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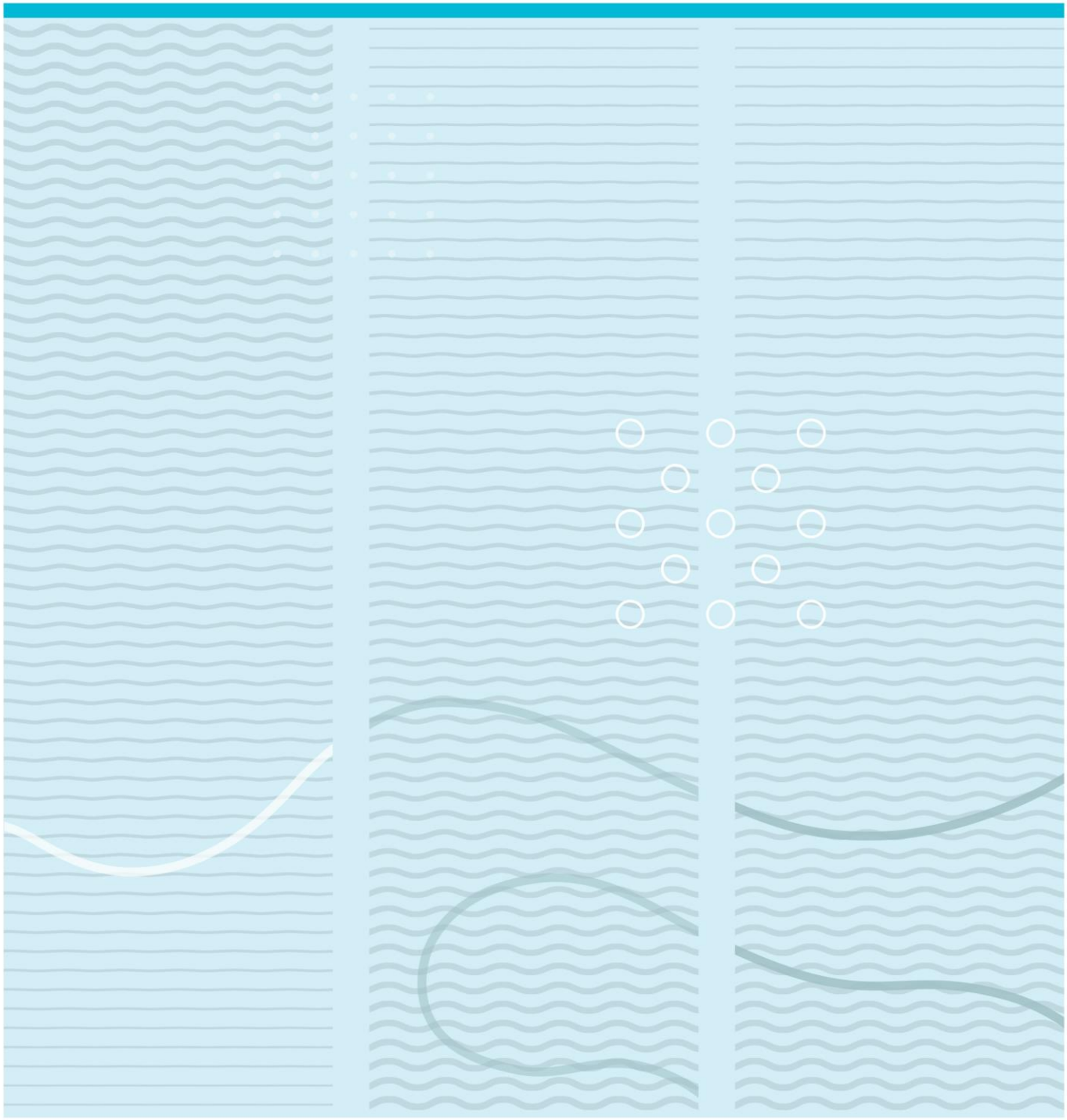
Katrine Kjellstrøm-Hoel

Harmful cyanobacteria and its toxic metabolites microcystin and saxitoxin in freshwater lakes of Southeast Norway.



University College of Southeast Norway
Faculty of Arts and Sciences
Department of Environment- and Environmental Health Studies
PO Box 235
NO-3603 Kongsberg, Norway

http://www.usn.no

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Harmful cyanobacteria and its toxic metabolites microcystin and saxitoxin in freshwater lakes of Southeast Norway.

Katrine Kjellstrøm-Hoel

Hellestvedtveien 30

3965 Herre

Norway

katrine.kjellstrom-hoel@mattilsynet.no

Abstract

Harmful cyanobacteria are a globally growing concern due to global warming and eutrophication. Cyanobacteria are capable of producing a variety of cyanotoxins, which could be harmful to animals and human health. In this study, the presence of cyanobacteria and its toxic metabolites microcystin and saxitoxin were investigated in six freshwater lakes in Vestfold, Southeast Norway, by using microscopic- , immunological- (ELISA) and molecular techniques (PCR). Cyanobacteria were detected in 11 out of 12 samples. *Dolichospermum* was the most frequently occurring genera, while *Microcystis* was the most abundant. Even though one lake showed N-limitation, which could favour the growth of N-fixing cyanobacteria, the lake was dominated by *Microcystis*, probably due to the high total concentrations of N and P. Microcystin and saxitoxin were detected in 8 and 4 samples, respectively, concentrations of microcystins ranging from 0,7-32,2 µg/L and saxitoxin from 0,05 to 0,146 µg/L. The mcyE gene was detected in five samples, while the sxtA gene was detected in only one sample. In all samples where mcyE and sxtA were detected, microcystin and saxitoxin were also detected. Even though microcystin and saxotoxin were detected in samples without mcyE/sxtA as well, there seemed to be a positive correlation between toxin concentration and gene detection. Based on microscopic findings and toxin- and gene-detection, there is a strong indication that the likely candidates responsible for microcystin and saxitoxin production in the samples belonged to the genera *Microcystis* and *Dolichospermum*, respectively.

1. Introduction

As the world´s population increases, large parts of the earth's surface are converted into agricultural and urban land, resulting in massive leakage of nutrients like nitrogen and phosphorus into freshwater systems (Jeppesen et al., 2014). Increased nutrient levels in combination with higher temperatures due to climate changes may exacerbate many symptoms of eutrophication in freshwater lakes. This means a higher risk of algal blooms and cyanobacterial dominance (Kernan et al., 2010). In temperate zones, these water blooms usually occur during late summer and early autumn, subsiding during the cold and dark autumn and winter period (Sivonen and Jones, 1999).

The cyanobacterial masses worsen the oxygen and light conditions in the water, which can result in reduction in the numbers and diversity of submerged plants, killing of aquatic animals, and alteration in food web dynamics (Stewart, 1973; Turner and Chislock, 2010).

Furthermore, a number of cyanobacterial species produce bioactive toxins that are harmful to humans and animals (Sivonen and Jones, 1999; Dittmann and Wiegand, 2006). The most common and widespread of these toxins are microcystins, associated with several bloom-forming cyanobacteria like *Dolichospermum, Microcystis* and *Planktothrix* (Codd et al., 2005; Falconer and Humpage, 2005). Acute exposure to microcystin can lead to liver failure and death (Kuiper-Goodman et al., 1999), while sub chronic exposure is associated with tumour promotion (Hitzfeld et al., 2000). Given their high toxicity, the World Health Organization (WHO) has placed microcystin on the list of potential health hazards, and has defined a drinking water guideline value of maximum 1 µg/L (WHO, 1998). WHO has established guidelines for recreational waters as well, which represent different levels of human health risks and correspond to expected microcystin concentrations of 4 µg/L (low risk), 20 µg/L (moderate risk) or even higher microcystin concentrations occurring during scum formation (high risk) (WHO, 2003).

Another type of cyanotoxins are saxitoxin, associated with cyanobacterial genera like *Aphanizomenon, Cylindrospermopsis, Dolichospermum and Planktolyngbya*. An oral dose of

between 1 to 4 mg saxitoxin is lethal for humans, resulting in cardiovascular failure and respiratory muscle paralysis (Aráoz et al., 2010).

Due to their great health threat, it is important to have simple, fast and cheap methods available to monitor waterbodies for the presence of these harmful cyanobacteria and their toxins.

A relatively simple and rapid method for cyanotoxin detection is enzymelinked immunosorbent assay (ELISA). Commercial ELISA kits are available, which are based on the recognition of among others saxitoxin and microcystin and its congeners by specific antibodies (Abraxis).

The method for identification of potential toxic cyanobacteria species have been, until recently, traditional identification by light microscopy. This method has its limitations as it does not differentiate between toxin-producing and non-toxin-producing cyanobacteria and is also laborious and time-consuming. Recently, several studies have applied molecular methods for monitoring the presence of harmful cyanobacteria and the genes involved in the synthesis of cyanotoxins. Screening based on genetic targeting using the Polymerase chain Reaction (PCR) is economically competitive, more precise and sensitive, less time-consuming, and able to distinguish toxic from nontoxic strains (Beltran et al., 2000; Pearson et al., 2008.)

In the county of Vestfold in Southeast Norway, a few waterbodies have been monitored due to massive phytoplankton blooms and fish deaths during the recent years (Berge, 2014). Even so, overall knowledge about the presence of cyanobacteria and the associated cyanotoxin production in popular recreational freshwater lakes in this area is scarce.

The main objectives of this work is therefore to achieve more knowledge about the phytoplankton communities in six freshwater lakes in Vestfold, Southeast Norway, by using microscopic- , immunological- (ELISA) and molecular techniques (PCR).

2. Materials and methods

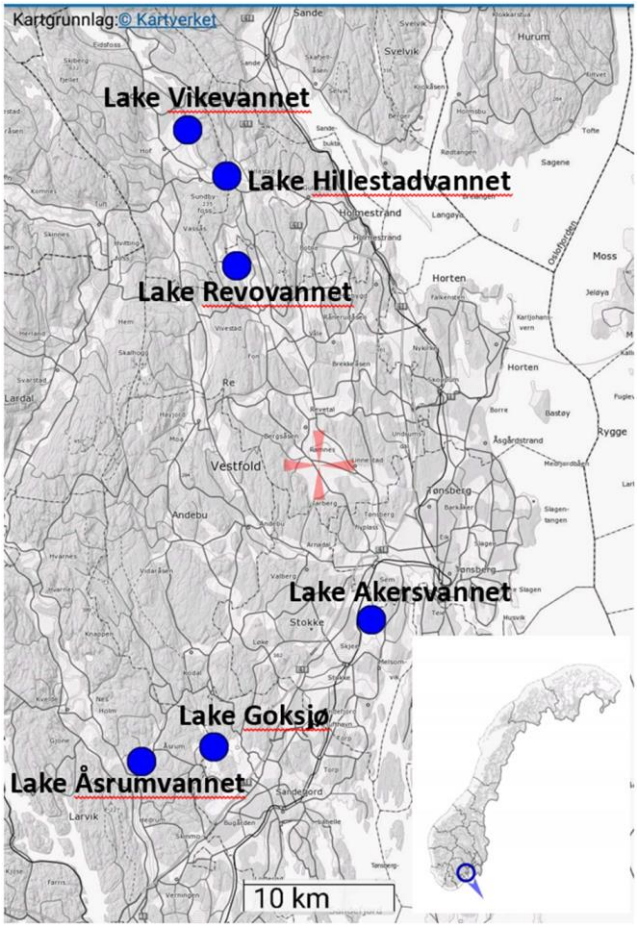


Figure 1. Location of the six investigated lakes in Vestfold county of Southeast Norway. (Source:Kartverket)

2.1 Study sites and water sampling

Samples for phytoplankton and water quality parameters were taken 5th of July and 23rd of August 2015 from six freshwater lakes in the county Vestfold, Southeast Norway; Lake Akersvannet, L., Goksjø, L. Hillestadvannet, L. Revovannet, L. Vikevannet and L. Åsrumvannet (Figure 1). Morphometrical parameters of these lakes are presented in Table 1. The main human impact on these lakes are drainage from agricultural land and human wastes (vann-nett.no). The lakes are mainly used for recreational purposes like swimming and fishing. L. Akersvannet is also used as a water source in agriculture. L. Vikevannet and L. Hillestadvannet have permanent warning signs which discourage swimming during phytoplankton blooms. Surface water was collected by boat approximately 50 meters from shore. Temperature and Secchi depth were recorded in situ.

Conductivity and pH were measured in the laboratory a few hours after water sampling. For phytoplankton species determination, surface water was collected by using a phytoplankton net (mesh size 25 µm). Quantitative phytoplankton samples were taken from the lake surface. All phytoplankton samples were immediately preserved with Lugol solution and stored at 4 °C in darkness.

Table 1. Morphometrical characteristics of the six investigated lakes in Vestfold county, Southeast Norway. (Berge and Brettum, 1999; Lunde and Kock, 2010; Johansen, 2014)

Lake	Latitude	Longitude	Surface area (km^2)	Maximum depth (m)	Elevations (masl)
Vikevannet	59, 54	10, 11	0,79	9	37
Hillestadvannet	59, 52	10, 15	1,57	3	37
Revovannet	59, 46	10, 17	1,66	Unknown	44
Akersvannet	59, 25	10, 33	2,40	13	16
Goksjø	59, 18	10, 14	3,40	25	29
Åsrumvannet	59, 16	10, 06	1,15	17	23

Surface water for toxin analysis was collected in glass containers and immediately deep-frozen (-21 °C). For DNA analysis, surface water was collected in 1 L plastic bottles and filtered through cellulose nitrate filters 0,2 µm pore size. The filters were stored at – 21 °C. Prior shipment to New Zealand, the filters were freeze dried in a Heto Lyolab 3000 freeze drier.

2.2 Phytoplankton determination

Samples were examined using an inverted microscope (Olympus CK2) at 100 and 400 x magnification, and 10 mL sedimentation chambers for phytoplankton cell counts. The taxonomic identification was based on microscopic observations of distinctive morphological features, using Växtplanktonflora by Tikkanen and Willén (1992). The phytoplankton volumes were calculated according to Räkningsförfarande av växtplankton vid laboratoriet för miljökontroll, Uppsala by Willén et al. (1985).

2.3 Toxin analysis using ELISA-technique

Frozen water samples were thawed, re-frozen and thawed again prior to analysis. Analysis were performed for microcystin and saxitoxin using commercial available ELISA kits (Microcystin ADDA ELISA kit, Saxitoxin PSP ELISA kit, Abraxis, Warminster, USA).

2.4 DNA extraction and analysis

DNA extraction and analysis were performed by dr. Susanna Wood and associates at Cawthron Institute, Nelson, New Zealand.

DNA was extracted from the freeze dried nitrate cellulose membrane filters using a Power Soil DNA Isolation Kit (MO BIO, USA) according to the protocol supplied by the manufacturer.

Polymerase Chain Reaction (PCR) of the following genes was undertaken: *mcyE* for microcystins using the HEPF/HEPR primers (Jungblut and Neilan, 2006), and *sxtA* for saxitoxins using the sxtaf/sxtar primers (Ballot et al. 2010). Reactions were carried out using 25 μL of i-Taq 2× PCR master mix (Intron, Korea), 0.4 μM of each primer, and template DNA (30-50 ng). The PCR conditions for the *mcyE* primers were 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 60 s, with a final extension of 72 °C for 5 min. Reactions for the *sxtA* were held at 94 °C for 2 min, followed by 30 cyclesof 94 °C for 30 s, 55 °C for 30 s, 72 °C for 60 s, and a final extension of 72 °C for 5 min. PCR products were visualized by 1% agarose gel electrophoresis with ethidium bromide staining and UV illumination.

2.5 Statistical analysis

Fisher Exact test of independence was used to determine the relationship between the presence/absence of mcyE/sxtA genes and microcystin/saxitoxin. This is a test of significance which is used instead of chi-square test in case of small sample size (McDonald, 2015).

3. Results

3.1 Physico-chemical parameters

Temperatures ranged from 18,5 °C to 19,9 °C in July, and from 20,0 °C to 21,4 °C in August in the six investigated lakes. The pH interval was between 7,2 and 10,1, with the maximum in L. Hillestadvannet and L. Vikevannet in July. Secchi-depth were <1 meter in all lakes, except L. Goksjø and L. Åsrumvannet with secchi-depth 2 and 3 meters, respectively. Total Nitrogen (TN) was at lowest 859 µg/L in L. Revovannet and maximum 3313 µg/L in L. Hillestadvannet, both measurementes from August (Table 2).

Table 2. Total Nitrogen (µg/L), Total Phosphorus (µg/L) and Total Nitrogen:Total Phosphorus ratio for the six investigated lakes in July and August 2015.

Lake	Date	pH	Total Nitrogen (µg/L)	Total Phosphorus (µg/L)	TN:TP
L. Akersvannet	05.07.2015	9,6	2458	64	38
	23.08.2015	9,5	2015	82	25
L. Goksjø	05.07.2015	7,7	2109	31	68
	23.08.2015	7,8	1571	26	60
L. Hillestadvannet	05.07.2015	10,1	1821	135	13
	23.08.2015	8,7	3313	57	58
L. Revovannet	05.07.2015	9,4	2101	49	68
	23.08.2015	7,8	859	31	28
L. Vikevannet	05.07.2015	10,1	1255	55	23
	23.08.2015	7,2	1194	31	39
L. Åsrumvannet	05.07.2015	8,5	1780	12	162
	23.08.2015	9	1464	21	70

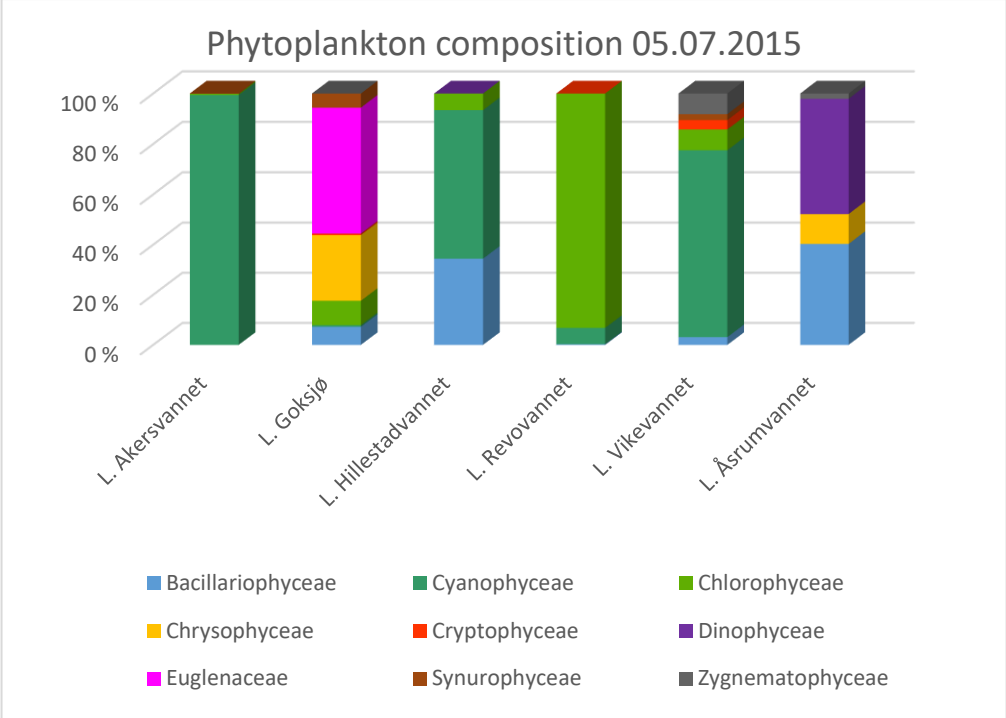
The maximum Total Phosphorus (TP) concentration was 135 µg/L, measured in L. Hillestadvannet in July, while the minmum was 12 µg/L, in L. Åsrumvannet in July. The lowest Chlorophyll-a concentration was measured in L. Åsrumvannet in July (3 µg/L), while the highest in L. Hillestadvannet in July (62 µg/L). See Appendix for detailed results.

3.2 Phytoplankton community

The total phytoplankton biovolume ranged from 0,024 mm^3/L in L. Åsrumvannet in July to 547 mm^3/L in L. Revovannet in July. Lake Akersvannet had a massive cyanobacteria bloom in August, with a total biomass of 286 mm^3/L (Figure 2).

Cyanobacteria were microscopically observed in 11 out of the total 12 samples collected (Table 3). The relative abundances of cyanobacteria ranged from <1 up to 98,7 %. Cyanobacteria was the dominant (≥50 %) group of phytoplankton in 5 samples; L. Akersvannet and L. Hillestadvannet in both July and August, and L. Vikevannet in July (Figure 3).

Figure 2. Massive phytoplankton bloom in L. Akersvannet in August 2015 (Photo: County governor of Vestfold).



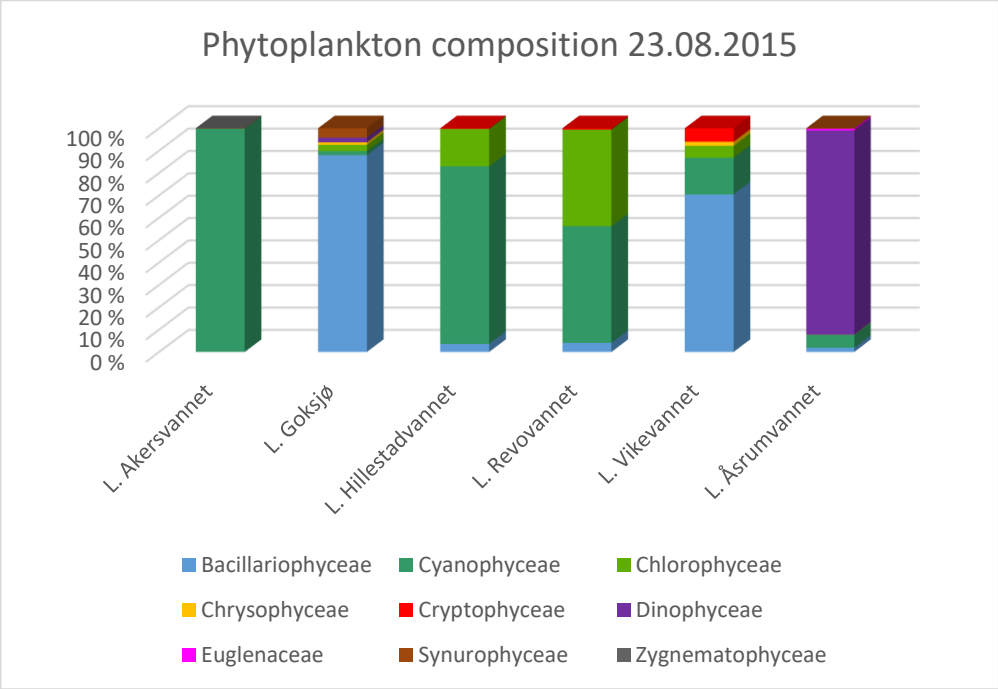


Figure 3.Composition (%) of the phytoplankton community in the six investigated lakes in July and August 2015.

The most abundant cyanobacterial genus, detected in 4 out of 6 locations and 8 out of 12 samples (66,7 %), was *Microcystis*. *Microcystis* was dominant (≥50 %) in 4 samples; L. Akersvannet in both July and August, L. Hillestad in August and L. Vikevannet in July. The most frequently occurring cyanobacterial genus in this study was *Dolichospermum*, which was found in all 6 lakes and 9 out of 12 samples (75 %). This genus dominated phytoplankton communities less frequently than *Microcystis*. Other cyanobacterial genera frequently observed were *Planktolyngbya* (5 out of 12 samples), *Chroococcus* and *Aphanizomenon* (both in 3 out of 12 samples).

3.3 Microcystin and saxitoxin detection by ELISA

Microcystin was detected by ELISA technique in 8 out of 12 samples (Figure 4). Lake Akersvannet, L. Hillestadvannet, L. Revovannet and L. Vikevannet all contained microcystin both in July and August. The concentrations ranged from 0,7 µg/L in L. Revovannet in August to 32,2 µg/L in L. Vikevannet in July.

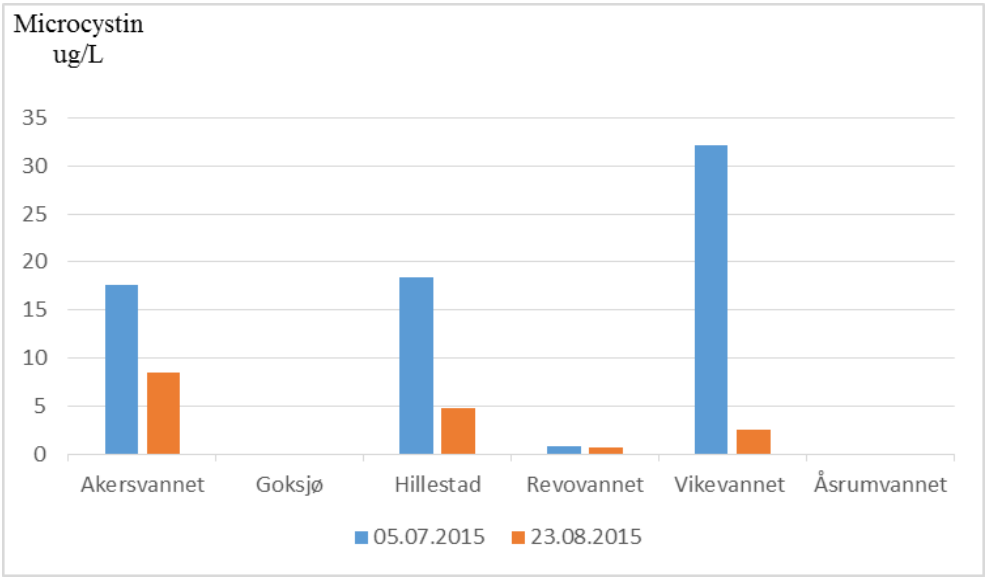


Figure 4. Dection of microcystin (µg/L) by ELISA-technique in the six investigated lakes in July and August 2015

*Table 3. Total biovolume (mm³/L) and composition/abundance of the cyanobacteria community in the six lakes investigated. Dominating species (≥50 %) in **bold**. M = microcystin, S = saxitoxin (Jančula, 2014).*

Lake	Date	Total biovolume (mm³/L)	Cyanobacteria detected	Potential toxins produced	Abundance (%)
Akersvannet	05.07.2015	188	*Aphanizomenon*	S	5,9
			Microcystis	M	93,8
			Snowella	M	0,06
	23.08.2015	286	*Aphanizomenon*	S	1,5
			Dolichospermum	M, S	0,07
			Microcystis	M	98,2
			Woronichinia	M	0,03
Goksjø	05.07.2015	0,9	*Dolichospermum*	M, S	0,7
			Snowella	M	0,003
	23.08.2015	6,6	*Woronichinia*	M	1,8
Hillestadvannet	05.07.2015	2,8	*Dolichospermum*	M, S	0,13
			Limnothrix	M	0,6
			Microcystis	M	47,8
			Planktolyngbya	M, S	0,01
			Pseudoanabaena	M	11,3
	23.08.2015	3,4	*Dolichospermum*	M, S	0,18
			Microcystis	M	79,11
Revovannet	05.07.2015	547	*Chroococcus*	Unknown	2,9
			Dolichospermum	M, S	0,8
			Microcystis	M	2,6
			Planktolyngbya	M, S	0,15
	23.08.2015	2,5	*Chroococcus*	Unknown	5,4
			Dolichospermum	M, S	14,8
			Merismopedia	M	5,4
			Microcystis	M	15,6
			Planktolyngbya	M, S	13,3
Vikevannet	05.07.2015	3,7	*Aphanocapsa*	M	0,1
			Chroococcus	Unknown	1
			Dolichospermum	M, S	0,4
			Microcystis	M	70,6
			Planktolyngbya	M, S	1
			Pseudoanabaena	M	1,3
	23.08.2015	1,3	*Aphanizomenon*	S	0,3
			Dolichospermum	M, S	4,3
			Microcystis	M	7,2
			Planktolyngbya	M, S	3,7
			Radiocystis	M	0,8

The occurrence of microcystins corresponded well with phytoplankton communities containing and/or being dominated by potential microcystin-producing cyanobacterial generas, i.e., *Microcystis* or *Dolichospermum*. Based on WHO guidelines for microcystin concentrations in recreational water, 58 % of samples indicated a low risk, 33,3 % a moderate risk and 8,3 % a high risk (Table 4).

Table 4. Microcystin concentrations (µg/L) in the six lakes and the risk assessment based on the WHO-guidelines for recreational waters; micocystin concentrations ≤ 4 µg/L (low risk), 4-20 µg/L (moderate risk) or >20 µg/L (high risk) (WHO, 2003).

Lake	Sample date	Microcystin concentration	Risk level based on WHO-guidelines
Akersvannet	05.07.2015	17,7	Moderate risk
	23.08.2015	8,5	Moderate risk
Goksjø	05.07.2015	N. D.	Low risk
	23.08.2015	N. D.	Low risk
Hillestadvannet	05.07.2015	18,4	Moderate risk
	23.08.2015	4,8	Moderate risk
Revovannet	05.07.2015	0,8	Low risk
	23.08.2015	0,7	Low risk
Vikevannet	05.07.2015	32,2	High risk
	23.08.2015	2,5	Low risk
Åsrumvannet	05.07.2015	N. D.	Low risk
	23.08.2015	N. D.	Low risk

Saxitoxin was detected in 4 out of 12 samples, concentrations ranged from 0,05 µg/L in L. Vikevannet in August to 0,146 µg/L in L. Revovannet in July (Figure 5). L. Hillestadvannet contained saxitoxin in both July and August, while L. Revovannet and L. Vikevannet contained saxitoxin in July and August, respectively. Neither microcystin nor saxitoxin were detected in L. Goksjø and L. Åsrumvannet.

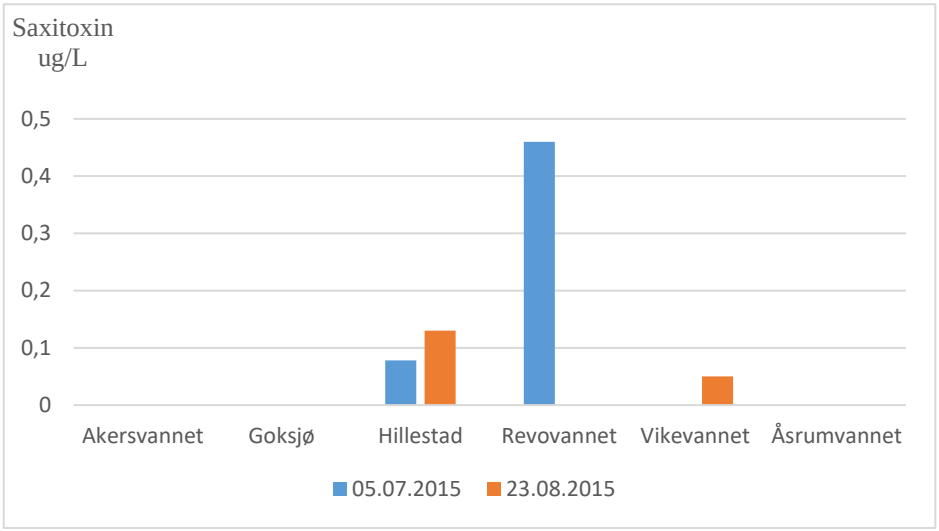


Figure 5: Dection of saxitoxin (µg/L) by ELISA-technique in the six investigated lakes in July and August 2015.

3.4 McyE and sxtA detection by PCR

The mcyE gene was detected in 5 of the 12 samples. L. Hillestad and L. Revovannet were mcyE-positive in both July and August, while L. Vikevannet was positive in July only. The samples were divided into two groups according to whether mcyE was detected (n= 5) or not detected (n=8), and the microcystin concentrations in these samples were compared (Figure 6).

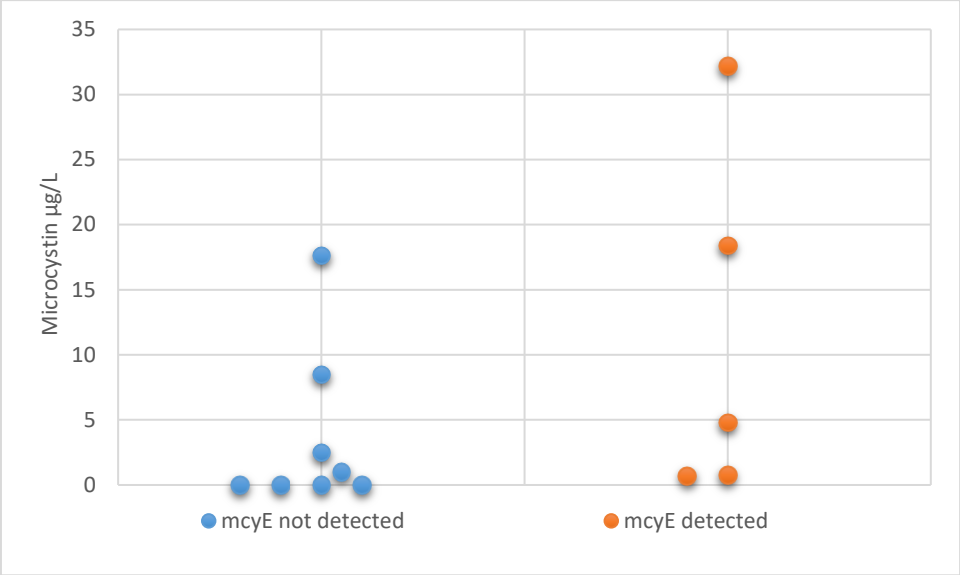


Figure 6. The distribution of microcystin concentration (µg/L) within the two sample groups 1) mcyE not detected (n=8) and 2) mcyE detected (n=5).

In all samples that were mcyE positive, microcystin was detected as well. Three samples with considerable microcystin concentration were mcyE negative. Although there is some overlapping, there is a significant correlation between microcystin detection and mcyE positive samples (Fisher exact test, level of significance = $P < 0,1$). The most abundant cyanobacterial genus, *Microcystis*, and the most frequently occurring genus, *Dolichospermum*, both co-occurred with microcystin and mcyE genes in 41,7 % of the samples as shown in Figure 7. In addition, *Aphanocapsa*, *Limnothrix*, *Merismopedia, Planktolyngbya and Pseudoanabena* are potential microcystin producers that co-occurred with both microcystin and mcyE.

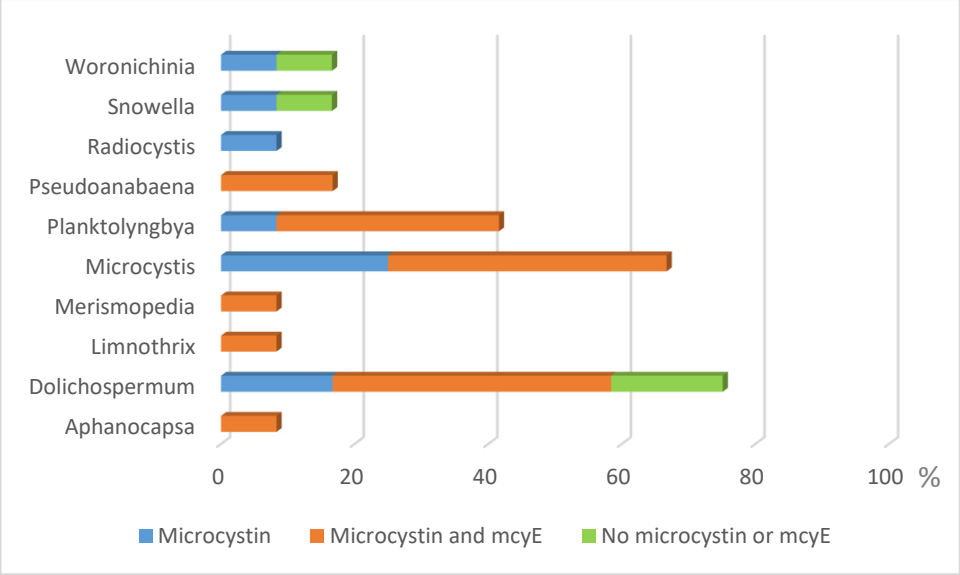


Figure 7. Prevalence (%) of potential microcystin-producing cyanobacterial genera in the samples studied (n= 12). The total percentage of samples containing each genus, divided into three categories containing either microcystin only, both microcystin and mcyE or neither of the two.

The stxA gene was detected in one sample only, L. Revovannet in July. The samples were divided into two groups according to whether sxtA was detected (n= 1) or not detected (n=11), and the saxitoxin concentrations in these samples were compared (Figure 8). The sample in which sxtA was detected, the highest concentration of saxitoxin was detected as well. In addition, three samples that contained saxitoxin were sxtA negative.

There was no significant correlation between saxitoxin detected and stxA positive samples (Fisher exact test, level of significance = $P < 0,1$). *Dolichospermum* and *Planktolyngbya* are potential saxitoxin-producing cyanobacteria that both co-occurred with saxitoxin and sxtA genes in one sample (L. Revovannet in July) (Figure 9). In addition, *Aphanizomenon* is a potential saxitoxin producer that co-occurred with saxitoxin in one sample (L. Vikevannet in August).

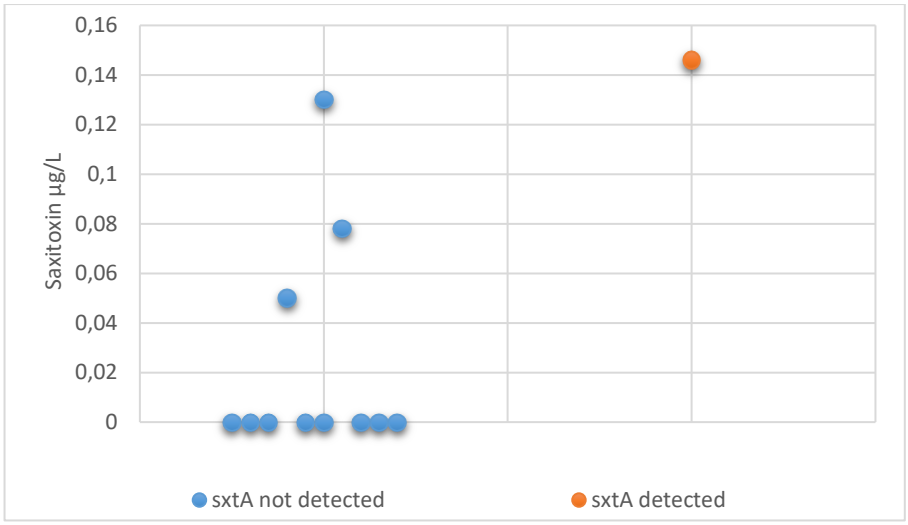


Figure 8. The distribution of saxitoxin concentration (µg/L) within the two sample groups 1) sxtA not detected (n=11) and 2) sxtA detected (n=1).

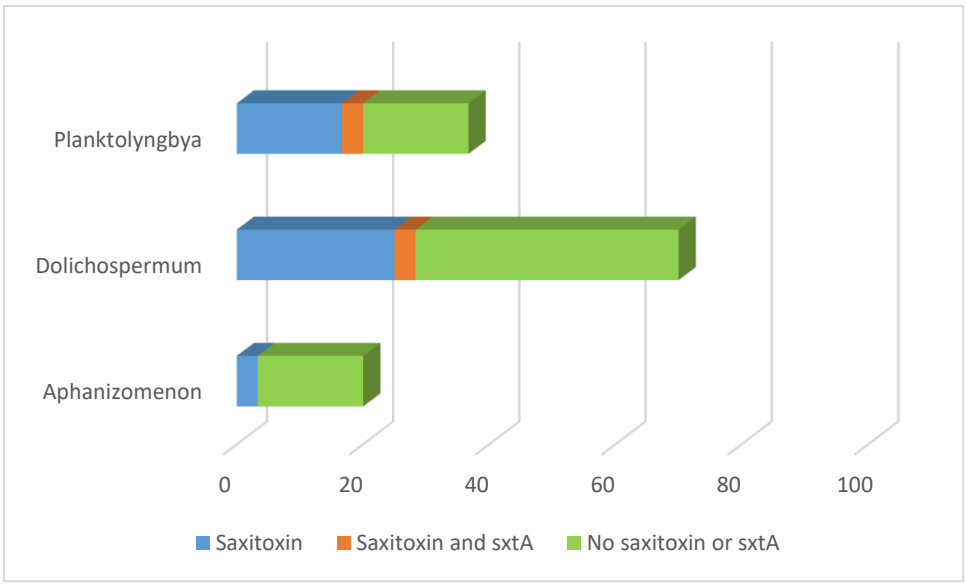


Figure 9. Prevalence (%) of potential saxitoxin-producing cyanobacterial genera in the samples studied (n= 12). The total percentage of samples containing each genus, divided into three categories containing either saxitoxin only, both saxitoxin and sxtA or neither of the two.

4. Discussion

Cyanobacteria and their toxins are becoming an increasing threat to human and animal health, it is therefore important to have simple, fast and cheap methods available for monitoring waterbodies (Dittmann and Wiegand, 2006; Jeppesen et al. 2014). In recent years, PCR has been more frequently used to screen environmental samples for the genes responsible for the production of cyanotoxins, because traditional microscopic detection of cyanobacteria cannot differentiate between toxic and non-toxic populations (Li et al., 2011; Savela et al., 2015).

In this study, a visible, dense bloom dominated by cyanobacteria was only observed in L. Akersvannet in August, but a pronounced dominance of cyanobacteria was found in lakes lacking visible blooms as well. All lakes had relatively high TN:TP ratio (TN:TP <23), except L. Hillestadvannet in July which showed N-limitation (TN:TP=13). With low N relative to P conditions, it is predicted that growth of N-fixing cyanobacteria like *Aphanizomenon* and *Dolichospermum* are favoured due to their ability to fix atmospheric N and produce ammonium necessary for growth (Dolman et al., 2012; Berge et al., 2014). An increase of TN and/or TN:TP ratio may initiate the decline and disappearance of *Aphanizomenon* and *Dolichospermum*, and trigger the growth and population development of *Microcys*tis (Wu et al., 2016).

Interestingly, *Microcystis* was, by far, the most dominant cyanobacterial genus in both L. Akersvannet and L. Hillestadvannet. Even though L. Hillestadvannet showed N-limitation in July, no *Aphanizomenon*, and only a small percentage of *Dolichospermum* (0,13 %), was detected. A study done in L. Hillestadvannet in 2013, revealed N-limitation in both July (TN:TP=12,3) and August (TN:TP=6,7), with *Microcystis* and *Aphanizomenon* as the dominant genera (Berge, 2014). The shift in cyanobacteria community to a solely *Microcystis* dominance in 2015, could probably be explained by the dramatic increase in both TN (from 665 µg/L to 2566 µg/L) and TP (from 70 µg/L to 96 µg/L) in this lake. This suggests that when the joint concentrations of TN and TP are high, the TN:TP ratio will no longer be significant for nutrient limitation. N-fixing is an energy-demanding process, which requires light. In a eutrophic lake with dense *Microcystis* masses, light may become a limiting factor (Ward and Wetzel, 1980). In addition, other factors like iron (Fe) and molybdenum (Mo)

concentrations, and temperature may affect the N-fixation and growth of cyanobacteria (Wurtsbaugh, 1988).

A study from 2013 in L. Akersvannet revealed a TN:TP ratio of 26,8 in July, with large amounts of *Aphanizomenon* (76,5%), and N-limitation (TN:TP=10,7) in August with a much lower concentration of the same cyanobacterial genus (6,6 %) (Johansen 2014). This is a contradiction to the hypothesis that N-fixing cyanobacteria are favoured by N-limited conditions. From a study done in German lakes, it was also observed that low N:P ratio did not always correspond to abundance of N-fixing cyanobacteria. It was therefore suggested that N-fixing cyanobacteria should not be treated as a single group regarding response to nutrient load (Dolman et al., 2013). Beversdorf et al. (2013), discovered that cyanobacteria community strongly correlated with dissolved inorganic nitrogen (DIN) concentrations, and that *Microcystis* and *Aphanizomenon* alternated dominance during growth season, depending on the level of DIN in the water. Furthermore, they suggested that relatively high concentrations of both N and P could allow *Microcystis* and *Aphanizomenon* to coexist at the same time.

Microcystin was the most abundant cyanotoxin in our study, which most likely corresponds to *Microcystis* being the most dominant cyanobacterial genus in the six investigated freshwater lakes. The microcystin concentration was above the WHO safety limit for drinking water (1 µg/L) in 50 % of the samples, and presenting a moderate to high risk regarding the recommended concentration for recreational waters (>4 µg/L) in L. Akersvannet and L. Hillestadvannet in both July and August, and L. Vikevannet in July. Surprisingly, the highest concentration of microcystin was detected in L. Vikevannet (32,2 µg/L), with no visible bloom. The water appeared clear, and children and dogs were swimming and playing in the lake. Already back in 1989, microcystin was detected in L. Akersvannet, L. Goksjø, L. Hillestadvannet, L. Revovannet, and L. Vikevannet (Skulberg, 1989). In 2013, microcystin concentrations ranged between 30-40 ug/L in L. Hillestadvannet (Berge, 2014), but was only detected in trace amounts in L. Akersvannet (Johansen, 2014).

Saxitoxins occurred in our samples at much lower frequency and at lower concentrations than microcystin. Saxitoxin was detected in L. Hillestadvannet in both July and August, with concentrations of 0,078 µg/L and 0,13 ug/L, respectively. In comaparison, saxitoxin was not detected

in this lake in either months by Berge in 2013. Saxitoxin was detected in 33,3 % of our samples. This is is a slightly higher prevalence of saxitoxin in the environment, but with similar concentration levels,compared to what have been reported from other European countries. In the Czech republic and Denmark, saxitoxin was detected in 7 % and 11 % of the samples, respectively, with concentrations ranging from 0,03-0,29 µg/L (Kaas and Henriksen, 2000; Jančula et al., 2014). Similar results were also detected in studies from Spain and Greece, were concentrations of saxitoxin ranged from 0,03-1,2 µg/L (Wörmer, 2011; Gkelis and Zaoutsos, 2013). In contrast to the latter results, the saxitoxin concentrations detected in a survey from Finland were noticeably higher than what was measured in other European countries, ranging from 33 to 1070 µg/L (Rapala et al., 2005).

The mcyE gene was detected in five out of twelve (42 %) samples and sxtA in one (8 %) sample. Previous studies from other countries have been yielding variable results. In a study in Australia, the sxtA gene was detected in 86-100 % of the samples (Bowling et al., 2013), while in studies from New Zealand and Japan all samples were negative (Hodoki et al., 2012; Wood et al., 2014). In a study from Greece, mcyA region was only amplified when the microcystin concentrations were >40 µg/L (Gkelis and Zaoutsos, 2014), which is a contrast to our results were the mcyE gene was amplified even in samples with micocystin concentrations as low as 0,7 µg/L.

In all samples were mcyE and sxtA were detected, there were microcystin and saxitoxin observed as well. On the other hand, we obtained samples with detectable amounts of microcystin and/or saxotoxin, with no manifestations of cyanobacterial mcyE or sxtA genes being present. In PCR analysis, false negatives can be caused by inhibition of amplification, a fact that needs to be taken into account when analysing environmental samples. Another explanation for this can be mutant genes, which was experienced by Fewer et al. (2008) who discovered microcystin synthetase deletion mutants capable of producing microcystin in *Dolichospermum* strains. On the contrary, a study from Finland reported the presence of the sxt gene in several environmental samples without detection of saxitoxin. This could be due to saxitoxin concentrations below detection limit or downregulation of the sxt gene expression (Savela et al., 2015).

The identification of the cyanobacteria responsible for cyanotoxin production in a lake is difficult because a number of different cyanobacteria can produce toxins. Cyanobacteria can have toxic and

non-toxic strains that may or may not co-occur, and the toxin production may vary due to environmental conditions (Kaebernick and Neilan, 2001). However, a prediction can be made based on the simultaneous presence of cyanobacteria, cyanotoxins and possibly the genes responsible for toxin production.

Microcystis and *Dolichospermum* co-occurred with only the microcystin toxin in 25 % and 16,7% of the samples, respectively, as well as with both the microcystin toxin and the mcyE gene in 41,7 % of the samples (Figure 7). *Planktolyngbya* was also frequently co-occurring with only microcystin (8,3 %) and both microcystin and mcyE (33,3 %). This suggests that microcystin may have been produced by a variety of cyanobacteria. However, *Microcystis* co-occurred with other cyanobacteria genera in all samples with detectable microcystin and mcyE. In samples with no detection of microcystin nor mcyE genes, no *Microcystis* was observed either. This strongly indicates that *Microcystis* may have been the main source of microcystin in these lakes.

In Portugal and France, saxitoxin have been frequently detected during *Aphanizomenon* blooms (Pereira et al., 2004; Ledreux et al., 2010). However, in Northern Europe, *Dolichospermum* have been more frequently linked to saxitoxin occurrence in the environment (Kaas and Henriksen, 2000; Rapala et al., 2005). Our findings agree with this, *Dolichospermum* being the most common genus in the saxitoxin-containing samples. *Dolichospermum* co-occurred with only the saxitoxin toxin in 25 % of the samples, and with both the saxitoxin toxin and the sxtA gene in 3,33% (Figure 8). Another interesting finding which also strongly suggests that *Dolichospermum* may be the main source of saxitoxin in these lakes, is that saxitoxin was not detected in L. Hillestadvannet in 2013, even though the phytoplankton community was dominated by the possible saxotoxin-producer *Aphanizomenon* (Berge, 2014). *Aphanizomenon* was not detected in L. Hillestadvannet in either July nor August, however the co-occurrence of saxitoxin and small amounts of *Dolichospermum* was detected in both months. The findings are additionally amplified by the fact that *Aphanizomenon* was not found in L. Revovannet, which was sxtA positive. In our study, *Planktolyngbya* was also frequently co-occurring in samples with only the saxitoxin toxin (16,7 %) and both the saxitoxin toxin and the sxtA gene (3,33 %).

There are a few limitations regarding the reliability of the results in this study. Samples were, of convenience, only collected at one point in each lake. A mixed sample with water collected from different points in each lake would give a more reliable result for the entire lake. However, the current study shows clearly that even samples taken at two selected dates and at one location can result in a detailed overview about the cyanobacterial and cyanotoxin composition in a certain part of a lake.

Regarding the cyanotoxin concentrations, these are only regarded as approximations. Firstly, ELISA may suffer from under- and overestimation of toxin concentrations, partly due to the variable cross-reactivities between the antibodies and different cyanotoxin variants, as well as organic material in the water (Hallengraeff, 2003). Secondly, it has been estimated that about 95 % of microcystins and saxitoxins are present inside cyanobacterial cells during the growth phase of the bloom, and may be released from cyanobacterial cells during a water bloom stagnation phase or collapse (Sivonen and Jones, 1999). However, this was taken into consideration in our study, as the water samples were frozen and thawed two times in order to burst cyanobacteria cells.

It has been proposed that future eutrophication and climate warming simultaneously will promote the dominance of cyanobacteria such as *Microcystis* and *Dolichospermum*, and may additively favour the growth of microcystin-producing populations of *Microcystis* (Davis et al., 2009; O'Neil 2012) The massive *Microcystis* blooms could be curtailed by reducing nutrient loads from agriculture into freshwater systems.

In conclusion, the mcyE and sxtA genes were readily detectable in the environmental samples, using qualitative PCR. In addition, saxitoxin and microcystin were commonly found in four of the six lakes investigated. The concentrations of microcystin detected in L. Akersvannet and L. Hillestadvannet presented a moderate risk based on WHO guidelines for recreational waters, while the concentration of microcystin in L. Vikevannet in July presented a high risk. Based on microscopic findings and toxin- and gene-detection, there is a strong indication that the likely candidates responsible for microcystin and saxitoxin production in the studied Norwegian lakes belonged to the genera *Microcystis* and *Dolichospermum*, respectively.

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Appendices

Appendix 1: Phytoplankton total biovolume in the six investigated lakes in 2015.

Appendix 2: Physical-chemical parameters in the six investigated lakes in 2015.

Appendix 3: Chlorofyll-a, microcystin, saxitoxin, mcyE and sxtA in the six investigated lakes in 2015.

Appendix 1. Phytoplankton total biovolume in the six investigated lakes in 2015.

L. Akersvannet 05.07.2015									
Genus	Number	Number/L	l	w	h	d	Formula	vol/ind	vol/L
								µm³	µm³
Cyanophyceae									
Aphanizomenon	203	796775	147,5	11			(3,14*h*d*d)/4	14010,3	1,1163E+10
Microcystis	865277	3396212225				4,5	(3,14*d*d*d)/6	52	1,766E+11
Snowella	100	6837026,2				3,13	(3,14*d*d*d)/6	16	109718195
Sum									1,8788E+11
Chlorophyceae									
Coccoid green algae	19	1195112,2				4,5	(3,14*d*d*d)/6	47,7	56993406
Schenedesmus large	1	62900,6	12,5	4,2			4*((π*l*d*d)/6)	461,6	29033677,9
Schenedesmus small	1	62900,6	9,4	2,5			4*((π*l*d*d)/6)	123	7735730,5
Monoraphidium	4	251602,6	4,2	2,5			(3,14*d*d*h)/12	6,9	1728195,1
Sum									95491009,5

Bacillariophyceae									
Amphora	3	188701,9	6,25	4,2			l*b*h	110,25	20804387
Sum									20804387
Synurophyceae									
Mallomonas	18	1132211,5	10	6,7			3,14*l*b*h/6	187,9	212787233
Sum									212787233
								µm³/L	1,882E+11
								mm³/L	188
L. Akersvannet 23.08.2015									
Genus	Number	Number/L	l	w	h	d	Formula	vol/ind	vol/L
								µm³	µm³
Cyanophyceae									
Aphanizomenon	120	471000	97,6	11			3,14*h*d*d/4	9270,5	4366422456
Microcystis	1374000	5392950000	4,5				(3,14*d*d*d)/6	52	2,8043E+11
Dolichospermum	10	39250	163	8			l*((π*d2)/6)	5459,4	214281973
Woronichinia	50	1572516,03	5				(3,14*d*d*d)/6	65,4	102868757
Sum									2,8512E+11
Chlorophyceae									
Coccoid green algae	12	754807,7	2,5				3,14*d*d*d/6	8,2	6172125,4
Sum									6172125,4

Bacillariophyceae									
Amphora	1	62900,6	15	4,13			l*b*h	255,9	16093349
Sum									16093349
Synurophyceae									
Mallomonas	6	377403,8	18,8	12,5			3,14*l*b*h/6	1229,8	464143830
Sum									464143830
Zygnematophyceae									
Closterium	1	62900,6	125	3,13			(3,14*d*d*h)/12	320,4	20155898
Sum									20155898
								µm³/L	2,8562E+11
								mm³/L	285,6
L. Goksjø 05.07.2015									
Genus	Number	Number/L	l	w	h	d	Formula	vol/ind	vol/L
								µm³	µm³
Cyanobacteria									
Dolichospermum	150	3120	62	8			l*((π*d2)/6)	2076,6	6478950
Snowella	75	750				4	(3,14*d*d*d)/6	33,5	12120
Sum									6491070
Chlorophyceae									
Coccoid green algae	11	691907,1				6,25	(3,14*d*d*d)/6	127,8	88402838
Pediastrum	1	10			25	100	(3,14*h*d*d)/4	196250	1962500
Sum									90365338

Bacillariophyceae									
Fragilaria	21	210	117	11			l*b*h	14157	2972970
Tabellaria	9	90	310	25			l*b*h	193750	17437500
Asterionella	1	10	100	5			7*(l*b*h)	17500	175000
Nitzchia	1	62900	18,8	6,25			l*b*h	734,4	46192658
Sum									66778128
Chrysophyceae									
Spiniferomonas	9	566105,8	9,4			6,25	(3,14*d*d*d)/6	431,9	244503170
Sum									244503170
Synurophyceae									
Mallomonas	9	566105,8	12,5	4,2			(3,14*l*b*h)/6	92,316	52260620
Sum									52260620
Dinophyceae									
Ceratium	1	10	25	5	11	5	3,14/12(D*D*D+l*b*b+2+h+d*d)	72033,7	720337
Sum									720337
Zygnematophyceae									
Closterium	1	10	125	16,5			(3,14*d*d*h)/12	8904,8	89048,4
									89048,4
Cryptophyceae									
Cryptomonas	1	#REF!	12,5	4,2		0,1	(3,14*l*b*h)6	92,3	5806735,6
									5806735,6
Euglenoidea									

Euglena	1	62900,6	50	18,8			(3,14*l*b*h)/6	7398,7	465381547
									465381547
								µm³/L	932395994
								mm³/L	0,9
Goksjø 23.08.2015									
Genus	Number	Number/L	l	w	h	d	Formula	vol/ind	vol/L
								µm³	µm³
Cyanophyceae									
Woronichinia	900	3532500				4	(3,14*d*d*d)/6	33,5	118315200
Sum									118315200
Chlorophyceae									
Coccoid green algae	23	1446715				6,25	(3,14*d*d*d)/6	127,8	184842297
Sum									184842297
Bacillariophyceae									
Tabellaria	2	125801,3	25	25			l*b*h	15625	1965645032
Asterionella	7	27475	200	10			7*(l*b*h)	140000	3846500000
Sum									5812145032
Chrysophyceae									
Dinobryon	36	2264423				4	(3,14*d*d*d)/6	33,5	75843076,9
Sum									75843076,9
Synurophyceae									
Mallomonas	1	62900,6	37,5	16,7			(3,14*l*b*h)/6	4378,6	275415049

Sum									275415049
Dinophyceae									
Ceratium	1	3925	75	10	75	10	3,14/12(D*D*D+l*b*b+2+h+d*d)	34717,4	136265834
Sum									136265834
								µm³/L	6602826489
								mm³/L	6,6
L. Hillestad 05.07.2015									
Genus	Number	Number/L	l	w	h	d	Formula	vol/ind	vol/L
								µm³	µm³
Cyanophyceae									
Dolichospermum 5 µm	48	480	109,5			5	l*((π*d2)/6)	1432,6	687660
Dolichospermum 8 µm	56	560	138			8,33	l*((π*d2)/6)	5011,3	2806353,7
Microcystis	103	80984575				3,13	(3,14*d*d*d)/6	16	1299612020
Pseudoanabaena	105	15725160	37,5	5			(3,14*d*d)/4	19,6	308606270
Planktolyngbya	1	62900,6	100	0,25			(3,14*d*d*h)/4	4,9	308606,3
Limnotrix	3	188701,9	29,2			2	(3,14*d*d*h)/4	91,6	17283926,2
Sum									1629304836
Chlorophyceae									
Staurastrum	2	125801,3	9,38	6,25		1,25	2*((√2/12)*b3)+(3d2*l)/4)	126,3	15887888,6
Schenedesmus large	10,5	660456,7	12,5			4,17	(π*l*d*d)/6	113,8	75128530
Schendesmus small	44	2767628	6,25			2,1	(π*l*d*d)/7	14,2	39164522,2
Tetraedron	1	62900,6	9,4	9,4	9,4		l*b*h	825,3	51911501
Sum									182092442

Bacillariophyceae									
Aulacoseira	323	32300	162	3,1	3,1		l*b*h	1587,1	51263258,9
Surirella	2	125801,3	46,9	9,4	9,4		(3,14*l*b*h)/4	3224,1	405596337
Nitzchia	2	125801,3	25	12,5	13		l*b*h	3903,3	491411258
Sum									948270854
Cryptophyceae									
Cryptomonas	1	62900,6	12,5			0,33	((3,14*d*d)/12)*((d/2)+l)	0,4	22929,9
Sum									22929,9
								µm³/L	2720526540
								mm³/L	2,7
L. Hillestad 23.08.2015									
Genus	Number	Number/L	l	w	h	d	Formula	vol/ind	vol/L
								µm³	µm³
Cyanophyceae									
Dolichospermum 8 µm	12	47100	62			2	l*((π*d2)/6)	129,8	6112952
Microcystis	119	20792156				6,25	(3,14*d*d*d)/6	127,8	2656549923
Sum									2662662875
Chlorophyceae									
Staurastrum	1	62900,6	12,5	6,25		1,25	2*((√2/12)*b3)+(3d2*l)/4)	1599,463	100607241
Schenedesmus large	5	314503,2	12,5			6,25	(π*l*d*d)/6	1022,1	321464865
Schendesmus small	2,5	157251,6	9,4			3,3	(π*l*d*d)/7	192,4	30249972,4
Tetraedron	3	188701,9	9,4	9,4	4,7		l*b*h	412,6	77867251,5
Chlamydocapsa	4	251602,6				6,25	(3,14*d*d*d)/6	127,8	32146486,5
Sum									562335816

Bacillariophyceae									
Aulacoseira	39	153075	33,4	0,8	0,8		l*b*h	20,3	3108707,1
Fragilaria	13	51025	27,9	3,1	3,1		l*b*h	273,1	13936844,6
Fragilaria sp.	5	314503,2	33,8	3,1			l*b*h	330,6	103989030
Sum									121034582
Cryptophyceae									
Cryptomonas	1	62900,6	12,5	6,25			((3,14*d*d)/12)*((d/2)+l)	159,7	10045777
Sum									10045777
Zygnematophyceae									
Closterium	1	62900,6	25	2				28,3	1780205,6
									1780205,6
								µm³/L	3357859256
								mm³/L	3,4
L. Revovannet 05.07.2015									
Genus	Number	Number/L	l	w	h	d	Formula	vol/ind	vol/L
								µm³	µm³
Cyanobacteria									
Dolichospermum straight	86	562583,3	117,5	8			l*((π*d2)/6)	3935,5	2214027956
Dolichospermum coiled	90	588750	111,5	8			l*((π*d2)/6)	3734,5	2198690800
Microcystis	28	1761218				25	(3,14*d*d*d)/6	8177,1	1,4402E+10
Planktolyngbya contorta	12	78500	200	5			l*((3,14*d*d/4))	3925	308112500
Planktolyngbya subtilis	22	143916,7	176,5	5			l*((3,14*d*d/4))	3463,8	498500349
Chroococcus	31	1949920	25	40			(3,14*d*d*d)/6	8177,1	1,5945E+10

Sum									3,5566E+10
Chlorophyceae									
Pediastrum	2	125801,3	87,5	235			(3,14*h*d*d)/4	3793267	4,772E+11
Schenedesmus large	21	1320913	12,5	6,25			(π*l*d*d)/6	1022,1	1350152431
Schendesmus small	30	1887019	6,25	3,1			(π*l*d*d)/6	128,2	241870781
Tetraedron	7	440304,5	41,7	33,3	17		l*b*h	23145,4	1,0191E+10
Coccoid green algae	40	2516026	25				(3,14*d*d*d)/6	8177,1	2,0574E+10
Sum									5,0955E+11
Bacillariophyceae									
Aulacoseira	2	13083,3	112,5	16,5			l*b*h	15314,1	200358984
Fragilaria	22	143916,7	114,5	5			l*b*h	1431,3	205980729
Asterionella	7	440304,5	50	3,1			l*b*h	489,8	215680952
Nitzchia	1	62900,6	100	12,5			l*b*h	15625	982822516
Sum									1604843181
								µm³/L	5,4673E+11
								mm³/L	547
L. Revovannet 23.08.2015									
Genus	Number	Number/L	l	w	h	d	Formula	vol/ind	vol/L
								µm³	µm³
Cyanophyceae									
Dolichospermum straight	13	51025	120	8			l*((π*d2)/6)	4019,2	205079680
Dolichospermum coiled	15	58875	85,5	8			l*((π*d2)/6)	2863,7	168599160
Microcystis	49	3082131				6,3	(3,14*d*d*d)/6	127,8	393794459

Planktolyngbya contorta	13	51025	73	5			l*((3,14*d*d/4))	1432,6	73099691
Planktolyngbya subtilis	13	51025	99	5			l*((3,14*d*d/4))	1942,9	99135197
Chroococcus	14	880609				8	(3,14*d*d*d)/6	267,9	235956239
Merismopedia	8	503205,1				8	(3,14*d*d*d)/7	267,9	134832137
Sum									1310496563
Chlorophyceae									
Pediastrum	3	11775	50	33,5			(3,14*h*d*d)/4	65743,8	7,74E+08
Schenedesmus large	3,2	201282,1	12,5	6,25			(π*l*d*d)/6	1022,1	205737513
Schendesmus small	5	314503,2	6,25	3,1			(π*l*d*d)/6	128,2	40311797
Tetraedron	3	188701,9	12,5	6,25			l*b*h	244,1	46069805
Chlamydomonas	3	188701,9				6,25	(3,14*d*d*d)/6	127,8	24109865
Sum									1,09E+09
Bacillariophyceae									
Aulacoseira	4	15700	43,8	12,5			l*b*h	3417	53662109
Fragilaria	10	39250	81	5			l*b*h	1012,5	39740625
Asterionella	42	164850	28				l*b*h	43,75	7212187,5
Sum									100614922
Cryptophyceae									
Cryptomonas	1	62900,6	12,5	6,25			3,14*l*d*d/6	255,5	16073243
									16073243
								µm³/L	2,52E+09
								mm³/L	2,5
L. Vikevannet 05.07.2015									

Genus	Number	Number/L	l	w	h	d	Formula	vol/ind	vol/L
								µm³	µm³
Cyanophyceae									
Dolichospermum	254	2540	180	8			l*((π*d2)/6)	6028,8	15313152
Microcystis	112	70448718				4,2	(3,14*d*d*d)/6	37,9	2673373610
Planktolyngbya	1	62900,6	125	2,5			l*((3,14*d*d/4))	613,3	38575783,8
Aphanocapsa	60	3774038				1,25	(3,14*d*d*d)/6	1,1	3857578,4
Pseudoanabaena	36	2264423	7,5	1,9		3,1	3,14*d*d/4	21,3	48127765
Sum									2779247889
Chlorophyceae									
Oocystis	1	62900,6				9,4	4*((3,14*l*d*d)/6))	1727,6	1,09E+08
Schenedesmus	14	880609	6,25	3,1			4*((3,14*l*d*d)/6)	128,2	108668075
Staurastrum	1	62900,6	12,5	6,3	1,3		2*((√2/12)*b3)+(3d2*l)/4)	466,3	29331109,5
Tetraedron	1	62900,6	12,5	12,5			l*b*h	976,6	61426407,3
Sum									3,08E+08
Bacillariophyceae									
Aulacoseira	69	690	121,7	25			l*b*h	76062,5	52483125
Fragilaria	125	1250	175	5			l*b*h	4375	5468750
Fragilaria sp.	27	270	62,8	12,5			l*b*h	211950	57226500
Sum									115178375
Zygnematophyceae									
Staurodesmus	1	62900,6	25	9,4			2*((3,14*√2*b*b*b)/12)	4868,3	306221058
Sum									306221058

Cryptophyceae									
Cryptomonas	13	817708,3	12,5	6,25			((3,14*d*d)/12))*((d/2)+l))	159,7	130595101
Rhodomonas	1	62900,6				6,25	((3,14*d*d)/12))*((d/2)+l))	134,2	8438452,7
Sum									139033554
Synurophyceae									
Mallomonas	2	125801,3	18,8	9,4			(3,14*l*b*h)/6	692,5	87119821,7
Sum									87119821,7
								µm³/L	3,73E+09
								mm³/L	3,7
L. Vikevannet 23.08.2015									
Genus	Number	Number/L	l	w	h	d	Formula	vol/ind	vol/L
								µm³	µm³
Cyanophyceae									
Dolichospermum	2	7850	137,5			10	l*((π*d2)/6)	7195,8	56487292
Microcystis	40	2516026				4,2	(3,14*d*d*d)/6	37,9	95477629
Planktolyngbya subtilis	33	2075721	4,4			2,5	l*((3,14*d*d/4))	21,5	44605950
Planktolyngbya contorta	3	188701,9	4,7			2,5	l*((3,14*d*d/4))	22,9	4323574
Aphanizomenon	6	377403,8	6,2	1,9		1,5	(3,14*d*d*h)/4	10,9	4112857
Radiocystis	20	1258010				2,5	(3,14*d*d*d)/6	8,2	10286857,7
Sum									215294160
Chlorophyceae									
Polyedriopsis	2	125801,3	12,5	6,3			l*b*h	244,1	3,07E+07
Schenedesmus	5	314503,2	7,6	6,3			4*((3,14*l*d*d)/6)	116	36475779,8
Tetraedron	1	62900,6	6,25	4,2			l*b*h	54,3	3418040,5

Sum									7,06E+07
Bacillariophyceae									
Aulacoseira	10	39250	82,5	12,5			l*b*h	12890,6	505957031
Fragilaria	47	184475	78	5			l*b*h	1950	359726250
Amphora	2	125801,3	12,5	6,3			l*b*h	488,3	61426407,3
Sum									927109689
Cryptophyceae									
Cryptomonas	4	251602,6	14,1	7,3			((3,14*d*d)/12))*((d/2)+l))	314,6	79149519,1
Sum									79149519,1
Chrysophyceae									
Uroglena	1	62900,6	12,5	6,3			(3,14*h*d*d)/4	383,3	24109864,9
Sum									24109864,9
								µm³/L	1,32E+09
								mm³/L	1,3
L. Åsrumvannet 05.07.2015									
Genus	Number	Number/L	l	w	h	d	Formula	vol/ind	vol/L
								µm³	µm³
Dinophyceae									
Ceratium	62	620	100	12,5	63	10	3,14/12(D*D*D+l*b*b+2+h+d*d)	17930,7	1,11E+07
Sum									1,11E+07
Bacillariophyceae									

Asterionella	7	70	200	10			7*(l*h*b)	140000	9800000
Sum									9800000
Zygnematophyceae									
Mougeotia	1	10	650	10			(3,14*h*d*d)/4	51025	510250
Sum									510250
Chrysophyceae									
Mallomonas	2	20	100	50			(3,14*l*b*h)6	130833,3	2616667
Dinobryon	52	520				10	(3,14*d*d*d)/6	523,3	272133
Sum									2888800
								µm³/L	2,43E+07
								mm³/L	0,02
L. Åsrumvannet 23.08.2015									
Genus	Number	Number/L	l	w	h	d	Formula	vol/ind	vol/L
								µm³	µm³
Dinophyceae									
Ceratium	1329	13290	100	12,5	38	10	3,14/12(D*D*D+l*b*b+2+h+d*d)	8213,8	1,09E+08
Sum									1,09E+08
Bacillariophyceae									
Asterionella	14	140	90	10			7*(l*h*b)	6300	882000
Nitzchia	3	68,5	20,8				l*b*h	29635,8	889075,2
Fragilaria	1	75	25				l*b*h	46875	468750
Sum									2239825,2

Cyanophyceae									
Dolichospermum	350	3500	150	5			l*(3,14*d*d)/6	1962,5	6868750
Sum									6868750
Chrysophyceae									
Dinobryon	20	200				10	(3,14*d*d*d)/6	523,3	104666,7
Sum									104666,7
Synurophyceae									
Mallomonas	5	50	42,5	16,7			(3,14*l*b*h)6	4962,4	248119,1
Sum									248119,1
Euglenaceae									
Euglena	1	75	37,5				(3,14*l*b*h)6	44156,3	441562,5
Sum									441562,5
								µm³/L	1,19E+08
								mm³/L	0,12

Appendix 2. Physical-chemical parameters in the six investigated lakes in 2015.

Lake	Sampling Date	Temperature (C°)	pH	Secchi-depth (m)	Conductivity	Tot-P (ug/L)	Tot-N (ug/L)
Akersvannet	05.07.2015	19,9	9,6	<1	182,5	64	2457,9
Akersvannet	23.08.2015	21,4	9,5	0,7	183,5	81,6	2014,5
Goksjø	05.07.2015	18,8	7,7	2	129,7	31,1	2108,5
Goksjø	23.08.2015	20,9	7,8	2	125,6	26,2	1574,1
Hillestad	05.07.2015	19,7	10,1	<0,5	159,4	134,9	1820,5
Hillestad	23.08.2015	20,7	8,7	0,7	140,9	56,8	3312,8
Revovannet	05.07.2015	18,5	9,4	<1	85,4	49,3	2100,9
Revovannet	23.08.2015	20,3	7,8	1	85,1	31	859,3
Vikevannet	05.07.2015	19,4	10,1	<1	129,3	55	1254,5
Vikevannet	23.08.2015	20	7,2	1,2	99,9	30,5	1193,8
Åsrumvannet	05.07.2015	19,6	8,5	3	89,4	11,4	1780,1
Åsrumvannet	23.08.2015	21,1	9	2	88,9	20,6	1463,9

Lake	Sampling Date	TOC	Fe (ug/L)	Cl- (ppm)	NO^3 (ppb)	SO4- (ppm)	Na+ (ppm)
Akersvannet	05.07.2015	7,5	23,6	17,56	1315	11,8	11,96
Akersvannet	23.08.2015	9,9	72,4	18,06	185	11,08	12,06
Goksjø	05.07.2015	5,5	77,4	15,88	1441	6,25	10,48
Goksjø	23.08.2015	6,5	100,6	14,45	1010	6,21	10,13
Hillestad	05.07.2015	10,9	77,3	14,36	9,2	4,81	10,84
Hillestad	23.08.2015	10,1	351	11,81	228	4,89	10
Revovannet	05.07.2015	6,9	<20	4,32	291	2,59	4,41
Revovannet	23.08.2015	7,4	<20	4,26	96,7	2,45	4,64
Vikevannet	05.07.2015	8,9	32,5	9,97	<5	4,3	7,41
Vikevannet	23.08.2015	9,1	42,6	7,86	179	3,84	6,42

Åsrumvannet	05.07.2015	5,6	49,2	9,42	1240	4,26	6,77
Åsrumvannet	23.08.2015	6,1	74,1	9,06	1022	4,48	6,88

Lake	Sampling Date	K+ (ppm)	Mg2+ (ppm)	Ca2+ (ppm)
Akersvannet	05.07.2015	3,98	5,02	14,08
Akersvannet	23.08.2015	4,31	5,57	14,87
Goksjø	05.07.2015	1,73	2,57	9,72
Goksjø	23.08.2015	1,66	2,73	9,41
Hillestad	05.07.2015	1,67	2,64	13,28
Hillestad	23.08.2015	1,46	3,21	14,14
Revovannet	05.07.2015	1,23	1,49	9,61
Revovannet	23.08.2015	1,24	1,78	9,95
Vikevannet	05.07.2015	1,45	2,14	10,29
Vikevannet	23.08.2015	1,27	2,27	10,32
Åsrumvannet	05.07.2015	1,27	1,93	6,89
Åsrumvannet	23.08.2015	1,22	2,07	6,99

Appendix 3. Chlorofyll-a, microcystin, saxitoxin, mcyE and sxtA in the six investigated lakes in 2015.

Lake	Sampling Date	mcyE	sxtA	Chlorofyll-a (ug/L)	Microcystin (ug/L)	Saxitoxin (ug/L)
Akersvannet	05.07.2015	N.D.	N.D	55,53	**17,65**	N.D
Akersvannet	23.08.2015	N.D.	N.D	11,82	**8,5**	N.D
Goksjø	05.07.2015	N.D.	N.D	12,36	N.D	N.D
Goksjø	23.08.2015	N.D.	N.D	6,79	N.D.	N.D
Hillestad	05.07.2015	**Pos**	N.D	61,75	**18,4**	**0,078**
Hillestad	23.08.2015	**Pos**	N.D	24,55	**4,8**	**0,13**
Revovannet	05.07.2015	**Pos**	**Pos**	29,84	**0,77**	**0,146**
Revovannet	23.08.2015	**Pos**	N.D.	20,04	**0,7**	N.D
Vikevannet	05.07.2015	**Pos**	N.D.	24,75	**32,2**	N.D.
Vikevannet	23.08.2015	N.D.	N.D.	16,17	**2,5**	**0,05**
Åsrumvannet	05.07.2015	N.D.	N.D.	3,13	N.D	N.D.
Åsrumvannet	23.08.2015	N.D.	N.D.	13,3	N.D	N.D.