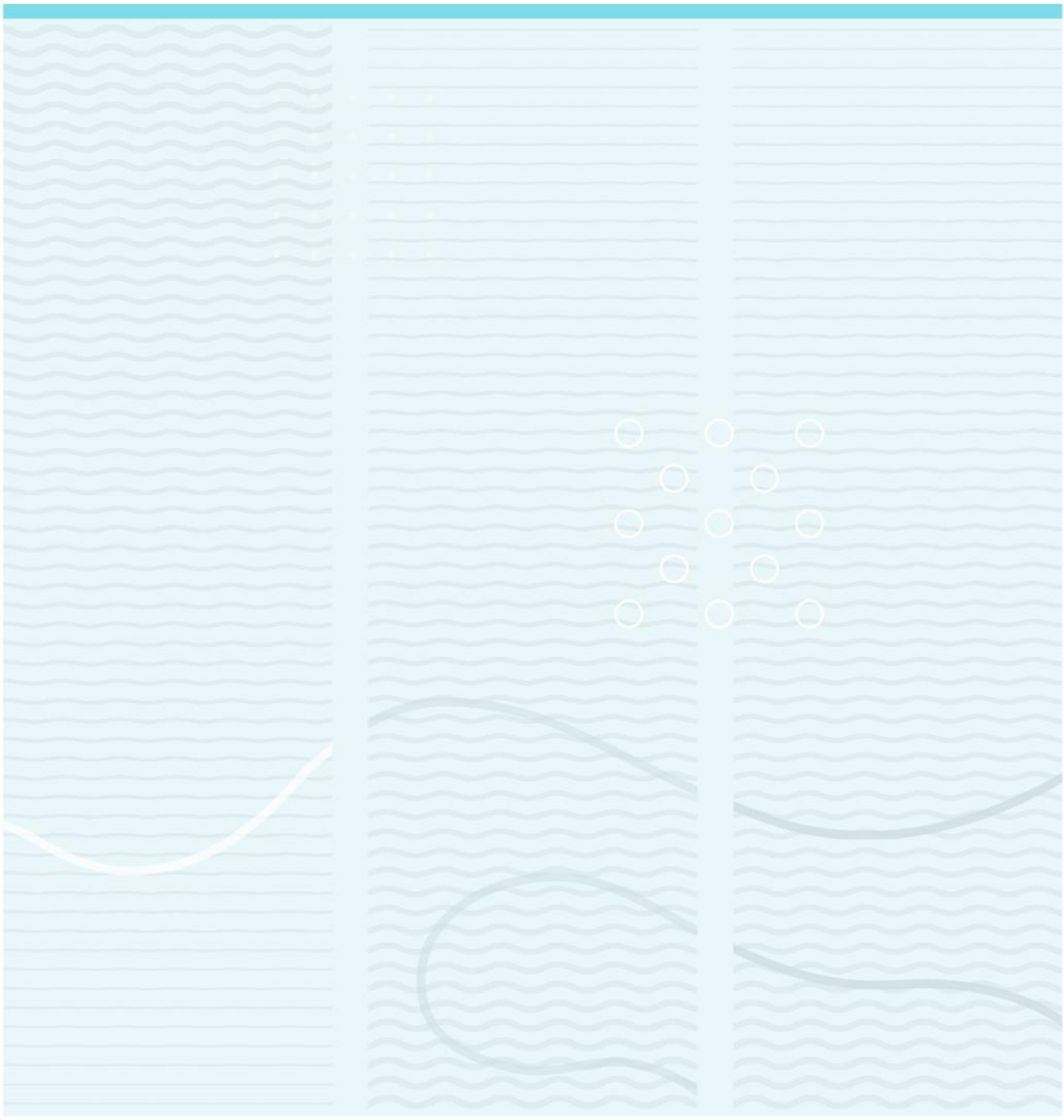
University of South-Eastern Norway
Faculty of Technology, Natural Sciences and Maritime Sciences

Master's Thesis
Master of Environmental Science
Spring 2022

Elisabeth Nordvik Eriksen

Detection of cyanobacteria in Lake Akersvannet (Southern Norway) using metabarcoding and light microscopy



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This thesis is worth 60 study points

Abstract

Cyanobacteria can be found in freshwater and drinking water systems almost all over the world and with climate change and increasing temperature and precipitation, cyanobacterial growth and blooms are favoured. One important concern related to cyanobacteria, is occurrence of cyanobacteria with abilities to produce metabolites and toxins which can be harmful to animals and humans. Some Norwegian lakes experience sporadic or annual cyanobacterial blooms, one of whom is Lake Akersvannet in Vestfold and Telemark County, in Southern Norway. Traditionally, inverted light microscope are used for quantitative and qualitative monitoring of cyanobacteria. However, molecular methods such as DNA metabarcoding are promising. This thesis will investigate the approach of these methods and if they are comparable. Water quality parameter such as total phosphorus was measured in L. Akersvannet with concentrations between 17 – 96 µg/L, giving the lake classifications of mesotrophic 9th of August and eutrophic 1st of July and 14th of September 2021. ELISA methods were used to analyse microcystin and saxitoxin concentrations, with microcystin being found in all samples from L. Akersvannet in concentrations from 0,22 – 324 µg/L. All samples were considered as negative for saxitoxin. 16S gene metabarcoding yielded 2675 OTUs in total, of which 2663 were kingdom of bacteria and 227 were cyanobacteria. Diversity tests showed that sampling date was a significant factor, and that July was separated from August and September. Microscopic approach identified eleven cyanobacteria taxa, and a cyanobacteria density constituting 83 % of total phytoplankton volume in July, which was clearly the highest cyanobacteria volume. Using Spearman´s rank correlation, it was found that the correlation of the data within the two methods had a significant and strong correlation, with p-value of 0,006 and rho of 0,8. Nine out of eleven cyanobacteria taxa identified by microscope, were identified with metabarcoding as well, but metabarcoding yielded a broader spectre of cyanobacteria with species not identified with microscopy. Based on the findings in this thesis, the use of eDNA metabarcoding and inverted light microscopy may work well as complementary methods, rather than one replacing the other.

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Foreword

This master thesis is written as a part of the master study of Environmental Science, at University of South-eastern Norway. The thesis is worth 60 study points and was written in the period of July 2021 to May 2022. I was lucky enough to have three supervisors, who all have shown great interest and enthusiasm in my thesis, and I want to express my greatest gratitude for their help. Associate professor Synne Kleiven has been of great help throughout the whole year, from planning the thesis, teaching me new laboratory methods and always being available for questions and help with her solid understanding of the academic. Associate professor Jørn Henrik Sønstebø has been a great support with the genetic part of the thesis, both in lab and theoretically, in addition to the statistical part which were performed in R. In the period of collecting water samples, I received good training and help from Drainage Basin Coordinator Miguel Angel Segarra Valls which I was fortunate to join in the field.

I would also like to thank members of our "Metabarcoding group", Peter Groth Farsund, Sofie Geck Sevatdal and Tora Camilla Eng Aune, for our meetings throughout the year. These meetings have been a good academic platform for discussing relevant articles, methods, and challenges, which have been very helpful.

Bø, 16th of May 2022
Elisabeth Nordvik Eriksen

1. Introduction

Cyanobacteria is a group of prokaryotes with an unique ability to perform photosynthesis, and is found in many different shapes and sizes as both single cells and colonies (Chorus & Welker, 2021). As a photosynthetic bacteria, light is a main source to derive energy and therefore one of the limiting factors of cyanobacterial growth (Oliver et al., 2012). These bacteria have distinctive properties which is favourable in different environments, and provides an advantage compared to other bacteria and algae found in the same environment. For example, several cyanobacterial taxa can produce aerotopes, which form gas vacuoles filled with air. Due to the low density of these gas vacuoles, they provide buoyancy and therefore these types of cyanobacteria can float in water as required (Chorus & Welker, 2021). Also, some cyanobacteria genera have heterocytes, specialised cells which can fixate atmospheric nitrogen (Adams, 2000; Chorus & Welker, 2021), and during a bloom the fixation process can increase the input of nitrogen and disturb the geochemical cycles in the system (Capone et al., 2005).

One of the main concerns when it comes to cyanobacteria, is the harmful cyanobacteria that can produce different metabolites and toxins (Cheung et al., 2013; Skulberg et al., 1993). Within the "main toxin-producing genera": *Anabaena* (now called *Dolichospermum* (Chorus & Welker, 2021)), *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*, *Microcystis*, *Nodularia*, *Nostoc* and *Planktothrix* (Carmichael, 2001), both toxic and non-toxic strains are found both toxic and non-toxic strains (Davis et al., 2009). Cyanotoxins can be harmful to both animals and humans as they can cause health problems as liver failure and muscle cramps, and in worst case scenarios, death (Carmichael et al., 2001; Samdal et al., 2021). Cyanobacteria can be found in freshwater almost all over the world, including drinking water systems, thus World Health Organization (WHO) have published recommended guidelines for some cyanotoxins in freshwater systems. For example, as for drinking water, the provisional guideline is 1 µg/L for microcystin-LR and 3 µg/L for saxitoxin, whereas for recreational use the provisional guideline value is 24 µg/L for microcystin-LR and 30 µg/L for saxitoxin (Chorus & Welker, 2021).

Some cyanobacterial genera can proliferate rapidly (Liu et al., 2020) and form so-called cyanobacterial blooms (Paerl & Huisman, 2009). The most important factors for cyanobacterial bloom is light, nutrients such as nitrogen and phosphate, and temperature (Behrenfeld et al., 2008). Human activities such as agricultural development with consequences of increased eutrophication, provides an environment which favour cyanobacterial dominance and

cyanobacterial blooms (Codd et al., 2005; Paerl & Huisman, 2009). In addition to human activities, climate change may also take part in the increasing eutrophication and cyanobacterial growth (Moss et al., 2011; Samdal et al., 2021). Two of the promoting factors of cyanobacterial and algal growth are higher temperature and changes in precipitation, especially in shallow lakes. Increased temperature gives an advantage for cyanobacteria as their growth optimum is at higher temperatures (Moss et al., 2011), and therefore cyanobacterial blooms may increase with rising temperatures (Paerl & Huisman, 2009). Changes in precipitation, with periodical heavy rain fall, may change the loading of nutrients and residence time in the lake and can lead to more nutrients being added to the lake (Moss et al., 2011).

From the middle of 20th century cyanobacterial blooms became a more frequent problem, due to industrialisation and urbanisation (Chorus & Welker, 2021), also in Norway (Aamodt et al., 2021). Norwegian lakes experienced large blooms, due to discharges from the industry, as well as sewage with nutrients and run-off from agriculture (Aamodt et al., 2021; Nashoug, 1999). These blooms reduced the water quality and several measures had to be initiated to limit the increased level of nutrients in the lakes (Aamodt et al., 2021). By limiting the nutritional supplement to the lakes, one could see that the frequency of blooms calmed down, even though some Norwegian lakes still experience sporadic or annual blooms (Aamodt et al., 2021: Solheim et al., 2020). Lake Akersvannet in Vestfold and Telemark County, in Southern Norway, is one of these lakes which still have sporadic cyanobacterial blooms. The lake is nutrient-rich and moderately calcareous lake (vann-nett.no; faktaark.naturbase.no). The area surrounding L. Akersvannet is characterized by agriculture and provides runoff with poor water quality and a high nutrient content (faktaark.naturbase.no). This extra supply of nutrients to the lake provides beneficial environment for cyanobacteria and algae to bloom. The ecological and environmental condition in L. Akersvannet is poor due to high algae and cyanobacterial production and the high amount of nutrients (vann-nett.no; faktaark.naturbase.no).

During the production season of algae and cyanobacteria, monthly water samples are collected from L. Akersvannet by Drainage Basin Coordinators in Horten-Larvik water area and studied. One of the methods used for phytoplankton studies, is inverted microscope for quantitative and qualitative investigations. In this method called Utermöhl´s counting technique, counting chambers and sedimentation tubes, chosen according to the density of plankton in the samples, are prepared overnight and studied with an inverted microscope at 100- or 400-times magnification. Despite the fact that microscopic identification can be time-consuming and in

need of an experienced person identifying (Chorus & Welker, 2021), it is considered a reliable method (Li et al., 2019a; MacKeigan et al., 2022).

Even though morphological methods for cyanobacterial studies are broadly used today, molecular methods are promising (Chorus & Welker, 2021). Molecular and genetic methods is continuously getting less time-consuming, less expensive, and more commercial kits are available than before (Dineen et al., 2010). One promising approach for detecting cyanobacteria and determinate species and genera, is 16S rRNA gene metabarcoding (Li et al., 2019b). This approach may be executed by taking, in this case, a water sample, extract and amplify target DNA, and sequence and determine species and genera. The 16S rRNA gene is present in all bacteria, and as it is highly conserved and have a length of approximately 1500 base pairs, it is widely used for bacterial identification (Clarridge, 2004; Patel, 2001).

This thesis will focus on investigation of cyanobacteria found in Lake Akersvannet, with two different approaches: a molecular genetic approach with DNA metabarcoding and a morphology approach with inverted light microscopy. The project will look at the possibility of using 16S rRNA gene metabarcoding approach to detect and analyse cyanobacteria. The sequencing results will be compared to the species- and genera determination of cyanobacteria by microscope. We will investigate if metabarcoding can supplement the current methods, how metabarcoding works as a tool for analysing cyanobacterial species present and if this method may be usable to determine taxa that are difficult to determine with microscopy. The two methods will be compared, their pros and cons investigated, as well as difference and overlap in species- and genera determination.

2. Material and methods

2.1 Sampling and study site

Water sampling for metabarcoding and microscopy analysis was conducted once a month in July, August, and September, respectively 1st of July, 9th of August and 14th of September 2021, from Lake Akersvannet (Fig. 1 and Fig. 2). The water samples were taken for investigation of cyanobacteria composition in L. Akersvannet. A boat was used to be able to take samples from approximately in the middle of the lake (Fig. 2). Morphometrical parameters for L. Akersvannet are presented in Table 1. Water samples for parameters such as pH and total phosphorous (TP) were taken and analysed by VestfoldLAB AS. Measurement of secchi depth were used to determine how far down in the water the mixed sample should be taken.

Figure 1. Location of Lake Akersvannet in Southern Norway, Vestfold and Telemark County (kartverket.no).

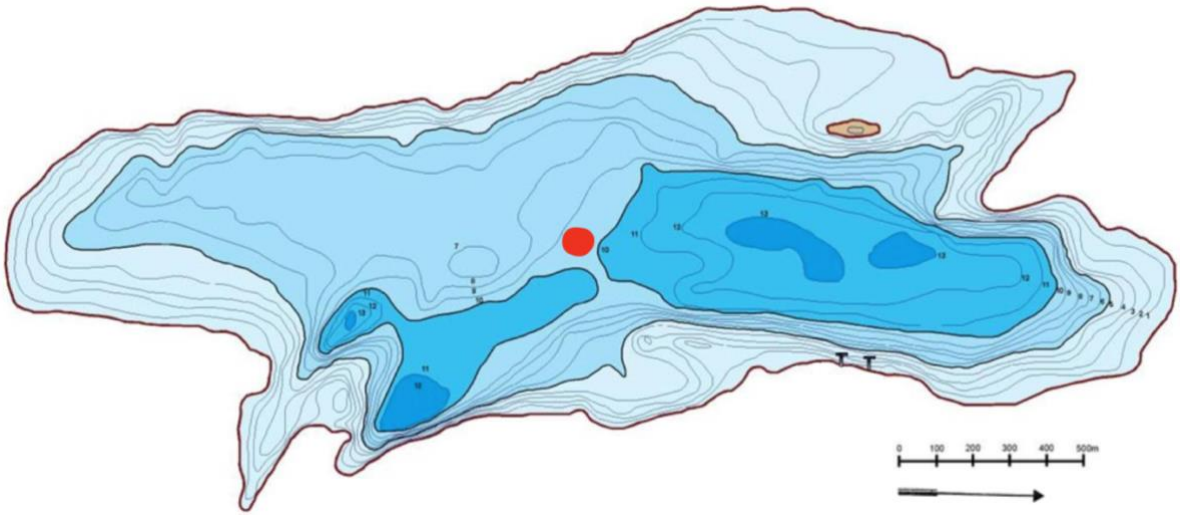

Figure 2. Bathymetric map of Lake Akersvannet (akersvannet.no). The red marking indicates sampling station.

Table 1. Morphometrical parameters of Lake Akersvannet (Berge (1986), norgeskart.no, vann-nett.no).

Latitude	Longitude	Surface area (km^2)	Maximum depth (m)	Average depth (m)	Elevation (masl)	Drainage area (km^2)
59.244	10.327	2,4	14	4	16	14

Samples were taken as mixed samples down to twice the secchi depth, and thereafter distributed to 50 mL[1] plastic containers for storage. For each of the sampling dates, two parallels for both metabarcoding and microscopic analysis were taken. Due to a possible cyanobacterial bloom in late August, additional water samples were collected at 5th and 14th of September. First, one mixed water sample was taken on 5th of September, by the Drainage Basin Coordinator in Horten-Larvik water area and sent to our laboratory for toxin analysis. Second, on the last sampling date, 14th of September, one mixed water sample and two phytoplankton samples were collected in addition to the water samples taken for metabarcoding and microscopy analysis. The two phytoplankton samples were taken to gain knowledge about cyanobacteria's morphology without the effect from Lugol solution, and to gain a slight picture of which genera of cyanobacteria that could be found in L. Akersvannet. These samples were taken from the water surface, one from the middle of the lake and the other from the shore, using a phytoplankton net with a mesh size of 25 µm.

[1] The samples from 01.07.21 was taken by the Drainage Basin Coordinator in Horten-Larvik water area and sent to our laboratory. These samples were taken in 200 mL plastic containers.

Samples for inverted microscopic analysis (quantitative phytoplankton analysis) were added Lugol solution while in field, for conservation of the cells and then stored in darkness in a refrigerator at approximately 4 degrees Celsius. The samples for metabarcoding and toxin analysis were frozen at -18 to -20 degrees Celsius. Back at the laboratory, both additional 50 mL phytoplankton samples were separated to two smaller containers. One was stored in darkness at 4 degrees Celsius for microscopy analysis, while the other was frozen and thawed two times and used for toxin analysis using ELISA (Enzyme-Linked Immunosorbent Assay) technique. Phytoplankton samples for microscopy analysis were studied shortly after, during the following days.

2.2 Toxin analysis

For toxin analysis of microcystin and saxitoxin, mixed water sample from 5th of September and 14th of September were analysed, as well as a net sample from 14th of September 2021. The mixed water samples from both dates were analysed without dilution, whereas the net sample were analysed as non-diluted and as 1:10 dilution. The net sample was diluted because of its visually green colour, which indicated high concentration of algae and cyanobacteria, and therefore possibly high concentration of toxins. The dilution was performed to ensure that the concentration of toxin was in the standard curve detection area of the kit. After the first ELISA analysis, the concentration of microcystin in the net samples, both diluted and non-diluted, was higher than standard 5 (measured to 4,3 µg/L (annex 4)) and therefore needed to be diluted even more. A new ELISA analysis of the net-sample was conducted, with dilutions 1:10, 1:20, 1:30, 1:50, 1:100, 1:200.

The cyanotoxins was analysed with commercial ELISA kits by ABRAXIS for microcystin (Microcystins/Nodularins (ADDA) ELISA kit, Lot No. 18J8148) and saxitoxin (Saxitoxin ELISA kit, Lot No. 19E9769) respectively. The test sensitivity of the kits provides a detection limit of the toxins, which for microcystin is based on microcystin-LR (MC-LR) with a limit of 0,1 µg/L, and for saxitoxin, a detection limit of 0,015 µg/L. Standards following each kit were analysed, as well as two parallels for each sample. For analysis of both microcystins and saxitoxins, the procedure following the kits were used. The absorbance was detected with AccuReader M965 (Metertech), at 450 nm, and the results was exported from the program Grabber to an excel-sheet to make a standard curve and calculate the toxin concentrations.

2.3 DNA isolation and PCR

The frozen water samples for DNA analysis were thawed and filtrated with a vacuum funnel, with 0,45 µm Cellulose Nitrate Membrane Filters (Whatman™). Seven samples were filtrated altogether. One sample with 100 mL water and two samples with 50 mL water from 1st of July, as well as two samples with 50 mL water from 9th of August and 14th of September respectively. For each sample, a new filter was used, and the equipment was washed with a chlorine solution. After filtration, each filter was placed in separate DNA Bead Tubes, and DNA isolation was performed by following the procedure for DNeasy PowerWater Kit by QIAGEN. After the DNA isolation process, the amount of DNA isolated was measured with NanoPore Lite Spectrophotometer (Thermo Scientific™).

After DNA isolation, 16S was amplified following the procedure for Barcoding Kit 1-24 (SQK-16S024) by Oxford Nanopore Technologies. The kit includes primers 24F (forward primer) and 1492R (reverse primer), where the primers numbers indicates where on the 16S gene the primers binds. The samples were assigned one barcode each, which came with the kit, from barcode 9 to 15. The enzyme LongAmp™ Hot Start Taq 2X Mastermix, by New Engand BioLabs ® Inc., were also added to the mix, which allows for greater PCR sensitivity. When following the protocol, one change was made to the PCR, by running 30 cycles instead of 25 which the protocol states. This change was done after recommendation from the laboratory due to their past experiences with the barcoding kit, to ensure that enough DNA was amplified during the PCR. When the PCR was completed, the DNA concentration was measured with Qubit 3.0 Fluorometer (Life Technologies), and each sample was diluted to provide samples with a DNA concentration of approximately 10 ng/µL.

2.4 Sequencing and metabarcoding

Before sequencing, the samples were pooled together to one library by following the 16S Barcoding Kit protocol and then sequenced with MinION flow cell, version 9.4 (Oxford Nanopore Technologies). Nanopore sequencing has the advantage of sequencing from short to long reads, as each base is read continuously as they pass through the pore (Branton et al., 2008). The flow cell measured the current for each DNA-base and the raw signals were translated to DNA-bases with basecalling by Guppy (Oxford Nanopore). The basecalling provided a FASTQ file which was filtered by length (1500 – 2000 base pairs) and quality (Q score > 8). The taxonomic classification system Kraken 2 (Wood et al., 2019) was used to

assign the taxonomy of the filtered reads to genus level by using the SILVA SSU Ref. Nr. 99 138.1 database (Quast et al., 2012) built with Kraken 2-build. Kraken 2 provided results files which were combined to a single biom file by using the kraken-biom script (Dabdoub, 2016). The biom file was then imported to R using the *import_biom* command in the phyloseq R package (McMurdie & Holmes, 2013).

2.5 Microscopic analysis

The fresh phytoplankton samples from 14th of September, without Lugol solution, were qualitatively studied with an Olympus CX21 microscope with both 100 and 400 magnification, to get an overview of the phytoplankton community that were present in L. Akersvannet. For determination of phytoplankton taxa Tikkanen and Willén (1992) was used as literature.

The Lugol preserved samples from 1st of July, 9th of August and 14th of September were prepared by filling 10 mL sedimentation chambers and set for sedimentation through the night, in a dark box in room temperature (Helcom, 2021). After sedimentation, the samples were quantitative studied with an inverted microscope (Olympus CK2), with 400 magnification. Plankton within 50 squares (Fig. 3) of each sample were counted, measured, and identified with Tikkanen and Willén (1992) as primary literature, and Komárek (2013); Blomqvist and Olsen (1981); Lepistö et al. (1994); Willén et al. (1985) as supporting literature. Biovolume of each sample was estimated in Excel with formulas from Räkningsförfarande av växtplankton vid laboratoriet för miljökontroll, Uppsala, by Willén et al. (1985).

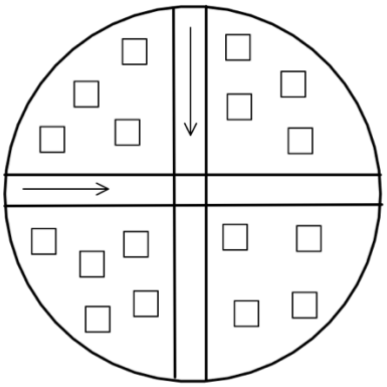

Figure 3. Illustration of the counting method used for quantitative analysis of phytoplankton samples with an inverted microscope. 10 squares down vertically, 10 squares horizontally and 30 squares distributed through remaining areas were counted.

2.6 Statistical analysis

Except from the calculations of microscopic analysis in Excel, all statistical analyses were performed in R Studio version 4.1.2 (R Core Team, 2021), with among others, the phyloseq package (McMurdie & Holmes, 2013). First, all the data were imported to R and the Operational Taxonomic Unit (OTU) table and taxa table were transformed to a phyloseq object, by using *phylosec()* function. With the vegan library (Oksanen et al., 2020) and *rarecurve()* function, rarefaction curves were made to explore the OTU richness vs. sequencing depth. Alpha diversity were explored by using the *plot_richness()* function from the phyloseq package, while beta diversity were studied by doing a *Principal Coordinates Analysis* (PCoA) with Bray Curtis dissimilarity with *plot_ordination()* function from phyloseq package (ggplot2 package are also in use for this function (Wickham, 2016)). After looking at the total taxa sequenced, the dataset were filtered to include only taxa of Phylum = Cyanobacteria, with the *subset_taxa()* function. Alpha and beta diversity of cyanobacteria were explored with the same functions as mentioned above. ANOVA using the *aov()* function, was used to test for difference in alpha diversity between sampling dates. The difference in community composition between sampling dates was tested with PERMANOVA using the *adonis ()* function in the vegan library. The last part of the statistical analyses was a test of correlation between the two methods, microscopic and genetic analysis. The excel datasheet from microscopic analysis were imported to R, and the abundance (cells/L) were tested against number of reads from the sequencing. Packages plyr (Wickham, 2011) and tidyverse (Wickham et al., 2019) were used for this last part. *Cor.test()* function with Spearman´s rank correlation was performed, as well as a plot of the result with "Relative sequence abundance" and "Estimated abundance of cyanobacteria cells/L".

3. Results

3.1 Water parameters

Secchi depth were measured to 1,0 m at 1st of July and 9th of August, and 1,4 m at 14th of September 2021, meaning mixed water samples were sampled down to 2,0 m in both July and August, and 2,8 m in September. Selected water quality parameters (Table 2 and annex 1-3) include pH, conductivity, turbidity, total organic carbon (TOC), total nitrogen (Tot-N) and total phosphorous (TP). pH ranged between 7,7 and 8,8 through the sampling depths and dates in L. Akersvannet, and conductivity from 17,4 mS/m to 19,0 mS/m. Both pH and conductivity were quite stable in the mixed samples, while turbidity had more variety from 4,2 FNU at 6 m 14th of September to 21,3 FNU in the mixed sample 1st of July. TOC had a concentration from 5,6 mg C/L to 6,2 mg C/L, while Tot-N varied some more with concentrations from 0,38 mg N/L to 1,0 mg N/L. TP concentration was at its lowest with 17 µg P/L at 4 m depth in August and highest with 96 µg P/L at 3 m depth 1st of July.

Table 2. Selected water quality parameters from Lake Akersvannet in the period from July to September 2021, analysed by VestfoldLAB AS. Measurements were taken at different depth intervals, as well a mixed sample taken down to twice the secchi depth (0-2 m at 1st of July and 8th of August, and 0-2,8 m at 14th of September) per sampling date. The water quality parameters include pH, conductivity, turbidity, total organic carbon (TOC), total nitrogen (Tot-N) and total phosphorous (TP).

Date	Depth (m)	pH	Conductivity at 25 °C (mS/m)	Turbidity (FNU)	TOC (mg/L)	Tot-N (mg/L)	TP (µg/L)
01.07.21	0,5	8,1	17,4	18,2	6,2	0,97	70
	3	8,3	17,6	18,8	6,0	1,0	96
	6	8,4	17,6	17,1	5,7	0,96	59
	Mixed (0-2)	8,1	17,6	21,3	5,8	1,0	74
09.08.21	1	8,7	17,8	15,2	5,8	0,45	22
	4	8,8	17,8	14,8	5,7	0,38	17
	6,8	8,6	17,8	15,1	5,6	0,46	24
	Mixed (0-2)	8,3	17,9	9,5	5,7	0,45	26
14.09.21	1	8,0	18,8	6,2	6,0	0,44	45
	6	7,7	19,0	4,2	5,9	0,44	46
	Mixed (0-2,8)	7,9	18,8	6,0	6,2	0,45	43

3.2 Toxin analysis

When analysing for microcystin (Table 3), the mixed water sample from 5th of September provided a mean concentration of 0,27 µg/L, and the mixed water sample from 14th of September provided a mean concentration of 0,22 µg/L. The net samples had high concentrations of microcystin, and only dilutions 1:100 and 1:200 showed a result within the standard curve (annex 6). The 1:100 diluted sample had a mean concentration of 3,2 µg/L, which were calculated to an actual concentration of microcystin of 324 µg/L. The 1:200 diluted sample showed a mean concentration of 0,99 µg/L, which were calculated to an actual microcystin concentration of 198 µg/L.

Table 3. Microcystin concentrations from ELISA analysis of samples from L. Akersvannet from July through September 2021.

Date	Sampling type	Dilution	Analysis results (µg/L)
05.09.21	Mixed water	–	0,39
		–	0,15
14.09.21	Mixed water	–	0,26
		–	0,18
14.09.21	Net sample	1:100	3,20
		1:100	3,29
14.09.21	Net sample	1:200	0,99
		1:200	0,99

The ELISA analysis of Saxitoxin, provided results ranging from 0,006 µg/L to 0,013 µg/L throughout the samples (annex 8). All results were below standard 1 (0,021 µg/L, annex 7) meaning they were outside the standard curve (annex 9). The procedure following the ELISA kit for Saxitoxin states that samples with concentration lower than standard 1 should be considered as negative for Saxitoxin.

3.3 DNA isolation and PCR

After DNA isolation of each sample, the DNA concentration measured on Nanodrop Lite varied from 3,0 ng/µL at 14th of September to 6,3 ng/µL 1st of July (Table 4), with a mean of 4,7 ng/µL. The A260/A280 ratio is a calculation often used to determine the purity of the sample, where ~1,8 (Thermo Fisher, 2012) is the generally accepted value for "pure" DNA. As seen in Table 4, the A260/A280 ratio is generally low (below 1,7) through the results. Nanodrop Lite

operates with a detection limit of 4,0 – 1500 ng/µL (Thermo Fisher, 2012) when analysing double stranded DNA (dsDNA). The results from sample F and G, is somewhat low considering the lower detection limit. The generally low concentration of DNA and A260/A280 ratio may be due to residues of polysaccharides, proteins and reagents which have not been completely removed and disturbs the DNA measures.

Table 4. Nanodrop Lite results of DNA concentration after DNA isolation of water samples from L. Akersvannet from July through September 2021. The results include a "purity parameter" A260/A280.

Date	Sample	A260/A280	ng/µL
01.07.21	A	1,67	6,3
	B	1,41	4,9
	C	1,38	4,7
09.08.21	D	1,56	5,4
	E	1,53	4,7
14.09.21	F	1,44	3,0
	G	1,29	3,9

Following the PCR procedure with primers and barcodes, the DNA concentration of the samples were measured with Qubit 3.0 Fluorometer. There was a large variation of measured DNA in the samples, with DNA concentrations from 39,8 ng/µL (sample G, 14th of September) to 370 ng/µL (sample D, 9th of August) (Table 5). Three samples had results < 100 ng/µL, while four samples had results > 100 ng/µL.

Table 5. Qubit 3.0 results of DNA concentration after PCR of water samples from L. Akersvannet from July through September 2021.

Date	Sample	Barcode	Sample concentration ng/µL
01.07.21	A	9	284
	B	10	59,2
	C	11	171
09.08.21	D	12	370
	E	13	155
14.09.21	F	14	62,6
	G	15	39,8

3.4 Sequencing and metabarcoding

The 16S gene metabarcoding yielded 2675 OTUs, of which 2663 were the kingdom of bacteria. The main phylum was proteobacteria, which is a large phylum of gram-negative bacteria. Out of the total 2675 OTUs, 2394 OTUs were assigned a family level, and 1940 OTUs were assigned a genus level. When sequencing, the different samples will have different number of reads (Fig. 4). The plot includes all the sequenced taxa, without rarefying of the data, and the sequencing depth ranged between 216 782 – 447 736 reads per sample. Barplot of the 10 most abundant phylum (Fig. 5) show a great amount of cyanobacteria in barcode 12 – 15, which are samples from 9th of August and 14th of September 2021.

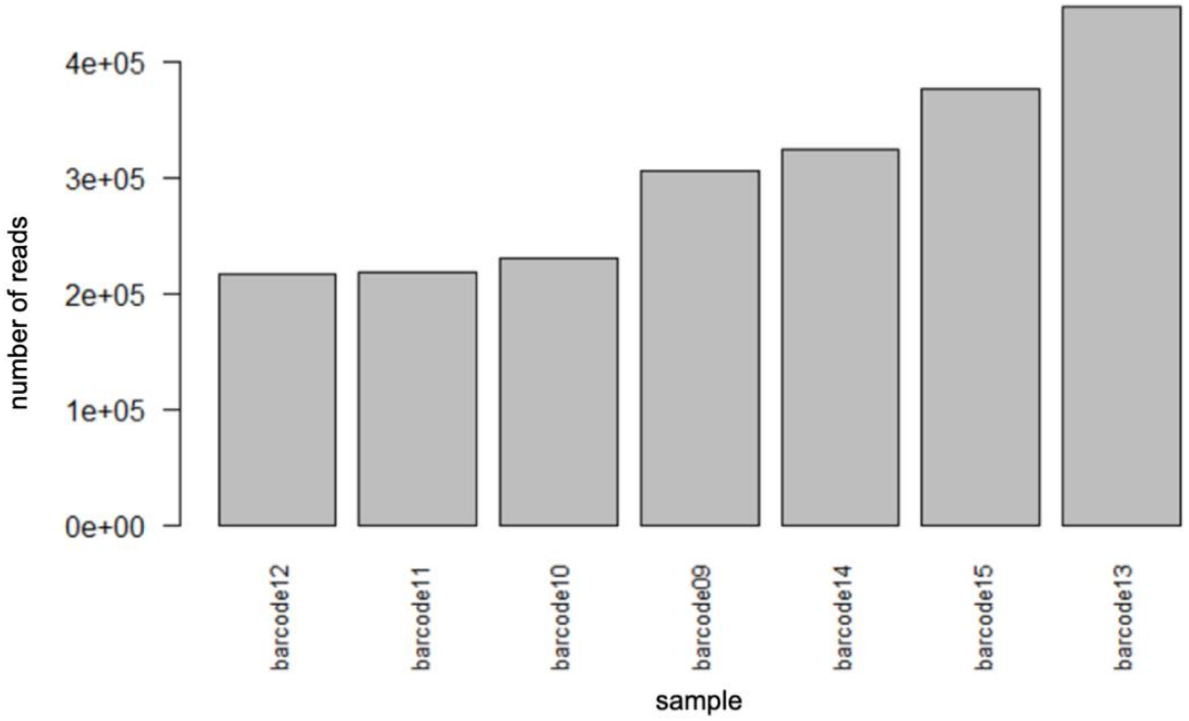

Figure 4. Barplot of variation in sequencing depth of all taxa from the sequenced water samples from July through September 2021 at Lake Akersvannet, made in R studio. The barcodes are placed in ascending order, based on the sequencing depth of each barcode.

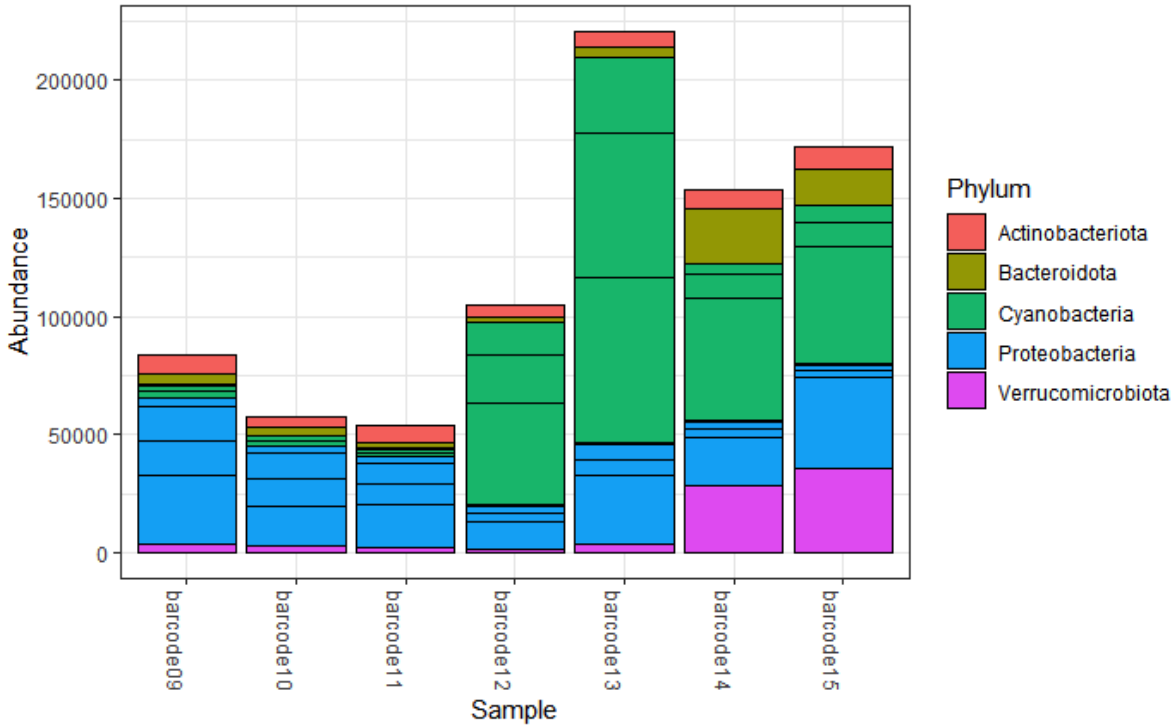

Figure 5. Barplot showing 10 most abundant cyanobacteria by phylum found in Lake Akersvannet in the sampling period July through September 2021.

Analysis of alpha diversity measures (Fig. 6) were done to investigate the diversity within the samples, and includes plots of observed, Chao1 index and Shannon index. The plot of observed, shows the number of species found in each sample and the colours indicates the date of sampling. The Chao1 index is an estimate of species richness and are reflections of the OUT abundance in the samples. The higher value Chao1 presents, the higher the expected species richness of the community. Results from Chao1 showed highest expected species richness in barcode 13, 14 and 15, and the lowest in barcode 9, 10, 11 and 12. Shannon index tells how diverse and even the species in the given community are, and the number will rise with number of species and the evenness of the species abundance. The Shannon plot shows that the samples from 1st of July had the highest diversity and evenness of taxa, even though the observed number of species and the expected species richness were lowest. In the plot of Shannon, the barcodes are relatively separated by date.

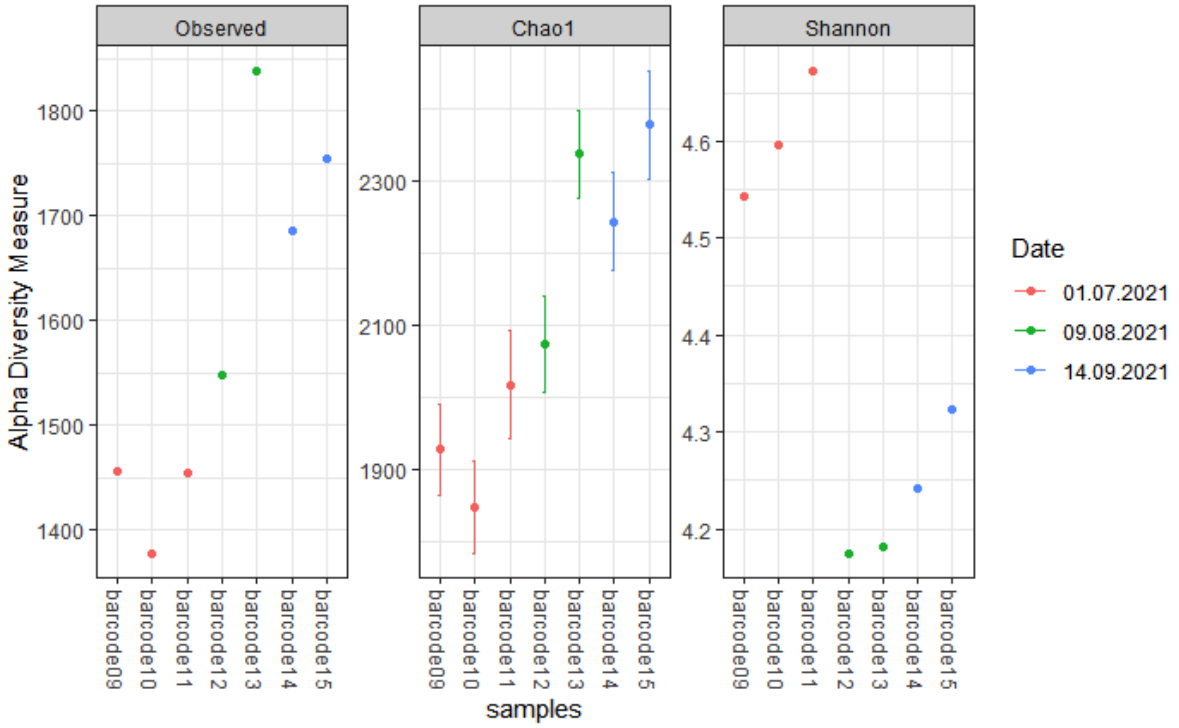

Figure 6. Plot of alpha diversity measures, i.e., observed, Chao1 and Shannon, interpreted on total taxa sequenced from water samples from July through September 2021 September at L. Akersvannet. The colours represent the sampling date for each of the barcodes. The plot of observed is merely a plot of the observed number of species in each sample. The Chao1 plot reflects the OTU abundance in each barcode, where a high value indicates high expected species richness. The Shannon plot reflects the evenness of OTUs in each barcode.

After studying the diversity within the samples, phyloseq were used to investigate the diversity between the samples, called beta diversity. Axis 1 at the PCoA plot (Fig. 7) separates the samples from July from the other two dates with an explanation of 72,2 %. Axis 2 separates the samples from August from the samples from September with an explanation of 16,3 %.

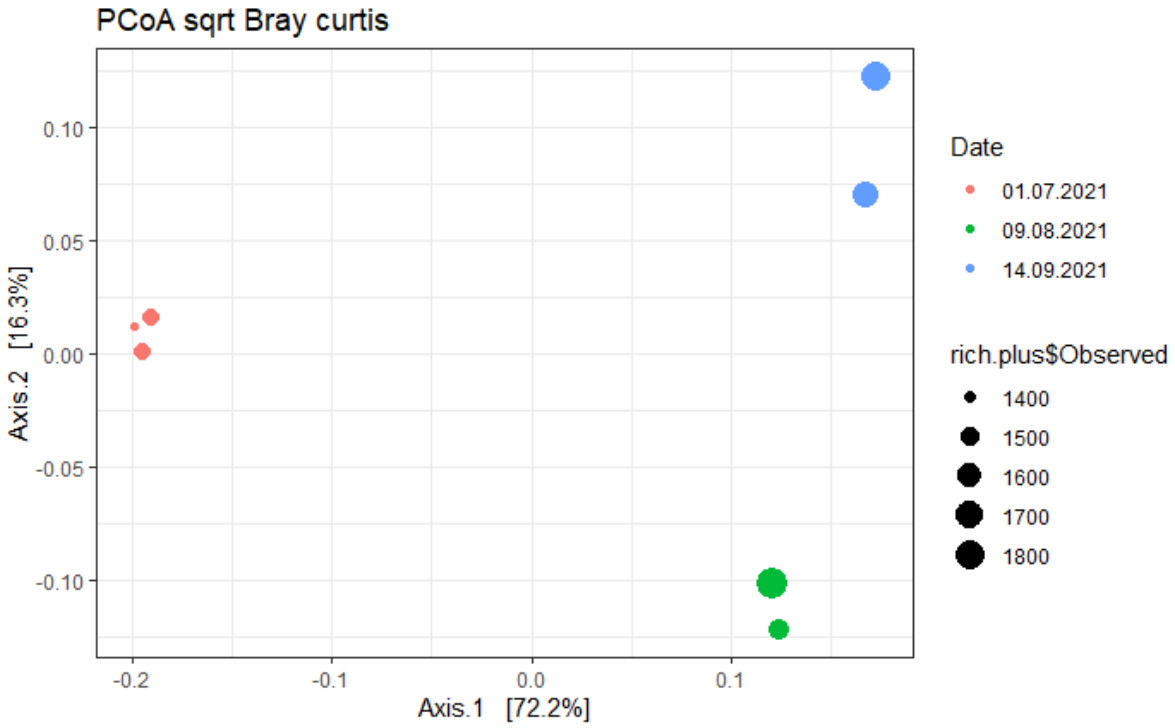

Figure 7. Plot of PCoA with Bray Curtis dissimilarity, on the total taxa sequenced from water samples taken at L. Akersvannet from July through September 2021. Axis 1 separates the parallels from July from the two other dates, and axis 2 separates the parallels from August and September.

Cyanobacterial diversity

Out of the 2663 OTUs identified as bacteria, 9 % were cyanobacteria, that is 227 OTUs. The main families of cyanobacteria were Nostocaceae (52 OTUs), Microcystaceae (19 OTUs), Chroococcidiopsaceae (11 OTUs), Cyanobacteriaceae (11 OTUs) and Phormidiaceae (11 OTUs). There were also 14 OTUs which did not get assigned a family level. A total of 157 cyanobacteria genera were sequenced, including known toxin-producing genera as *Anabaena*, *Dolichospermum*, *Aphanizomenon*, *Nodularia* and *Microcystis*. After filtering out all but cyanobacteria, the sequencing depth (Fig. 8) ranged between 4926 – 186 098 reads per sample. Barplot of the 10 most abundant genera (Fig. 9) show *Snowella* and *Microcystis* as most abundant genera.

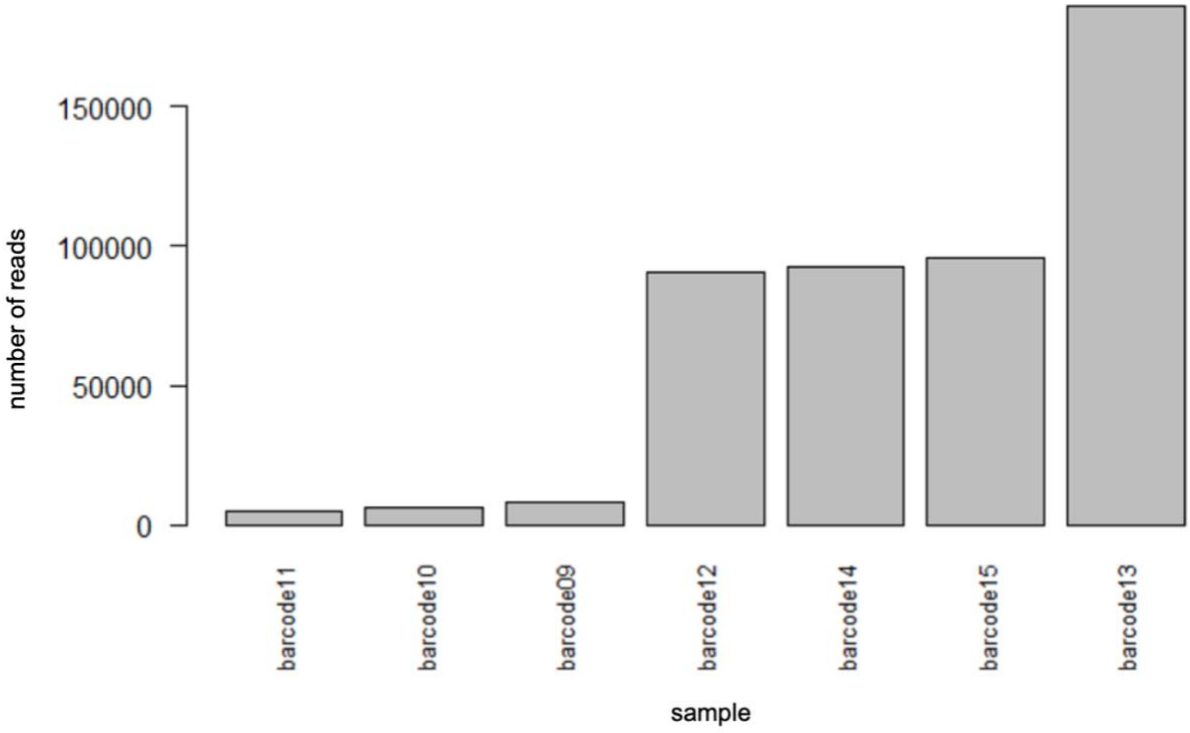

Figure 8. Barplot of variation in number of reads of the barcodes, including only cyanobacterial taxa sequenced from water samples taken at L. Akersvannet during July through September 2021. The barcodes are placed in ascending order, based on the sequencing depth of each barcode.

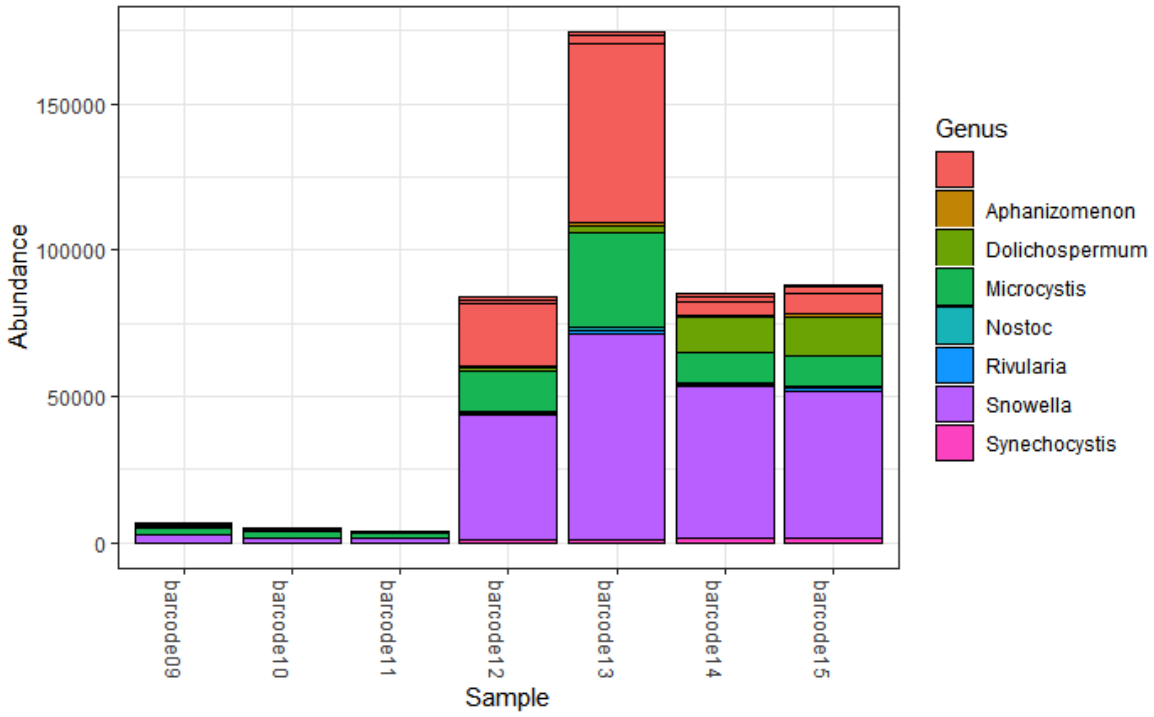

Figure 9. Barplot showing 10 most abundant cyanobacteria by genus found in Lake Akersvannet in the sampling period July through September 2021. Due to the low sequencing depth of barcode 9 - 11, these barplots have quite low abundance compared to the rest of the barcodes.

Rarefaction curves (Fig. 10) are plots presenting the number of species against sample size and gives a picture of the species richness in the samples. The rarefaction curve of the cyanobacteria in the samples shows a steep start where the most common species are found, also where barcodes 9, 10 and 11 stopped, and then the curve flattens slightly, where barcodes 12, 14 and 15 are found. Barcode 13 has the longest curve, therefore most species found, including rare species. Based on the curve, one cannot predict how the curves of barcodes 9, 10 and 11 would look like with larger sample size, but barcodes 12, 14 and 15 seems to have a relatively similar curve as barcode 13, indicating that the diversity is comparable.

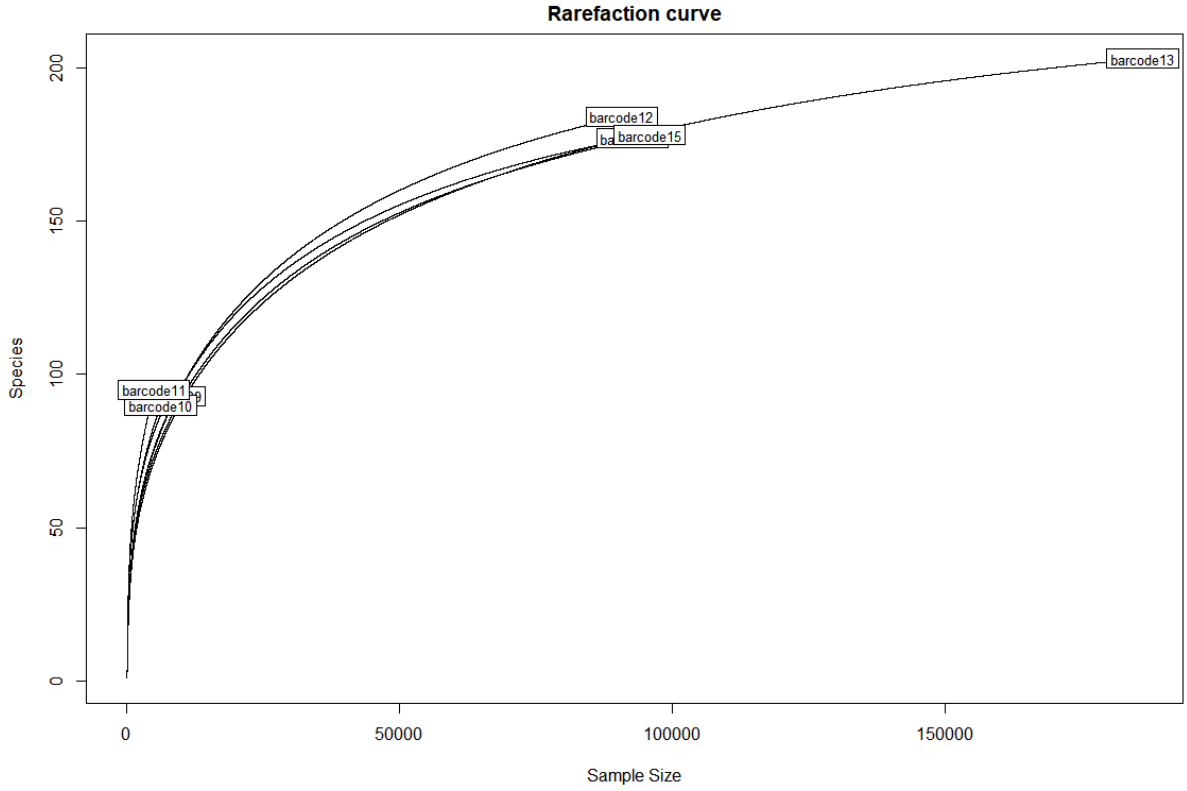

Figure 10. Rarefaction curve i.e., OUT richness vs. sequencing depth of cyanobacterial taxa sequenced from water samples taken during July through September 2021 at L. Akersvannet. Barcodes 9, 10 and 11 are found in the steep start where the most common species are found, barcodes 12, 14 and 15 are found after the curve starts flattening with a sample size at approximately 100 000, and barcode 13 have the longest curve with the largest sample size > 150 000.

As for the complete sequenced taxa, alpha diversity measures of the cyanobacteria taxa (Fig. 11) were implemented. In all plots, the parallels within each date are grouped close together. The plot of observed species shows results where the sampling from 1st of July have almost half the observed species as the other two dates. Chao1 results show highest expected species richness in the samples from 9th of August and 14th of September. As for the total taxa, the Shannon index shows the highest evenness in the samples from 1st of July. The alpha

diversity (i.e., observed, Chao1 and Shannon) at the different sampling dates were visualized using box plots (Fig.12). All the box plots illustrate that the samples from July have the largest distance from the other sampling dates.

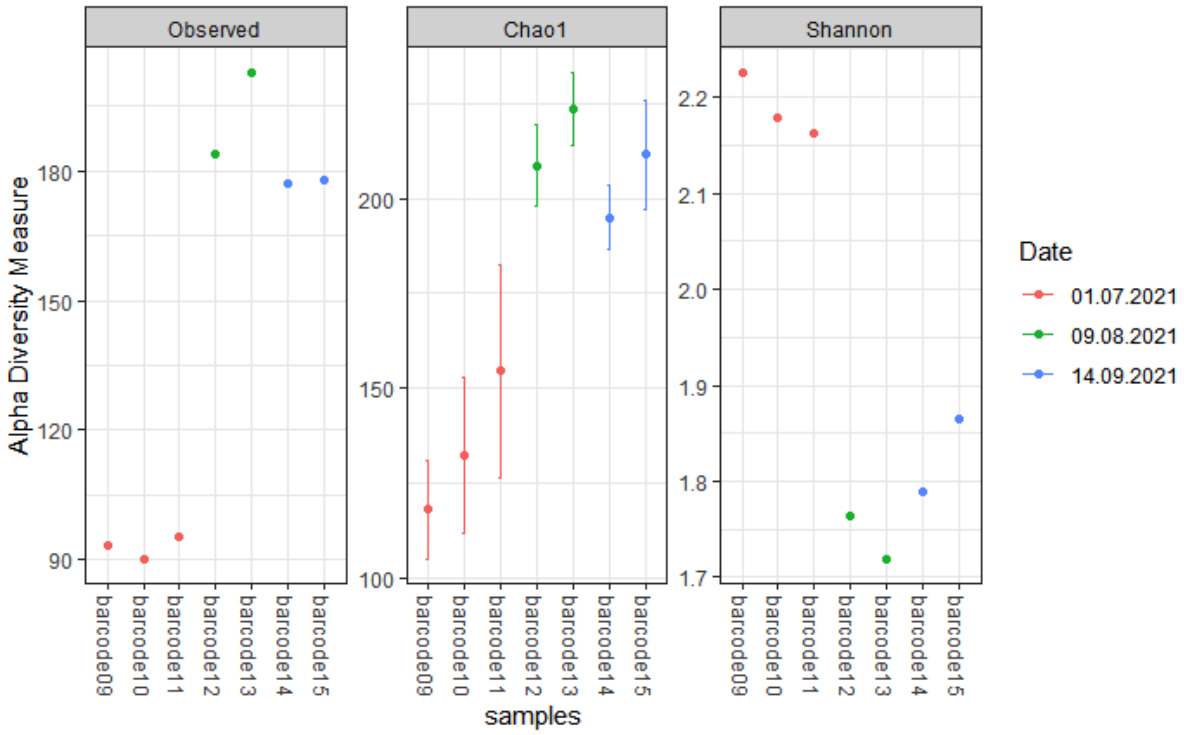

Figure 11. Plot of alpha diversity measures i.e., observed, Chao1 and Shannon for cyanobacteria taxa from water samples taken at L. Akersvannet July through September 2021. The colours represent the sampling date for each of the barcodes. The plot of observed is merely a plot of the observed number of species in each sample. The Chao1 plot reflects the OTU abundance in each barcode, where a high value indicates high expected species richness. The Shannon plot reflects the evenness of OTUs in each barcode.

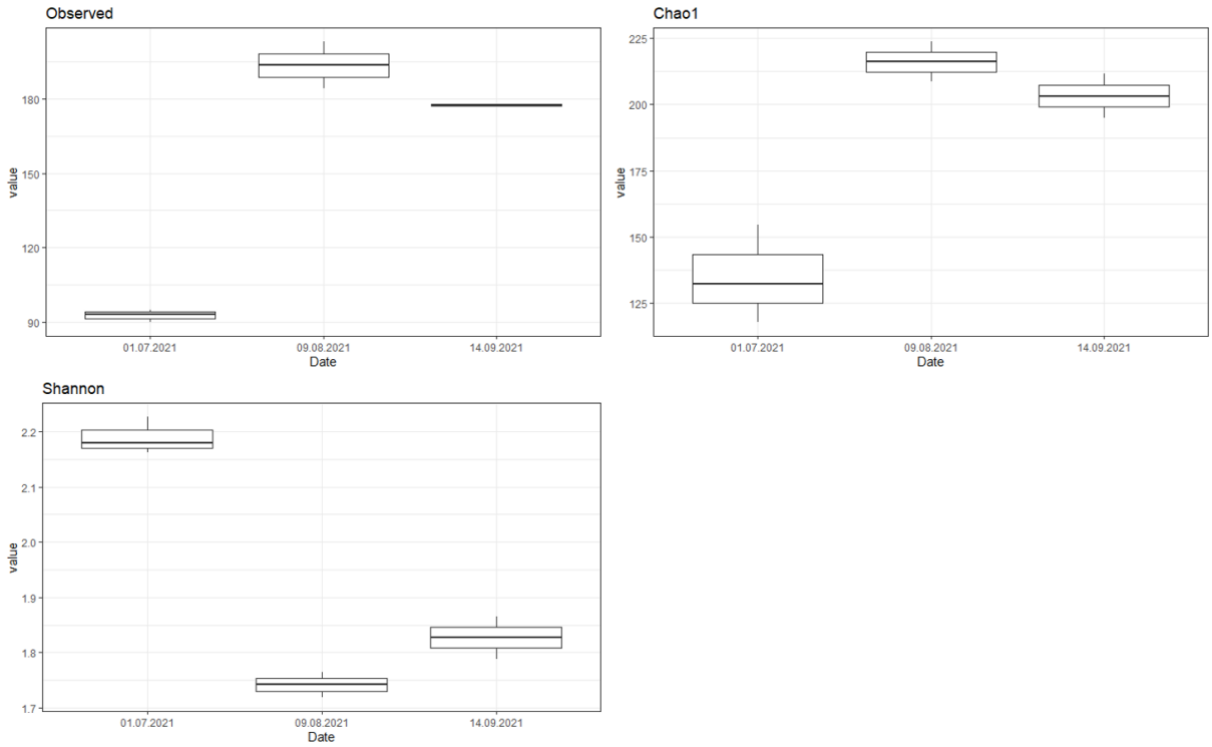

Figure 12. Boxplot of alpha diversity measures i.e., observed, Chao1 and Shannon, of cyanobacteria taxa sequenced from water samples taken in July through September 2021 at Lake Akersvannet.

The one-way ANOVA test (Table 6) gave p-values < 0,05 for observed, Chao1 and Shannon which indicates a significant difference between the sampling dates. The one-way ANOVA does not say anything about which pairs of groups are significantly different, but the boxplots indicates that the samples from July are significantly different from the samples from August and September. Tukey multiple comparisons of means (annex 11) of observed, Chao1 and Shannon, confirmed the assumptions made from boxplots and demonstrated that there was no significant difference between the samples from August and September in neither of the plots.

Table 6. One-way ANOVA test of alpha diversity measures, i.e., observed, Chao1 and Shannon, of cyanobacteria taxa sequenced from water samples taken in July through September 2021 at Lake Akersvannet. The Pr(>F) value corresponds to the p-value of the test, and the results shows all the alpha diversity measures have a p-value < 0,05.

	Df	Sum sq.	Mean sq.	F-value	Pr(>F)
Observed					
Date	2	15030	7515	155,2	0,000162
Residuals	4	194	48		
Chao1					
Date	2	9747	4874	21,03	0,00754
Residuals	4	927	232		
Shannon					
Date	2	0,28875	0,14437	93,88	0,000435
Residuals	4	0,00615	0,00154		

After analyses of alpha diversity measures, calculation of beta diversity by ordination analysis were conducted. Axis 1 at the PCoA plot (Fig. 13) separates the July samples from the other sampling dates with an explanation of 69,4 %. Axis 2 separates the August samples from the September samples with an explanation of 16,5 %.

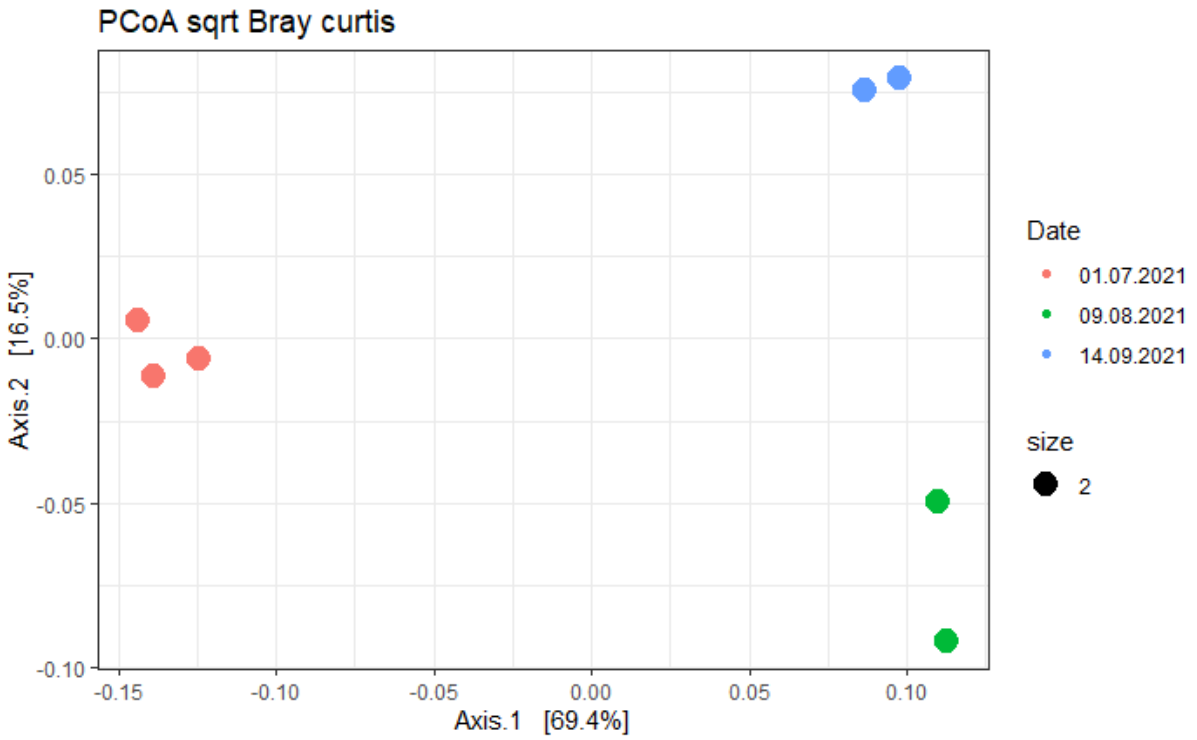

Figure 13. PCoA plot with Bray Curtis dissimilarity, on cyanobacteria sequenced from water samples taken in July – September 2021 at Lake Akersvannet. Axis 1 explains 69,4 % of the difference between July and the other two dates, and axis 2 explains 16,5 % of the difference between August and September.

Following up on the beta diversity analysis, a Permutation Based Analysis of Variance (PERMANOVA) test were used to test for significant difference in community composition between the sampling dates, based on Bray Curtis dissimilarity. In this case, the test was used to see if date was a significant variable which determines the community. PERMANOVA test includes calculations of R^2 value and p-value. R^2 calculations were at 0,85, meaning that 85 % of the variance can be explained by date. The p-value from the PERMANOVA test were 0,01, which indicates that there is a significant dissimilarity between the groups.

3.5 Microscopic analysis

The inverted microscope analysis provided results of total volume of phytoplankton with 1,5 mm^3/L at 1st of July, 3,4 mm^3/L at 9th of August, and 2,3 mm^3/L at 14th of September (Table 7, full results in annex 12-14). The cyanobacteria abundance varied during the sampling dates.

In the first sample in July 2021, 83 % of the total phytoplankton volume was cyanobacteria. In August and September, the result was at 28 % and 24 %, respectively.

Table 7. Total volume of phytoplankton and cyanobacteria (volume and %) in L. Akersvannet 2021 in the period from July to September 2021 based on microscopic analysis. The results includes both volume mm^3/L and cells/L.

Date		Volume mm^3/L	Cells/L
01.07.21	Total phytoplankton	1,5	25 056 089
	Cyanobacteria	1,2	13 044 552
	% Cyanobacteria	83 %	52 %
09.08.21	Total phytoplankton	3,4	28 260 000
	Cyanobacteria	0,93	17 759 840
	% Cyanobacteria	28 %	63 %
14.09.21	Total phytoplankton	2,3	23 537 440
	Cyanobacteria	0,55	8 691 520
	% Cyanobacteria	24 %	37 %

A total of eleven cyanobacteria taxa were identified with microscopy, seven cyanobacteria 1st of July, seven cyanobacteria 9th of August, and six cyanobacteria taxa 14th of September. Four of the cyanobacteria taxa, were found in samples from all three dates: *Woronichinia compacta*, *Snowella lacustris*, *Snowella septentrionalis* and *Aphanizomenon sp*.

The cyanobacteria with highest density (volume/L) 1st of July were *Aphanizomenon* sp. which consituted 78 % of the total cyanobacterial volume. On the 9th of August *Microcystis wesenbergii* had highest density with 36 % of total volume, and at 14th of September *Aphanizomenon* sp. were dominant constituting 46 % of total volume.

3.6 Comparing 16S rRNA gene metabarcoding and light microscopy

Direct comparisons between the 16S rRNA gene metabarcoding and light microscopy by number of reads and estimated abundance of cyanobacteria (cells/L) were done. The Spearman´s rank correlation test provided p-value of 0,006 and a rho of 0,8. The p-value indicate that there is a significant correlation between the two methods, and the rho indicates that the correlation is strong. A plot (Fig. 14) was made to visualize the results from the Spearman´s rank correlation test. The metabarcoding approach did not assign any cyanobacteria at species level, so the plot of correlation includes only genus name. *Snowella* genera are all

seen to be found middle right in the plot, whereas *Dolichospermum* and *Aphanizomenon* are found bottom left. The Spearman´s rank correlation test provided results of strong and significant correlation, even with the outlier "*Microcystis* 09.08.2021".

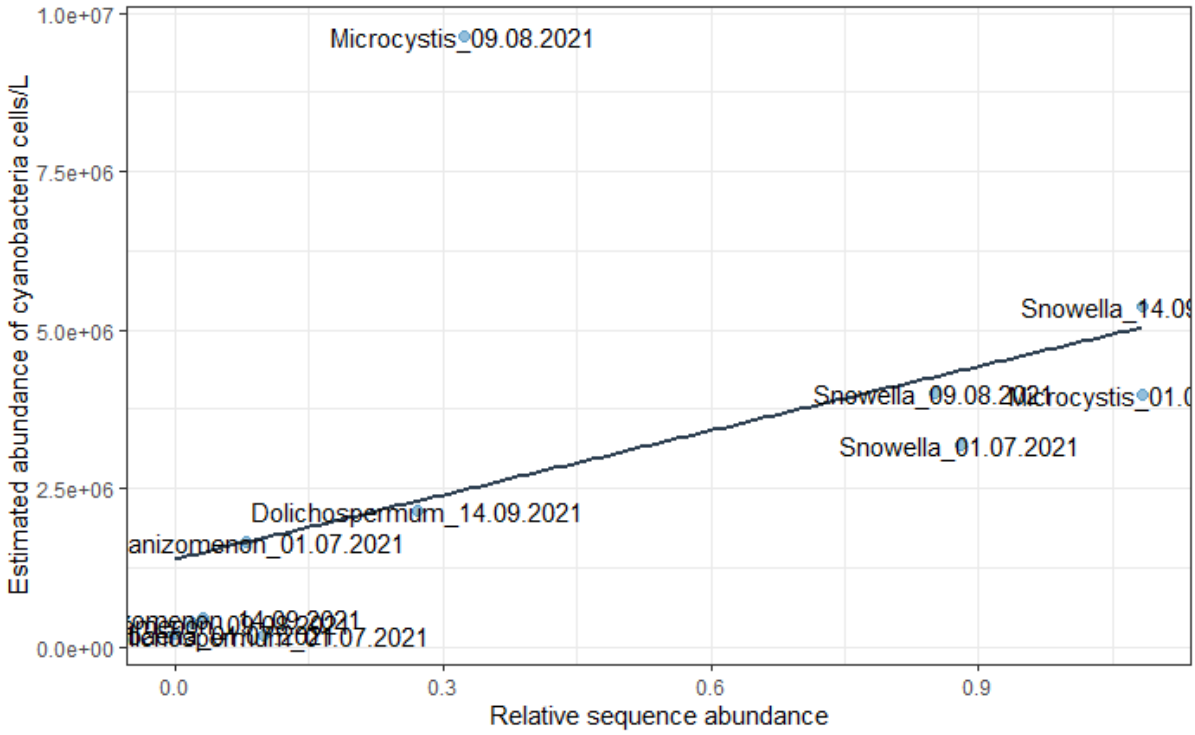

Figure 14. Correlation between relative sequence abundance and estimated abundance of cyanobacteria (cells/L) in water samples from L. Akersvannet taken in July through September 2021. Microcystis 09.08.2021 seems to be an outlier, whereas the rest of the plot looks more correlating.

4. Discussion

16S rRNA gene metabarcoding of water samples from Lake Akersvannet collected in the period of July through September 2021, provided a total of 157 genera of cyanobacteria. None of the sequenced cyanobacteria were assigned at species level. Out of the eleven cyanobacterial taxa, found with microscopy, only two were not found with metabarcoding. As a tool for analysing cyanobacteria, metabarcoding yielded a broader spectre of genera than microscopy. Correlation test revealed a strong and significant correlation between the methods.

Cyanobacteria were found in varying amounts in all samples from Lake Akersvannet, using both microscopic and metabarcoding approach. When the samples were collected in September, there was a visible bloom in the lake and colonies could be seen floating at the shore. Water quality parameters were analysed to investigate the environmental conditions. Concentration of total phosphorus can be used as a classification parameter for the trophic state of lakes (Chorus & Welker, 2021). A study by Vuorio et al. (2020) showed that cyanobacteria in general needs a TP level of approximately 20 µg/L for the biomass to strongly increase. Except from one sample, taken 9th of August at 4 m depth, all samples showed a TP concentration > 20 µg/L. According to the results, Lake Akersvannet can be given the classification of mesotrophic (10 – 35 µg TP/L) at 9th of August 2021, and eutrophic (35 – 100 µg TP/L) (Chorus & Welker, 2021) at 1st of July and 14th of September 2021.

Cyanobacteria in the environment can include both toxin-producing and non-producing strains (Davis et al., 2009), and cyanotoxins are not necessarily present in a bloom (Solheim et al., 2020). Microcystin analysis of net samples (table 3) provided results above WHOs provisional guideline for drinking water (1 µg microcystin/L) and waters for recreational use (24 µg microcystin/L). The most important difference between the mixed water samples and net samples, are the sampling method. When using a net, the accumulation of cyanobacteria will be larger as the method is used specifically for collecting phytoplankton. This is in opposite to the mixed water sampling which does not concentrate the sample and collects water from surface down to twice the secchi depth. Natural movement in the lake and wind, are factors which may have affected the buoyant cyanobacteria to accumulate at the shore. Investigation with microscopy and metabarcoding confirmed that well known microcystin producers were present in Lake Akersvannet, such as *Dolichospermum sp.* and *Microcystis sp.*

All samples were considered as negative for saxitoxin, as the concentrations were all below standard 1 (0,021 µg/L) of the saxitoxin ELISA kit. These results indicates that the known saxitoxin producers found with metabarcoding and microscopy, *Aphanizomenon sp.* and *Dolichospermum sp.*, were probably non-producing strains. A similar situation was seen during the bloom in the late July in 2019 in Lake Mjøsa (eastern Norway), where *Dolichospermum lemmermannii* were the dominating species, but no cyanotoxins were detected (Solheim et al., 2020).

When studying communities of microorganisms, it is common to rarefy the reads from each sample (McMurdie & Holmes, 2014), in order to standardize the sample size. However, given the relatively even number of sequence reads found in all samples and the potential bias rarefaction may introduce (McMurdie & Holmes, 2014) it was decided not to rarefy the samples.

Diversity measures showed a large difference in cyanobacterial taxa depending on sampling date, in particular the samples from July were significantly different from the other sampling dates (Fig. 11-12, and annex 8-9). July samples scored highest on evenness, indicating a community where the species have similar abundance. Samples from August and September scored high on estimated species richness but low on evenness, indicating that there are a few dominating species in the community. Changes in the algal and cyanobacterial community may occur due to natural cycles in the lake, changes in weather or supply of nutrients, as cyanobacteria species compete with each other and other phytoplankton for the available lights and nutrients (Chorus & Welker, 2021).

Microscopic identification of cyanobacteria is a traditional method still used today (Li et al., 2019a; MacKeigan et al., 2022), but phenotypic variations can be misleading (Li et al., 2019b) and the method needs an experienced person identifying (Chorus & Welker, 2021). Positive result of microcystin from the toxin analysis from 14th of September, contributed to expectations of finding microcystin producing cyanobacteria in samples from this date. *Microcystis* were not found on this date (only with metabarcoding), but *Dolichospermum crassum* (*Anabaena crassa*) and *Dolichospermum lemmermannii* (*Anabaena lemmermannii*) were found. *Microcystis* are small cells, there is a possibility that they have been placed outside the squares counted, or that there has been a personal error of misidentification. Misidentification and being able to recognise the different cyanobacteria and phytoplankton,

are one of the challenges of species determination with light microscopy (Li et al., 2019b). A factor which can complicate the identification of the cells, are the possibility of cells and colonies laying on top of each other due to the sedimentation process. This may cause error in counting, which can give a wrong biovolume, and error in species identification as the stacking of cells can "change" the look of the cells. Other factors that contribute to identification challenges are the fact that the morphology of colonies may change naturally throughout the seasonal cycle, as well as fixation with Lugol solution may disintegrate the distribution of aerotopes within the cells (Chorus & Welker, 2021).

The results of the quantitative microscopy analyses of cyanobacteria were presented as volume (mm^3/L) in the results but can also be presented by abundance (cells/L). When looking at the most frequently occurring cyanobacteria by cells/L, the result from each date will change as this approach only consider the number of cells, not their volume. For example, *Microcystis aeruginosa* were most frequent with 30 % of cells/L 1st of July, compared to *Aphanizomenon* sp. which dominated 78 % of the volume the same date. There is a large size gap between these two species, with *Microcystis aeruginosa* having a cell diameter of 4-6 µm, while *Aphanizomenon* can be up to 150 µm long (Tikkanen & Willén, 1992). These facts explain the change of dominating species when comparing number of cells/L and biovolume.

Direct comparisons of the results from 16S rRNA metabarcoding and inverted light microscopy was one of the main focuses of investigation in this thesis. Spearman´s rank correlation test showed that there was a significant correlation between the methods and that the correlation was strong. Visualization by plot (Fig. 14) showed "*Microcystis* 09.08.21" as a possible outlier, due to the high abundance of cells/L. A possible explanation for this might be because the correlation was made at genus level, meaning that the abundance of *Microcystis aeruginosa* and *Microcystis wesenbergii* from microscopy analysis were merged. The total estimated abundance of *Microcystis* from 9th of August constituted 55 % of the total cyanobacteria cells/L.

Out of the eleven cyanobacteria taxa identified using light microscopy, nine of them were also found with metabarcoding, with *Woronichinia compacta* and *Planktolyngbya* as exceptions. Metabarcoding yielded a broader spectre of identified cyanobacteria than light microscopy, with genera such as *Nodularia* and *Planktothrix*, which are genera formerly found in Norway (Samdal et al., 2021). *Nodularia* are mostly found in brackish and coastal waters (Chorus & Welker, 2021), but are found in a fresh water lake in Turkey (Akcaalan et al., 2009). *Anabaena*

and *Dolichospermum* were both identified with metabarcoding. Most of the planktonic members of *Anabaena* are now included in the genus of *Dolichospermum*, but some specific isolates are still known as *Anabaena* (Li et al., 2016). Chorus and Welker (2021) confirmed that the *Dolichospermum* species identified with light microscope are indeed included in the *Dolichospermum* genus, despite the fact that some of the older literature used for species determination, for example Tikkanen and Willén (1992) uses *Anabaena*. Such change in taxonomy classification may be a source of challenge with metabarcoding if the reference sequence databases are not updated continuously with new species or change of names (MacKeigan et al., 2022).

Barplot of 10 most abundant genera (Fig. 9) identified with metabarcoding showed that *Aphanizomenon* were among top 10, but still seemed to be underrepresented compared to the microscopy results where *Aphanizomenon* had highest density at two of three dates. This may be explained by the measurement of total cells/L versus total volume/L. The results from microscopic analysis were presented with volume/L, in which *Aphanizomenon* were dominating due to the large volume of each individual cell. The percentage of *Aphanizomenon* were much lower looking at the abundance, ranging between 2,1 % of total cells/L at 9th of August and 12,5 % of total cells/L at 1st of July, which could be a reason of low abundance in metabarcoding results. It is formerly found that some strains of planktic *Anabaena* were indistinguishable from *Aphanizomenon* due to clustering of strains (Sarma, 2013). Five *Anabaena* strains and two *Aphanizomenon* strains were found with metabarcoding approach, and a clustering of strains could explain the low abundance of *Aphanizomenon*. Another explanation may be that the method used to isolate DNA were not optimal for *Aphanizomenon* genera. Tillett and Neilan (2000) proposed xanthogenate-SDS nucleic acid isolation as a method yielding high quality DNA of cyanobacteria, including strains of *Aphanizomenon flosaquae*.

5. Conclusion

This thesis provided results where the metabarcoding approach found nine out of eleven cyanobacteria demonstrated by light microscope and did also identify a greater spectre of cyanobacteria than light microscopy. Other studies as MacKeigan et al. (2022) and Li et al. (2019b) both specified that metabarcoding approach detected more species and more rare taxa than with morphological identification. Spearman´s rank correlation test resulted in a significant and strong correlation between the methods. Both methods have their pros and cons. With microscopic approach, several cyanobacteria were identified down to species level, and calculations of biovolume provided a good picture of the lakes condition. The metabarcoding approach identified more cyanobacteria genera than the microscopic approach, but the taxa were not assigned at species level. Abundance of *Aphanizomenon* from metabarcoding seemed to be underrepresented compared to the microscopy results, and if this is due to difficulty isolating DNA, a more specific method is needed. To improve the understanding of cyanobacterial communities, a combination of methods using genetics and morphology are hopeful (Li et al., 2019b). Based on the results in this thesis, the use of DNA metabarcoding and light microscopy may work well as complementary methods, rather than one replacing the other.

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Annexes

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Annex 1: Analysis result of selected water parameters sampled from VestfoldLAB AS sampled 1st of July 2021 (Norwegian)

ANALYSERESULTATER

Analyseperiode: 02/07/21 - 29/07/21, Uttaksprosedyre: Enkel stikkprøve Prøvetaker: Tatt ut av leverandør

21/4098-1 Vannforekomster,overvåkning Akersvannet 0,5m — Tatt ut: 01/07/21 014-38222

Analyse	Metode	Ref	Resultat	Benevning	Usikkerhet
pH, surhetsgrad	NS-EN ISO 10523	N)	**8.11**		±0.2e
Konduktivitet v/25°C	NS-ISO 7888	L)	**17.4**	mS/m	±10%
Turbiditet	NS-ISO 7027-1		**18.2**	FNU	±15%
SS,suspendert stoff	NS-EN 872		**18**	mg/l	±25
Fargetall filtrert	NS-EN ISO 7887		**22**		±15%
Kalsium, AES	NS-EN ISO7980		**13.0**	mg Ca/l	±10%
Totalt organisk karbon	NS 1484		**6.2**	mg C/l	±20%
Ammonium-nitrogen	Intern/ISO 11732		**0.021**	mg N/l	25
Nitrat, IC	ISO 10304-1		**0.44**	mg N/l	±15%
Orto fosfat	ISO 15681-2 2005		**0.017**	mg P/l	±30%
Totalfosfor	NS-EN-ISO 15681-2:18		**0.070**	mg P/l	±15%
Totalnitrogen	Int/ISO 29441:2010		**0.97**	mg N/l	±20%
Intestinale enterokokker	ISO 7899		**10**	/100 ml	(6 - 15)
E.coli *)	ISO 9308-1:2014		**<1**	/100ml	

21/4098-2 Vannforekomster,overvåkning Akersvannet 3m — Tatt ut: 01/07/21 014-38222

Analyse	Metode	Ref	Resultat	Benevning	Usikkerhet
pH, surhetsgrad	NS-EN ISO 10523	N)	**8.26**		±0.2e
Konduktivitet v/25°C	NS-ISO 7888	L)	**17.6**	mS/m	±10%
Turbiditet	NS-ISO 7027-1		**18.8**	FNU	±15%
SS,suspendert stoff	NS-EN 872		**20**	mg/l	±25
Fargetall filtrert	NS-EN ISO 7887		**23**		±15%
Kalsium, AES	NS-EN ISO7980		**12.9**	mg Ca/l	±10%
Totalt organisk karbon	NS 1484		**6.0**	mg C/l	±20%
Ammonium-nitrogen	Intern/ISO 11732		**0.021**	mg N/l	25
Nitrat, IC	ISO 10304-1		**0.47**	mg N/l	±15%
Orto fosfat	ISO 15681-2 2005		**0.019**	mg P/l	±30%
Totalfosfor	NS-EN-ISO 15681-2:18		**0.096**	mg P/l	±15%
Totalnitrogen	Int/ISO 29441:2010		**1.0**	mg N/l	±20%
Intestinale enterokokker	ISO 7899		**<10**	/100 ml	(0 - 15)
E.coli *)	ISO 9308-1:2014		**<1**	/100ml	

21/4098-3	Vannforekomster,overvåkning Akersvannet 6m			Tatt ut: 01/07/21 014-38222	
Analyse	**Metode**	**Ref**	**Resultat**	**Benevning**	**Usikkerhet**
pH, surhetsgrad	NS-EN ISO 10523	N)	**8.40**		±0.2e
Konduktivitet v/25°C	NS-ISO 7888	L)	**17.6**	mS/m	±10%
Turbiditet	NS-ISO 7027-1		**17.1**	FNU	±15%
SS,suspendert stoff	NS-EN 872		**16**	mg/l	±25
Fargetall filtrert	NS-EN ISO 7887		**23**		±15%
Kalsium, AES	NS-EN ISO7980		**13.1**	mg Ca/l	±10%
Totalt organisk karbon	NS 1484		**5.7**	mg C/l	±20%
Ammonium-nitrogen	Intern/ISO 11732		**0.050**	mg N/l	25
Nitrat, IC	ISO 10304-1		**0.44**	mg N/l	±15%
Orto fosfat	ISO 15681-2 2005		**0.013**	mg P/l	±30%
Totalfosfor	NS-EN-ISO 15681-2:18		**0.059**	mg P/l	±15%
Totalnitrogen	Int/ISO 29441:2010		**0.96**	mg N/l	±20%
Intestinale enterokokker	ISO 7899		**<10**	/100 ml	(0 - 15)
E.coli *)	ISO 9308-1:2014		**2**	/100ml	

21/4098-4	Vannforekomster,overvåkning Blandeprøve			Tatt ut: 01/07/21 014-38222	
Analyse	**Metode**	**Ref**	**Resultat**	**Benevning**	**Usikkerhet**
pH, surhetsgrad	NS-EN ISO 10523	N)	**8.12**		±0.2e
Konduktivitet v/25°C	NS-ISO 7888	L)	**17.6**	mS/m	±10%
Turbiditet	NS-ISO 7027-1		**21.3**	FNU	±15%
SS,suspendert stoff	NS-EN 872		**22**	mg/l	±25
Fargetall filtrert	NS-EN ISO 7887		**21**		±15%
Kalsium, AES	NS-EN ISO7980		**12.9**	mg Ca/l	±10%
Totalt organisk karbon	NS 1484		**5.8**	mg C/l	±20%
Ammonium-nitrogen	Intern/ISO 11732		**0.029**	mg N/l	25
Nitrat, IC	ISO 10304-1		**0.44**	mg N/l	±15%
Orto fosfat	ISO 15681-2 2005		**0.011**	mg P/l	±30%
Totalfosfor	NS-EN-ISO 15681-2:18		**0.074**	mg P/l	±15%
Totalnitrogen	Int/ISO 29441:2010		**1.0**	mg N/l	±20%
Intestinale enterokokker	ISO 7899		**<10**	/100 ml	(0 - 15)
E.coli *)	ISO 9308-1:2014		**2**	/100ml	

*) markerer "Ikke akkreditert analyse".

N) Prøvene er målt ved 24±1 °C. pH >2 og <12 er akkreditert.
L) Målt og korrigert ved romtemperatur

Annex 2: Analysis result of selected water parameters sampled from VestfoldLAB AS sampled 9th of August 2021 (Norwegian)

ANALYSERESULTATER

Analyseperiode: 10/08/21 - 20/08/21, Uttaksprosedyre: Enkel stikkprøve Prøvetaker: Tatt ut av leverandør

21/4867-1	Vannforekomster,overvåkning Akersvannet 1 m	Tatt ut: 09/08/21 014-38222

Analyse	Metode	Ref	Resultat	Benevning	Usikkerhet
pH, surhetsgrad	NS-EN ISO 10523	N)	**8.69**		±0.2e
Konduktivitet v/25°C	NS-ISO 7888	L)	**17.8**	mS/m	±10%
Turbiditet	NS-ISO 7027-1		**15.2**	FNU	±15%
SS,suspendert stoff	NS-EN 872		**8**	mg/l	±35%
Fargetall filtrert	NS-EN ISO 7887		**17**		±15%
Kalsium, AES	NS-EN ISO7980		**13.9**	mg Ca/l	±10%
Totalt organisk karbon	NS 1484		**5.8**	mg C/l	±20%
Ammonium-nitrogen	Intern/ISO 11732		**0,020**	mg N/l	50
Nitrat, IC	ISO 10304-1		**<0.01**	mg N/l	±30%
Orto fosfat	ISO 15681-2 2005		**<0.002**	mg P/l	±30%
Totalfosfor	NS-EN-ISO 15681-2:18		**0.022**	mg P/l	±15%
Totalnitrogen	Int/ISO 29441:2010		**0.45**	mg N/l	±20%
Intestinale enterokokker	ISO 7899		**<10**	/100 ml	(0 - 15)
E.coli *)	ISO 9308-1:2014		**<30**	/100ml	

21/4867-2	Vannforekomster,overvåkning Akersvannet 4 m	Tatt ut: 09/08/21 014-38222

Analyse	Metode	Ref	Resultat	Benevning	Usikkerhet
pH, surhetsgrad	NS-EN ISO 10523	N)	**8.77**		±0.2e
Konduktivitet v/25°C	NS-ISO 7888	L)	**17.8**	mS/m	±10%
Turbiditet	NS-ISO 7027-1		**14.8**	FNU	±15%
SS,suspendert stoff	NS-EN 872		**6**	mg/l	±35%
Fargetall filtrert	NS-EN ISO 7887		**18**		±15%
Kalsium, AES	NS-EN ISO7980		**13.4**	mg Ca/l	±10%
Totalt organisk karbon	NS 1484		**5.7**	mg C/l	±20%
Ammonium-nitrogen	Intern/ISO 11732		**0,017**	mg N/l	50
Nitrat, IC	ISO 10304-1		**<0.01**	mg N/l	±30%
Orto fosfat	ISO 15681-2 2005		**<0.002**	mg P/l	±30%
Totalfosfor	NS-EN-ISO 15681-2:18		**0.017**	mg P/l	±30%
Totalnitrogen	Int/ISO 29441:2010		**0.38**	mg N/l	±20%
Intestinale enterokokker	ISO 7899		**<10**	/100 ml	(0 - 15)
E.coli *)	ISO 9308-1:2014		**<30**	/100ml	

21/4867-3	Vannforekomster,overvåkning Akersvannet 6,8m			Tatt ut: 09/08/21 014-38222	
Analyse	Metode	Ref	Resultat	Benevning	Usikkerhet
pH, surhetsgrad	NS-EN ISO 10523	N)	**8.63**		±0.2e
Konduktivitet v/25°C	NS-ISO 7888	L)	**17.8**	mS/m	±10%
Turbiditet	NS-ISO 7027-1		**15.1**	FNU	±15%
SS,suspendert stoff	NS-EN 872		**6**	mg/l	±35%
Fargetall filtrert	NS-EN ISO 7887		**18**		±15%
Kalsium, AES	NS-EN ISO7980		**14.5**	mg Ca/l	±10%
Totalt organisk karbon	NS 1484		**5.6**	mg C/l	±20%
Ammonium-nitrogen	Intern/ISO 11732		**0,017**	mg N/l	50
Nitrat, IC	ISO 10304-1		**<0.01**	mg N/l	±30%
Orto fosfat	ISO 15681-2 2005		**<0.002**	mg P/l	±30%
Totalfosfor	NS-EN-ISO 15681-2:18		**0.024**	mg P/l	±15%
Totalnitrogen	Int/ISO 29441:2010		**0.46**	mg N/l	±20%
Intestinale enterokokker	ISO 7899		**<10**	/100 ml	(0 - 15)
E.coli *)	ISO 9308-1:2014		**<30**	/100ml	

21/4867-4	Vannforekomster,overvåkning Akersvannet blandeprøve			Tatt ut: 09/08/21 014-38222	
Analyse	Metode	Ref	Resultat	Benevning	Usikkerhet
pH, surhetsgrad	NS-EN ISO 10523	N)	**8.30**		±0.2e
Konduktivitet v/25°C	NS-ISO 7888	L)	**17.9**	mS/m	±10%
Turbiditet	NS-ISO 7027-1		**9.5**	FNU	±15%
SS,suspendert stoff	NS-EN 872		**4**	mg/l	±35%
Fargetall filtrert	NS-EN ISO 7887		**18**		±15%
Kalsium, AES	NS-EN ISO7980		**14.2**	mg Ca/l	±10%
Totalt organisk karbon	NS 1484		**5.7**	mg C/l	±20%
Ammonium-nitrogen	Intern/ISO 11732		**0,014**	mg N/l	50
Nitrat, IC	ISO 10304-1		**<0.01**	mg N/l	±30%
Orto fosfat	ISO 15681-2 2005		**<0.002**	mg P/l	±30%
Totalfosfor	NS-EN-ISO 15681-2:18		**0.026**	mg P/l	±15%
Totalnitrogen	Int/ISO 29441:2010		**0.45**	mg N/l	±20%
Intestinale enterokokker	ISO 7899		**<10**	/100 ml	(0 - 15)
E.coli *)	ISO 9308-1:2014		**<30**	/100ml	

*) markerer "Ikke akkreditert analyse".

L) Målt og korrigert ved romtemperatur
N) Prøvene er målt ved 24±1 °C. pH >2 og <12 er akkreditert.

Annex 3: Analysis result of selected water parameters sampled from VestfoldLAB AS sampled 14th of September 2021 (Norwegian)

ANALYSERESULTATER

Analyseperiode: 15/09/21 - 30/09/21, Uttaksprosedyre: Enkel stikkprøve Prøvetaker: Tatt ut av leverandør

21/5833-1	Vannforekomster,overvåkning Akersvannet blandprøve			Tatt ut: 14/09/21 014-38222	
Analyse	Metode	Ref	Resultat	Benevning	Usikkerhet
pH, surhetsgrad	NS-EN ISO 10523	N)	**7.94**		±0.2e
Konduktivitet v/25°C	NS-ISO 7888	L)	**18.8**	mS/m	±10%
Turbiditet	NS-ISO 7027-1		**6.0**	FNU	±15%
SS,suspendert stoff	NS-EN 872		**<2**	mg/l	±35%
Fargetall filtrert	NS-EN ISO 7887		**21**		±15%
Kalsium, AES	NS-EN ISO7980		**29.2**	mg Ca/l	±10%
Totalt organisk karbon	NS 1484		**6.2**	mg C/l	±20%
Ammonium-nitrogen	Intern/ISO 11732		**0.012**	mg N/l	25
Nitrat, IC	ISO 10304-1		**<0.01**	mg N/l	±30%
Orto fosfat	ISO 15681-2 2005		**0.009**	mg P/l	±30%
Totalfosfor	NS-EN-ISO 15681-2:18		**0.043**	mg P/l	±15%
Totalnitrogen	Int/ISO 29441:2010		**0.45**	mg N/l	±20%
Intestinale enterokokker	ISO 7899		**<10**	/100 ml	(0 - 15)
E.coli *)	ISO 9308-1:2014		**<30**	/100ml	

21/5833-2	Vannforekomster,overvåkning Akersvannet 1 m			Tatt ut: 14/09/21 014-38222	
Analyse	Metode	Ref	Resultat	Benevning	Usikkerhet
pH, surhetsgrad	NS-EN ISO 10523	N)	**8.01**		±0.2e
Konduktivitet v/25°C	NS-ISO 7888	L)	**18.8**	mS/m	±10%
Turbiditet	NS-ISO 7027-1		**6.2**	FNU	±15%
SS,suspendert stoff	NS-EN 872		**5**	mg/l	±35%
Fargetall filtrert	NS-EN ISO 7887		**21**		±15%
Kalsium, AES	NS-EN ISO7980		**30.8**	mg Ca/l	±10%
Totalt organisk karbon	NS 1484		**6.0**	mg C/l	±20%
Ammonium-nitrogen	Intern/ISO 11732		**0.008**	mg N/l	25
Nitrat, IC	ISO 10304-1		**<0.01**	mg N/l	±30%
Orto fosfat	ISO 15681-2 2005		**0.009**	mg P/l	±30%
Totalfosfor	NS-EN-ISO 15681-2:18		**0.045**	mg P/l	±15%
Totalnitrogen	Int/ISO 29441:2010		**0.44**	mg N/l	±20%
Intestinale enterokokker	ISO 7899		**<10**	/100 ml	(0 - 15)
E.coli *)	ISO 9308-1:2014		**<30**	/100ml	

21/5833-3	Vannforekomster,overvåkning Akersvannet 6 m		Tatt ut: 14/09/21 014-38222		
Analyse	Metode	Ref	Resultat	Benevning	Usikkerhet
pH, surhetsgrad	NS-EN ISO 10523	N)	**7.66**		±0.2e
Konduktivitet v/25°C	NS-ISO 7888	L)	**19.0**	mS/m	±10%
Turbiditet	NS-ISO 7027-1		**4.2**	FNU	±15%
SS,suspendert stoff	NS-EN 872		**7**	mg/l	±35%
Fargetall filtrert	NS-EN ISO 7887		**22**		±15%
Kalsium, AES	NS-EN ISO7980		**31.0**	mg Ca/l	±10%
Totalt organisk karbon	NS 1484		**5.9**	mg C/l	±20%
Ammonium-nitrogen	Intern/ISO 11732		**0.089**	mg N/l	25
Nitrat, IC	ISO 10304-1		**<0.01**	mg N/l	±30%
Orto fosfat	ISO 15681-2 2005		**0.021**	mg P/l	±30%
Totalfosfor	NS-EN-ISO 15681-2:18		**0.046**	mg P/l	±15%
Totalnitrogen	Int/ISO 29441:2010		**0.44**	mg N/l	±20%
Intestinale enterokokker	ISO 7899		**<10**	/100 ml	(0 - 15)
E.coli *)	ISO 9308-1:2014		**<30**	/100ml	

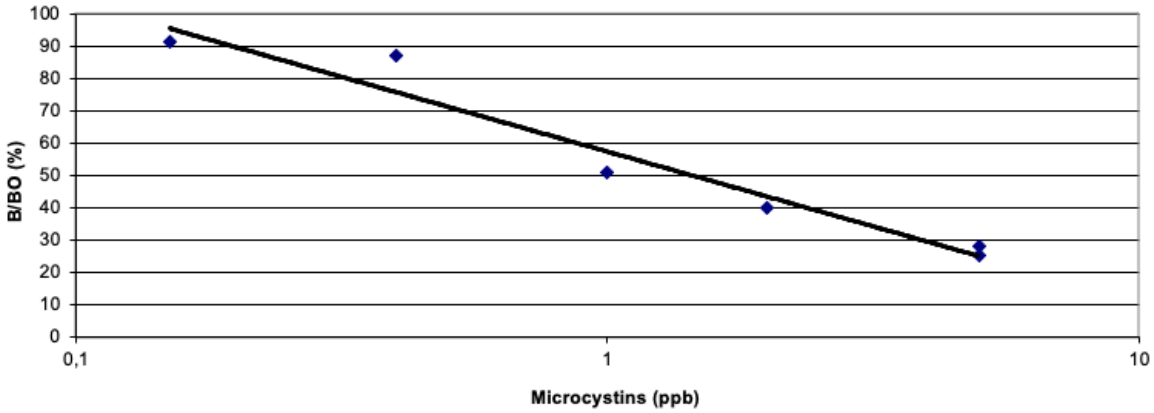
Annex 4: Results of standards from ELISA analysis of microcystin, including absorbance and results in µg/L

Standard	Absorbance	Results (µg/L)
Std 0	0,919	–
Std 0	0,898	–
Std 1	0,83	0,201
Std 2	0,789	0,249
Std 3	0,462	1,374
Std 4	0,362	2,317
Std 5	0,254	4,071
Std 5	0,230	4,615

Annex 5: Results of samples from ELISA analysis of microcystin in net samples from 14th of September, including dilution and results in µg/L

Dilution	Results (µg/L)
1:10	8,86
	8,82
–	11,15
	10,75
1:10	7,75
	7,05
1:20	6,20
	7,01
1:30	6,89
	6,23
1:50	5,09
	4,68

Annex 6: Standard curve generated from microcystin analysis with ELISA method



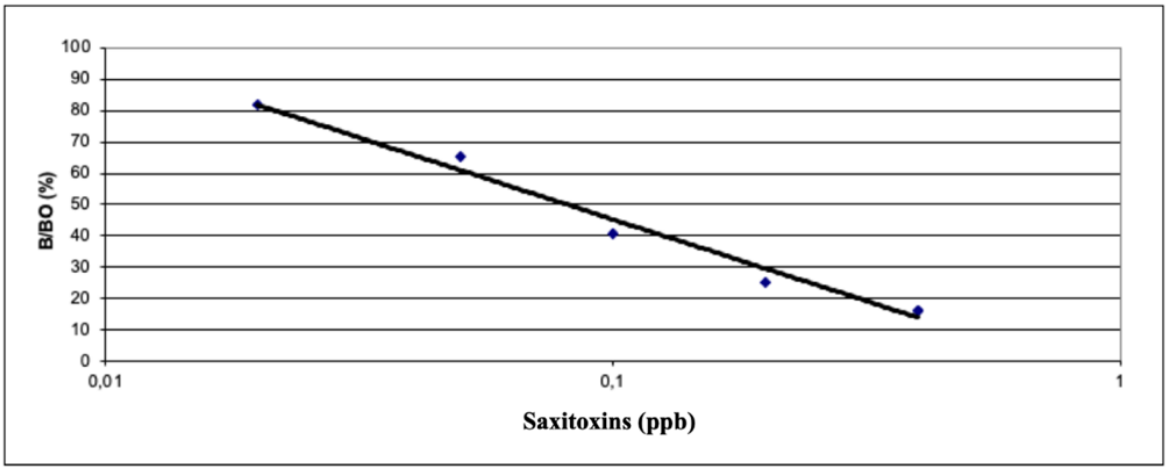
Annex 7: Results of standards from ELISA analysis of saxitoxin, including absorbance and results in µg/L

Sample	Absorbance	Results (µg/L)
Std 0	1,089	–
Std 0	1,09	–
Std 1	0,89	0,021
Std 2	0,713	0,042
Std 3	0,445	0,122
Std 4	0,275	0,239
Std 5	0,175	0,356
Std 5	0,174	0,357

Annex 8: Results of samples from ELISA analysis of saxitoxin in samples from 5th and 14th of September, including dilution and results in µg/L

Date	Sampling type	Dilution	Analysis results (µg/L)
05.09.21	Mixed water	–	0,008
		–	0,008
14.09.21	Mixed water	–	0,007
		–	0,007
14.09.21	Net sample	1:10	0,006
		1:10	0,006
14.09.21	Net sample	–	0,009
		–	0,013

Annex 9: Standard curve generated from saxitoxin analysis with ELISA method



Annex 10: Results from alpha diversity measures, i.e., observed, Chao1 and Shannon for cyanobacterial taxa

Observed			
Date	**Sample**	**Value**	**SE**
01.07.21	Barcode 9	93	*NA*
	Barcode 10	90	*NA*
	Barcode 11	95	*NA*
09.08.21	Barcode 12	184	*NA*
	Barcode 13	203	*NA*
14.09.21	Barcode 14	177	*NA*
	Barcode 15	178	*NA*
Chao1			
Date	**Sample**	**Value**	**SE**
01.07.21	Barcode 9	118,0000	13,013253
	Barcode 10	132,2727	20,690029
	Barcode 11	154,5000	28,183794
09.08.21	Barcode 12	208,6667	10,675785
	Barcode 13	223,6667	9,635145
14.09.21	Barcode 14	194,8846	8,506743
	Barcode 15	211,4762	14,333301
Shannon			
Date	**Sample**	**Value**	**SE**
01.07.21	Barcode 9	2,226365	*NA*
	Barcode 10	2,179357	*NA*
	Barcode 11	2,162284	*NA*
09.08.21	Barcode 12	1,764024	*NA*
	Barcode 13	1,718914	*NA*
14.09.21	Barcode 14	1,788511	*NA*
	Barcode 15	1,865084	*NA*

Annex 11: Tukey multiple comparisons of means of alpha diversity measures,

Table includes the difference (diff) between compared groups, lower (lwr) and upper (upr) of the 95 % confidence interval, and p-value adjusted for multiple comparisons (p adj).

Observed				
Date	**diff**	**lwr**	**upr**	**p adj**
09.08.21-01.07.21	100,8333	78,1950	123,471599	0,0002111
14.09.21-01.07.21	84,83333	62,19507	107,471599	0,0004066
14.09.21-09.08.21	-16,00000	-40,79898	8,798977	0,1668554
Chao1				
Date	**diff**	**lwr**	**upr**	**p adj**
09.08.21-01.07.21	81,24242	31,71893	130,76592	0,0093673
14.09.21-01.07.21	68,25616	18,73266	117,77966	0,0173608
14.09.21-09.08.21	-12,98626	-67,23654	41,26401	0,6941433
Shannon				
Date	**diff**	**lwr**	**upr**	**p adj**
09.08.21-01.07.21	-0,4478661	-0,57545513	-0,3202770	0,0005242
14.09.21-01.07.21	-0,3625382	-0,49012723	-0,2349491	0,0011907
14.09.21-09.08.21	0,0853279	-0,05443891	0,2250947	0,1895378

Annex 12: Results from phytoplankton analysis by light microscope of water samples from L. Akersvannet sampled 1st of July 2021

				01.07.2021	50 ml										
Akersvannet															
Art		antall	ruter	antall/l	l	b	h	d	formel		vol/ind um3	vol/l um3	% av tot vol		
				40x											
Microcystis aeruginosa		316	50	3968960					3,1	3,14xd3/6		16,0	63387728		
Woronichinia compacta		306	50	3843360	5	2,5				3,14xkxd2/6		16,4	62854950		
Snowella lacustris		6	50	75360					3,13	3,14xd3/6		16,0	1203564		
Snowella septentrionalis		248	50	3114880					4,16	3,14xd3/6		37,7	117566194		
Pseudanabaena		16	50	200960	9,38	2,5				3,14xkxd2/4		46,0	9243375		
Aphanizomenon		100	38	1652632	75	3,13				3,14*h*d2/4		575,0	950182463		
Dolichospermum flosaquae/lemmermannii		15	50	188400	6,25	3,13				3,14xkxd2/4		47,9	9026733		
sum cyanobacteria				**13044552**									**1213465007**	**83 %**	
Cryptomonas		4	50	50240	11,72	7,29	5,83			3,14xkxbxh/6		260,8	13101735		
Rhodomonas		21	50	263760	10,29	5,76				3,14xd2/12x(d/2+l)		114,3	30152757		
Chrysophyceae sp. oval		7	50	87914	12,40	7,29				3,14xkxd2/6		344,9	30322682		
Chrysophyceae sp. round		2	50	25118					5,21	3,14xd3/6		73,9	1855006		
Chrysophyceae monade		1	50	12559					6,25	3,14xd3/6		127,8	1604650		
Dinobryon (cyst - chrysophyceae) oval		1	50	12559	9,38	6,25				3,14xkxd2/6		191,7	2406975		
Dinobryon (cyst - chrysophyceae) round		1	50	12559					9,38	3,14xd3/6		431,2	5415693		
Cyclotella sp.		1	50	12560	12,5	6,25				3,14xkxd2/4		383,3	4814258		
Aulacoseira islandica		1	50	12560	62,5	12,5				3,14xkxd2/4		7666,0	96285156		
Chlorophyceae sp. oval		2	50	25120	12,5	6,25				3,14xkxd2/6		255,5	6419010		
Chlorophyceae sp. round		7	50	87920					7,81	3,14xd3/6		249,5	21939977		
Coelastrum sphaericum		12	50	150720					4,16	3,14xd3/6		37,7434	5688687		
Small cell		100	6	10466667					1,25	3,14xd3/6		1,0	10698351		
Medium cell		54	50	678240					3,13	3,14xd3/6		16,0	10832080		
Large cell		9	50	113040					6,25	3,14xd3/6		127,8	14442773		
sum algae				**12011537**									**25597972**	**17 %**	
	total number/L									total vol um3/l			**1469444799**		
	25056089									mm3/l			1,3000		
										mm3/m3			1300,0000		

Annex 13: Results from phytoplankton analysis by light microscope of water samples from L. Akersvannet sampled 9th of August 2021

Akersvannet			09.08.2021	50 ml										
Art	antall	ruter	antall/l	l	b	h	d	formel		vol/ind um3	vol/l um3	% av tot vol		
		40x												
Microcystis aeruginosa	560	50	7033600				3,125	3,14xd3/6		16,0	112332682			
Microcystis wesenbergii	207	50	2599920				6,25	3,14xd3/6		127,8	332183789			
Woronichinia compacta	298	50	3742880	5	2,5			3,14xkxd2/6		16,4	61211683			
Snowella lacustris	286	50	3592160	8,85	3,38			3,14xkxd2/6		53,1	190628409			
Snowella septentrionalis	32	50	401920				3,125	3,14xd3/6		16,0	6419010			
Planktolyngbya	1	50	12560	125	2,5			3,14xkxd2/4		613,3	7702813			
Aphanizomenon	30	50	376800	69,38	3,26			3,14xd2/4		580,0	218527217			
sum cyanobacteria			**17759840**								**929005603**	**28 %**		
Cryptomonas	20	50	251200	28,44	12,97	10,38		3,14xkxbxh/6		2002,4	503008901			
Rhodomonas	36	50	452160	8,76	5,67			3,14xd2/12x(d/2+1)		97,5	44086047			
Chrysophyceae sp. (gullalge) ovale	5	50	62800	21,5	11,38			3,14xkxd2/6		1455,9	91428019			
Chrysophyceae sp. (gullalge) runde	1	50	12560				9,38	3,14xd3/6		431,2	5416040			
Aulacoseira sp. (kiselalge)	6	50	75360	91,67	5,09			3,14xkxd2/4		1865,8	140608957			
Aulacoseira tenella?	6	50	75360	17,5	9,375			3,14xkxd2/4		1207,4	90989473			
Fragilaria sp.	57	50	715920	54,17	4,16	8,33		lxbxh		1877,0	1343801424			
Asterionella formosa (kiselalge)	1	50	12560	62,5	3,64	3,64		lxbxh		829,8	10422235			
Chlorophyceae sp. (grønnalge)	1	50	12560	9,38	6,25			3,14xkxd2/6		191,7	2407129			
Cosmarium depressum	1	50	12560	25	27,5			3,14xkxd2/6		9894,3	124272042			
Staurastrum sp.	1	50	12560	25	12,5		4,163	2(r0r2/12xb3+3x3,1		2500,5	31406786			
Small cell	100	8	7850000				1,25	3,14xd3/6		1,0	8023763			
Medium cell	64	50	803840				3,13	3,14xd3/6		16,0	12838021			
Large cell	12	50	150720				6,25	3,14xd3/6		127,8	1925031			
sum algae			**10500160**								**2427965868**	**72 %**		
	total number/L							total vol um3/l			**335697471**			
		28260000						mm3/l			1,3000			
								mm3/m3			1300,0000			

Annex 14: Results from phytoplankton analysis by light microscope of water samples from L. Akersvannet sampled 14th of September 2021

Akersvannet			14.09.2021	50 ml									
Art	antall	ruter	antall/l	l	b	h	d	formel	vol/ind um3	vol/l um3	% av tot vol		
			40x										
Woronichinia compacta	57	50	715920	5	2,5			3,14xlxd2/6	16,4	11708275			
Snowella lacustris	377	50	4735120	5,73	3,64			3,14xlxd2/6	39,8	188459927			
Snowella septentrionalis	51	50	640560				3,64	3,14xd3/6	25,3	16217501			
Aphanizomenon	37	50	464720	62,67	3,32			3,14xd2/4	542,7	252187876			
Dolichospermum crassum	102	50	1281120				9,38	3,14xd2/6	46,0	58926516			
Dolichospermum lemmermannii	68	50	854080				7,64	3,14xd2/6	30,5	26072303			
sum cyanobacteria			**8691520**							**553572398**	**24 %**		
Cryptomonas	5	50	62800	22,5	10,63	8,5		3,14xlxbxh/6	1063,4	66783384			
Rhodomonas	13	50	163280	10,26	7,52			3,14xd2/12x(d/2+l)	207,2	33825039			
Chrysophyceae sp. (gullalge) ovale	9	50	113040	10,55	6,46			3,14xlxd2/6	230,1	26007285			
Chrysophyceae sp. (gullalge) runde	6	50	75360				12,67	3,14xd3/6	1065,1	80269217			
Fragilaria sp.	21	50	263760	47,5	4,1625	8,33		lxbxh	1646,0	434151227			
Chlorophyceae sp. (grønnalge) ovale	2	50	25120	6,25	4,1625			3,14xlxd2/6	56,7	1423595			
Chlorophyceae sp. (grønnalge) runde	57	50	715920				10,98	3,14xd3/6	693,5	496518286			
Oocystis sp.	4	50	50240	10	6,25			3,14xlxd2/6	204,4	10270417			
Korschikoviella limnetia	1	50	12560	25	2,1			3,14xd2xh/12	28,7	360041			
Cosmarium depressum	4	50	50240	30,83	26,67			3,14xlxd2/6	11474,6	576482300			
Staurastrum	1	50	12560	25	12,5		4,16	2(r*d2/12xb3+3x3,1·	2500,5	31406786			
Small cell	100	5	12560000				1,25	3,14xd3/6	1,0	12838021			
Medium cell	52	50	653120				3,13	3,14xd3/6	16,0	10430892			
Large cell	7	50	87920				6,25	3,14xd3/6	127,8	11233268			
sum algae			**14845920**							**1791999758**	**76 %**		
sum totalt			23537440					total vol um3/l		**2345572156**			
								mm3/l		1,3000			
								mm3/m3		1300,0000			