University of South-Eastern Norway
Faculty of Technology, Natural Sciences and Maritime Sciences
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Master's Thesis
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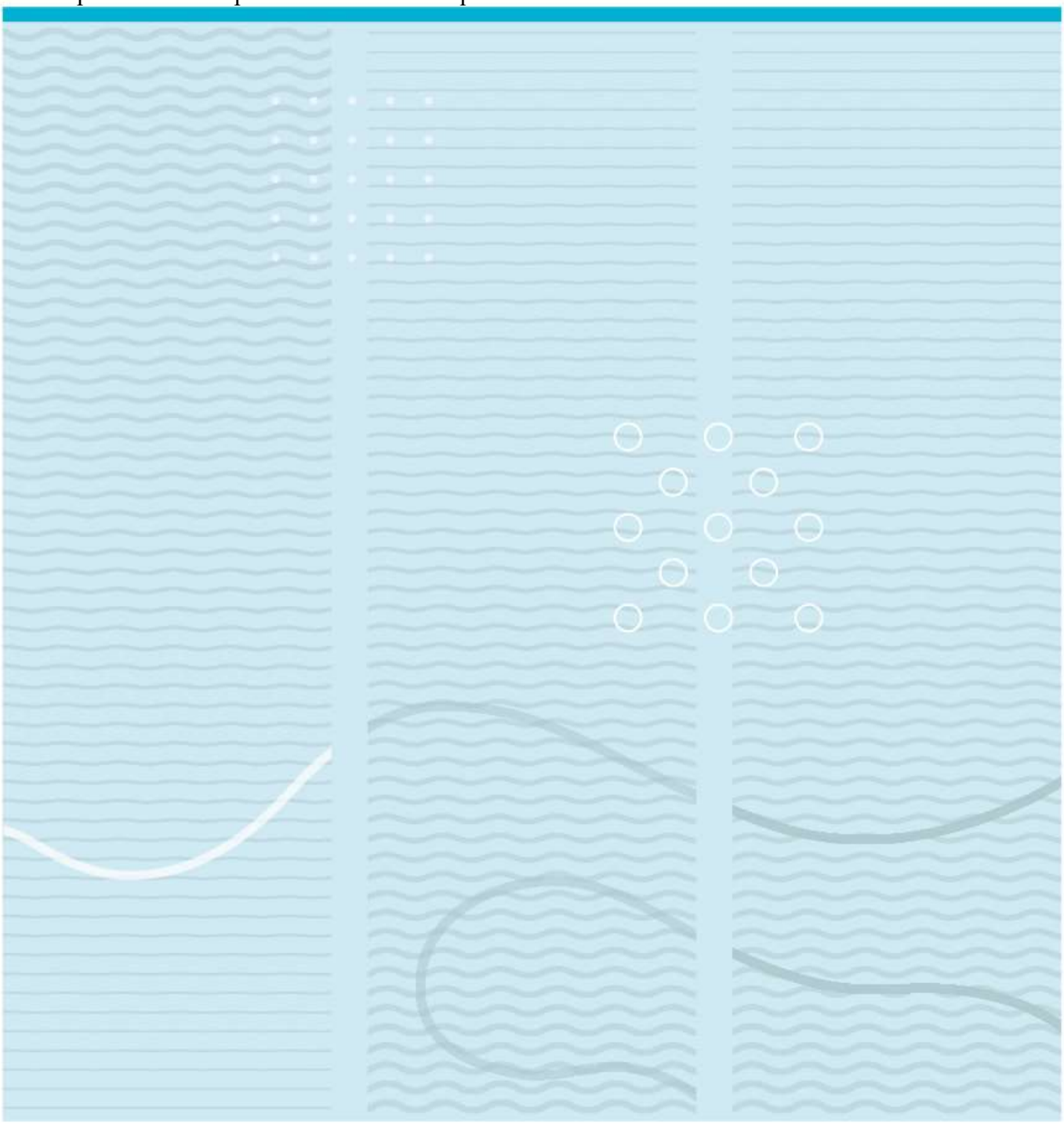
Blooms of cyanobacteria and toxin production in Lake Vikevannet, Lake Haugestadvannet and Lake Hillestadvannet 2019

Occurrence of cyanobacteria in three lakes in Holmestrand municipality, South-East Norway 2019 – Toxin production and potential health consequences.



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This thesis is worth 60 study points

Summary

The occurrence of cyanobacteria in water used for drinking and recreational purposes is a widespread problem around the world. Cyanobacteria have the ability to produce a variety of toxins and this has led to a greater focus on the effects concerning human health. This thesis examines the importance of environmental factors for the occurrence of various cyanobacterial taxa and the production of microcystins and saxitoxin in Lake Vikevannet, Lake Haugestadvannet and Lake Hillestadvannet in Holmestrand municipality in South-East Norway. Potential health risks related to cyanotoxins are also assessed. These lakes are mainly influenced by run-off from agricultural areas and wastewater from dispersed households. The water samples were taken once a month from June to September 2019 (26th June, 29th July, 27th August and 24th September). The water samples were analysed for physical, chemical and biological parameters and the methods were conducted in accordance with the Norwegian standards. The toxins were analysed using a commercial enzyme linked immunosorbent assay (ELISA) technique. Microcystins were detected in all the samples, where the highest concentration was measured in L. Hillestadvannet in June (20,7 µg/L). This exceeds the guideline value for microcystins in recreational waters (10 µg/L) set by the World Health Organization (WHO). According to a risk assesment based on WHO guidelines for microcystins in recreational waters, L. Hillestadvannet shows high risk for adverse health effects in June, while moderate risk in July and August. L. Vikevannet shows mainly low risk, however little is known about the effect of lower concentrations over a long period of time. Saxitoxin concentrations were highest in July, where the highest concentration was measured in L. Hillestadvannet (0,12 µg/L), however there are no guideline values for saxitoxin yet. Chlorophyll a gives an estimate of the phytoplankton biomass in the lakes, and L. Hillestadvannet has the highest average concentration (37,6 µg chl a/L) compared to both L. Vikevannet (16,1 µg chl a/L) and L. Haugestadvannet (24,2 µg chl a/L). This may explain the high toxin concentrations in L. Hillestadvannet. The physical/chemical results indicate good growth conditions for cyanobacteria as they were above the optimum value for cyanobacterial growth, thus leading to dominance of cyanobacteria, especially *Microcystis* sp., *Dolichospermum* sp., and *Aphanizomenon* sp. which were the main species identified in the lakes. L. Hillestadvannet showed N-limitation from June to August, which could lead to a dominance of N_2-fixating cyanobacteria, however *Microcystis* sp. were the most abundant species independent of the total nitrogen and total phosphorus ratio. Considering the results

and studies reporting annual recurring toxin concentrations, it is not justifiable to use the lakes for recreational purposes as there is a possibility of health-related consequences.

Sammendrag

Forekomsten av cyanobakterier i vann brukt til drikke- og rekreasjonsformål er et utbredt problem rundt om i verden. Cyanobakterier har evnen til å produsere en rekke giftstoffer, og dette har ført til et større fokus på effektene som berører menneskers helse. Denne avhandlingen undersøker viktigheten av miljøfaktorer for forekomst av forskjellige cyanobakterielle taxa og produksjonen av mikrocystiner og saxitoksin i Vikevannet, Haugestadvannet og Hillestadvannet i Holmestrand kommune. Og vurdere den potensielle helserisikoen knyttet til cyanotoksiner. Disse innsjøene er hovedsakelig påvirket av avrenning fra jordbruksarealer og avløpsvann fra spredte husholdninger. Vannprøvene ble tatt en gang i måneden fra juni til september 2019 (26 juni, 29 juli, 27 august og 24 september). Vannprøvene ble analysert for fysiske, kjemiske og biologiske parametere, og metodene ble utført i samsvar med de norske standardene. Toksinanalysene ble analysert ved bruk av en kommersiell enzymbundet immunosorbentanalyse (ELISA) teknikk. Mikrocystiner ble påvist i alle prøvene, der den høyeste konsentrasjonen ble målt i Hillestadvannet i juni (20,7 µg/L). Denne verdien overskrider retningslinjeverdien for mikrocystiner i rekreasjonsvann (10 µg/L) satt av Verdens helseorganisasjon (WHO). Ifølge en risikovurdering basert på WHOs retningslinjer for mikrocystiner i rekreasjonsvann, viser Hillestadvannet høy risiko for uheldige helseeffekter i juni, mens moderat risiko i juli og august. Vikevannet viser hovedsakelig lav risiko, men det er lite kjent om effekten av lavere konsentrasjoner over lengere tid. Saxitoksinkonsentrasjoner var høyest i juli, hvor den høyeste konsentrasjonen ble målt i Hillestadvannet (0,12 µg/L), men det er ikke satt noe retningslinjeverdi på saxitoksin ennå. Klorofyll a gir et estimat av algebiomassen i innsjøene, og Hillestadvannet (37,6 µg kl a/L) har den høyeste gjennomsnitt konsentrasjonen av klorofyll a sammenlignet med Vikevannet (16,1 µg kl a/L) og Haugestadvannet (24,2 µg kl a/L). Dette kan forklare de høye giftstoffkonsentrasjonene i Hillestadvannet. De fysiske/kjemiske resultatene indikerte gode vekstbetingelser for cyanobakterier, da de var over den optimale verdien for cyanobakteriell vekst. Dette førte dermed til en dominans av cyanobakterier, spesielt *Microcystis* sp., *Dolichospermum* sp., og *Aphanizomenon* sp. som var de viktigste artene som ble indentifisert i innsjøene. Hillestadvannet viste N-begrensning fra juni til august, noe som kan føre til en dominans av N_2-fikserende cyanobakterier, men *Microcystis* sp. var den mest tallrike arten

uavhengig av forholdet mellom nitrogen og fosfor. Tatt i betraktning av resultatene og tidligere studier som rapporterer årlig tilbakevennende toksinkonsentrasjoner, er det ikke forsvarlig å bruke innsjøene til rekreasjonsbruk, da det mest sannsynlig vil få helserelaterte følger.

Foreword

I would like to pay my special regards to my supervisor, Synne Kleiven, who has been there for me through the entire master process and guided me when the road got tough. I really appreciate the enormous interest she has shown me towards my thesis, and I could not have accomplished this without her help. I would also like to express my gratitude to Karin Brekke Li who helped me with the water analyses at the laboratory. Thanks to the University of South-Eastern Norway for lending me equipment and access to their laboratories. I wish to acknowledge the tremendous support of my family and friends. They have been motivating me throughout this process.

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Contents

1 Introduction

The primary water quality issue in the world is eutrophication (over-enrichment of nutrients) (Smith and Schindler 2009). Eutrophication often leads to challenges regarding certain phytoplankton species, which can dominate when conditions are right and possibly lead to cyanobacterial blooms (figure 1.1 and 1.2). It is predicted that climate change may cause increased frequencies of cyanobacterial blooms as cyanobacteria have an advantage as they are very competitive at elevated temperatures (Lürling et al. 2018). A cyanobacterial bloom is known as the biomagnification of cyanobacteria. This formation can be seen at the lake surface as scum and can be a threat to human health due to the impact on the quality of freshwaters used for both drinking and recreational purposes, as many cyanobacterial taxa are toxin producers (Cheung et al. 2013). Globally, the occurrence and intensity of such blooms are increasing (Paerl et al. 2011). Their living conditions are good as there has been an increase in the availability of nutrients caused by eutrophication, agricultural and industrial activities, as well as climate change (Edwards 1998; Lürling et al. 2018). There is, however, not only one factor that defines the development of such blooms, but a complex series of interactions between several environmental factors. Temperature is known to be one of the main environmental factor affecting the growth and bloom development of cyanobacteria and is expected to change with changes in the climate (Wells et al. 2015).

Figure 1.1: Cyanobacterial bloom in L. Hillestadvannet, June 2019 (private).

Figure 1.2: Cyanobacterial bloom in L. Hillestadvannet, July 2019 (private).

Trophic status is a way of characterizing lakes based on how well they have access to nutrients such as nitrogen and phosphorus and the production of plant material (Hongve 2018). There are three main trophic statuses; oligotrophic, mesotrophic and eutrophic. Oligotrophic status represents low production of plant material due to low concentrations of nutrients. Mesotrophic is intermediate between oligotrophic and eutrophic when it comes to production and content of nutrients. In contrast to oligotrophic, eutrophic conditions represents high production of both plant material and content of nitrogen and phosphorus (Hongve 2018). The trophic status can change due to changes in the supply of nutrients. The trophic status and the corresponding chlorophyll a concentration (µg chl a/L), phosphorous (µg/L) and nitrogen concentrations (µg/L) are shown in table 1.1.

Table 1.1: Trophic status and the corresponding concentrations of chlorophyll a (µg chl a/L), phosphorous (µg/L) and nitrogen (µg/L) in water bodies (Kalff 2002; Tundisi and Tundisi 2011).*

Trophic status	**P (µg/L)**	**N (µg/L)**	**Chlorophyll a* (µg chl a/L)**
Oligotrophic	<10	<350	0-4
Mesotrophic	10-30	350-650	4-10
Eutrophic	30-100	650-1200	10-100

1.1 Cyanobacteria

Cyanobacteria, formerly referred to as blue-green algae, are gram-negative prokaryotes that perform oxygenic photosynthesis. They are related to bacteria as their cell structure is similar. They do not have a nucleus or membrane-bound organelles (Hoiczyk and Hansel 2000; Percival et al. 2014). The gram-negative cyanobacterial cell structure consists of a cell membrane and an outer membrane with a peptidoglykan membrane located in between these two membranes. The membranes are surrounded by a mucoid sheath (Percival et al. 2014) (figure 1.3). Cyanobacteria contain several pigments such as chlorophyll a, phycocyanin and phycoerythrin which they use to capture light most efficiently at low light intensities (Mur et al. 1999). These pigments are situated in the thylakoids (Mur et al. 1999). Morphologically, cyanobacteria are composed of three main types; unicellular (e.g. *Chroococcales*), colonial (e.g. *Microcystis*) and multicellular filamentous forms. The filamentous forms are split into those with specialized cells capable of nitrogen fixation (e.g. *Dolichospermum*) and those

without (e.g. *Oscillatoria*) (Mur et al. 1999; Manisha 2016). Heterocysts are specialized cells which are capable of nitrogen fixation during N-limitation, using an enzyme called Nitrogenase. This enzyme converts nitrogen gas into ammonium which is included in amino acids and proteins. Another type of specialized cell is akinetes (resting cells) which contain reserve materials which allows cyanobacteria (e.g. *Dolichospermum*) to grow under unfavorable conditions (Mur et al. 1999; Kumar et al. 2010).

Cyanobacteria are classified within the kingdom Prokaryota, division Eubacteria, class Cyanobacteria (Percival et al. 2014). There are approximately 150 genera, with over 1500 species described, of which 40 species produce toxins (Norwegian Institute of Public Health 2010). They are known to be one of the oldest fossils on earth, dating back to 3500 million years ago. Due to their long evolutionary history, they can be found in many habitats including freshwater, marine ecosystems and terrestrial habitats. They can even be found in extreme conditions such as hot springs and frozen lakes (Percival et al. 2014; Henn 2019). Many cyanobacteria possess gas vesicles which enables them to regulate buoyancy and move through the water column seeking optimal light and nutrient conditions (World Health Organization 2003; Edwin et al. 2005). Such buoyant cells may accumulate at the surface where the wind may drive them towards the shoreline where they can form scums which can be very toxic to both humans and animals (World Health Organization 2003). Different species possess different characteristics and thus favor different environmental conditions (Edwin et al. 2005). The most favorable conditions in which cyanobacteria tend to dominate are high temperature and high light intensity combined with increased pH. This occurs during summer, however key nutrients such as nitrogen and phosphorus also play a crucial role in determining whether cyanobacteria become dominant or not (Dignum et al. 2005).

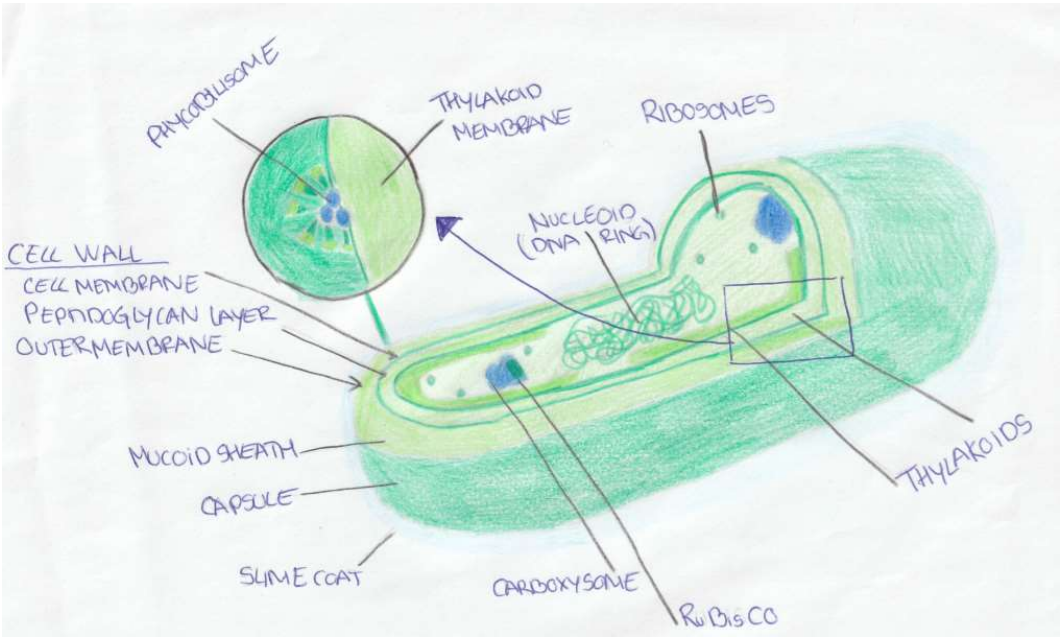

Figure 1.3: Cyanobacterial cell structure. Inspired by "Cyanobacteria: Definition and Examples" (2019).

1.2 Cyanotoxins

Cyanotoxins are produced by many different cyanobacteria genera; *Microcystis*, *Cylindrospermopsis*, *Dolichospermum* and *Aphanizomenon*. There are four main types of cyanotoxins: hepatotoxins (microcystins), neurotoxins (anatoxin-a, anatoxin-a (s) and saxitoxin), lipopolysaccharide endotoxins and dermatotoxins (Norwegian Institute of Public Health 2010). The most common and widespread type of cyanotoxins are hepatotoxins and neurotoxins (World Health Organization 2003). Two of the toxins being analysed in this thesis is microcystins and saxitoxin. Microcystins are potent hepatotoxins that form an irreversible covalent bond which inhibits the protein phosphatases. Saxitoxin is an toxic alkaloid which can disrupt the signalling between neurones in the nervous system causing paralysis and death by respiratory arrest (World Health Organization 2003; Gjølme et al. 2010). Some species can produce many different cyanotoxins; *Dolichospermum* species (e.g. *D. circinale*) are able to produce all the different cyanotoxins; BMAA, microcystins, cylindrospermopsin, anatoxin-a, anatoxin-a (s) and saxitoxin. Another extremely toxic species is *Aphanizomenon flos-aquae*, which produce all the cyanotoxins except anatoxin-a (s) (Berg and Sutula 2015).

1.2.1 Microcystins

Microcystins are cyclic heptapeptides, and as the name implies, they contain seven amino acids (figure 1.4). Microcystins are named after the various amino acids present on the peptide structure (Butler et al. 2009). There are various types of microcystins, however in this thesis, microcystin-LR is the type being focused on. Microcystin-LR is named for the leucine and arginine amino-acids and is among the most commonly studied cyanotoxins. Microcystins are primarily produced by cyanobacteria genera *Microcystis*, however *Dolichospermum* and *Planktothrix agardhii* are also potential microcystin producers (Sivonen and Jones 1999). When the cyanobacterial cells die, the toxin is released into the water. Microcystins are very resistant towards several chemical breakdowns such as hydrolysis or oxidation, thus extremely stable in the water (Butler et al. 2009). Microcystins are hepatotoxins (liver toxins). This toxin mainly attacks the liver; however, it can also be a skin, eye and throat irritant. Exposure to humans and animals occurs most frequently through dermal contact, drinking or during recreational activities in which the water is accidentally swallowed (Falconer et al. 1999). These toxins inhibit liver function and are inhibitors of the protein phosphatases and may therefore act as tumor promoters (Whitton and Potts 2000). To protect consumers from negative health effects by microcystins, the World Health Organization (WHO) has proposed guideline values for microcystin-LR of 1,0 µg/L in drinking water and 10 µg/L in waters used for recreational purposes (World Health Organization 2003). Microcystins occurs most frequently when there are adequate levels of both phosphorus and nitrogen in the water, temperatures from 15-30°C and pH within the range of 6 to 9 (Whitton and Potts 2000).

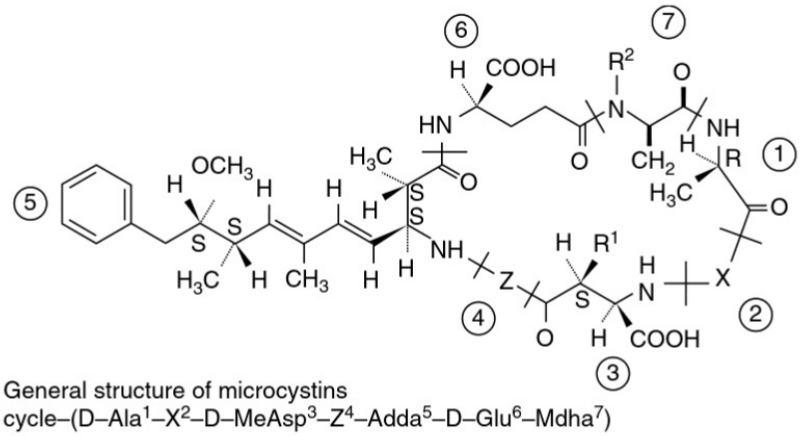

Figure 1.4: General structure of microcystins (Tundisi and Tundisi 2011).

1.2.2 Saxitoxin

Saxitoxin is a neurotoxic alkaloid (figure 1.5) produced by dinoflagellates in the marine ecosystem and cyanobacteria in freshwater environments. Cyanobacteria genera which could produce this kind of toxin are *Dolichospermum*, *Cylindrospermopsis*, *Aphanizomenon, Planktothrix* and *Lyngbya* (Wiese et al. 2010). This toxin is best known as paralytic shellfish toxin (PST) and is acutely toxic. Saxitoxin and anatoxin-a (s) are known to be among the most toxic substances known (World Health Organization 2003). Exposure to saxitoxin is likely to happen through consumption of shellfish contaminated with this toxin, drinking water or during recreational activities in waters infested with cyanobacteria. However reports of illness caused by saxitoxin is scarce (World Health Organization 2019). The biological mechanism of saxitoxin is to block the sodium channels to inhibit the sodium entry through the cell membrane. In contrast to microcystins, the blocking mechanism is reversible (Mur et al. 1999).

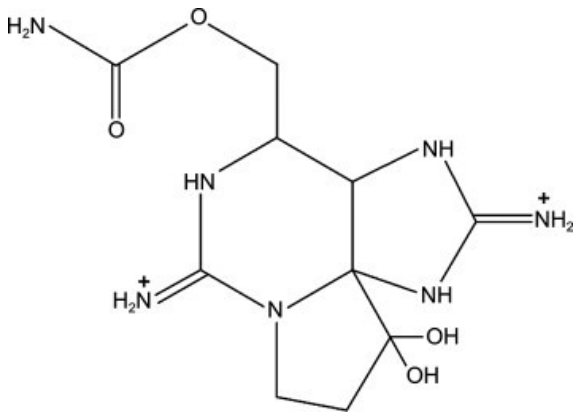

Figure 1.5: Structure of saxitoxin (Solter and Beasley 2013).

2 Main goal and issues

The main goal for this master thesis is to examine the importance of environmental factors for the occurrence of various cyanobacterial taxa and the production of microcystins and saxitoxin in L. Vikevannet, L Haugestadvannet and L. Hillestadvannet in Holmestrand municipality. Potential health risks concerning cyanotoxins are also assessed as the occurrence of cyanobacterial blooms in the lakes is an increasing problem. Based on previous studies reporting annual blooms, cyanobacterial blooms are expected in the three investigated lakes during the sampling period in 2019. The blooms of cyanobacteria will be responsible for the production of both microcystins and saxitoxin.

3 Methods

3.1 Study site

Water samples were taken in the period from June to September 2019 from three adjoining lakes (figure 3.1) in Holmestrand municipality, Vestfold county, South-East Norway. The lakes and their coordinates are Lake Vikevannet (59,542699˚ N, 10,111020˚ E), Lake Haugestadvannet (59,529055˚ N, 10,118125˚ E) and Lake Hillestadvannet (59,516476˚ N, 10,155210˚ E). The sampling was carried out monthly (26th June, 29th July, 27th August and 24th September). Each sample was taken at the shoreline at the exact same position each time, however location 8 was changed due to incorrect measurements on the first sampling date. This was due to difficulties in performing the water sampling and to a river coming down into the water which interfered with the results. The 'Veileder 02:2018' was used to characterize water type for the lakes studied. According to Veileder 02:2018, the water type for L. Vikevannet, L. Haugestadvannet and L. Hillestadvannet is moderately calcareous and humic. The national water type number is L108 (Direktoratsgruppen vanndirektivet 2018).

Figure 3.1: The geographical position of L. Vikevannet, L. Haugestadvannet and L. Hillestadvannet in Holmestrand municipality (Norges vassdrags- og energidirektorat 2019). Red circles with numbers show sampling stations.

3.1.1 Geology and watershed description

The area consists mainly of sea deposits as most of the area is situated below the marine boundary (blue line) (figure 3.2). It is also composed of weathering material and marine deposits which accordingly can explain the moderately calcareous conditions of the lakes. The watershed area of L. Vikevannet (figure 3.3 a) is 132 km² and the inflow is 600 mm/year. The watershed area for L. Haugestadvannet (figure 3.3 b) is 125 km² and the inflow is almost the same as for L. Vikevannet; 606 mm/year. The watershed area for L. Hillestadvannet (figure 3.3 c) is 48 km² and has an inflow of 525 mm/year (NEVINA 2019). Most of the watersheds consists of forest (average 68%) and agricultural land (average 16%) which can explain why these lakes are eutrophicated.

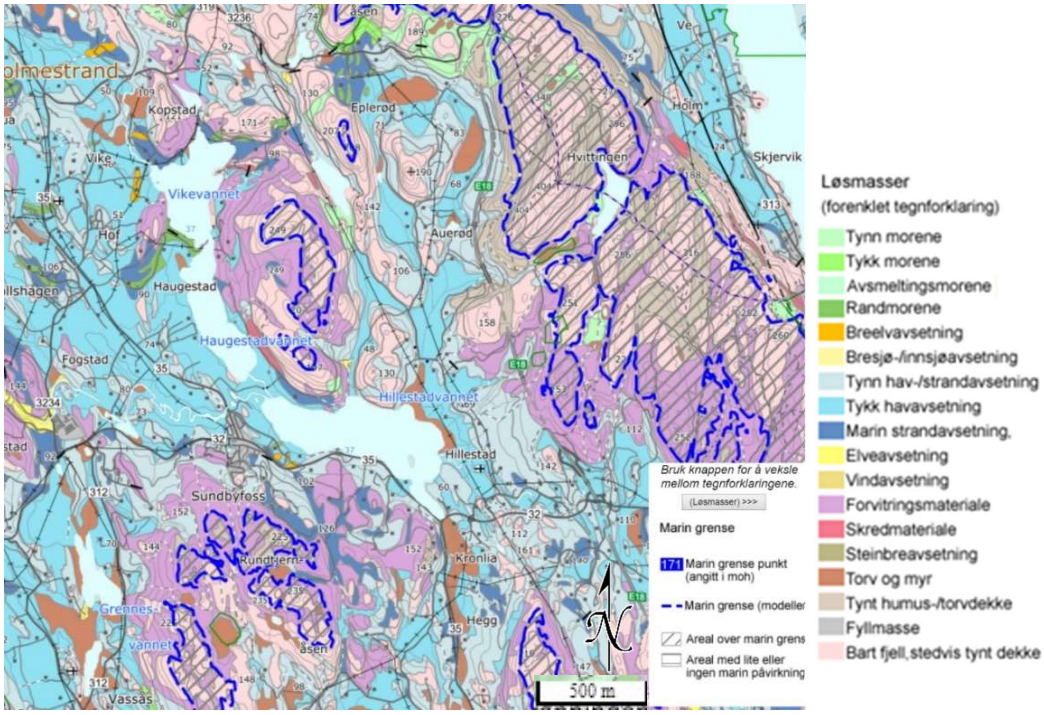

Figure 3.2: Surficial deposits around L. Vikevannet, L. Haugestadvannet and L. Hillestadvannet. Shaded area represents areas above the marine boundary (Norges geologiske undersøkelse 2019).

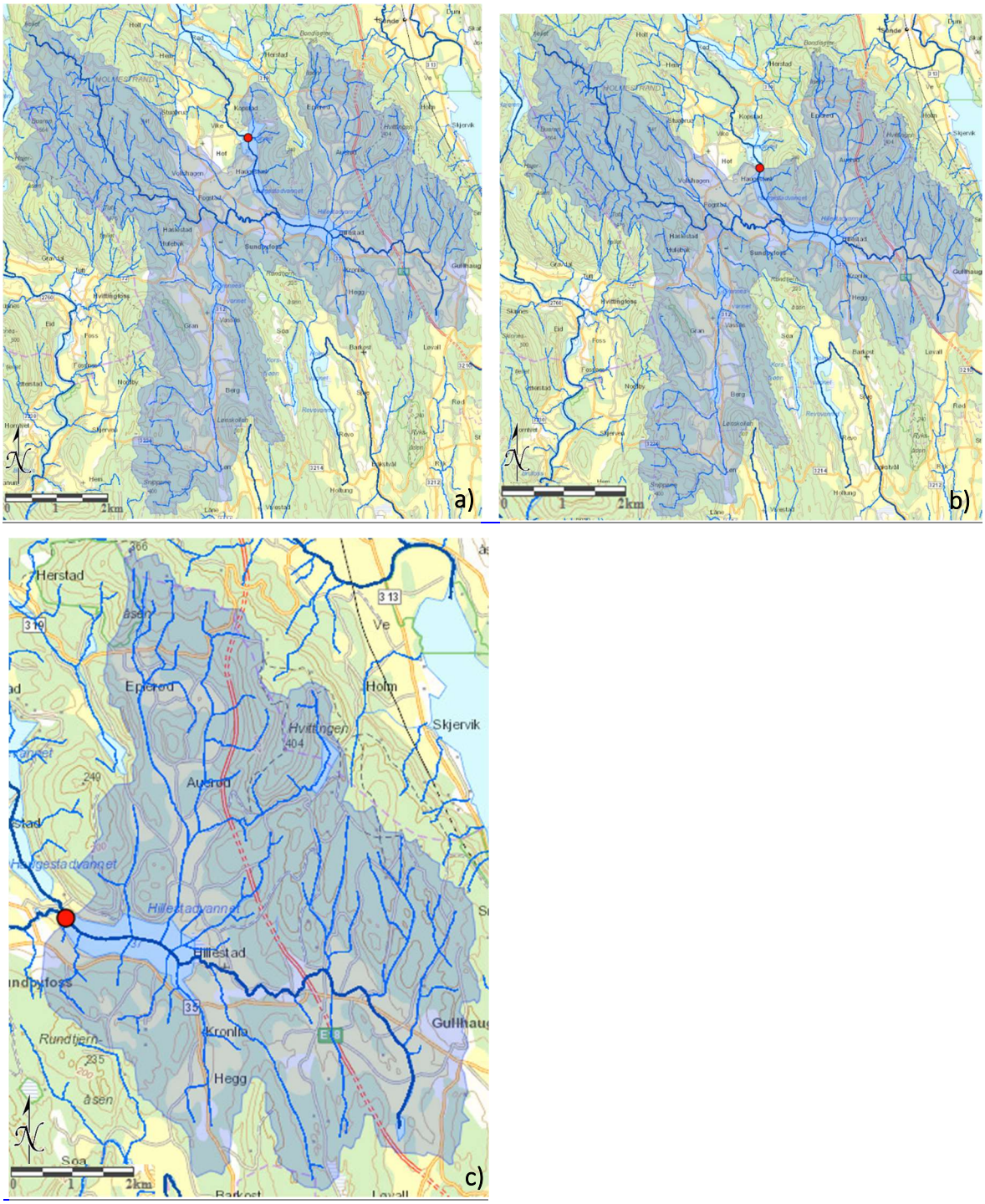

Figure 3.3: The watershed area of L. Vikevannet (a), L. Haugestadvannet (b) and L. Hillestadvannet (c). The inflow values are mean values for the period 1961-1990 (NEVINA 2019).

3.1.2 Morphology of the lakes

Morphological parameters and ecological status of each lake are presented in table 3.1 and bathymetric map of each lake is presented in figure 3.4.

Table 3.1: Morphological parameters and ecological status of L. Vikevannet, L. Haugestadvannet and L. Hillestadvannet. Data from "VannNett" 2019 and Berge 1990.*

Lake	*Vikevannet*	*Haugestadvannet*	*Hillestadvannet*
Lake area km²	0,793	1,565	1,568
Max depth (m)	9,1	2.2	3
Elevation above sea level (m)	37	37	37
*Retention Time (year)**	0,031	0,0091	0,033
Calcium (mg/L)	Moderately calcareous Ca > 4-20	Moderately calcareous Ca > 4-20	Moderately calcareous Ca > 4-20
Watercolor (mg Pt/L)	Humic 30-90	Humic 30-90	Humic 30-90
Turbidity	Clear	Clay influenced	Clay influenced
Ecological status	Poor	Very bad	Very bad

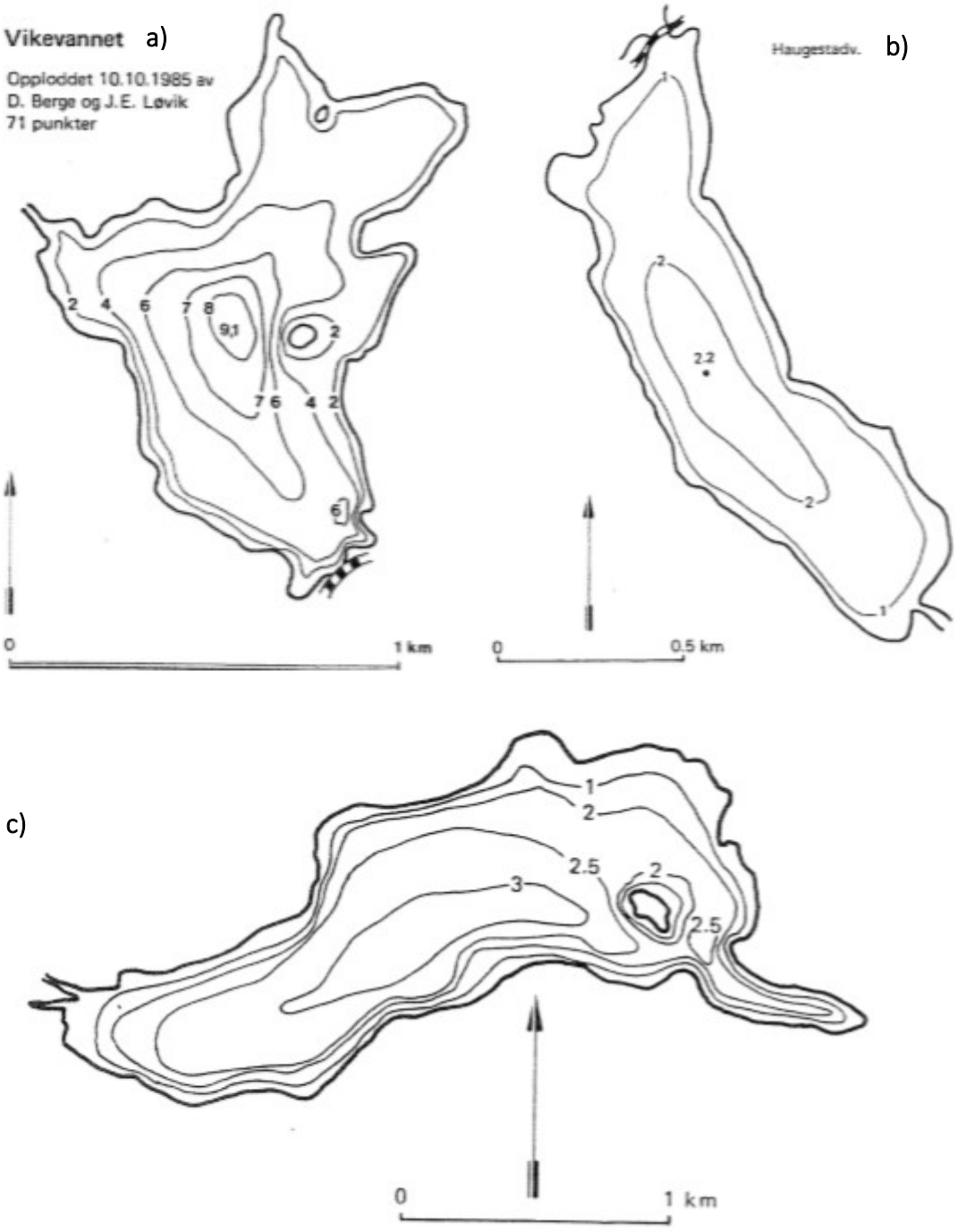

Figure 3.4: Bathymetric map of L. Vikevannet (a), L. Haugestadvannet (b) and L. Hillestadvannet (c) (Berge 1990).

3.1.3 Pollution/main impacts

The main impacts on the lakes are mostly anthropogenic with diffuse run-off from agricultural areas and wastewater from dispersed households as some are not connected to the municipal sewage system ("VannNett" 2019). The road Fv 35 is adjacent to L. Hillestadvannet and E18 has discharges from treatment ponds which cause pollution from transport and infrastructure. The lakes are primarily used for recreational purposes such as swimming and kayaking, there is also a lot of hiking terrain around the lakes (Birgit Vildalen 2019 pers.com). Arriving at L. Vikevannet and L. Hillestadvannet, there are warning signs which discourage swimming and any other contact with the water during cyanobacterial blooms.

3.2 Sampling and storage of the water samples

When conducting the water sampling, a 1 L plastic bottle was filled with lake water to analyse for chlorophyll a. Another 1 L plastic bottle was filled to analyse for turbidity, watercolor, pH, alkalinity, total phosphorus (tot-P) and total nitrogen (tot-N). For microcystins and saxitoxin samples, 15ml plastic centrifuge tubes were filled with surface water up to 5 ml to avoid cracks when freezing the samples. 100 ml glass bottles were filled with surface water to analyse for phytoplankton. All the phytoplankton samples were fixed with Lugol solution to preserve the phytoplankton community. The temperature was measured in situ using a thermometer. Table 3.2 shows type of containers and how the samples were stored and processed before being analysed in the laboratory. The approximate storage time for watercolor, tot-P, tot-N, chlorophyll a and phytoplankton samples were two months.

Table 3.2: Overview over parameters, containers and storage of samples before analyses.

Parameters	*Containers*	*Storage*
pH, turbidity, watercolor, and alkalinity	1 L plastic bottle	Stored at 4 °C until further procedures.
Tot-N and tot-P	100 ml glass bottles	Preserved with 1 mL of 4M sulfuric acid (H_2SO_4) and stored at 4 °C.
Microcystin/saxitoxin	15 ml plastic tube	Stored in the freezer
Chlorophyll a	1 L plastic bottle	Filtrated with GF/C 47mm filter. The filter is then covered with aluminium foil and stored in the freezer.
Phytoplankton	100 ml glass bottle	Fixed with Lugol solution and stored at 4 °C in darkness until further analysis.

3.3 Analytical methods

3.3.1 Water quality analyses

The laboratory analysis was performed at the University of South-Eastern Norway (USN), Bø campus. The pH and alkalinity were analysed within 24 hours after the sampling. Chlorophyll a was filtered within the same timeframe. The water quality analyses were conducted in accordance with the Norwegian standards. The water quality parameters with its assigned Norwegian standards and instruments used in the analysis are shown in table 3.3.

Table 3.3: Physical/chemical parameters, Norwegian standards and instruments used for water quality analyses.

Parameters	*Standard*	*Instruments*	*Note*
pH	NS-EN ISO 10523	*Mettler Toledo SevenCompact™ pH meter S210*	
Turbidity	NS-EN ISO 7027-1	*Merck TurbiQuant 1100 IR.*	
Watercolor	NS-EN ISO 7887	*Lambda 25 spectrophotometer (410nm)*	Filtered with 0,47mm cellulose nitrate filter.
Alkalinity	NS-EN ISO 9963	*Mettler Toledo G20S Compact titrator with electrode glass-D5 115 and 0,0100 M HCL.*	The value is corrected when below 0,7 by this formula: ALK (corrected) = ALK (measured) – (0,0316-[H_3O^+]).
Nitrogen (tot-N)	NS-EN 4743	Flow injector analyser (FIAlab®)	10 ml of the sample was pipetted out and an oxidation solution was added (10g $K_2S_2O_8$ + 16g NaOH). Samples were autoclaved at 121 ˚C
Phosphorous (tot-P)	NS-EN 6878	*Lambda 25 spectrophotometer with cuvette length of 1 cm and wavelength of 880nm.*	15ml of the sample was pipetted out and kaliumperoxodisulphate ($K_2S_2O_2$) added before autoclaving at 121 ˚C. Ammonium molybdate and ascorbic acid was added before analysing.
Chlorophyll a	NS 4766	*Lambda 25 spectrophotometer, cuvette length of 5 cm and wavelength of 650 and 750nm.*	Spectrophotometric determination in acetone extract

3.3.2 Cyanobacterial toxins analysis

To analyse for cyanobacterial toxins, a commercial enzyme linked immunosorbent assay (ELISA) technique was performed. This is an analytical biochemistry technique (Edvotek Inc 2015) which is based on the presence of antibodies and antigens to detect toxins. The amount of toxins present is inversely proportional to colour developing in the wells. The colour is then measured at 450 nm using an ELISA plate reader. The analysis was done according to the method descriptions in the Abraxis-kits.

To test for microcystins, an indirect competitive *Microcystins/Nodularins (ADDA) ELISA kit (*Product No. 520011) and a direct competitive *Saxitoxin ELISA kit* (Product No. 52255B) were used. Both kits are from Abraxis, Warminster. The samples were thawed and frozen two times to break the cell walls to allow the toxins to enter the water solution before conducting the analysis. The results were read using an *ELISA Accu Reader* and a program called *M965 Grabber*, the results were then transferred to excel. One example of calculating the concentrations from a standard excel sheet available at the laboratory is shown in appendix 1. Since the standards of saxitoxin differs from microcystin, these were manually plotted in the excel sheet to get the correct results. For microcystin, a value below 0,15 µg/L is negative and samples with values above the highest standard (5 µg/L) must be diluted and analysed again. In this project, some of the microcystin samples were diluted 1:10. Positive saxitoxin results have values higher than 0,02 µg/L.

3.3.3 Phytoplankton determination

The determination of phytoplankton was performed using a microscope (Olympus Cx22 LED) at 100x and 400x magnification. A few drops of the sample were added on top of a microscope slide and prepared by placing a coverslip over it. New slides of the sample were studied until there were no more new species to detect. Minimum five drops per sample were analysed. The identification of genera was based on morphological structures, using Växtplanktonflora by Tikkanen and Willén (1992), Växtplanktonkompendium by Blomquist and Olsen (1981), Diversity of *Aphanizomenon*-like cyanobacteria by Komárek and Komárková (2006) and Planktic morphospecies of the cyanobacteria genus *Anabaena* by Komárek and Zapomělová (2007). The results in the form of tables and appendixes are according to the taxonomic order presented in Växtplanktonflora by Tikkanen and Willén (1992). Species names were updated according to Nordicmicroalgae.org and Algaebase.org.

4 Results

4.1 Physical-chemical parameters

The temperature in the lakes varied from 11°C to 26°C in the period from June to September 2019. The highest temperature was recorded in L. Vikevannet and L. Haugestadvannet (26°C) in July. The pH interval was between 7,2 and 10,0 with the maximum pH value measured in L. Vikevannet in July (table 4.1).

The highest turbidity (61,9 NTU) was shown for L. Hillestadvannet in August. Watercolor varied in the lakes from 20 to 55 mg Pt/L. with highest value (55,1 mg Pt/L) measured in June in L. Vikevannet.

The highest tot-P concentration was measured in L. Hillestadvannet in July (120 µg/L), and the highest amount of tot-N was detected in L. Hillestadvannet in August (1856 µg/L). See Appendix 2 for more detailed results of the water quality parameters.

Table 4.1: Physical and chemical results from L. Vikevannet, L. Haugestadvannet and L. Hillestadvannet 2019, given as maximum, minimum and average values.

Lake		**°C**	**pH**	**Alkalinity (mmol/L)**	**Turbidity (NTU)**	**Watercolor (mg Pt/L)**	**Tot-P (µg/L)**	**Tot-N (µg/L)**
Vikevannet	**Average**	19,5	8,3	0,61	12,1	36	34	1062
	Min	12,0	7,2	0,49	4,6	20	22	662
	Max	26,0	10,0	0,83	21,6	55	47	1630
Haugestad-vannet	**Average**	18,9	7,9	0,63	19,4	37	63	1178
	Min	11,5	7,3	0,39	7,5	20	37	726
	Max	26,0	8,8	0,78	33,3	44	95	1723
Hillestadvannet	**Average**	18,9	8,8	0,87	32,3	30	78	1454
	Min	11,0	7,6	0,78	10,7	21	47	1055
	Max	25,5	9,9	0,98	61,9	46	120	1856

4.2 Chlorophyll a

The concentration of chlorophyll a gives an approximate estimate of the phytoplankton biomass in the lakes. Figure 4.1 illustrates the chlorophyll a concentration (µg chl a/L) in the three investigated lakes during the sampling period from June to September 2019.
L. Hillestadvannet (sampling stations 6, 7 and 8) had the highest chlorophyll a concentration of the lakes in August (94 µg chl a/L) giving poor/very poor ecological status (red color). The chlorophyll a concentration was generally higher in L. Hillestadvannet with an average of 37,6 µg chl a/L compared to L. Vikevannet (16,1 µg chl a/L) and L. Haugestadvannet (24,2 µg chl a/L).

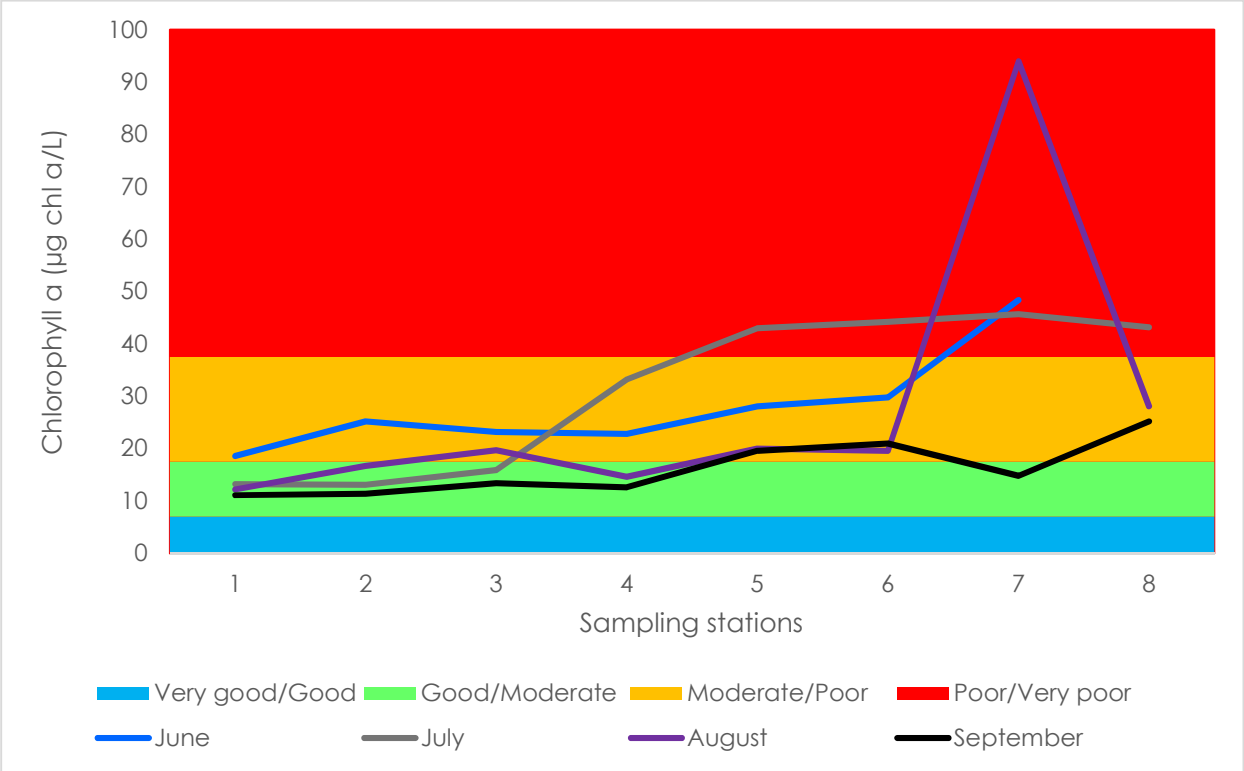

Figure 4.1: Chlorophyll a concentrations in L. Vikevannet (sampling stations 1, 2 and 3), L. Haugestadvannet (sampling stations 4 and 5) and L. Hillestadvannet (sampling stations 6, 7 and 8) during the sampling period from June to September 2019 with its respective ecological status (Direktoratsgruppen vanndirektivet 2018).

Average chlorophyll a concentrations for the lakes in the period from 2005 to 2019 are shown in figure 4.2. The source of the data is from Vannmiljø.no, however the results from this study (2019) are included. There was no recorded data from 2019 at vannmiljø.no. The highest concentration of chlorophyll a during the period from 2005 to 2019 was measured for L. Haugestadvannet 2015 (76,5 µg chl a/L) and in L. Hillestadvannet 2017 (75,8 µg chl a/L). Generally, the chlorophyll a concentrations are within the ecological status 'Moderate/poor'

and ‘Poor/very poor’ for both of the lakes. The chlorophyll a concentrations increased from 2005 to 2015 and decreased from 2017 to 2019.

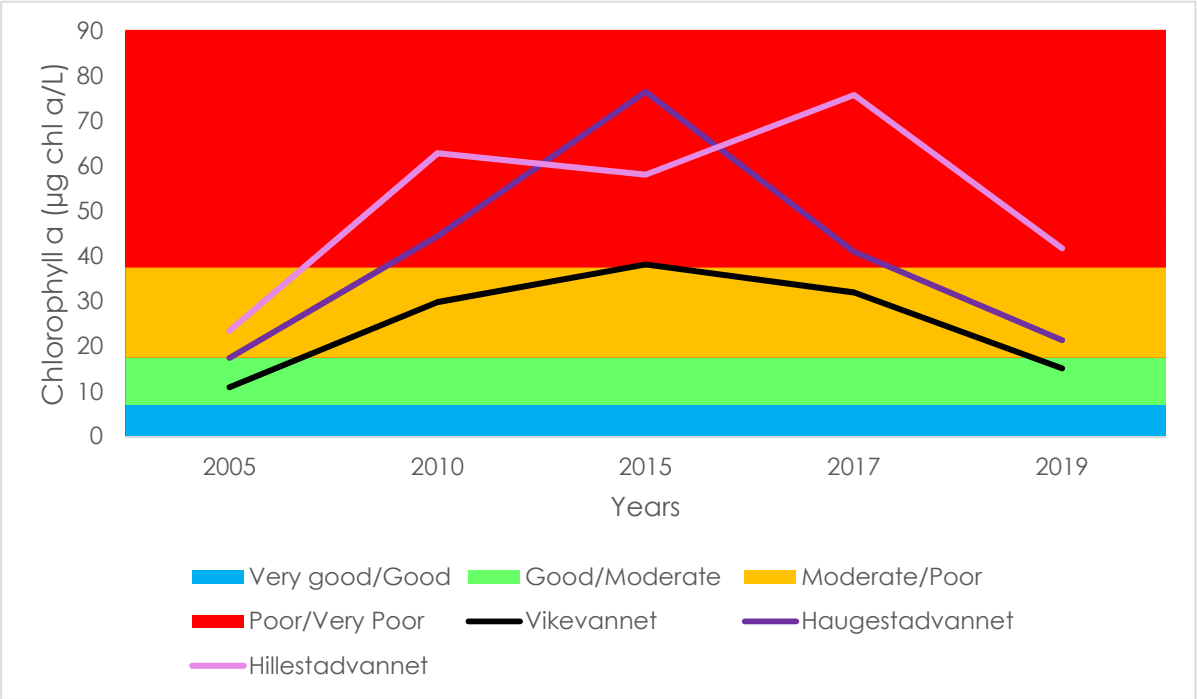

Figure 4.2: Average concentrations of chlorophyll a during the period from 2005-2019 in L. Vikevannet, L. Haugestadvannet and L. Hillestadvannet with its respective ecological status. Data collected from Vannmiljø.no (Miljødirektoratet 2019).

4.3 Correlations between chlorophyll a, tot-P and tot-N

Pearson’s correlation between chlorophyll a, tot-P and tot-N, gives an indication of the strength of the relationship between the variables. The indication of the strength of the relationship is given as values between -1 and 1. Closer to 1 indicates a strong relationship between the variables (Løvås 2013). Coefficient of determination (R^2) shown in figures 4.3 and 4.4 will have a value between 0 to 1. A value near 1 indicates that the variation of the response variable can be attributed to the explanatory variable, whereas a value close to 0 indicates that a small proportion of the variation is explained by the explanatory variable (Kasuya 2018). The correlation coefficient (r) shows a strong correlation between chlorophyll a and tot-P (r = 0,77) in the period of sampling (figure 4.3), however, if the outlier is removed, the correlation coefficient is shown to be even higher (r= 0,85). The correlation coefficient between chlorophyll a and tot-N (figure 4.4) shows less correlation as the r value is 0,54 and an even lower correlation when the outlier is removed (r = 0,44).

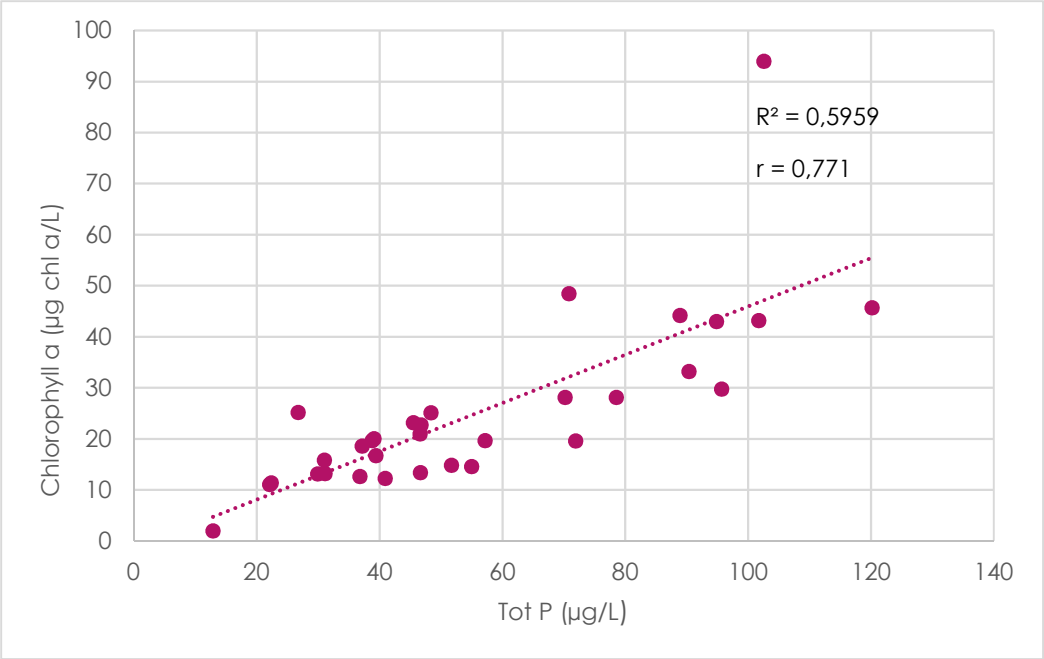

Figure 4.3: Correlation between chlorophyll a and tot-P in L. Vikevannet, L. Haugestadvannet and L. Hillestadvannet in the period from June to September 2019.

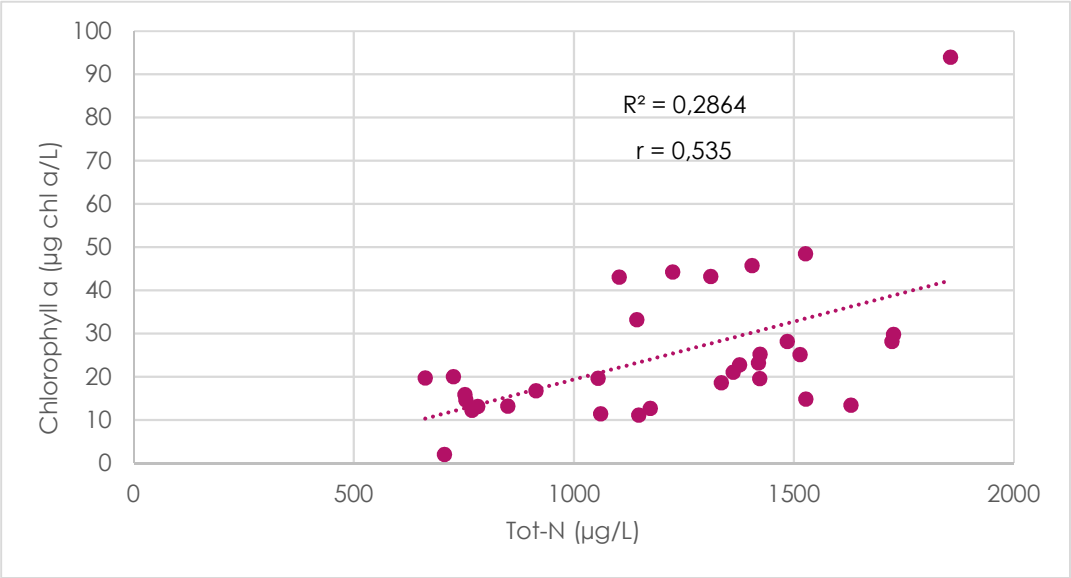

Figure 4.4: Correlation between chlorophyll a and tot-N in L. Vikevannet, L. Haugestadvannet and L. Hillestadvannet in *the period from June to September 2019.*

4.4 N:P ratio and the correlation to chlorophyll a concentration

The relationship between tot-N and tot-P indicates which of the nutrients are limiting the primary production in the lakes (table 4.2). The ratio varies between 12 in L. Haugestadvannet in July to 45 in L. Vikevannet in September. The correlation between chlorophyll a and the N:P ratio is shown in figure 4.5. The correlation coefficient (r) is -0,47 which indicates that the correlation is negative and of moderate strength. High phytoplankton biomass is shown to be present at lower N:P ratios. When the N:P ratio increases, the biomass decreases (figure 4.5).

Table 4.2: Average N:P ratio in L. Vikevannet, L. Haugestadvannet and L. Hillestadvannet in the period from June to September 2019.

MONTH	VIKEVANNET (N:P)	HAUGESTADVANNET (N:P)	HILLESTADVANNET (N:P)
JUNE	40	27	20
JULY	26	12	13
AUGUST	20	16	19
SEPTEMBER	45	26	30

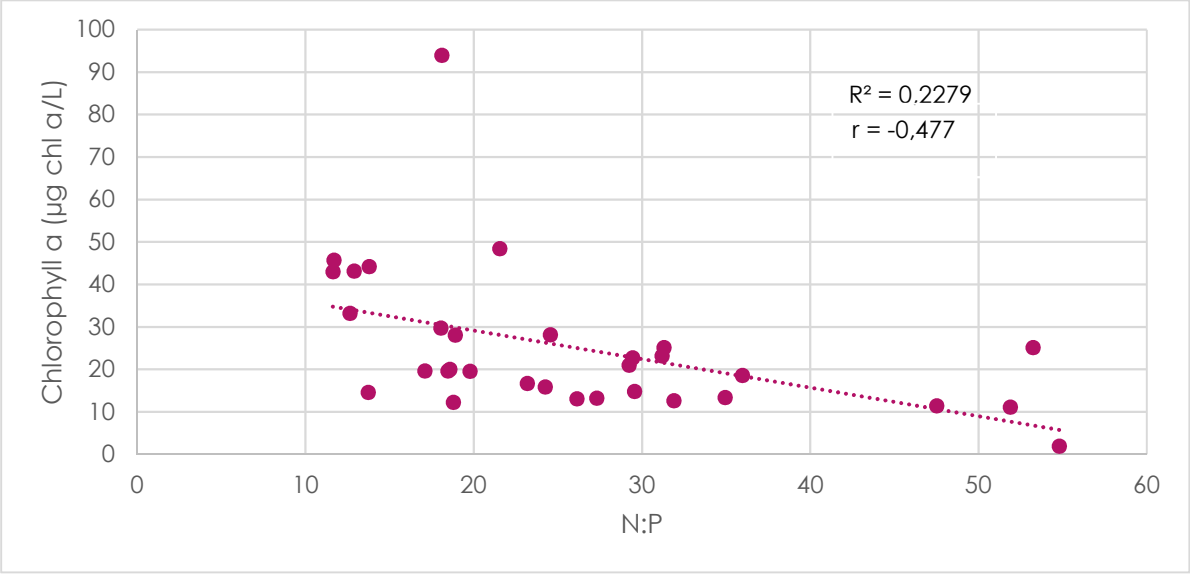

Figure 4.5: Correlation between chlorophyll a and the N:P ratio in L. Vikevannet, L. Haugestadvannet and L. Hillestadvannet in the period from June to September 2019.

4.5 Cyanobacterial toxins

4.5.1 Microcystins

Microcystins were detected in all the samples (figure 4.6). The concentrations were generally higher in L. Hillestadvannet compared to the two other lakes. The highest microcystin concentration was recorded in L. Hillestadvannet in June (20,7 µg/L and 11,2 µg/L) and August (14,1 µg/L) which was above the guideline value for recreational waters. For more detailed results, see appendix 2.

Table 4.3 shows the risk assessment for L. Vikevannet, L Haugestadvannet and L. Hillestadvannet based on the WHO's guidelines for microcystins in recreational waters. Using this risk assessment, L. Hillestadvannet had ''high risk'' in June (20,7 µg/L microcystins) and ''moderate risk'' in July and August. September remains at low risk for all the lakes.

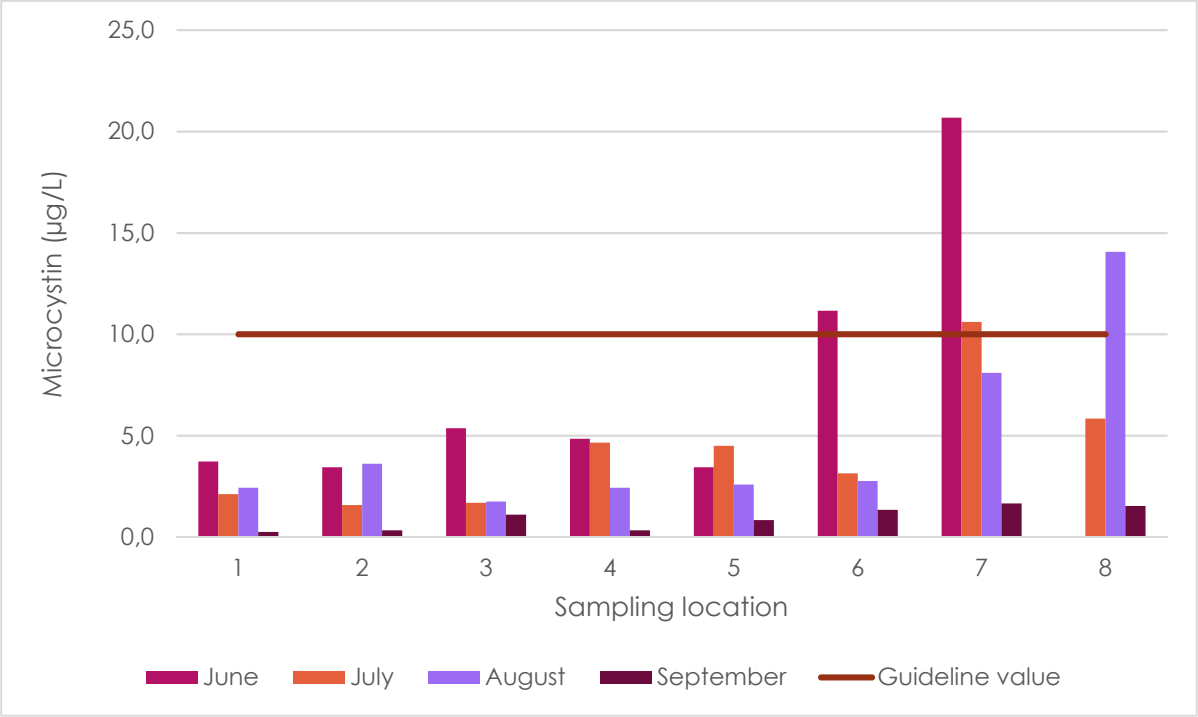

Figure 4.6: Microcystin concentrations (µg/L) in L. Vikevannet (sampling stations 1, 2 and 3), L. Haugestadvannet (sampling stations 4 and 5) and L. Hillestadvannet (sampling stations 6, 7 and 8) 2019. The horizontal line represents the WHO's guideline value for microcystins in recreational waters (World Health Organization 2003).

Table 4.3: Risk assessment of L. Vikevannet (1,2 and 3), L. Haugestadvannet (4 and 5) and L. Hillestadvannet (6,7 and 8) based on the WHO guidelines for microcystins (µg/L) in recreational waters; microcystin concentrations ≤ 4 µg/L (low risk=green), 4-20 µg/L (moderate risk=orange) and >20 µg/L (high risk=red) (World Health Organization 2003).

Lake	Sample date	Sampling stations	Microcystin concentration (µg/L)	Risk level based on WHO guidelines
Vikevannet	26.06.2019	1	3,7	Low risk
		2	3,4	Low risk
		3	5,4	Moderate risk
	29.07.2019	1	2,1	Low risk
		2	1,6	Low risk
		3	1,7	Low risk
	27.08.2019	1	2,4	Low risk
		2	3,6	Low risk
		3	1,8	Low risk
	24.09.2019	1	0,2	Low risk
		2	0,3	Low risk
		3	1,1	Low risk
Haugestadvannet	26.06.2019	4	4,8	Moderate risk
		5	2,9	Low risk
	29.07.2019	4	4,7	Moderate risk
		5	4,5	Moderate risk
	27.08.2019	4	2,4	Low risk
		5	2,6	Low risk
	24.09.2019	4	0,3	Low risk
		5	0,8	Low risk
Hillestadvannet	26.06.2019	6	11,2	Moderate risk
		7	20,7	High risk
	29.07.2019	6	3,1	Low risk
		7	10,6	Moderate risk
		8	5,8	Moderate risk
	27.08.2019	6	2,8	Low risk
		7	8,1	Moderate risk
		8	14,1	Moderate risk
	24.09.2019	6	1,3	Low risk
		7	1,7	Low risk
		8	1,5	Low risk

4.5.2 Saxitoxin

The highest concentration of saxitoxin was measured in L. Hillestadvannet in July (0,12 µg/L). There are generally higher concentrations in L. Hillestadvannet (average of 0,05 µg/L) compared to the other lakes (average of 0,02 µg/L) (figure 4.7). All results from September are negative as are most results from August except from L. Hillestadvannet.

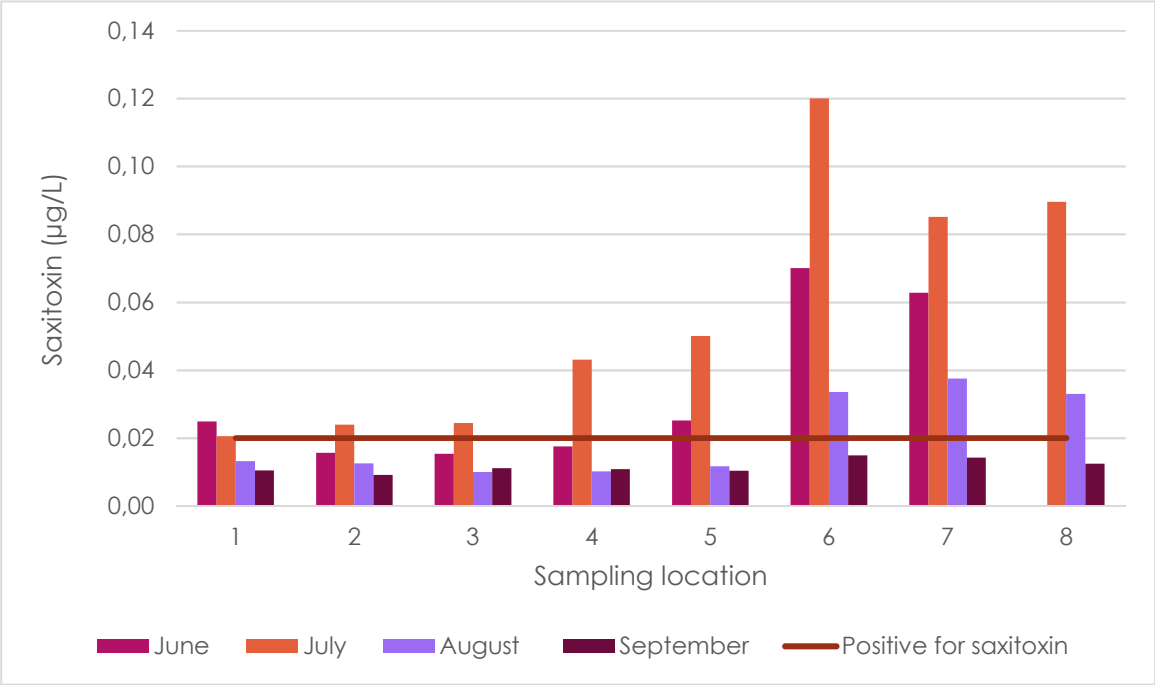

Figure 4.7: Saxitoxin concentrations in L. Vikevannet (sampling stations 1, 2 and 3), L. Haugestadvannet (sampling stations 4 and 5) and L. Hillestadvannet (sampling stations 6, 7 and 8) 2019. The horizontal line represents the limit for positive samples for saxitoxin (0,02 ug/L) according to the manual following the ELISA kit (Product No. 52255B) from Abraxis.

4.6 Phytoplankton composition

The number of identified phytoplankton taxa in the samples are shown in table 4.4. The total number of identified taxa increase from June to September in L. Vikevannet, in contrast to L. Haugestadvannet and L. Hillestadvannet where approximately the same number of identified taxa where observed throughout the sampling period. According to table 4.5, it is the genus *Microcystis (M. aeruginosa M. viridis*, *M. wesenbergii)* that dominates most of the period, however the genus *Dolichospermum* is also frequently occurring from June to August in the lakes. *Aphanizomenon* dominates together with *Microcystis* in August and September in L. Hillestadvannet. *Aphanizomenon* sp. co-dominates with *Planktothrix agardhii* in September in L. Vikevannet. The diatom *Fragilaria ulna* and the green algae *Pediastrum sp.* are known to be eutrophication indicators together with the cyanobacteria mentioned above. Other cyanobacteria taxa like *Chroococcus, Snowella, Anathece clathrata and Planktolyngbya limnetica* occurred throughout the whole period, however *Planktolyngbya limnetica* was not found in L. Vikevannet in June. From July to September there was a lot of *Planktolyngbya contorta* in the lakes co-occurring with positive saxitoxin samples in L. Hillestadvannet. See Appendix 3-6 for more detailed results about the phytoplankton communities in the lakes.

Table 4.4: Number of identified taxa in each phytoplankton taxonomic class in L. Vikevannet (L.V), L. Haugestadvannet (L.H) and L. Hillestadvannet (L.Hi) from June to September 2019.

Class	**June**			**July**			**August**			**September**		
	L.V	L.H	L.Hi	L.V	L.H	L.Hi	L.V	L.H	L.Hi	L.V	L.H	L.Hi
Cyanophyta	19	21	21	25	26	27	25	23	24	28	21	21
Cryptophyta	1	2	1	1	1	2	1	1	1	1	1	1
Dinophyta	1	2	1	2	1	1	2	2	2	2	2	2
Chrysophyta	0	0	0	1	0	1	1	1	1	1	1	2
Chrysophyceae	1	1	0	1	1	1	1	1	1	1	1	1
Diatomophyceae	2	4	3	4	4	4	4	6	2	5	5	5
Tribophyceae	0	0	0	0	0	0	0	0	1	0	0	2
Chlorophyceae	5	10	7	9	7	9	7	8	6	11	8	11
Conjugatophyceae	2	2	1	1	2	2	1	2	2	4	2	1
Total	**31**	**42**	**34**	**44**	**42**	**47**	**42**	**44**	**40**	**53**	**41**	**46**

*Table 4.5: Identified Cyanophyta in L. Vikevannet (L.V), L. Haugestadvannet (L.H) and L. Hillestadvannet (L.Hi) from June to September 2019. (- = not present, X = present and **D** = dominant)*

Cyanophyta	June			July			August			September		
	L.V	L.H	L.Hi	L.V	L.H	L.Hi	L.V	L.H	L.Hi	L.V	L.H	L.Hi
Snowella	X	X	X	X	X	X	X	**D**	**D**	**D**	**D**	**D**
Coelosphaerium	-	-	-	-	-	X	-	-	-	-	-	-
Microcystis	X	**D**	**D**	**D**	**D**	**D**	**D**	**D**	**D**	X	**D**	**D**
Aphanocapsa	X	-	X	X	X	X	-	X	X	X	X	**D**
Aphanocapsa conferta	-	**D**	-	-	-	-	-	-	-	-	-	-
Chroococcus	X	X	X	X	X	X	X	X	X	X	X	X
Anathece clathrata	X	**D**	X	**D**	X	X	X	X	X	**D**	X	**D**
Achroonema proteiforme	-	-	-	X	X	X	X	X	X	**D**	**D**	X
Planktothrix agardhii	-	-	-	X	**D**	**D**	X	X	X	**D**	**D**	X
Pseudanabaena limnetica	X	-	X	-	-	X	X	X	X	X	X	X
Limnothrix planctonica	-	-	-	-	-	-	-	X	X	X	X	X
Planktolyngbya limnetica	-	X	X	X	X	**D**	X	X	X	X	X	X
Planktolyngbya contorta	-	-	-	X	X	X	X	X	**D**	X	X	X
Pseudanabaena mucicola	-	-	-	X	X	X	X	X	X	X	X	X
Aphanizomenon	X	X	X	X	X	X	X	X	**D**	**D**	X	**D**
Dolichospermum	X	**D**	**D**	X	**D**	**D**	**D**	**D**	**D**	X	X	X

5 Discussion

5.1 Environmental factors regulating occurrence and growth of cyanobacteria

Visible blooms dominated by cyanobacteria were observed in L. Hillestadvannet in June, July and August. A dominance of cyanobacteria was also found in L. Vikevannet and L. Haugestadvannet without visible blooms. There are many factors that play a critical role when it comes to regulation of cyanobacterial blooms and the amount of cyanotoxins. The occurrence of cyanotoxins depends highly on the taxonomic composition of the cyanobacteria communities because different species can produce different toxins (Dolman et al. 2012). Microcystin production is affected by several environmental factors like temperature, light availability and nutrient availability (Edwin et al. 2005), however genetics also play a role in toxin production as environmental factors may provoke and regulate gene activity in cyanobacteria. One study showed that transcription of the gene complex for producing microcystins increases at high light intensities (Kaebernick et al. 2000). However, Pearson et al. (2008) states that some studies have indicated that microcystin production is higher at lower light intensities. Nutrients may also play a role in the transcription of microcystins (Ginn et al. 2010).

Temperature is one of the major factors controlling the growth rate of cyanobacteria and the concentration of toxins in the water. Cyanobacteria usually have an optimum temperature where the growth rate is at its highest. The cyanobacterial growth is favored by temperatures above 15°C, however many cyanobacteria species have optimal temperatures above 20°C (Paerl et al. 2001). According to a study by Robarts and Zohary (1987) it has previously been stated that the dominance of cyanobacteria usually occurs at higher temperatures (>20°C) and that the temperature optimum for other phytoplankton taxa tend to be lower. Compared with other phytoplankton taxa, cyanobacteria typically have lower growth rates at lower temperatures and higher growth rates at higher temperatures (Berg and Sutula 2015).

Since the water sampling was conducted from June to September, the temperature ranged from 11 to 26°C with the highest measured temperature in July. The results from phytoplankton identification clearly confirmed that cyanobacteria, especially *Microcystis* sp. dominated the phytoplankton population. Interestingly they also dominated at lower temperatures all the way down to 11°C, but at lower temperatures, the diversity of other phytoplankton species also increased. Robarts and Zohary (1987) discovered that the growth

rate for *Microcystis* sp. declined when the temperatures were below a critical temperature (15°C). On the other hand, the dominance of *Microcystis* sp. at low temperatures in this study can be due to the fact that the samples were taken from the shore. When the cyanobacteria floats to the surface, the wind can transport them to shallow areas where the concentration in the water may reach high levels (Norwegian Institute of Water Research 2014). It is known that cyanobacteria grow best under relatively high pH. They are considered to be alkalophiles and are favored by alkaline conditions. The pH growth optimum for cyanobacteria is between 7,5 and 10 (Giraldez-Ruiz et al. 1997). The pH has also been discovered to be the main factor that influence the growth of *Microcystis* sp. (Takarina and Wardhana 2017). According to a study on the regulatory effect of external pH on the intracellular pH in alkalophilic cyanobacteria, *Microcystis aeruginosa* showed an optimal growth rate at pH 10 (Dwivedi et al. 1994). Interestingly, *Microcystis aeruginosa* was one of the species that dominated under high pH values (pH>7,7) in the lakes in Vestfold.

Most of the carbon is in the form of bicarbonate (HCO_3^-) at high pH and different species can be capable of utilizing this carbon fraction (Harris 1986). Cyanobacteria and other microalgae possess a mechanism which converts bicarbonate (HCO_3^-) to carbon dioxide (CO_2) using an enzyme called carbonic anhydrase (Gjølme et al. 2010). This effective carbon concentrating mechanism in cyanobacteria make them highly competitive at elevated pH, which is one of the characteristics of eutrophic waters (Edwin et al. 2005). According to the measured alkalinity in L. Vikevannet (0,61 mmol/L), L. Haugestadvannet (0,63 mmol/L) and L. Hillestadvannet (0,87 mmol/L), the buffer capacity is good, hence the high pH. The pH stayed more or less at the pH growth optimum for cyanobacteria and can be one of the many factors explaining the dominance of *Microcystis aeruginosa*, *M. viridis* and other cyanobacteria species in the lakes.

The taxonomic composition of the phytoplankton communities depends on the ratio between nitrogen concentration and the phosphorous concentration in the water (Huisman and Hulot 2005). Low ratios between nitrogen and phosphorous concentrations (N:P) may favor the development of cyanobacterial blooms (Mur et al. 1999). There are several studies focusing on explaining N:P ratios and indication of nitrogen deficiency in the water. An analysis showed that eutrophic lakes had a N:P ratio less than 15 (Harris 1986), and according to Reynolds (1984) N:P ratios lower than 5 promotes a dominance of N_2-fixating cyanobacteria.

On the other hand, N:P ratios are considered to be relatively high when over 23 (N:P>23) (Kjellstrøm Hoel 2016). According to the N:P ratios in this study,
L. Hillestadvannet shows N-limitation in June (N:P=19,8), July (N:P=12,8) and August (N:P=18,5), while L. Haugestadvannet had N-limitation in July (N:P=12,1) and August (N:P=16,2) and L. Vikevannet only in August (N:P=19,7). The abundance of heterocysts observed during phytoplankton identification can also indicate N-limitation in the lakes as these are specialized cells which carry out the nitrogen fixation (Kumar et al. 2010). The biomass of phytoplankton measured as chlorophyll a increases at low N:P ratios. This probably indicates an increase of N_2-fixating cyanobacteria as they dominate over the non-N_2-fixing and may therefore develop high biomasses. During periods of low N:P ratios (e.g. <20), nitrogen is taken in from the air. At high N:P ratios (>50), the lakes are most certainly P-limited. According to Dolman et al. (2012), N_2 fixation can only occur in Nostocales, which may explain the abundance of *Dolichospermum* sp. and *Aphanizomenon* sp. in the lakes during N-limitation as they are capable of N_2 fixation. In theory nitrogen fixating cyanobacteria should dominate during high phosphorus and low nitrogen concentrations due to their N-fixation ability. The study by Dolman et al. (2012) on the influence of nitrogen and phosphorus, did not support the view that N_2 fixing taxa were more abundant in lakes with high concentrations of phosphorus compared to nitrogen. One reason for this could be that nitrogen fixation requires high amounts of energy (Mur et al 1999) and due to enrichment of nutrients, the cyanobacteria biomass increases and light intensity decreases. There has been a suggestion that N_2 fixers are not capable of dominance in turbid waters (Dolman et al. 2012) and this could possibly be explained by the need for light (energy) to fix nitrogen. This corresponds well to the results in this thesis and may explain why *Aphanizomenon* sp. and *Dolichospermum* sp. were more abundant in L. Vikevannet and L. Haugestadvannet with turbidity values of 12,1 NTU and 19,4 NTU respectively compared to L. Hillestadvannet with higher turbidity (32,3 NTU).

During conditions with high N:P ratios (N:P=40-45), *Microcystis* sp. dominates. They were also co-dominating with *Dolichospermum* sp. and *Aphanizomenon* sp. at lower N:P ratios (N:P=12). *Microcystis* sp. is a genus that does not have the ability to produce heterocysts or to perform N_2 fixation. However, in the case of phosphorus deficiency, M*icrocystis* has a large capacity to store phosphorus (Whitton and Potts 2000), which will allow them to grow even if the phosphorus concentrations in the lakes are low (Edwin et al. 2005). Altogether,

Microcystis sp. was the most abundant and dominant taxa present in this study independent of N:P ratio.

Since many cyanobacteria species do possess gas vesicles and can control buoyancy, they often tend to sink during the daytime, and slowly regain their buoyancy at night and move upwards to the surface (Visser et al. 2005). This buoyancy trait may have been the reason for why there was only scum formations in L. Hillestadvannet and not in L. Vikevannet nor L. Haugestadvannet. The water sampling in L. Hillestadvannet were conducted during early morning and in L. Vikevannet during early afternoon. In July and August one could barely see traces of a thin green layer that might have been a scum formation of cyanobacteria in L. Vikevannet. According to Mur et al. (1999), gas vesicles tend to become more abundant when the light intensity is reduced, and growth rate slows down. This may also explain why blooms were more abundant in L. Hillestadvannet where the turbidity values were highest. Since the growth rate of cyanobacteria is low, the retention time of the lakes must be long enough for the cyanobacteria to form a bloom (Mur et al. 1999). One study report that high retention time (low flow) and high temperatures favors the dominance of cyanobacteria (Elliott 2010), which appears clearly in L. Hillestadvannet with the longest retention time and visible blooms.

Two factors tend to affect the quality and quantity of light in aquatic environments; the turbidity and depth of epilimnion (Renaud et al. 2011). However, watercolor also affects the light quality as high values are normally due to high humic content (Norwegian Institute of Public Health 2018). These factors will be important in determining the taxonomic composition of the phytoplankton community and dominant taxa because some phytoplankton require more light than others. *Microcystis* sp., *Dolichospermum* sp. and *Aphanizomenon* sp. were the most abundant and important taxa found in this study. Usually in eutrophic waters, the phytoplankton biomass will be excessive, thus causing high turbidity. Therefore organisms adapted to lower light intensities are favored. According to Mur et al. (1999), exposure of intense light is lethal for many species, however if cyanobacteria are exposed occasionally, they will grow at their maximum rate. Since the maintenance of cyanobacterial cells requires low amounts of energy, they can maintain cell functions with low light intensities, therefore, cyanobacteria can have higher growth rates at lower light intensities compared to other phytoplankton species. Hence, they will out-compete other phytoplankton species when the lakes are turbid (Mur et al. 1999).

According to the turbidity and the watercolor of the lakes, the values are high (average of 21,2 NTU and 34 mg Pt/L respectively). The phytoplankton biomass is probably the main reason for the high turbidity values. *Microcystis* sp. have high light requirements (Edwin et al. 2005), and this is supported by a study which found that high light intensity increased growth of *Microcystis aeruginosa* (Singh and Singh 2015). They both stated that *Microcystis* uses its ability of buoyancy regulation to migrate between layers with different light intensities and nutrient availability as they have high light requirements.

5.2 Toxin production

''Microcystis are the chief among microcystin producing taxa'' (Berg and Sutula 2015). *Dolichospermum* is also known to produce microcystins (World Health Organization 2003), however *Aphanocapsa*, *Limnothrix*, *Planktothrix*, *Planktolyngbya* and *Pseudoanabaena* are also potential microcystin producers (Sivonen and Jones 1999; Kjellstrøm Hoel 2016). All of the above-mentioned taxa co-occurred in the lakes when the microcystin concentrations were high. Higher biomass of *Microcystis* sp. and other microcystin producing taxa will probably result in higher microcystin concentrations. Changes in other environmental factors and gene composition also give rise to variations in the microcystin content. LeBlanc Renaud (2011) suggests that more than 50% of the cyanobacterial blooms produce toxins. It is difficult to determine exactly what controls the cyanotoxin concentrations in the lakes, but studies show that cyanobacteria tend to produce more toxins when the conditions are most favorable (Edwin et al. 2005). According to the microcystin results, the concentrations in L. Vikevannet and L. Haugestadvannet are below the WHO guideline value for microcystin concentrations in recreational waters. L. Hillestadvannet has concentrations that exceeds the guideline value, especially in June, July and August. One explanation for this may be that there are interactions between several factors leading to favorable environmental conditions, and the phytoplankton biomass (chlorophyll a) was generally higher in L. Hillestadvannet compared to the other lakes. The WHO has derived a number of guidelines associated with human health effects from cyanobacteria. It consists of three levels; mild, moderate and high probability of adverse health effects (Falconer et al. 1999). Mild or low probability of adverse health effects involves irritative or allergenic effects. Moderate probability of adverse health effects involves increased irritative symptoms while high probability of adverse health effects involves severe health hazards. High probability is associated with scum formations. When it comes to possible misleading information or warnings, L. Vikevannet and L. Hillestadvannet

have warnings about not swimming in the water during a bloom. However one species that does not form scum and is known to contain high concentrations of microcystins is *Planktothrix agardhii*. They can still be present in the water at high concentrations without visible blooms (Sivonen and Jones 1999). The *Planktothrix* species is known to be a major reason for high microcystin concentrations in Norwegian lakes (Chorus 2001). *Microcystis* and *Dolichospermum* species are also a major reason for high microcystin concentrations in lakes and are known to form blooms. These blooms can increase the toxicity by a factor of 1000 or more within few hours. Accordingly, the lake may go from moderate to high risk in a very short time and could be a potential threat for people who swim or do water sports in the lakes (Falconer et al. 1999).

According to this risk assesment based on the WHO guidelines for microcystin in recreational waters (table 4.3), there is moderately risk for all the lakes (4-20 µg/L microcystin).
L. Vikevannet shows moderate risk in June and L. Haugestadvannet in June and July.
L. Hillestadvannet shows high risk (>20 µg/L microcystin) in June and moderate risk in July and August. Although L. Hillestadvannet shows high risk due to high microcystin concentrations, little is known regarding the effects during exposure to low concentrations over a longer period. Earlier studies from L. Hillestadvannet (Skjelbred 2016) show that the concentrations exceed the guideline value as the microcystin concentrations are above 10 µg/L. The concentration of microcystin was 40 µg/L and 25 µg/L in July and August 2013 respectively. In 2014 the microcystin concentrations were 31,2 µg/L and 34,7 µg/L. Another study of cyanotoxins in L. Vikevannet and L. Hillestadvannet (Kjellstrøm Hoel 2016) shows that L. Vikevannet had a maximum microcystin concentration of 32,2 µg/L in August 2015. Comparing these results to the microcystin results in this study, the overall concentrations are lower in 2019 than in earlier years. However it is difficult to find an explanation for this as there are many factors that play a role in determining the toxin concentrations in the lakes at certain periods.

Potential saxitoxin producers are *Aphanizomenon, Dolichospermum*, *Planktolyngbya*, *Cylindrospermopsis* and *Planktothrix* (Sivonen and Jones 1999; Norwegian Institute of Public Health 2010). According to the saxitoxin results and phytoplankton identification, *Planktothrix*, *Planktolyngbya limnetica, Planktolyngbya contorta*, *Dolichospermum* and *Aphanizomenon* were either present or dominant when the saxitoxin analyses were positive. This may be an indication of why saxitoxin was present in higher concentrations especially in

L. Hillestadvannet in June (0,07 µg/L) and July (0,12 µg/L). On the other hand, many of these cyanobacteria species also co-occurred when the saxitoxin concentrations were low, so to find the reasons for high saxitoxin concentrations seems nearly impossible. *Aphanizomenon* co-occurred with positive saxitoxin samples in L. Vikevannet in August 2015 (Kjellstrøm Hoel 2016). *Aphanizomenon* were also co-occurring with positive samples of saxitoxin in L. Vikevannet in June and July (0,2 µg/L) 2019, however *Planktolyngbya contorta*, *Planktolyngbya limnetica* and *Planktothrix* were also present in L. Vikevannet in July 2019. Nevertheless, the saxitoxin concentrations in L. Vikevannet were barely positive. L. Hillestadvannet is the lake with the highest saxitoxin concentrations in June (0,07 µg/L), July (0,12 µg/L) and August (0,04 µg/L) 2019. According to the phytoplankton identification, potential saxitoxin producers as *Planktolyngbya*, *Planktothrix, Dolichospermum* and *Aphanizomenon* were the most abundant species in L. Hillestadvannet and may therefore explain the high saxitoxin concentrations.

The health aspects concerning toxin production needs to be addressed as these lakes (primarily L. Vikevannet and L. Hillestadvannet) are used for recreational purposes. There are not many reports of health problems related to recreational exposure of cyanotoxins. Humans are less likely to be exposed to lethal doses of cyanotoxins compared to animals. There have been reports of illness attributed to cyanotoxins in recreational waters (World Health Organization 2003). One report is from Canada 1959, where people swam in a lake with cyanobacteria. Thirteen people became ill with headache and nausea. *Microcystis* sp. and *Dolichospermum circinale* were found in the excreta from one person who had accidentally ingested the water (World Health Organization 2003; Gjølme et al. 2010). In Pennsylvania during the period 1980-1981, people swam or did watersports such as canoeing in a cyanobacterial (*Dolichospermum* and *Aphanizomenon*) infested lake. Over 100 people got either eye or skin irritations, earache, cough, diarrhea, vomiting and blisters in their mouth (Gjølme et al. 2010). In Australia 1995, a study on epidemiological evidence of adverse health effects after recreational water contact, involving 852 participants showed an increased frequency of vomiting, diarrhea, skin rashes, fevers, eye/ear irritations, as well as flu symptoms. The symptoms showed a significant correlation with duration of water contact and density of cyanobacterial cells (World Health Organization 2003). It is difficult to know if people have been exposed to microcystins in the three investigated lakes as the symptoms do not appear immediately and there are no reports of such incidents. People may have experienced sickness or allergic reactions, however they may not have been aware that the

toxins in the water may be the reason for it. People living near the lakes used to use the lakes for recreational purposes. They do not use them for swimming anymore as there has been an increase in awareness regarding cyanobacterial blooms and potential health risk concerning them. Local media has shown interest for the results from this thesis and the conditions regarding cyanobacteria, toxins and water quality of the lakes today (Hordnes 2019).

The three major routes of exposure are through direct contact, accidental swallowing and inhalation of water. Inhalation, contact with nasa mucosa and swallowing is considered to be the important routes of exposure during recreational activities in the water (Falconer et al. 1999). In fact when swimming a person can accidentally swallow 100-200 ml of cyanobacterial infested water (World Health Organization 2003). L. Vikevannet and L. Hillestadvannet consist of beaches where people can be exposed to these cyanobacterial toxins, however all of the lakes are also used for canoeing and therefore aerosols of infested water can also be inhaled. The blooms can be mistaken for pollen as they seem quite similar if dispersed out on the surface, so young children can be tempted to swim anyway. Young children are more prone to cyanotoxins and the lethal dose is lower as they weigh less. In fact, it has been calculated that a child, playing in a bloom of *Microcystis* for a period of time, could possibly receive a lethal dose if ingesting a significant volume of water. Based on the results from several animal studies, a human of 10 kg, who accidentally ingesting 2 mg of microcystins can get liver injury (World Health Organization 2003).

6 Conclusion

The trophic status of L. Vikevannet, L. Haugestadvannet and L. Hillestadvannet is eutrophic as the concentrations of phosphorus (>30 µg/L), nitrogen (>650 µg/L) and chlorophyll a (>10 µg chl a/L) exceed the limits for mesotrophic conditions. It is not uncommon for eutrophic lakes to occur in the area where the water samples were taken as there is a lot of agricultural land around the lakes and excessive run-off of nutrients.

Microcystins was the most frequent type of cyanobacterial toxin observed during the production period 2019. Hepatotoxins are usually more common in the investigated lakes than neurotoxins. Altogether, L. Hillestadvannet comes out worst, and has the highest microcystin and saxitoxin concentrations, however previous studies have shown L. Vikevannet to have high microcystin concentrations as well. This confirms that there must be many environmental factors controlling the taxonomic composition and the production of toxins. Therefore, concentrations of these toxins are difficult to predict.

It is not justifiable to use the lakes, especially L. Hillestadvannet for recreational uses as there was high concentrations of microcystins and saxitoxin, and recurring high concentrations reported annually. Warnings at the shore may also be misleading as there are cyanobacteria which contain high concentrations of microcystins but do not form blooms. Even though the odds of getting a lethal dose are low, the possibility of having long-term effects after swimming in a cyanobacterial infested water is likely. A cyanobacterial bloom is always to be considered as a potential health risk when occurring in recreational waters such as L. Vikevannet, L. Haugestadvannet and L. Hillestadvannet.

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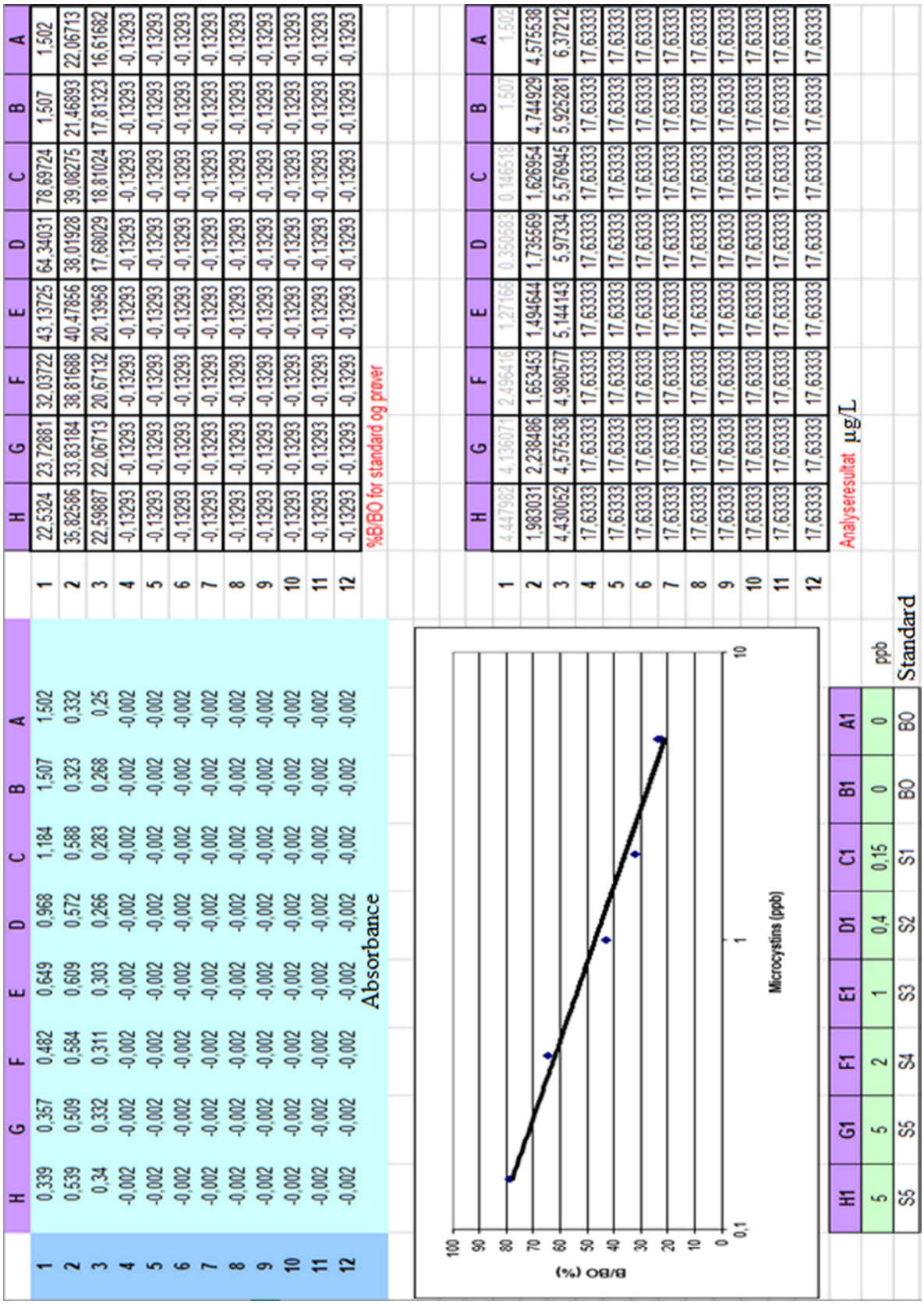
Personal communication

Birgit Vildalen. 2019, May 27.

Appendix

Appendix 1: Example of calculating microcystin concentrations from a standard excel sheet.



	H	G	F	E	D	C	B	A
1	0,339	0,357	0,482	0,649	0,968	1,184	1,507	1,502
2	0,539	0,509	0,584	0,609	0,572	0,588	0,323	0,332
3	0,34	0,332	0,311	0,303	0,266	0,283	0,268	0,25
4	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002
5	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002
6	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002
7	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002
8	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002
9	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002
10	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002
11	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002
12	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002	-0,002

Absorbance

	H	G	F	E	D	C	B	A
1	22,5324	23,72881	32,03722	43,13725	64,34031	78,69724	1,507	1,502
2	35,82586	33,83184	38,81688	40,47856	38,01928	39,082275	21,46893	22,06713
3	22,59887	22,06713	20,67132	20,13958	17,68029	18,81024	17,81323	16,61682
4	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293
5	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293
6	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293
7	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293
8	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293
9	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293
10	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293
11	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293
12	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293	-0,13293

%Bi/BO for standard og prøver

	H	G	F	E	D	C	B	A
1	4,447982	4,136071	2,496416	1,27166	0,350583	0,146518	1,507	1,502
2	1,983031	2,238486	1,653453	1,494644	1,735569	1,626954	4,744929	4,575538
3	4,430052	4,575538	4,980577	5,144143	5,97334	5,576945	5,925281	6,37212
4	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333
5	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333
6	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333
7	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333
8	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333
9	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333
10	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333
11	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333
12	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333	17,63333

Analyseresultat µg/L

H1	G1	F1	E1	D1	C1	B1	A1
5	5	2	1	0,4	0,15	0	0
S5	S5	S4	S3	S2	S1	B0	B0

ppb

Standard

Appendix 2: Physical/chemical results from L. Vikevannet (sample 1,2 and 3), L. Haugestadvannet (4 and 5) and L. Hillestadvannet (6,7 and 8) 2019.

(red colour = corrected alkalinity).

Sample	Temperature °C	pH	Colour (mg Pt/L)	Turbidity (NTU)	Tot-P (µg/L)	Tot-N (µg/L)	Chlorophyll (µg/L)	Microcystin (µg/L)	Saxitoxin (µg/L)	Alkalinity (mmol/L)	correction mmol/L
jun.19											
1	21	8,7	55	13,47	37	1335	18,6	3,7	0,02	0,54	0,5
2	21	8,5	51	10,27	27	1423	25,2	3,4	0,02	0,53	0,5
3	20	8,2	39	15,10	46	1420	23,2	5,4	0,02	0,54	0,5
4	20	7,7	44	25,55	47	1376	22,8	4,8	0,02	0,39	0,4
5	19	8,4	42	22,57	70	1723	28,1	3,4	0,03	0,61	0,6
6	19	9,7	38	39,89	96	1726	29,8	11,2	0,07	0,78	0,78
7	19	9,9	46	33,71	71	1526	48,4	20,7	0,06	0,81	0,81
8	12,5	7,9	38	3,03	13	706	2,0	0,04	0,00	1,08	1,08
jul.19											
1	26	9,6	25	13,57	31	850	13,2	2,1	0,02	0,67	0,64
2	26	9,8	20	11,96	30	781	13,1	1,6	0,02	0,67	0,64
3	25,5	10,0	20	11,78	31	752	15,9	1,7	0,02	0,67	0,64
4	26	8,8	20	31,82	90	1143	33,2	4,7	0,04	0,78	0,78
5	25	8,4	27	33,27	95	1103	43,0	4,5	0,05	0,78	0,78
6	25,5	9,6	23	37,45	89	1225	44,2	3,1	0,12	0,97	0,97
7	25	8,8	21	39,76	120	1405	45,7	10,6	0,09	0,98	0,98
8	25	9,5	26	39,53	102	1311	43,2	5,8	0,09	0,98	0,98
aug.19											
1	19,5	7,4	30	10,63	41	768	12,2	2,4	0,01	0,54	0,51
2	19,5	7,6	25	21,57	39	914	16,7	3,6	0,01	0,55	0,52
3	19	7,5	39	12,65	39	662	19,7	1,8	0,01	0,50	0,47
4	19	7,3	42	12,41	55	755	14,6	2,4	0,01	0,49	0,46
5	19	7,5	42	13,15	39	726	20,0	2,6	0,01	0,50	0,45
6	19,5	9,1	25	22,54	57	1055	19,6	2,8	0,03	0,84	0,84
7	19,5	8,6	25	61,87	103	1856	94,0	8,1	0,04	0,84	0,84
8	19,5	8,9	26	44,27	79	1485	28,1	14,1	0,03	0,82	0,82
sep.19											
1	12	7,2	44	6,17	22	1147	11,1	0,2	0,01	0,53	0,5
2	12	7,2	40	4,64	22	1061	11,4	0,3	0,01	0,83	0,83
3	12	7,5	38	13,78	47	1630	13,4	1,1	0,01	0,72	0,72
4	12	7,7	37	7,54	37	1174	12,6	0,3	0,01	0,74	0,74
5	11,5	7,7	40	9,01	72	1422	19,6	0,8	0,01	0,75	0,75
6	13	7,6	33	12,15	47	1362	21,0	1,3	0,01	0,83	0,83
7	11	7,7	33	10,70	52	1527	14,8	1,7	0,01	0,88	0,88
8	12	7,9	36	13,53	48	1514	25,2	1,5	0,01	0,85	0,85

Appendix 3: Identified phytoplankton species in L. Vikevannet (1,2 and 3) L. Haugestadvannet (4 and 5) and L. Hillestadvannet (6,7 and 8) June 2019.

(X = present, D = dominant)

Cyanophyta	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Gomphosphaeria aponina							
Snowella lacustris		X		X	X	X	
Snowella septentrionalis	X		X	X			
Coelosphaerium sp.							
Microcystis viridis	X	X	X	X	X D	X D	X
Microcystis aeruginosa	X	X		X	X D	X	X
Microcystis wesenbergii	X	X	X	X	X D	X D	X
Snowella atomus							
Aphanocapsa sp.	X				X	X	X
Aphanocapsa conferta					X D		
Aphanocapsa reinboldii	X	X	X		X	X D	X
Chroococcus limneticus		X		X	X	X	
Chroococcus minimus		X	X		X	X	X
Chroococcus dispersus		X			X		
Anathece clathrata		X	X	X	X D	X	
Achroonema proteiforme	X		X	X	X		X
Pseudanabaena catenata						X	

Cyanophyta continued	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Planktothrix agardhii							
Pseudanabaena limnetica							X
Limnothrix planctonica							
Planktolyngbya limnetica	X	X	X	X	X	X	
Planktolyngbya contorta			X				
Pseudanabaena mucicola						X	
Aphanizomenon sp.					X	X	
Aphanizomenon flos-aquae	X	X	X		X		
Aphanizomenon gracile				X	X		
Dolichospermum sp.				X	X D	X D	
Dolichospermum spiroides	X					X	X
Dolichospermum affine					X		X
Dolichospermum crassum	X	X	X	X	X	X D	
Anabaena inaequalis	X	X	X	X	X	X D	X
Anabaena cylindrica							X
Dolichospermum sigmoideum							
Dolichospermum delicatulum			X				

Cyanophyta continued	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Dolichospermum danicum							
Cryptophyta							
Cryptomonas sp.	X	X	X	X	X	X	X
Rhodomonas lacustris					X		
Dinophyta							
Ceratium hirundinella				X			
Peridinium sp.			X	X	X	X	
Chrysophyta							
Uroglena sp.							
Bicosoeca petiolata							
Chrysophyceae (Golden algae)							
Spiniferomonas sp.	X	X			X		
Diatomophyceae (Diatoms)							
Cyclotella sp.							
Aulacoseira islandica	X	X	X	X	X	X D	
Fragilaria sp.				X		X D	
Fragilaria ulna	X	X	X		X		X
Tabellaria sp.							
Tabellaria flocculosa					X		
Asterionella formosa							
Amphora sp.							
Tribophyceae							
Euglena sp.							
Trachelomonas sp.							
Chlorophyceae *(green algae)*							
Eudorina elegans				X			

Chlorophyceae continued	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Ankistrodesmus fusiformis							
Golenkinia radiata		X			X		
Tetraëdron minimum				X			X
T.caudatum				X			
Micractinium sp.							
Micractinium pusillum							
M. quadrisetum							
Pediastrum simplex						X	
P.duplex	X	X	X		X	X	
P.duplex var. gracillimum				X			
P. boryanum		X	X	X	X	X	X
Stauridium tetras							
Stauridium privum							
Scenedesmus sp.	X	X		X	X	X D	
Scenedesmus dimorphus							
Scenedesmus quadricauda		X	X				X
Desmodesmus subspicatus							
Desmodesmus abundans							X
Desmodesmus spinosus				X			
Tetradesmus wisconsinensis							

Chlorophyceae continued	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Coelastrum microporum					X		
Kirchneriella sp.							
Selenastrum bibraianum							
Messastrum gracile							
Selenastrum capricornutum							
Conjugatophyceae							
Euastrum sp.							
Staurodesmus sp.	X			X			X
S. aristiferus					X		
Staurastrum sp.							
Staurastrum chaetoceras			X				

Appendix 4. Identified phytoplankton species in L. Vikevannet (1,2 and 3) L. Haugestadvannet (4 and 5) and L. Hillestadvannet (6,7 and 8) July 2019.

(X = present, M = large amount present and D = dominant)

Cyanophyta	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Gomphosphaeria aponina								
Snowella lacustris		X	X			X	X	
Snowella septentrionalis			X	X	X			X
Coelosphaerium sp.							X	
Microcystis viridis	X D	X	X D	X D	X D	X D	X D	X D
Microcystis aeruginosa	X D	X D	X D	X D	X D	X D	X D	X D
Microcystis wesenbergii	X D	X D	X D	X D	X D	X D	X D	X D
Snowella atomus	X	X						
Aphanocapsa sp.	X	X		X	X	X	X	X
Aphanocapsa conferta			X	X	X	X		
Aphanocapsa reinboldii	X	X	X	X D	X D	X D		X D
Chroococcus limneticus	X	X		X	X	X		
Chroococcus minimus		X	X	X	X	X	X	
Chroococcus dispersus	X	X	X	X		X	X	X
Anathece clathrata	X D	X D	X D	X	X		X	
Achroonema proteiforme	X	X	X	X	X	X		X
Pseudanabaena catenata								
Planktothrix agardhii	X			X	X M	X	X D	X
Pseudanabaena limnetica						X		

Cyanophyta continued	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Limnothrix planctonica								
Planktolyngbya limnetica	X	X	X M	X	X	X	X D	X
Planktolyngbya contorta		X	X	X	X	X		
Pseudanabaena mucicola	X	X	X	X	X	X		X
Aphanizomenon sp.			X	X	X		X	X
Aphanizomenon flos-aquae	X	X M	X M	X	X	X	X	
Aphanizomenon gracile	X	X	X	X	X	X	X	
Dolichospermum sp.				X D		X D		X M
Dolichospermum spiroides								
Dolichospermum affine		X	X	X D	X			
Dolichospermum crassum	X	X	X	X M	X M	X	X	X
Anabaena inaequalis	X M	X D	X D	X D	X D	X D	X	X
Anabaena cylindrica								
Dolichospermum sigmoideum			X M	X M	X	X		X
Dolichospermum delicatulum								
Dolichospermum danicum							X	
Cryptophyta								
Cryptomonas sp.	X	X	X	X	X		X	
Rhodomonas lacustris							X	
Dinophyta								
Ceratium hirundinella	X		X					
Peridinium sp.	X M	X D	X D	X	X	X		X D

Chrysophyta	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Uroglena sp.	X	X	X				X	
Bicosoeca petiolata								
Chrysophyceae *(Golden algae)*								
Spiniferomonas sp.		X	X	X		X	X	
Diatomophyceae *(Diatoms)*								
Cyclotella sp.								
Aulacoseira islandica	X	X	X	X	X	X M	X D	X
Fragilaria sp.	X	X	X		X	X	X	X
Fragilaria ulna	X	X	X	X	X	X		X
Tabellaria sp.		X						
Tabellaria flocculosa								
Asterionella formosa				X	X	X		X
Amphora sp.								
Tribophyceae								
Euglena sp.								
Trachelomonas sp.								
Chlorophyceae *(green algae)*								
Eudorina elegans								
Ankistrodesmus fusiformis			X					
Golenkinia radiata								
Tetraëdron minimum	X	X	X	X	X	X	X	
T.caudatum		X					X	
Micractinium sp.								
Micractinium pusillum								
M. quadrisetum								
Pediastrum simplex								
P.duplex	X	X		X		X		
P. duplex var. gracillimum	X			X	X			
P. boryanum	X	X	X	X	X	X	X	X

Chlorophyceae continued	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Stauridium tetras								
Stauridium privum								
Scenedesmus sp.	X D	X D	X D	X D	X D	X D		X
Scenedesmus dimorphus		X						
Scenedesmus quadricauda							X	
Desmodesmus subspicatus								
Desmodesmus abundans							X	
Desmodesmus spinosus							X	
Tetradesmus wisconsinensis								
Coelastrum microporum								
Kirchneriella sp.								
Selenastrum bibraianum					X			
Messastrum gracile	X	X	X	X			X	
Selenastrum capricornutum								
Conjugatophyceae								
Euastrum sp.								
Staurodesmus sp.	X	X	X D	X			X	
S. aristiferus				X	X		X	X
Staurastrum sp.								
Staurastrum chaetoceras								

Appendix 5. Identified phytoplankton species in L. Vikevannet (1,2 and 3) L. Haugestadvannet (4 and 5) and L. Hillestadvannet (6,7 and 8) August 2019.

(X = present, M = large amount present and D = dominant)

Cyanophyta	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Gomphosphaeria aponina								
Snowella lacustris			X					X
Snowella septentrionalis	X	X	X	X	X M	X	X	X M
Coelosphaerium sp.								
Microcystis viridis	X D	X D	X D	X D	X D	X D	X D	X D
Microcystis aeruginosa	X D	X D	X D	X D	X D	X D	X D	X
Microcystis wesenbergii	X D	X D	X D	X D	X D	X D	X D	X D
Snowella atomus								
Aphanocapsa sp.				X	X			X
Aphanocapsa conferta	X		X					X
Aphanocapsa reinboldii	X D	X	D	X	X D	X D	X D	X D
Chroococcus limneticus					X			
Chroococcus minimus	X	X	X			X		
Chroococcus dispersus	X	X	X	X	X			X
Anathece clathrata	X	X		X	X	X	X	X
Achroonema proteiforme	X	X	X	X	X	X	X	X
Pseudanabaena catenata								

Cyanophyta continued	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Planktothrix agardhii	X	X	X	X	X	X		X
Pseudanabaena limnetica	X	X	X	X	X		X	X
Limnothrix planctonica				X		X		
Planktolyngbya limnetica	X	X (R)	X	X	X	X	X	X
Planktolyngbya contorta		X	X	X	X	X M	X	X M
Pseudanabaena mucicola	X		X	X	X	X	X	
Aphanizomenon sp.	X	X	X		X		X M	X M
Aphanizomenon flos-aquae	X			X	X	X	X	X M
Aphanizomenon gracile	X	X	X	X	X	X	X	X
Dolichospermum sp.	X D	X D	X	X M	X		X	X
Dolichospermum spiroides	X	X M						
Dolichospermum affine	X	X D	X M	X	X		X	X M
Dolichospermum crassum	X	X						
Anabaena inaequalis	X D	X D	X M	X	X		X	
Anabaena cylindrica								
Dolichospermum sigmoideum	X	X			X			
Dolichospermum delicatulum								

Cyanophyta continued	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Dolichospermum danicum								
Cryptophyta								
Cryptomonas sp.	X	X	X	X D	X D	X	X D	X
Rhodomonas lacustris								
Dinophyta								
Ceratium hirundinella		X	X	X	X		X	
Peridinium sp.	X	X	X	X	X	X		X
Chrysophyta								
Uroglena sp.			X			X		
Bicosoeca petiolata				X				
Chrysophyceae *(Golden algae)*								
Spiniferomonas sp.			X		X	X		X
Diatomophyceae *(Diatoms)*								
Cyclotella sp.								
Aulacoseira islandica	X	X	X	X M	X D	X M	X	X
Fragilaria sp.	X	X	X	X	X			
Fragilaria ulna	X	X	X	X	X D	X	X	X
Tabellaria sp.					X			
Tabellaria flocculosa								
Asterionella formosa		X	X	X	X			
Amphora sp.					X			
Tribophyceae								
Euglena sp.								X
Trachelomonas sp.								
Chlorophyceae *(Green algae)*								
Eudorina elegans								

Chlorophyceae continued	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Ankistrodesmus fusiformis	X					X		
Golenkinia radiata								
Tetraëdron minimum	X			X	X	X	X	X
T.caudatum								
Micractinium sp.								
Micractinium pusillum								
M. quadrisetum								
Pediastrum simplex			X					
P.duplex	X	X	X	X	X	X	X	X
P.duplex var. gracillimum						X	X	
P. boryanum	X	X	X	X	X	X	X	
Stauridium tetras				X				
Stauridium privum					X			
Scenedesmus sp.	X	X	X D	X D	X D	X D	X D	X D
Scenedesmus dimorphus								
Scenedesmus quadricauda								
Desmodesmus subspicatus								
Desmodesmus abundans								
Desmodesmus spinosus								
Tetradesmus wisconsinensis								

Chlorophyceae continued	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Coelastrum microporum								
Kirchneriella sp.								
Selenastrum bibraianum	X	X	X	X				
Messastrum gracile								
Selenastrum capricornutum				X				
Conjugatophyceae								
Euastrum sp.								
Staurodesmus sp.	X	X		X	X	X	X	X
S. aristiferus					X	X		
Staurastrum sp.								
Staurastrum chaetoceras								

Appendix 6. Identified phytoplankton species in L. Vikevannet (1,2 and 3) L. Haugestadvannet (4 and 5) and L. Hillestadvannet (6,7 and 8) September 2019.

(X = present, M = large amount present and D = dominant)

Cyanophyta	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Gomphosphaeria aponina			X	X	X			
Snowella lacustris	X	X				X		
Snowella septentrionalis	X M	X M	X	X M	M	X	X	X M
Coelosphaerium sp.								
Microcystis viridis	X	X	X	X	X	X	X D	X M
Microcystis aeruginosa	X	X	X	X	X	X	X D	X M
Microcystis wesenbergii	X	X L	X D	X M	X M	X M	X D	X M
Snowella atomus								
Aphanocapsa sp.		X	X	X	X	X M	X	X
Aphanocapsa conferta	X							
Aphanocapsa reinboldii	X	X	X D	X	X M	X	X D	X M
Chroococcus limneticus		X	X					
Chroococcus minimus	X	X			X	X	X	
Chroococcus dispersus	X	X	X		X	X	X	X
Anathece clathrata	X M	X	X	X	X M	X M	X	X M
Achroonema proteiforme	X M	X	X	X M	X	X	X	X
Pseudanabaena catenata								

Cyanophyta continued	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Planktothrix agardhii		X	X M	X	X M	X		X
Pseudanabaena limnetica			X	X	X			
Limnothrix planctonica								
Planktolyngbya limnetica	X	X	X	X	X	X	X	X
Planktolyngbya contorta	X	X M	X	X	X M	X M	X	X
Pseudanabaena mucicola	X		X	X	X			X
Aphanizomenon sp.	X	X	X	X	X			X
Aphanizomenon flos-aquae	X		X D	X	X	X L	X	X
Aphanizomenon gracile	X	X	X D	X		X L		
Dolichospermum sp.		X	X		X	X		
Dolichospermum spiroides			X					
Dolichospermum affine	X L	X	X M	X	X	X	X	X
Dolichospermum crassum			X					
Anabaena inaequalis	X		X					
Anabaena cylindrica								
Dolichospermum sigmoideum			X				X	
Dolichospermum delicatulum								

Cyanophyta continued	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Dolichospermum danicum								
Cryptophyta								
Cryptomonas sp.	X D	X D	X	X D	X D	X D	X D	X D
Rhodomonas lacustris								
Dinophyta								
Ceratium hirundinella	X		X	X	X			X
Peridinium sp.		X	X	X	X		X	X
Chrysophyta								
Uroglena sp.						X	X	X
Bicosoeca petiolata		X	X	X M	X		X	X
Chrysophyceae *(Golden algae)*								
Spiniferomonas sp.	X	X			X	X	X	X
Diatomophyceae *(Diatoms)*								
Cyclotella sp.		X				X	X	X
Aulacoseira islandica	X M	X	X	X	X D	X D	X D	X D
Fragilaria sp.	X	X	X	X	X	X	X	X
Fragilaria ulna	X M	X	X	X D	X D	X	X M	X
Tabellaria sp.								
Tabellaria flocculosa				X				
Asterionella formosa	X	X			X	X	X	X
Amphora sp.								
Tribophyceae								
Euglena sp.								X
Trachelomonas sp.						X		
Chlorophyceae *(Green algae)*								
Eudorina elegans								

Chlorophyceae continued	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Ankistrodesmus fusiformis						X		X
Golenkinia radiata								
Tetraëdron minimum		X	X	X M	X	X	X	X
T.caudatum								
Micractinium sp.						X		
Micractinium pusillum		X						
M. quadrisetum			X					X
Pediastrum simplex								
P.duplex	X	X		X	X	X	X	
P.duplex var. gracillimum				X	X			X
P. boryanum	X	X	X	X	X	X		X
Stauridium tetras	X	X		X	X			
Stauridium privum					X			
Scenedesmus sp.	X D	X D	X	X D	X D	X D	X	X
Scenedesmus dimorphus						X		
Scenedesmus quadricauda								
Desmodesmus subspicatus			X					
Desmodesmus abundans								
Desmodesmus spinosus								
Tetradesmus wisconsinensis		X						

Chlorophyceae continued	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Coelastrum microporum								
Kirchneriella sp.						X		
Selenastrum bibraianum			X	X	X		X	
Messastrum gracile			X					
Selenastrum capricornutum								
Conjugatophyceae								
Euastrum sp.	X							
Staurodesmus sp.	X	X	X	X	X	X	X	X
S. aristiferus	X	X		X				
Staurastrum sp.		X						
Staurastrum chaetoceras								