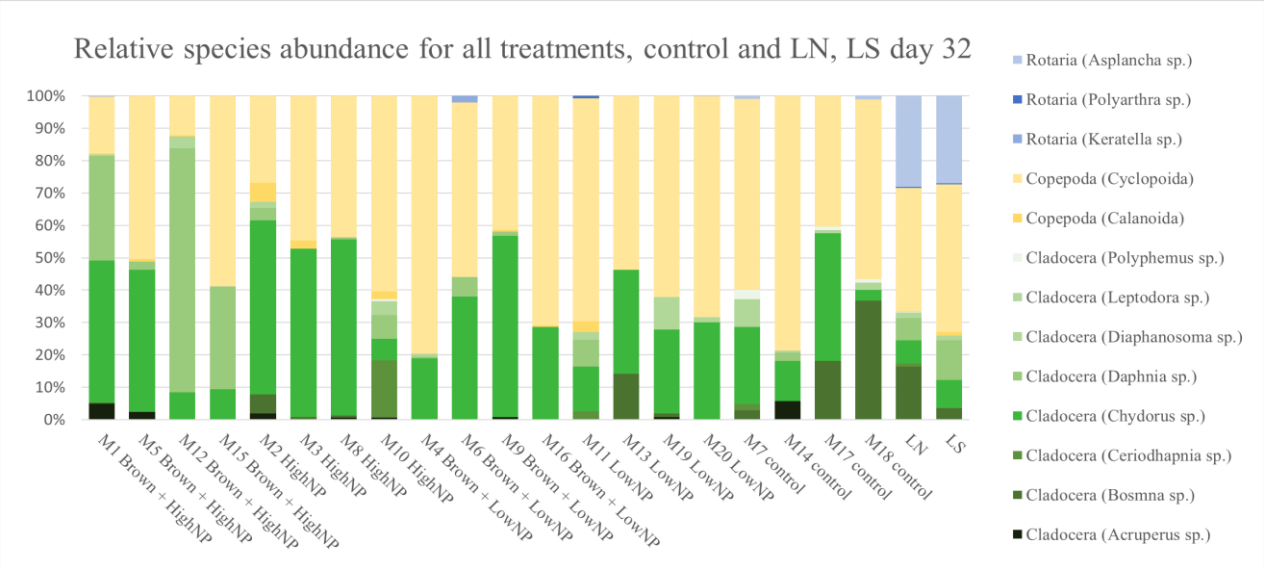

Figure B1. Relative abundance day 32 for all treatments and LN & LS. Rotaria are different shades of blue, Copepoda shades of yellow and Cladocera are different shades green

Appendix C.

Shows Differences in abundance of phytoplankton. Results gathered from Post - hoc (tukey) on One-way ANOVA.

Table C1. Significant differences in abundance between treatments for Green algae on day 2. Differences seen between treatments simulating brownification against eutrophication

Treatment	Brown + HighNP	HighNP	Brown + LowNP	LowNP	Control
Brown + HighNP					
HighNP	0,011				
Brown + LowNP		0,023			
LowNP	0,011		0,024		
Control	0,019		0,041		

Table C2. Significant differences in abundance between treatments for Cyanobacteria on day 16. Differences is seen between high and low nutrient addition.

Treatment	Brown + HighNP	HighNP	Brown + LowNP	LowNP	Control
Brown + HighNP					
HighNP	0,008				
Brown + LowNP	0,033	< 0,001			
LowNP		<0,001			
Control		<0,001			

Appendix D.

Shows Differences in abundance of phytoplankton. Results gathered from Post - hoc (tukey) on One-way ANOVA.

Table D1. Significant differences in abundance between treatments for Diatoms on day 16. Differences between high and low addition of nutrients.

Treatment	Brown + HighNP	HighNP	Brown + LowNP	LowNP	Control
Brown + HighNP					
HighNP					
Brown + LowNP					
LowNP	0,002	0,038			
Control	<0,001	0,005	0,008		

Table D2. Significant differences in abundance between treatments for Diatoms on day 32. Differences seen between treatments simulating brownification against eutrophication.

Treatment	Brown + HighNP	HighNP	Brown + LowNP	LowNP	Control
Brown + HighNP					
HighNP	<0,001				
Brown + LowNP		<0,001			
LowNP	<0,001		<0,001		
Control	<0,001		<0,001		