Norwegian University
of Life Sciences

Master's Thesis 2022 30 ECTS
Faculty of Environmental Sciences and Natural Resource Management

Road salt-induced lake stratification: Effects on nutrient availability and consequences for the phytoplankton community in Lake Kutjern

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Natural resource management

PREFACE

This thesis marks the end of my master’s degree in Natural Resource management at NMBU. I want to thank my advisors Thomas Rohrlack and Gunnhild Riise for their guidance and feedback in this process. Additionally, I want to thank Daria Dedikova for great collaboration in the field and in the laboratory. I am also grateful to the lunch crew at Sørhellinga. At last, I want to thank my family and friends who have supported me throughout my degree and who have given me feedback on my thesis.

Ås, 16.05.2022
Vida Steiro

ABSTRACT

In Norway, the use of road salt for de-icing highways has increased by more than 200 percent in the last two decades. This is partly due to climate change and increased traffic volumes, and hence may continue to rise. Salt is readily transported with runoff into the environment, where it can harm ecosystems, including lakes. In lakes, dense, salty water that sinks to the bottom may develop a chemical density gradient that prevents water circulation. Several Norwegian lakes are subject to road salt pollution and consequent changes in lake physicochemical conditions. Despite this, the impact of road salt on phytoplankton community composition in these lakes has not been extensively studied. This study aims to improve the understanding of the consequences of road salt runoff for algal community composition through examining a range of parameters in a humic, boreal lake highly affected by road salt. Measurements were conducted in Lake Kutjern from mid-May through mid-September 2021.

The study finds that road salt has led to chemical stratification, bottom water oxygen depletion, and likely caused meromixis (permanent stratification) in Lake Kutjern. This is the probable cause of bottom accumulations of iron and manganese, and depletion of sulfate and nitrate in the lake. The results also suggest that meromixis has led to ammonium accumulation in the bottom layer and nitrogen limitation at the top. Pigment analyses indicate that such conditions have led to the dominance of the nuisance alga *Gonyostomum semen* (Ehr.) Diesing (Raphidophyceae) and to green sulfur bacteria (Chlorobiaceae) blooms. These are potentially affecting nutrient distributions in the lake. Overall, this study contributes to the understanding of what consequences road salt may have on humic, boreal lakes, even after road relocation, and of the factors affecting *G. semen* growth. Such knowledge may be of importance for freshwater management into the future, as more lakes may be affected by road salt because of climate change and increased traffic.

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1. Introduction

Runoff from roads contains various pollutants that are harmful to the environment. One of these is salt, which affects lake systems in the northern hemisphere, including Norway (Bækken & Haugen, 2011; Hintz et al., 2022; Kaushal et al., 2005; Müller & Gächter, 2012). In Norway, road salt in the form of sodium chloride (NaCl) is used as a de-icing agent during wintertime (Norwegian Public Roads Administration, 2021). The road salt consumption in Norway has increased in the past two decades, and in recent years around 250 000 metric tons of salt were added to national and county highways each season (**Figure 1**). The rise in salt consumption is partly due to increased traffic volumes and climate change leading to longer periods of temperatures around 0 degrees (Bazilchuk, 2019; NPRA, 2021; NPRA 2019). Norway will continue to build new roads and climate will continue changing (Ministry of Transport, 2021). Therefore, understanding the consequences of road salt on Norwegian lake systems will be increasingly important. More knowledge on the negative consequences may also encourage a moderation on the use of road salt, which is already higher than necessary for safety measures (Bazilchuk, 2019).

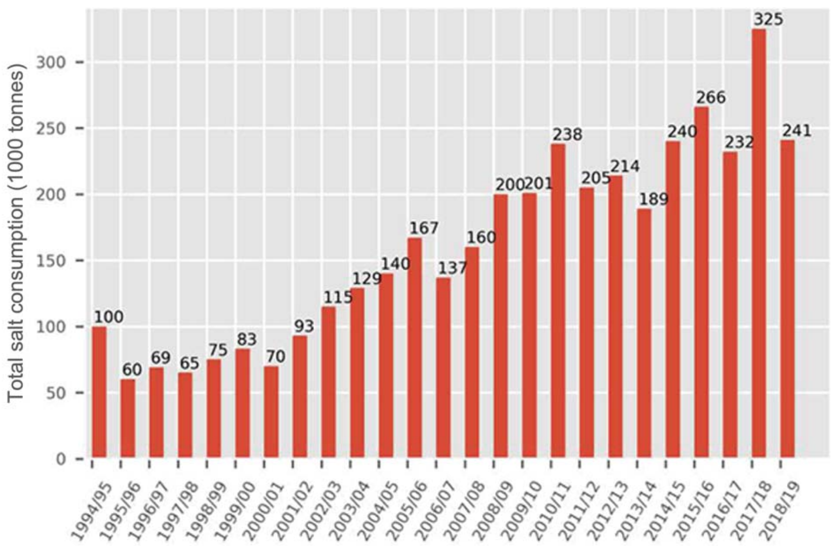

Figure 1. *Salt consumption on national highways and county road networks in Norway. Translation of figure obtained from: Norwegian Public Roads Administration. (n.d). Hvor mye salt brukes i Norge? Available at: https://www.vegvesen.no/fag/veg-og-gate/vinterdrift/salting/sporsmal-og-svar/hvor-mye-salt/ (accessed: Feb 18).*

Although de-icing is beneficial for traffic safety and accessibility, road salt runoff can cause damage to lake ecosystems and reduce drinking water quality (Amundsen et al., 2008; Hintz et al., 2022; Hintz & Relyea, 2019; Howard & Maier, 2007; Sivertsen et al., 2012). Sodium chloride is highly soluble in water and is readily transported as Na^+ and Cl^- ions with rainwater or snowmelt and into soil and water systems, where it can cause harm (Amundsen et al., 2008; Hintz & Relyea, 2019). Due to their smaller dilution effect, lakes with smaller catchments have a greater risk of salt harm than those with large catchments (Ramakrishna & Viraraghavan, 2005; Saunes et al., 2018). Road salt can cause changes to lake species composition and a loss of biodiversity as a result of direct toxicity, and indirectly through physicochemical effects (Amundsen et al., 2008; Bækken & Færøvig, 2004; Færøvig et al., 2006; Hintz & Relyea, 2019; Ramakrishna & Viraraghavan, 2005). Exposure to high Cl^- levels can cause both acute and chronic effects in aquatic organisms (Amundsen et al., 2008; Lopatina et al., 2021). Studies of Norwegian lakes have shown that phytoplankton communities change when chloride concentrations reach above 20-30 mg/L (Haugen et al., 2010). Although road salt is known to affect biota in lakes, its consequences on Norwegian lakes are not well known (Saunes & Værøy, 2017). Given that more lakes may be subject to salt stratification in the future, it is important to improve our understanding of its effects on algal communities. This study aims to contribute to this knowledge by looking at algal community composition in Lake Kutjern, Marker municipality.

One of the ways in which road salt may indirectly affect algal communities, is through the development of a vertical density gradient in the water column, with consequent changes to water chemistry (Bækken & Haugen, 2006; Judd, 1970; Sivertsen et al., 2012). The chloride concentration in lakes is normally around 2 to 10 mg/L, while in runoff it can be up to 10 g/L (Amundsen et al., 2008; Saunes & Værøy, 2017). Dense, salty water sinks to the bottom, and may stratify the lake into layers with specific terminology (**Figure 2**). Stratification inhibits water circulation, which prevents the distribution of oxygen and nutrients throughout the water column (Bækken & Haugen, 2006). With no gas exchange with above layers, the bottom layer becomes oxygen depleted (Ramakrishna & Viraraghavan, 2005). Anoxia at the sediment/water interface can lead to the release of metals such as manganese and iron from sediments due to reduction of complexes (Golterman, 2001; Hongve, 1997; Mortimer, 1941; Mortimer, 1971). Phosphate, which commonly adsorbs to these complexes (in oxic conditions), is then released. Additionally, compounds used as alternative electron acceptors in anaerobic respiration (sulfate, sulfur, nitrate, ferric iron) will be found in reduced form in the anoxic layer (Boehrer et al., 2017; Gulati et al., 2017; Su et al., 2019; Wetzel, 2001). These changes to the water chemistry have biological consequences, especially if they become permanent.

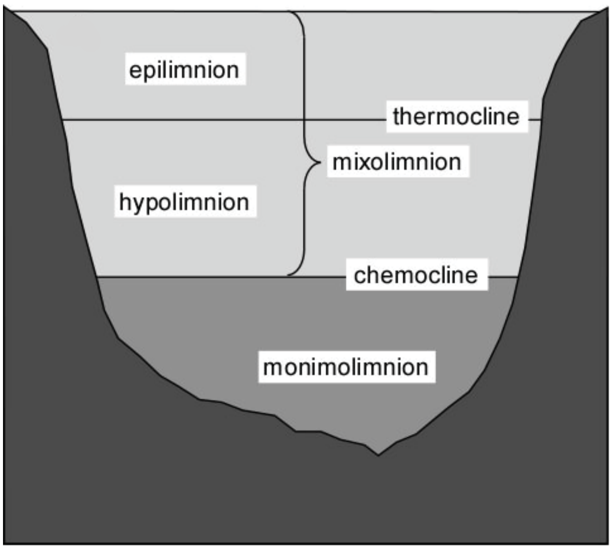

Figure 2. *Cross-section illustrating the terminology of thermal and chemical stratification in meromictic lakes during summer stagnation in a temperate lake. Layers formed by the thermal gradient are called epilimnion (above the thermocline) and hypolimnion (below the thermocline). Layers formed by a strong chemical gradient, the chemocline, are denoted mixolimnion (top) and monimolimnion (bottom). Source: Boehrer, B., & Schultze, M. (2006). On the relevance of meromixis in mine pit lakes. 7th International Conference on Acid Rock Drainage (ICARD), St. Louis MO.*

When road salt causes permanent stratification (meromixis), algal communities are affected by long-term accumulation of nutrients and other reduced compounds. Stratification caused by temperature is normal in temperate lakes during summer (Wetzel, 2001). It commonly leads to the accumulation of nutrients and reduced compounds in the hypolimnion, unavailable to surface algae (Boehrer et al., 2017; Gulati et al., 2017; Wetzel, 2001). As a result, inorganic nutrients important to algae (NO_3^- and NH_4^+, and PO_4^{3-}) become restricted in the euphotic layer. Stratification can therefore lead to shifts in the phytoplankton community, where motile algae become dominant (Salonen et al., 1984; Salonen & Rosenberg, 2000). Motility is advantageous for obtaining nutrients from the hypolimnion and avoiding predators (Rohrlack, 2020a, 2020b; Salonen et al., 1984; Salonen & Rosenberg, 2000). They have the ability to “close the gap” between light availability in the euphotic zone and nutrient availability in the hypolimnion through diel vertical migration (DVM) (Salonen et al., 1984).

Temperate lakes typically have two overturns each year, in fall and spring, leading to reoxygenation and reversions of the nutrient accumulations (Xing et al., 2021). A meromictic lake can also develop summer stagnation, but does not experience complete overturn (**Figure 2**). This is because the density gradient from salt is greater than that induced by temperature, thus more difficult to destabilize by wind action. The lack of complete overturn leads to long-term changes in lake chemistry and nutrient availability. Several Norwegian lakes are stratified due to road salt, including Lake Kutjern (Amundsen et al., 2008; Bækken & Haugen, 2011; Saunes & Værøy, 2017; Saunes et al., 2018). Despite this, the impacts of long-term salt stratification on phytoplankton communities in Norwegian lakes have not been extensively studied (Saunes & Værøy, 2017).

This study will investigate the effect of salt stratification on nutrient availability and algal composition in Lake Kutjern - a humic lake in Norway. Lake Kutjern represents an ideal study site because it has been highly affected by road salt runoff, having the greatest chloride gradient out of 23 roadside lakes studied in Norway (Saunes & Værøy, 2017). The highway that has contributed with road runoff into Lake Kutjern has been relocated and is now further away from the lake. The current level and stability of the stratification is uncertain. Moreover, the motile, flagellated alga *Gonyostomum semen* (Ehr.) Diesing (Raphidophyceae) was observed in the lake in 2017 (Rohrlack, T. personal communication, 2021). *G. semen* tends to become dominating in stratified lakes, likely due to its ability to perform diel vertical migration (DVM) (Hagman et al., 2015; Hagman et al., 2020; Johansson, 2013; Rohrlack, 2020a, 2020b; Salonen & Rosenberg, 2000; Sassenhagen et al., 2014). If this alga outcompetes other species in Lake Kutjern, it could lead to water management challenges both due to its nuisance effect on bathers, its potential to clog water treatment plants, and, if permanent, a potential loss of biodiversity (Cronberg et al., 1988; Hongve et al., 1988; Lepisto et al., 1994; Rengefors et al., 2008). However, the effect of the stratification on the phytoplankton community in Lake Kutjern has not yet been examined. Therefore, parameters such as oxygen, temperature, conductivity, and nutrients measured from mid-May to mid-September 2021 will be examined to assess stratification dynamics, and pigment analyses will be performed to look at algal community composition in the lake.

The hypothesis of this study is that road salt still contributes to a permanent stratification (meromixis) in Lake Kutjern. It is expected that accumulated salt in the bottom water creates a steep density gradient, thereby preventing vertical mixing and redistribution of substances. It hypothesized that meromixis is causing anoxic bottom waters with resulting monimolimnic accumulations of phosphate, ammonium, iron, and manganese, among other reduced compounds. The hypothesized effect of these conditions on the phytoplankton community is a dominance of the alga *Gonyostomum semen* (*G. semen*), due to its competitive advantage under stratified conditions.

2. MATERIALS AND METHODS

2.1. STUDY SITE

Lake Kutjern is a small, boreal lake located at 222 m.a.s.l. in Marker municipality, southeastern Norway (**Figure 3**). It is a humus rich, nutrient poor bog lake, with little wind exposure and a pH right below 7 (Saunes & Værøy, 2017). Its surface area is approximately 0.015 km^2 (Bækken & Haugen, 2011). Water measurements and samples in this study were taken at the location of maximum depth, which is about 9 m (59.48380 N, 11.74714 E).

Since there is no registered catchment area for this specific lake, it was approximated by combining two automatically generated catchment areas that together cover the lake (**Figure S1**). The lake and most of the catchment area are located above the marine limit (Geological survey of Norway, n.d.). Superficial deposits consist of bare rock and thin marine deposits (**Figure S2**). The generated catchment area is about 2.28 km^2 and dominated by forest cover (98.5%) (**Figure S1**). The small size of the catchment area and relatively great depth of the lake, indicate that the water residence time in the lake is long.

A few buildings exist in the lake's vicinity, including a ski sport center (Norwegian Public Roads Administration (NPRA), 2012). All the buildings are connected to the municipal treatment, except one that has a sludge separator (Heed, R. personal communication, 2022). The lake is located close to the old highway stretch E18 Riksgrensen – Ørje (now called Svenskeveien), which has been part of its catchment area since 1959 **(Figure 3**) (Wiik & Johansen, 2015).

Previous reports found that the lake was strongly affected by road salt with a sharp chemocline and a salt-induced oxygen gradient at 2 m in June 2016 (Bækken & Haugen, 2011; Saunes & Værøy, 2017). The chloride concentration at 8 m was 400 mg/L, which is very high for a freshwater lake. A strong smell of H_2S from the bottom water was also registered. Most of the biomass consisted of the cyanobacteria *Aphanocapsa reinboldii* (68%), which tolerates high salt concentrations and is considered a marine species. This indicates that the high salt content likely affects the lake's biology (Saunes & Værøy, 2017). In 2017, a new and relocated E18 highway opened, this one further away (68 m at the shortest) (Amundsen, 2017). The effect of the road relocation on the salt levels of Lake Kutjern has not yet been studied.

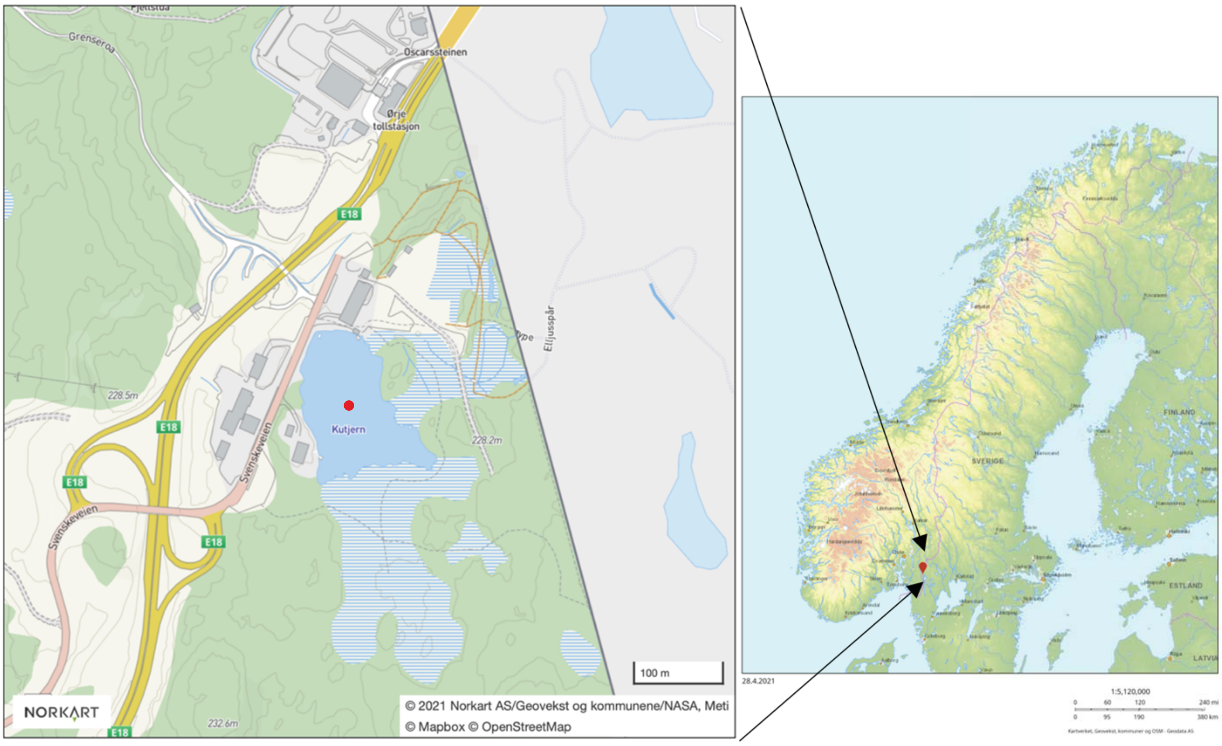

Figure 3. Map of field site (red dot) in Lake Kutjern, Marker municipality (left), located near the newly built E18 highway (yellow) and the previous highway, Svenskeveien (pink). On the right is an overview map of Norway showing the location of the lake with regards to the whole country. Source: Norkart webklient 3.0.3. (2021). Kutjern, Marker kommune. Norkart AS. https://kommunekart.com/

2.2. DATA COLLECTION

Weather data for the study period was collected from the *Norwegian Centre for Climate Services*' website (NCCS, n.d.). This includes daily precipitation data measured at Ørje station SN1950 (the closest to Lake Kutjern) and daily temperature data gathered at E18 Ørje Vest station SN1960. Monthly temperature data, however, were not available at any of the Ørje stations, and were therefore obtained from the closest station, Rakkestad (SN3290).

Most of the fieldwork was conducted with a two-week interval starting on May 20 and ending on September 12, 2022. This was done in collaboration with Daria Dedikova, another student. Temperature, oxygen, and conductivity were measured in situ using a WTW MultiLine Multi-parameter portable meter, at 0.5 m and at one-meter intervals from one through eight meters depth. Temperature is measured to determine thermal stratification, while conductivity is used for detecting salt stratification, together with Cl^- concentration. If the difference in chloride concentration between the bottom and the surface layer of a lake exceeds 10 mg/L, the lake is considered chemically stratified (Haaland et al., 2012). The limit value to define an oxygen gradient is a top-bottom O_2 difference of 6 mg/L.

Water samples taken for lab analysis of water chemistry and pigment content were obtained using a plastic Ruttner sampler at 0.5, 1, 2, 4, 6, and 8 meters. These were stored in 500 mL plastic bottles that were rinsed three times with water from their assigned depths, and transported to the lab in a cooler, keeping out sunlight. As a control sample, a bottle with distilled water was subjected to similar treatments as the water samples throughout field and lab work.

2.3. Laboratory analyses

All lab work was performed in the laboratories of the Soil Science Building at the Norwegian University of Life Sciences.

Water samples were filtered through 0.45 µm cellulose acetate membrane and GF/C fiberglass filters and stored in a freezer before analysis of ions in the filtrate. The GF/C filters were immediately stored in airtight 15 mL plastic containers at -21℃ for later analysis of pigments. 50 mL of untreated water samples were saved for analysis of metals and sulfur. For later analyses of Tot-P and Tot-N, 10 mL of unfiltered water samples each were added to scintillation vials and decomposed by adding potassium persulfate solution ($K_2S_2O_8$) before autoclaving for 25 minutes at 121 °C. 5 mL and 2 mL of the oxidation solution were added to Tot-N and Tot-P samples, respectively.

2.3.1. Nitrate, sulfate, chloride, and total nitrogen

Nitrate as N (NO_3-N), sulfate (SO_4^{2-}), and chloride (Cl^-) concentrations were determined through ion chromatography, running filtrate samples through an ion chromatograph (Lachat IC5000) from Zellweger Analytics using an auto sampler (ASX-500 series). This was done according to Norwegian Standard NS-EN ISO 10304-1. Diluted certified standard samples (ION-96,4 and REF-IC) were used as references. The limits of quantification (LOQ) for NO_3-N, SO_4, and Cl^- were 0.02 mg/L, 0.12 mg/L, and 0.08 mg/L, respectively. The same instrument was used to determine total nitrogen (Tot-N) according to Norwegian Standard (NS 4743), but for this, the autoclaved, unfiltered water samples were analyzed. The LOQ for Tot-N is 0.02 mg/L.

2.3.2. *Ammonium, total phosphorus, and orthophosphate*

To measure ammonium as N (NH_4-N), total phosphorus (Tot-P), and orthophosphate as P (PO_4-P), reagents were added to the different samples to create a color reaction. The absorbances of the color complexes were then measured. These are proportional to the concentration of the substances.

Ammonium-N was determined according to a modified Norwegian standard (NS 4746) using salicylic acid instead of the very toxic phenol. To 3 mL of each filtered water sample and to 20x diluted ammonium standards (QC3198), 0.2 mL salicylate and 0.5 mL hypochlorite were added. Samples were vortexed between reagent additions and left to react for at least one hour before reading off results from a 1 cm cuvette. This reaction forms monochloramine, which is green in color.

Total phosphorous and orthophosphate P were analyzed according to NS-EN 1189. 0.2 mL ascorbic acid (5%) and 0.2 mL molybdate were added to 5 mL of each of the autoclaved samples for Tot-P analysis. The same was done to 5 mL of filtered water samples for PO_4-P analysis, and to a 4x diluted certified standard (QC3198). The samples were vortexed before the addition of the second reagent. These were analyzed in 2 cm cuvettes immediately after a 20-minute rest to allow for reaction into a molybdate complex with blue color. In this study, phosphate (PO_4-P) refers to orthophosphate (PO_4^{3-}).

NH_4-N, Tot-P, and PO_4-P were then determined through spectrophotometry on an UH5300 spectrophotometer (Hitachi). NH_4-N was measured at 655 nm wavelength, while Tot-P and PO_4-P were measured at 880 nm. The LOQ for NH_4-N is 0.02 mg/L, for PO_4-P it is 0.0005 mg/L and for P it is 0.01 mg/L.

2.3.3. *Dissolved organic carbon*

Dissolved organic carbon (DOC) in filtrated samples was quantified according to Norwegian Standard (NS-EN 1484) using a Shimadzu Total Organic Carbon Analyzer (TOC-VCPN) and ASI-V autosampler. At first, inorganic carbon was removed by acidifying the samples with HCl and purging with purified air. The Non-purgeable organic carbon (NPOC) then went through oxidative combustion at 680 ℃ by a platinum catalyst. DOC was detected in the form of CO_2(g) by a non-destructive infrared analyzer (Direktoratsgruppen vanndirektivet). Certified reference material Sangamon was used as a control. The LOQ for DOC is 0.3 mg/L.

2.3.4. Pigment extraction

The GF/C algal culture filters were lyophilized, and pigments were extracted by adding 3 mL acetone to each sample. In order to prevent degradation in the acetone, the samples were stored in a refrigerator (4 ℃) for 24 hours. The samples were then centrifuged at 3000 rpm for 10 minutes to exclude particles. The supernatant was diluted with water at a 1:4 ratio before analysis. The pigment analysis was performed according to a modified High Performance Liquid Chromatography (HPLC) procedure of Wright et al. (1991) as described in Hagman, Rohrlack, Uhlig and Hostyeva (2019). Pigments were identified and quantified by translating peak absorbance areas into concentrations using standards of pure pigments with known concentrations. The pigments heteroxanthin and bacteriochlorophylls are expressed as their peak areas in milli absorbance per liter (mAU/L), due to the lack of standards for these pigments. Heteroxanthin and diadinoxanthin are pigments commonly used as proxies for *Gonyostomum semen* (*G. semen)* since in Norwegian lakes, other algae do not normally contain these (Rohrlack, T. personal communication, 2021). Alloxanthin and violaxanthin are other pigments that likely originate from *G. semen* (Sassenhagen et al., 2014).

Microscopy was also done to look at what algal groups were present and to confirm the presence of *G. semen*.

2.3.5. Iron and manganese

Fe and Mn were determined through inductively coupled plasma optical emission spectrometry (ICP-OES) on unfiltered samples, according to Norwegian Standard (NS-EN ISO 11885). The ICP was conducted by qualified laboratory personnel on an Agilent 8800 ICP Triple Quad. Prior to analysis, the samples were digested with nitric acid (5% HNO3) in an UltraClave. The limit of quantification for both Fe and Mn is 0.000 mg/L. A standard (1643H) was used as control.

2.4. Data processing and statistical analyses

The significance of difference in abiotic parameters at the top and bottom of the water column was determined by the non-parametric Wilcoxon/Mann-Whitney U test. Relationships between all variables were first evaluated from a principal component analysis (PCA) biplot after performing a PCA in RStudio version 1.4.1103. Interesting relationships were then evaluated by calculating Spearman's rank correlation coefficients for relevant parameters. Statistical tests were conducted in RStudio. Standard deviations are denoted in parentheses. Figures were created in Microsoft Excel version 16.58 and RStudio.

3. RESULTS

3.1. WEATHER CONDITIONS DURING STUDY PERIOD

The mean monthly temperatures were between 0 and 3.3 degrees warmer than the 1961-1990 normal throughout the months of field work (**Table 1**). The highest temperatures were measured in July, with a maximum of 20.9 °C on July 3 and 15 (**Figure 4**). July was also the month with the greatest temperature deviation from the normal, while May had the smallest temperature deviation (Table 1). The lowest temperature within the study period was measured on May 26 (7.6°C).

Table 1. Mean monthly temperature as a deviation from the 1961-1990 normal, normal monthly precipitation (1961-1990), and monthly precipitation for the period May 2021 through September 2021. Precipitation data is collected from the SN1950 station in Ørje, while temperature data is measured at the SN3290 in Rakkestad. Source: Norwegian Centre for Climate Services. (n.d.). Seklima Observations and weather statistics. NCCS. https://seklima.met.no/observations/

Month	Mean temperature (deviation in °C from the 1961-1990 normal)	Monthly precipitation (mm)	1961-1990 monthly precipitation normals (mm)
May	0	74.8	56
June	2	41.4	69
July	3.3	168.1	79
August	0.5	46.6	91.9
September	1.9	82.8	95

Precipitation was greater than the 1961-1990 normal in May and July, and lower in the other months, although September precipitation was also close to the normal (**Table 1**). It rained a couple of days before the first field day (May 20), while it was dry for over a week before the second, fourth, and eight sampling dates (June 6, July 3, and August 28) (**Figure 4**). It was also dry for five days before July 18. July 31 was the sampling date that had the most precipitation preceding it, including the days with the most and second-most precipitation of the period. August was characterized by relatively low temperatures compared to the other summer months. There is a comparatively large increase in temperature the first weeks of September, before it decreases again. Precipitation was third greatest on the last field day (Sept 12), and this was the only field day it rained during sampling.

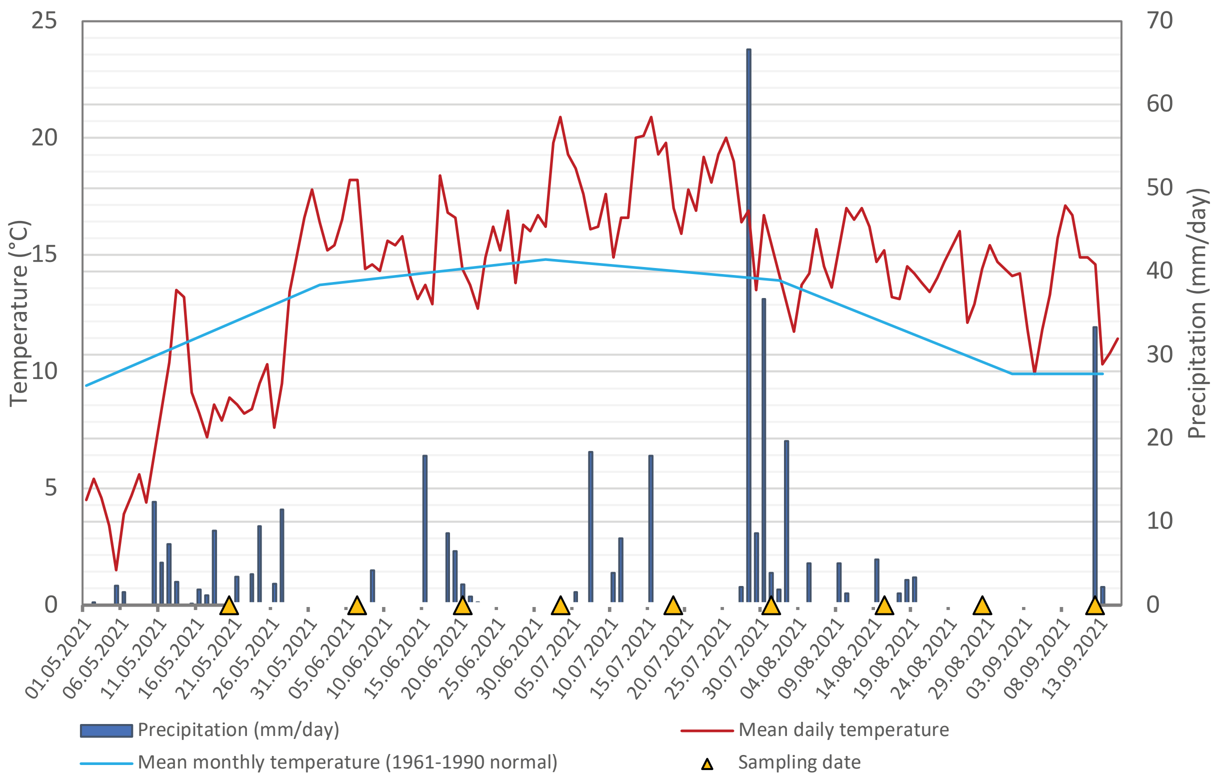

Figure 4. Mean daily temperature and daily precipitation (mm) measured at weather stations E18 Ørje Vest (SN1960) and Ørje (SN1950), respectively. Source: Norwegian Centre for Climate Services. (n.d.). Seklima Observations and weather statistics. NCCS. https://seklima.met.no/observations/.

3.2. SALT-INDUCED AND THERMAL STRATIFICATION

The temperature measurements indicate that a thermal stratification began developing at the end of May and was at its steepest in mid-July (**Figure 5**). Once the thermal stratification was established, the thermocline was located at about 2 m depth, and deepened at the end of July (3 m). Beyond that, the thermocline became less steep. The greatest temperature difference between the top (0.5 m) and bottom (8 m) was 17.8°C and occurred on July 18. This was also when the highest temperatures were measured at the top (22.4°C) and in most other depths of the water column. Throughout the study period, the bottom two meters of the lake had temperatures below 5°C, the lowest being 4.2°C in May. The mean temperature of the top four meters was 12.2 (± 5.2) °C, while that of the bottom four meters was 4.9 (± 0.4) °C, a significant difference ($p < 0.01$). The greatest temperature measured at 8 m depth was 4.8°C, on July 31, August 28, and September 12, when the thermal gradient was weakening.

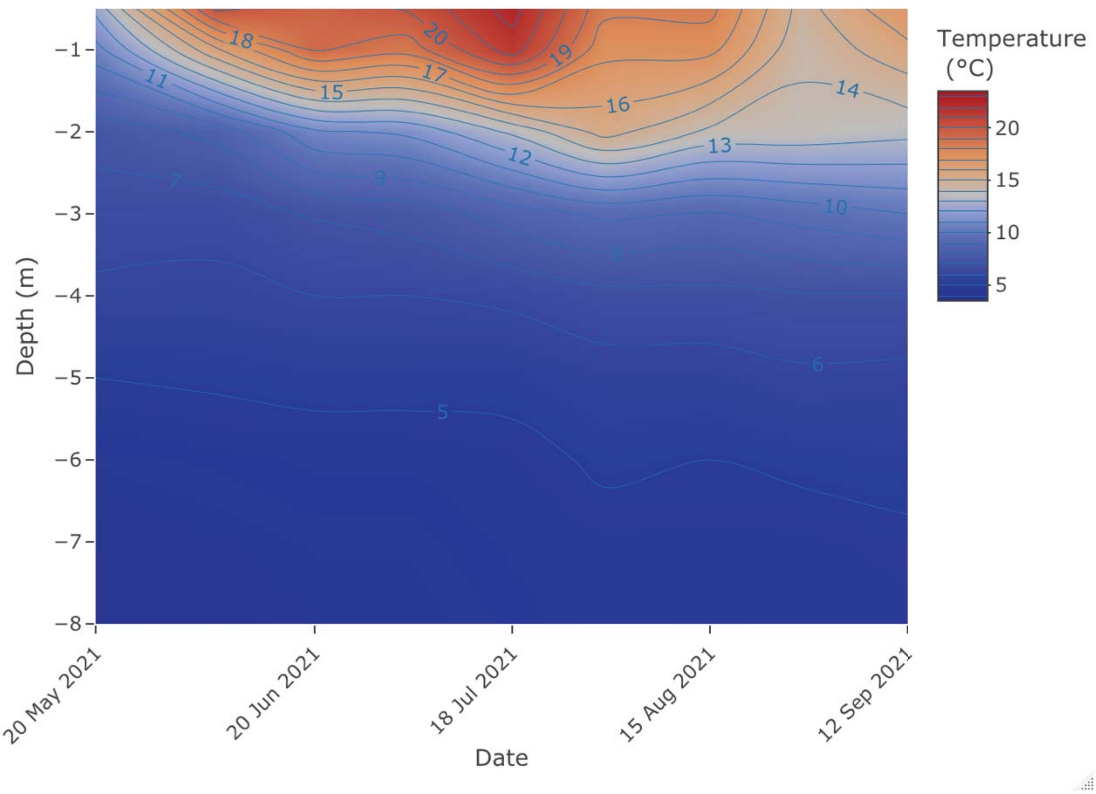

Figure 5. Contour plot showing the temperatures measured at 0.5 m, and every meter from 1 through 8 m in Lake Kutjern every other week from May 20 through September 12, 2021.

The conductivity measurements indicate that there was a clear and quite stable chemical divide between the top and bottom water column throughout the study period (**Figure 6)**. The chemocline had a depth of about 4.5 meters until the end of August when it shifted down to 5 - 6 meters. The difference between the 0.5 m and 8 m of the lake was 1326 µS/cm on average and there was a significant difference between the conductivity measurements of the top (0.5 - 4 m) and bottom (5 - 8 m) water layers (top mean: 114.8 ± 29.6 µS/cm, bottom mean: 1269 ± 234.7 µS/cm, p-value < 0.001). The bottom layer had almost 10 times greater conductivity than the top throughout the study period. From the start to the end of the study period, especially on the last two measuring dates, the conductivity gradient in the water column became gradually less steep.

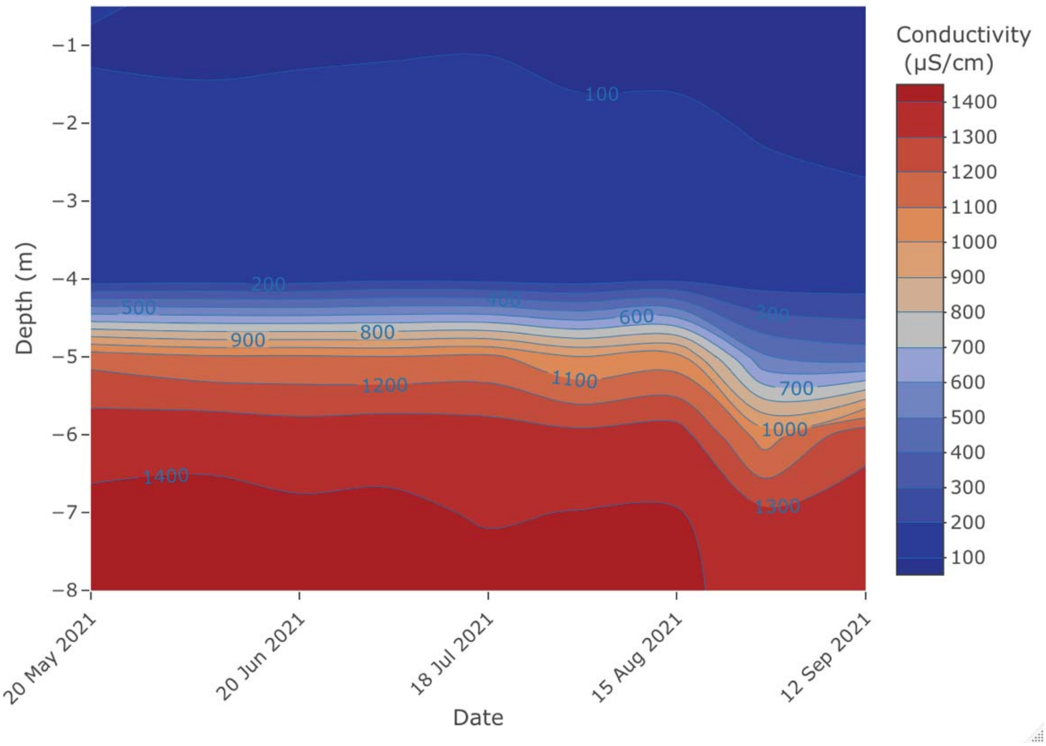

Figure 6. Contour plot showing conductivity measured in Lake Kutjern at 0.5 m and every meter from 1 through 8 m between May 20 and September 12, 2021.

Throughout the period there was a clear oxygen gradient (top – bottom > 6 mg/L). On the first field date (May 20), the oxygen saturation was 97% at 0.5 m (**Figure 7**). This increased to a maximum of 115.8% on June 6, but then decreased and fluctuated between 94.6% and 104.1%. Oxygen was at its minimum on July 31 (64.7%), before it again increased and fluctuated between 87.7% and 73% until the last field day (Sept 12). At the beginning of the study period the lake was anoxic from five meters and below. Then the anoxia gradually increased in extent, until the oxygen conditions followed the thermal stratification that established at the end of June. There were anoxic conditions below 2 m on the 18th and 31st of July. From August 15, when thermal stratification disassociated, the anoxic conditions decreased in extent and were present at 3 m and below. However, it became near-anoxic again at 2 meters in September. The oxygen percentage in the bottom four meters is significantly lower than the top four meters (p-value < 0.01).

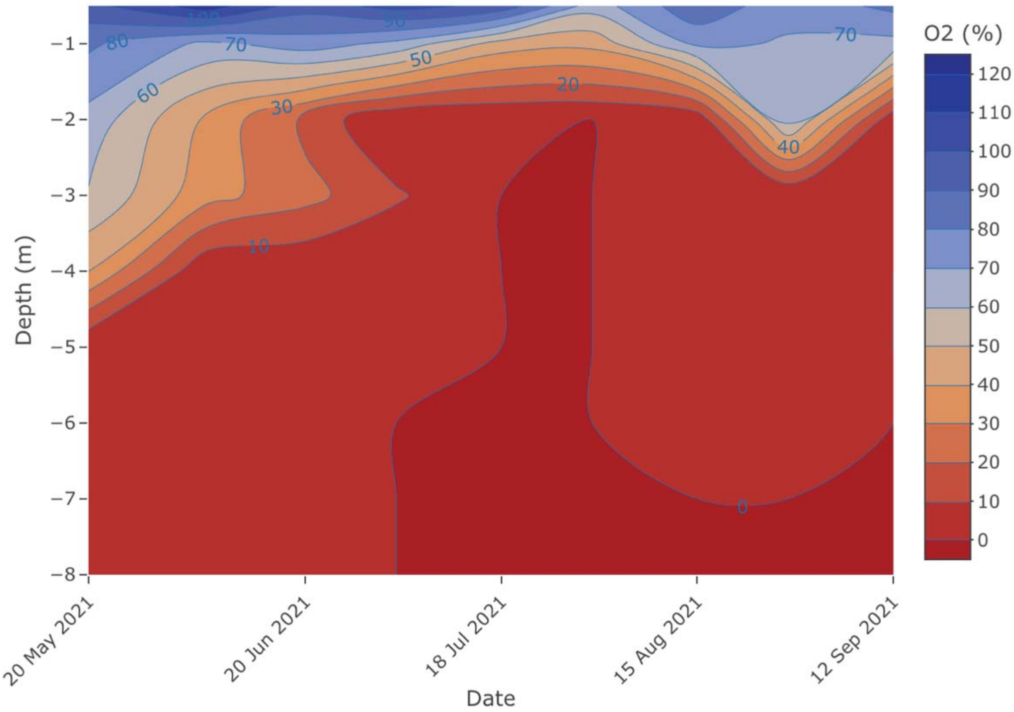

Figure 7. Contour plot showing oxygen percentage measured in Lake Kutjern at 0.5 m and every meter from 1 through 8 m every other week between May 20 and September 12, 2021.

Chloride concentrations correlate well with conductivity and indicate that there is a clear divide between 4- and 6-meters depth (**Figure 8**). The spearman rho correlation coefficient for conductivity and chloride is 0.94 ($p < 0.001$). Average chloride concentrations were 18 times greater in the bottom 6 - 8 meters compared to the top four meters – a significant difference ($p < 0.001$). Hence, the lake is chemically stratified. The chloride concentration at 8 m was the lowest in May, then fluctuated some. It was the greatest on July 3rd at 354 mg/L. On average, the top layer (0.5 - 4 m) chloride concentration was 17 (± 6) mg/L, while the bottom layer (6 - 8 m) average was 300 (± 35) mg/L.

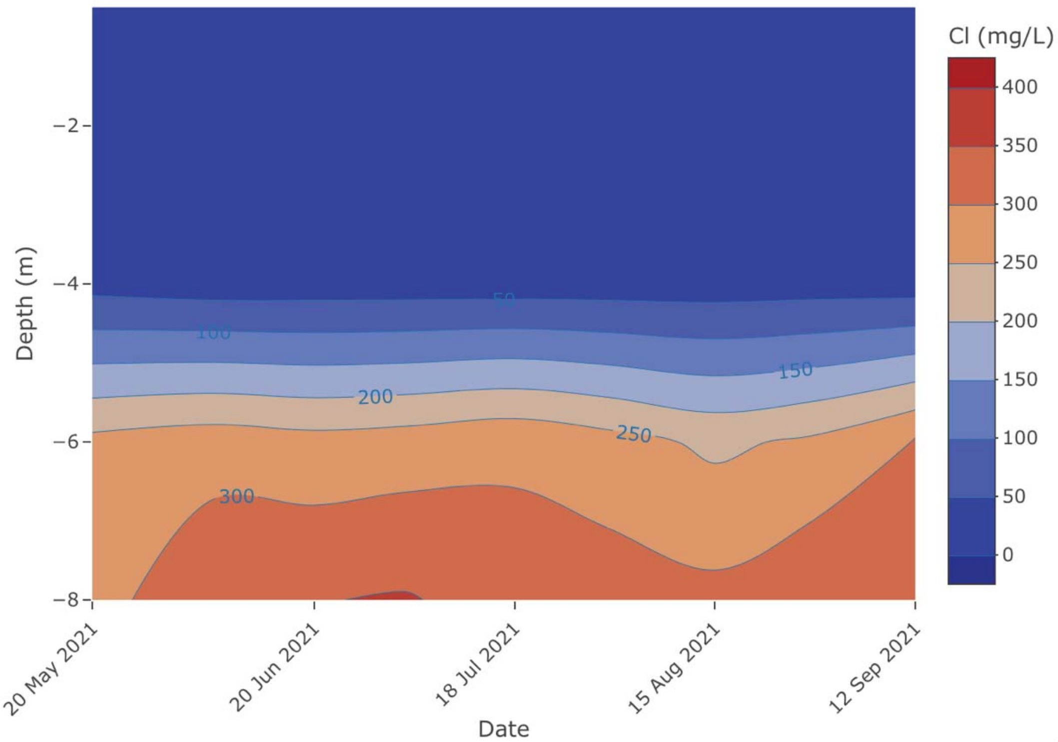

Figure 8. Contour plot showing chloride concentrations measured in water samples gathered in Lake Kutjern at 0.5, 1, 2, 4, 6, and 8 meter every other week between May 20 and September 12, 2021.

3.3. *TEMPORAL AND SPATIAL DISTRIBUTION OF CHEMICALS*

The measurements of iron concentrations were generally high below 4 meters and indicated that there is a boundary at around 5 meters throughout the study period, with a significant accumulation below ($p < 0.01$) (**Figure 9**). The top 0.5 - 4 meters had values below 1 mg/L iron and 0.5 (± 0.2) mg/L on average throughout the study period, except on the two last field dates (Aug 28 and Sept 12) when the concentration at 4 meters was 1.1 mg/L. At 6 meters and below, the concentrations fluctuated little around the average of 8.9 (± 0.3) mg/L throughout the period. The maximum iron concentration was 9.2 mg/L measured at 8 meters on the last three field dates (Aug 15 & 28, & Sept 12).

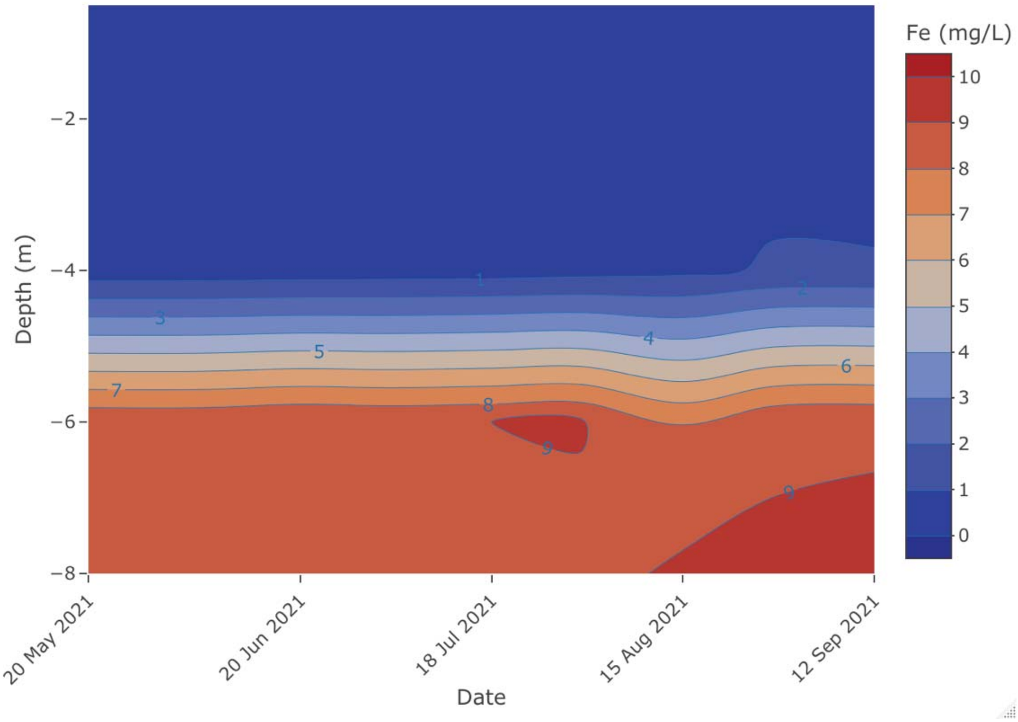

Figure 9. Contour plot showing iron concentrations measured in water samples gathered in Lake Kutjern at 0.5, 1, 2, 4, 6, and 8 meter every other week between May 20 and September 12, 2021.

Measurements of manganese concentrations indicate a similar boundary layer as iron concentration does, at about 5 meters (**Figure 10**). Above and below this layer the values were relatively stable throughout the study period, with higher values at the bottom (bottom layer mean = 0.5 ± 0.02 mg/L) and lower at the top (top layer mean = 0.09 ± 0.05 mg/L). The maximum concentration of 0.55 mg/L was measured at 6 meters on August 28. There was a small increase in the bottom layer concentrations from start of June until the end of the study period.

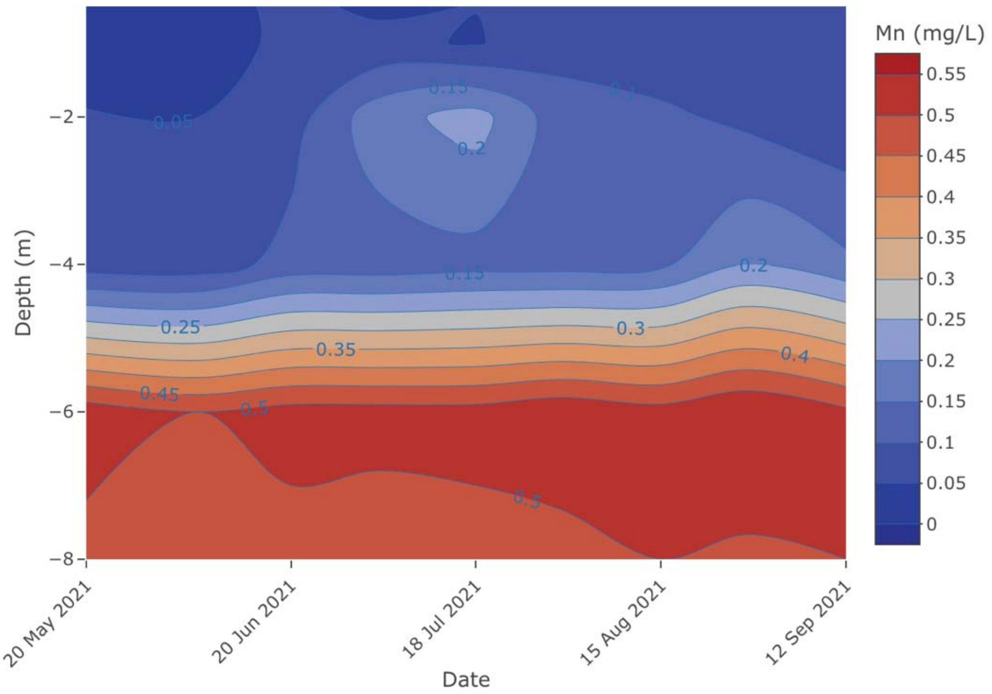

Figure 10. Contour plot showing manganese concentrations measured in water samples gathered in Lake Kutjern at 0.5, 1, 2, 4, 6, and 8 meter every other week between May 20 and September 12, 2021.

Sulfate (SO_4) concentrations varied both temporally and spatially, although this salt was distributed with a boundary between 4 and 6 meters, below which concentrations were low and stable (bottom mean = 0.17 ± 0.12 mg/L) **(Figure 11)**. Sulfate concentrations decreased at 2 and 4 meters from August 15 and onwards. There was a short period of lower concentrations near the surface (0.5 - 1 m) between June 20 and July 18. The maximum sulfate concentration was 4.18 mg/L, measured at 0.5 m on May 20. The average of the top 4 meters was 3.15 ± 0.54 mg/L.

In addition to the sulfate measurements, a strong smell of hydrogen sulfide (H_2S) was observed in the field throughout the study period, mostly for water samples taken from 6 and 8 meters.

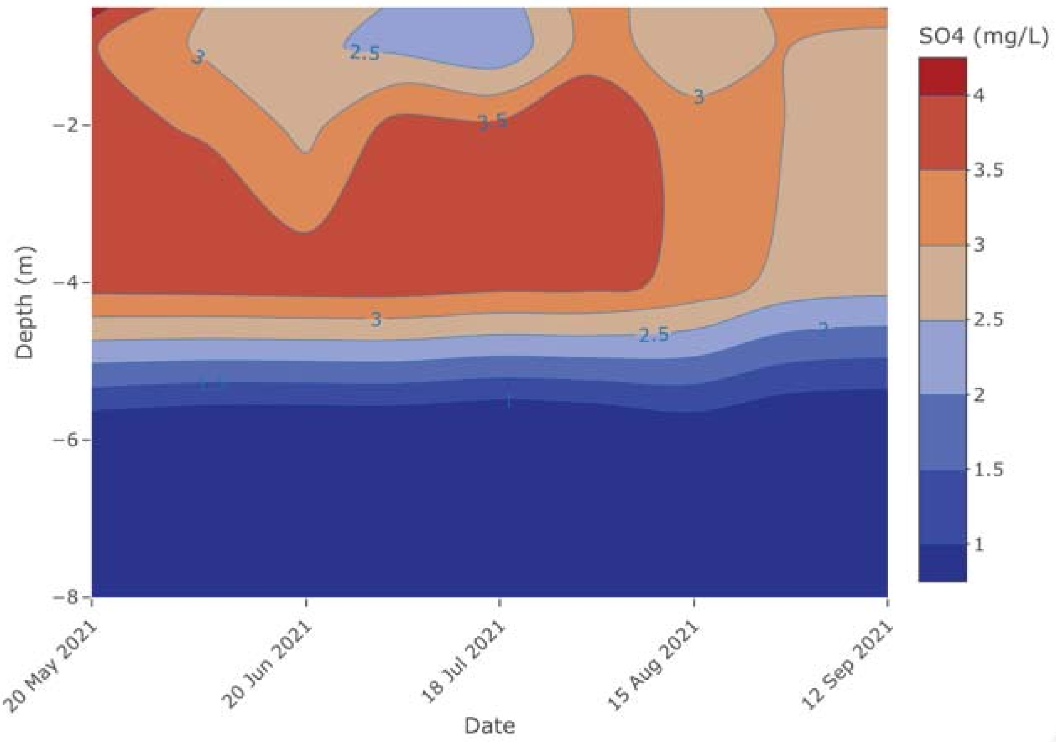

Figure 11. Contour plot showing sulfate concentrations measured in water samples gathered in Lake Kutjern at 0.5, 1, 2, 4, 6, and 8 meter every other week between May 20 and September 12, 2021

Dissolved organic carbon (DOC) was distributed with a general decrease with depth and an increase with time in the top layer (0.5-4 m). The maximum concentration of DOC (24 mg/L) was measured at 0.5, 1 and 2 meters on August 15. Before this there was a pocket of higher concentrations at 1 meter on July 3 (20 mg/L) and at 0.5-1 meters on June 6 (19 mg/L). The average DOC concentration of the lake was 16.4 (± 4.3) mg/L.

3.4. Nutrient availability

The majority of nitrate (NO_3-N) measurements were below detection limit (0.02 mg/L), and the mean of the values above the LOQ was 0.04 mg/L (Table S2). The top 0.5 - 4 meters had an average of 0.02 mg/L, while the bottom 6 - 8 meters had an average concentration of 0.01 mg/L, when half LOQ was used for calculation (0.01 mg/L). Thus, a large fraction of Tot-N is presumably NH_4.

Total nitrogen and ammonium concentrations correlate well throughout the study period (**Figure 12**). There were elevated concentrations of both total nitrogen (Tot-N) and ammonium (NH_4-N) in the bottom water throughout the study period. Both these nutrients' measurements also indicate a boundary layer between 4 and 6 meters, with a significant increase at the bottom (p < 0.01). Above this, total nitrogen has some more variation in concentration than ammonium does, with generally higher values above 3 meters throughout, and a small pocket of more than 1 mg/L on July 3 at 1 m. There is also a small pocket of 0.97 mg/L Tot-N at 1 m on September 12. The maximum concentration of both Tot-N and NH_4-N was 5.4 mg/L, measured at 8 meters on August 15 and September 12 (Tot-N) and on August 28 (NH_4-N). Top layer (0.5-4 m) Tot-N concentrations were 0.56 (± 0.17) mg/L on average, while those of the bottom layer (6-8 m) were 4.04 (± 1.19) mg/L. For ammonium, the top and bottom averages were 0.014 (± 0.009) mg/L and 3.945 (± 1.302) mg/L, respectively. The means for ammonium were, however, calculated with values below detection limit (0.02 mg/L) being counted at half limit value (0.01 mg/L). There were two small "dips" in the concentrations at 6 m of both ammonium and total nitrogen on June 20 and August 15.

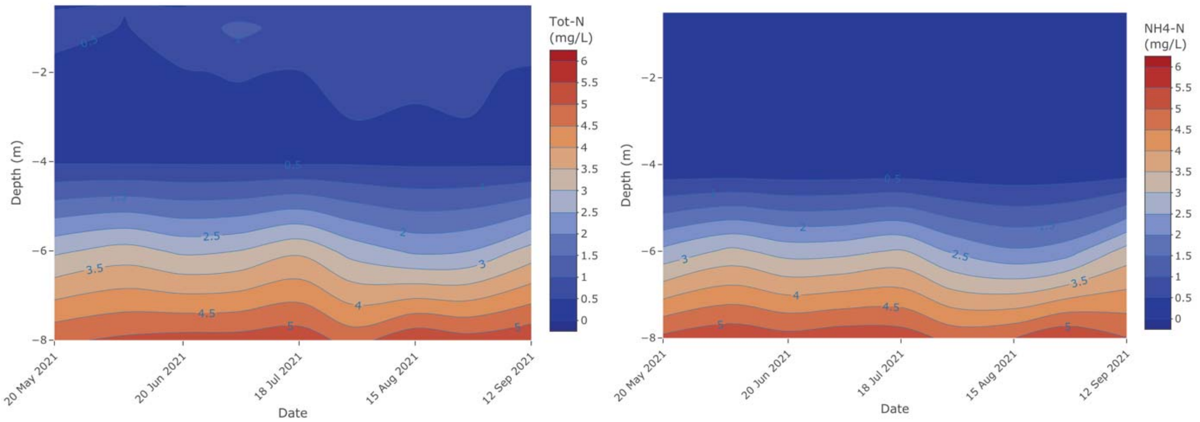

Figure 12. Contour plots showing total nitrogen (left) and ammonium (right) concentrations measured in water samples gathered in Lake Kutjern at 0.5, 1, 2, 4, 6, and 8 meter every other week between May 20 and September 12, 2021.

Like nitrogen and other parameters, total phosphorus measurements indicate a boundary at about 5 meters, but with a general increase both above and below **(Figure 13)**. The bottom layer was, however, significantly greater in average concentration than the top layer ($p < 0.01$). The averages for top and bottom were 31.1 (± 19.5) µg/L and 58.6 (± 21.3) µg/L, respectively. The mean across all depths was 40.3 (± 23.8) µg/L. The ratio of top average Tot-N and Tot-P is below 20, and the sum of ammonium and nitrate is below the detection limit more than once, indicating nitrogen limitation in the top layer (Direktoratsgruppen vanndirektivet, 2018).

Throughout the period, the concentration in the top 4 meters fluctuated. The maximum concentration of Tot-P was 122.5 µg/L, measured at 1 meter on July 3, similar to surface Tot-N. There was also an increase in concentration near the surface at the end of the study period.

The minimum value of 14.2 µg/L was measured at 4 meters on May 20. There were also elevated concentrations of Tot-P at 8 meters in the beginning of the study period, and an increase on August 28 at the bottom, the same day as other parameters change noticeably.

Orthophosphate (PO_4-P) measurements correlate well with Tot-P measurements, especially in the top layer (top: $r = 0.84$, $p < 0.01$, bottom: $r = 0.7$, $p < 0.01$). In contrast with Tot-P, PO_4-P concentrations were significantly greater in the top layer than in the bottom ($p < 0.01$) (**Figure 13**). The top layer average PO_4-P concentration was 3.9 (± 1.7) µg/L and bottom layer average was 2.4 (± 0.7) µg/L. Like Tot-P, PO_4-P's maximum value (9.26 µg/L) was measured at 1 m on July 3. PO_4-P concentrations also increased near the surface towards the end of the study period and reached 9 µg/L at 1 m on September 12. Although not as great as for Tot-P, there were elevated PO_4-P concentrations at the bottom on the first field day. It was 4.4 µg/L at 8 m, a maximum for this depth. The minimum PO_4-P concentration was 1.6 µg/L at 6 m on July 18.

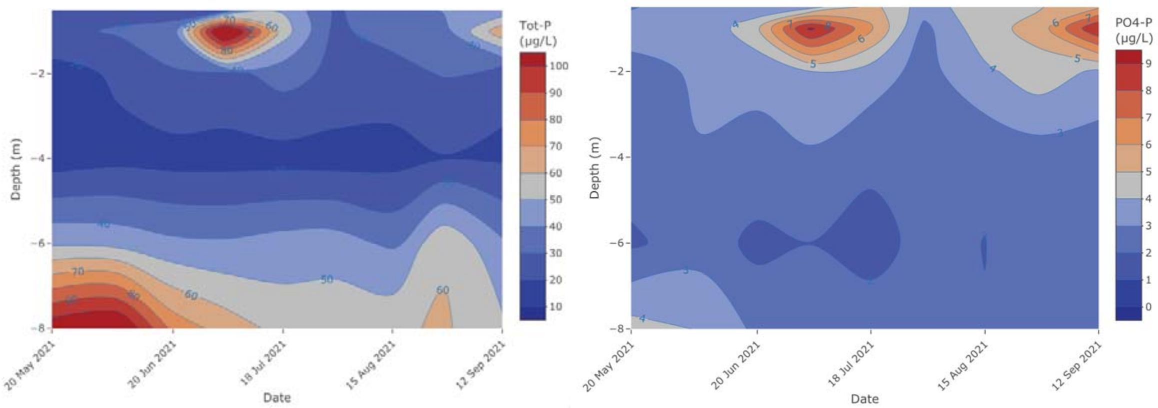

Figure 13. Contour plots showing total phosphorus (left) and orthophosphate (right) concentrations measured in water samples gathered in Lake Kutjern at 0.5, 1, 2, 4, 6, and 8 meter every other week between May 20 and September 12, 2021.

3.5 Spatial distribution and succession of phytoplankton

The pigment analysis resulted in the detection of pigments corresponding to the algal groups *G. semen*, dinoflagellates, and *Synura* (golden-brown algae). Microscopy helped confirm that *G. semen* was present in the samples, confirming that heteroxanthin and diadinoxanthin can be used as its proxy. Fucoxanthin is a pigment found in diatoms and *Synura* (Ehr.). Diatoms were not observed in microscopy, but *Synura* were. Therefore, fucoxanthin is assumed to be from *Synura*, an algal group with motility, rather than from diatoms. Another group with some presence was dinoflagellates, which contain the pigment peridinin. In addition, bacteriochlorophylls a, b, and c were detected. The filters were green, not purple, in color, which indicates that the bacteriochlorophylls in Lake Kutjern come from green sulfur bacteria

(GSB) (Chlorobiaceae) rather than purple sulfur bacteria (PSB). Due to the three types of bacteriochlorophylls being similar in distribution, an analysis of one of them only is provided in these results.

Chlorophyll-a measurements indicate that the phytoplankton in Lake Kutjern were distributed with the greatest density near the surface (0.5 - 2 m) and decreasing with depth (**Figure 14**). In most of the samples, the greatest concentration of chlorophyll-a was found at 1 meter. At the beginning of the study period, there was no chlorophyll-a present at 6 meters and below, and barely any at 4 meters. The concentrations at depths below the surface started increasing on July 3 until September 12, following which they decreased at 6 and 8 meters. The average concentration in the top layer (0.5-4 m) was 28.9 (± 36.3) µg/L, and in the bottom layer (6-8 m) it was 4.4 (± 4.2) µg/L. For this parameter, there was a boundary between two and four meters rather than four and six, which it was for others.

The pigment analyses suggest that two main blooms occurred during the study period. The first one on July 3, when chlorophyll-a concentrations reached 131.2 µg/L at 1 m, and the second one on Sep 12, when concentrations reached a maximum of 168.3 µg/L, also at 1 m depth. There were also gradual, elevated concentrations both temporally and spatially surrounding these points of bloom. Furthermore, there was a general increase in chlorophyll-a concentrations in the bottom water masses from the start to the end of the study period.

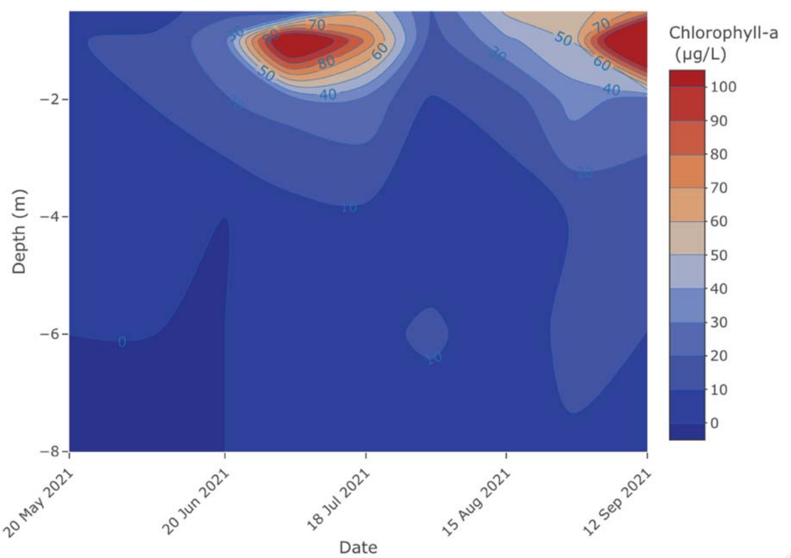

Figure 14. Contour plot showing chlorophyll-a concentrations measured in water samples gathered in Lake Kutjern at 0.5, 1, 2, 4, 6, and 8 meter every other week between May 20 and September 12, 2021. Chlorophyll-a is an indication for phytoplankton activity.

Heteroxanthin values are only presented in milli absorption units per liter (mAU/L) due to the lack of a standard for conversion into mg/L. Hence, these measurements are an indication of the relative changes in *G. semen* concentrations in Lake Kutjern. The heteroxanthin measurements correlated well with chlorophyll-a concentrations, both temporally and spatially. Like chlorophyll-a, the greatest values were measured on July 3 and September 12 (**Figure 15**). There was no heteroxanthin present in the water column until June 20. The bottom water masses had no presence of this pigment until July 18. From then on there was a presence in the bottom layer (with some exceptions at 8 meters), however, never reaching the same levels as the surface. This could be the result of dead biomass settling in the water column.

The other pigments which most likely stem from *G. semen* (violaxanthin, diadinoxanthin and alloxanthin) were present in small densities. At the beginning of the field period, their concentrations were very low, but they increased in density through the study period. However, there were more of these pigments in the lake than there were pigments that represent other algae. All of the presumed *G. semen*-pigments have maximums on the same dates and depths as do heteroxanthin and chlorophyll-a. The pigment that correlates best with heteroxanthin is diadinoxanthin (**Figure 15**). Diadinoxanthin had an average concentration of 4.3 (± 7.1) µg/L in the top layer.

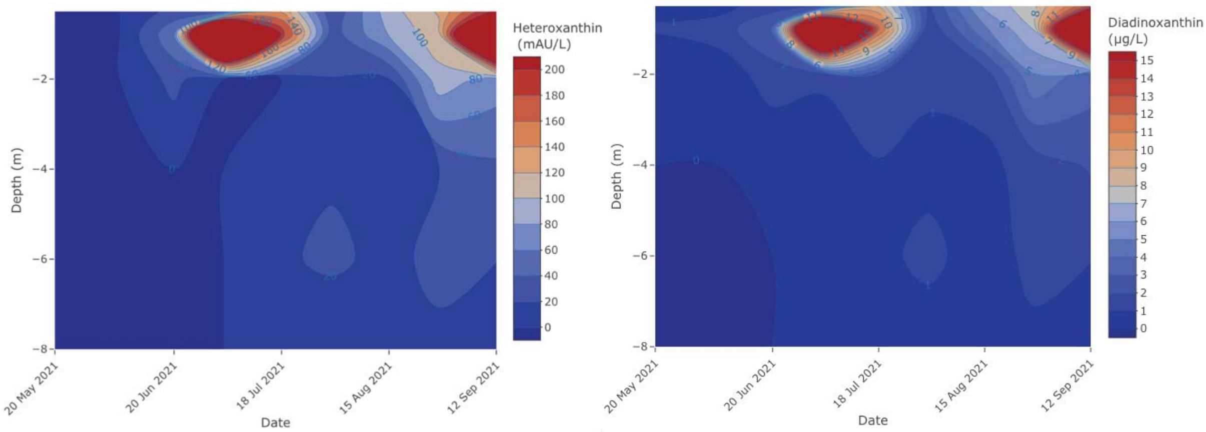

Figure 15. Contour plots showing heteroxanthin (left) and diadinoxanthin (right) concentrations measured in water samples gathered in Lake Kutjern at 0.5, 1, 2, 4, 6, and 8 meter every other week between May 20 and September 12, 2021. Heteroxanthin and diadinoxanthin are proxies for Gonyostomum semen.

Similar to heteroxanthin, bacteriochlorophylls were not converted to mass per liter due to the lack of a standard equation, and are therefore presented in mAU/L. The concentration of bacteriochlorophyll 1 did not have any general trend, but varied throughout the study period, both temporally and spatially (**Figure 16**). There was no detection of bacteriochlorophyll at 4 meters and above before July 3. Until July 18 there was a greater concentration below 4 meters than above, but then it increased in concentrations both around 2-4 meters and 6-8 meters. Towards the end of the study period there was an increase at 4 meters, as well as between 6 and 8 m.

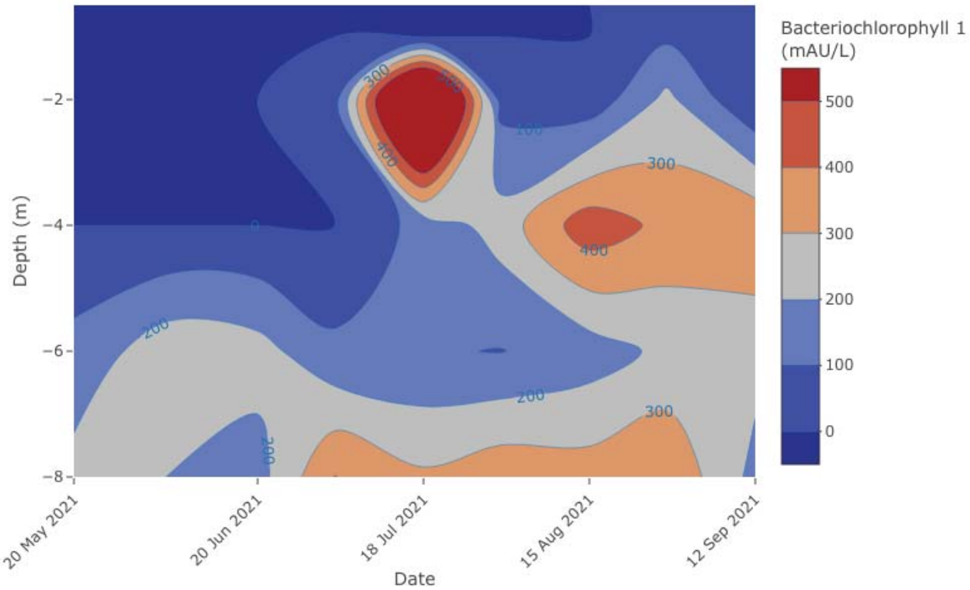

Figure 16. Contour plot showing bacteriochlorophyll concentrations measured in water samples gathered in Lake Kutjern at 0.5, 1, 2, 4, 6, and 8 meter every other week between May 20 and September 12, 2021. Bacteriochlorophyll a is an indication of green sulfur bacteria.

The other pigments present, peridinin and fucoxanthin represent dinoflagellates and *Synura* (golden-brown algae), respectively. These two pigments are mostly present in the top 0.5-2 meters, most of the time *only* above this. The concentrations of peridinin for the 0.5-2 m water samples indicate that there was a small presence of dinoflagellates at the surface from the first field day (**Figure 17**). It gradually decreased in concentration to a minimum of 0.0 µg/L on July 31, before a gradual increase to 1.0 µg/L at 0.5 m on September 12. Measurements of fucoxanthin indicate a presence of *Synura* that fluctuated throughout the period, with a maximum of 2.4 µg/L at 0.5 m on August 15. The lowest concentrations for fucoxanthin in the 0.5-2 m water layer were measured on July 3.

On July 3, when *G. semen* was at its maximum, the concentrations of peridinin and fucoxanthin at 1 m depth were both at their minimums, while diadinoxanthin (*G. semen*) was at its maximum concentration. The days when dinoflagellates and *Synura* were at their greatest densities were mostly when *G. semen* pigments were low compared to its blooms. An exception was the last field day, Sept 12, when many of the pigments were elevated.

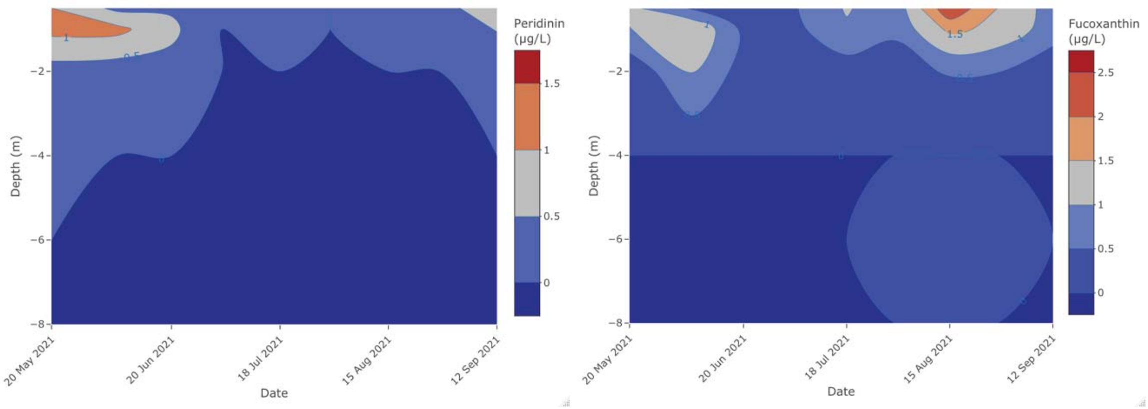

Figure 17. Contour plots showing peridinin (left) and fucoxanthin (right) concentrations measured in water samples gathered in Lake Kutjern at 0.5, 1, 2, 4, 6, and 8 meter every other week between May 20 and September 12, 2021. The pigments peridinin and fucoxanthin are in this study proxies for dinoflagellates and golden-brown algae (Synura (Ehr.)).

3.6 Principal component analysis (PCA)

The PCA biplot of the top layer (at and above 4 m) shows that principal components 1 and 2 explain 67.43% of the variation in the data in total (**Figure 18**). Conductivity and Cl^- have a strong positive correlation. These also have some positive correlation with Fe and Mn. The bacteriochlorophylls do not correlate with the nutrients Tot-N, PO_4-P, and Tot-P, but they correlate negatively with oxygen, and have a small negative correlation with NH_4-N. They also correlate positively with Fe and Mn. Oxygen correlates negatively with conductivity and Cl, and has a less strong (positive) correlation with temperature. DOC and temperature have a strong positive correlation in the top layer. There is a positive correlation between chlorophyll-a and the *G. semen* pigments heteroxanthin, violaxanthin, diadinoxanthin, and alloxanthin. These pigments correlate positively with PO_4-P, Tot-P, and Tot-N. The other pigments – peridinin and fucoxanthin – correlate positively with NH_4-N. Sulfate correlates negatively with these parameters.

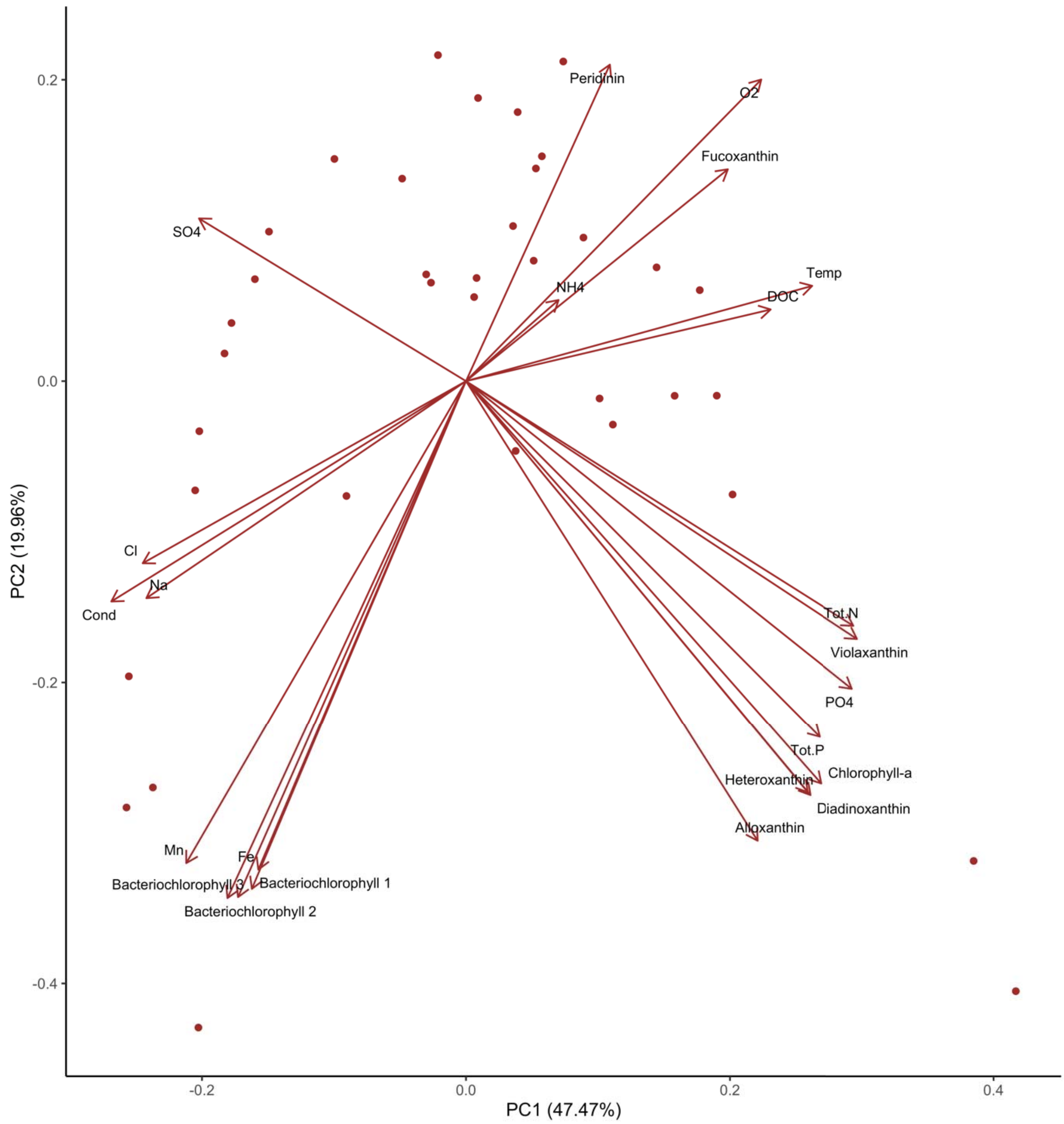

Figure 18. Biplot from principal component analysis (PCA). Parameters include oxygen, conductivity, and temperature measured in situ and other parameters measured in water samples obtained at 0.5, 1, 2, and 4 meter in Lake Kutjern between May 20 and September 12, 2021. The closer the arrows, the more positively correlated two parameters are, and vice versa. A ninety-degree angle between arrows of two parameters indicates that there is no correlation between them.

4. Discussion

4.1. Key findings

4.1.1. Salt stratification is present and stable in Kutjern

As expected, a stable chemical density gradient is present in Lake Kutjern due to road salt runoff. A significant elevation in conductivity levels at and below 5 m correlated with accumulations of chloride throughout the study period indicates that road salt runoff continues to affect the lake. At the top, these parameters are somewhat greater in concentration during the first field dates, presumably due to leftover salt in the catchment brought into the lake after rain. The high concentration of DOC in the lake contributes to the stability of the salt stratification through thermal stratification. Humus steepens the thermal gradient through its contribution to color (Hongve, 1997). However, thermal stratification is less stable than that of salt due to a smaller contribution of temperature to density change. On August 28, the chemical stratification shifts down somewhat, presumably due to a mixing event induced by low temperatures and a temporary weakening of the thermal stratification. As such, road salt has created a more stable stagnation than DOC would have induced alone.

The road salt in Lake Kutjern prevents full circulation of the water column, causing meromixis. This is indicated by the elevated concentrations of reduced substances below the chemocline throughout the study period. Significant accumulations of iron and manganese in the bottom layer of Lake Kutjern indicates reductive desorption of Fe and Mn compounds in anaerobic sediments, releasing these substances into the water column (Wetzel, 2001). The absence of circulation also prevents them from being distributed. These accumulations were present before anoxia by thermal stratification was established and must result from continuous bottom water anoxia. Otherwise, they would have started accumulating at the onset of thermal stratification, and then increased in extent (Wetzel, 2001). The lack of measurable nitrate and sulfate at the bottom throughout the study period, further supports the argument for meromixis in Kutjern (Wetzel, 2001). It indicates that the monimolimnion has been anoxic for a longer time period causing denitrification of nitrate and reduction of sulfate during anaerobic respiration (Beaulieu et al., 2014; Beutel et al., 2008; Su et al., 2019). These findings show that road salt has caused meromixis in lake Kutjern, preventing spring and fall mixing and thus suppressing the oxygen supply to the bottom waters and accumulating nutrients in the otherwise nutrient poor lake.

Road relocation may have reduced salt levels and deepened the chemocline in Kutjern. Compared to 2015 and 2016, chloride concentrations are lower, and bottom conductivity has decreased (Saunes & Værøy, 2017). The chemocline has shifted downwards by about 2 meters compared to June 2016 and by 1 meter compared to November 2016. November should be the month of deepest chemocline position, due to wind having the best chance to destabilize the stratification when temperatures are low. As the chemocline was still deeper during the 2021 summer, the relocation of the E18 highway in 2017 may have lowered the salt content and stratification. The nearby sport center has never used salt, and there are no other likely salt sources around the lake (N. Skogstad, personal communication, March 30, 2022). However, we only have previous data from 2015 and 2016, and cannot conclude the significance of difference between these years. Nevertheless, even if road relocation may reduce runoff pollution and improve conditions in the long term, previously added salt continues to greatly alter the lake biogeochemistry.

4.1.2. Effects of meromixis on nutrient distribution

The hypothesis that meromixis in lake Kutjern has led to accumulations of ammonium and phosphate is partly confirmed by the results. Both ammonium and total nitrogen measurements show clear and significant accumulations in the bottom waters. The elevated top layer concentrations of total nitrogen correspond with phytoplankton blooms and likely stem from phytoplanktonic and bacterial organic matter. Forest lakes like Lake Kutjern typically have low nitrogen availability due to a nutrient poor catchment area and high demands for this nutrient. This, in combination with a long water residence time, normally leads to low nitrogen content due to fast biological uptake, burial, and subsequent denitrification during anaerobic respiration (Wetzel, 2001). Assumably, this would have been the case for Lake Kutjern if it did not receive road salt, at least before the onset of thermal stratification. The strong density gradient prevents the distribution of ammonium liberated by microbial decomposition of organic matter in the monimolimnion and sediments (Boehrer et al., 2017). Tot-N and NH_4-N accumulations correspond with the depth of the chemocline before the thermal stratification developed, supporting the hypothesis that salt is the cause. Otherwise, they would distribute in the water column through mixing, and nitrate would form through nitrification of ammonium.

Phosphate on the other hand, had not accumulated in the bottom layer as hypothesized. Surprisingly, the concentration was significantly greater in the top layer than in the bottom. The greater surface concentrations of phosphate seem to be especially associated with remineralization of organic matter during phytoplankton blooms. Total phosphorous (Tot-P), however, had accumulated in the bottom.

The bottom accumulation of Tot-P and the lack of that of PO_4^{3-} could be due to sorption of PO_4^{3-} to ferric hydroxide forming in the oxic zone, before sinking into the anoxic zone (Hongve, 1997). The reductive desorption of these compounds in the anoxic bottom waters may be prevented due to a redox coupling of Fe(II) as an electron donor and Mn(VI) as an electron acceptor in mixed potentials redox reactions. Hongve (1997) showed that ferric oxide formed in the anoxic zone of a meromictic lake due to interactions with manganese cycling, constraining phosphorus recycling from the sediments. This was a more important process in the lake than was the formation of Fe(II)sulfide, and is likely what is happening in Lake Kutjern as well.

The presence of green sulfur bacteria (GSB) and *G. semen* could also be an important factor affecting PO_4^{3-} distribution in Kutjern. For instance, the formation of FeS could be competing with H_2S uptake by GSB, further restricting PO_4^{3-} release (Wetzel, 2001). The formation of FeS tends to be more efficient in PO_4^{3-} release (when SO_4^{2-} is available) than the microbial reduction of Fe(III) to Fe(II), so if this process is restricted, it may be lowering the rate of PO_4^{3-} release. One study found that under continuous light, H_2S utilization by *Chromatium* (purple sulfur bacteria) was more effective than ferric iron reduction by H_2S (van Gemerden, 1967). Additionally, some strains of GSB may use ferrous iron as an electron donor for growth (Frigaard & Bryant, 2008). The resulting ferric iron would react with PO_4^{3-} to form Iron(III)phosphate. Hence, GSB utilization of ferrous iron could potentially precipitate PO_4^{3-} and together with the manganese interactions, prevent its re-release.

A more important consequence of GSB presence is likely their uptake of PO_4^{3-} as a nutrient. As indicated by the bacteriochlorophyll measurements, the GSB were present at 2-4 m on July 18, but they already started increasing at 6 m on June 6. Before the GSB increased, Tot-P and PO_4-P concentrations were elevated in the monimolimnion. PO_4-P concentration was at its minimum at 6 m on July 18, when the largest GSB bloom occurred. The GSB require PO_4^{3-} as a P source in anoxygenic photosynthesis and thus probably contribute to its depletion in the monimolimnion. The stability of the water column due to meromixis creates conditions that are optimal for producers with specific niches or competitive advantages, such as GSB and *G. semen,* respectively (Chapin et al., 2004; Pfennig, 1975; Salonen & Rosenberg, 2000). These organisms may be of importance to nutrient distribution in the lake.

Furthermore, *G. semen* started appearing on June 20th, which is when monimolimnic PO_4^{3-} concentrations decreased. This could potentially be due to hypolimnetic assimilation. Such a scenario has previously been found in a small, stratified humic lake in Finland (Salonen & Rosenberg, 2000). Increases in Tot-P at the surface indicates *G. semen* migrating up and supplying the surface waters with P (Salonen et al., 1984). As blooms senesce, and the carbon to P ratio increases, microbes in the monimolimnion would also take up PO_4^{3-}, potentially contributing to the lack of PO_4^{3-} accumulation (Hessen, 1992). Since the supply of phosphate from the nutrient poor catchment area is low, any phosphate released from sediments by anoxic desorption seem to be exhausted by *G. semen*- and GSB-uptake. This, together with manganese interactions with ferric hydroxide formation are likely what prevent PO_4^{3-} from accumulating in the monimolimnion of Lake Kutjern.

Another potential reason for the low bottom phosphate concentration could be the absence of microfaunal bioturbation due to anoxia (Anderson et al., 1985; Villnäs et al., 2012). This leads to reduced resuspension and mineralization of organic matter, and reduced burrowing, thus could reduce phosphate release into the water column. However, a study showed that anoxia had a much greater influence on P release than did bioturbation (Gautreau et al., 2020). Thus, lack of bioturbation could be of minor importance to the absence of phosphate accumulation.

4.1.3. Dissolved organic carbon (DOC)

G. semen blooms contribute to DOC while also taking advantage of it. The increase in epilimnetic DOC on July 3rd corresponds with increased phytoplanktonic activity and not with precipitation ahead of sampling. Precipitation in the catchment area brings both nutrients and organic matter (including DOC) into lakes (Wetzel, 2001). Hence, when DOC increases outside of precipitation events, it is likely autochthonous DOC. *G. semen* produces slime through trichocyst expulsion, and this contributes to DOC (Rengefors et al., 2008; Rengefors et al., 2012). The DOC content in Lake Kutjern is high and likely contributes to a steep thermal gradient, which *G. semen* is known to thrive in.

4.1.4. Effects of road salt-induced stratification on the phytoplankton community

Meromixis has consequences for the composition of the algal community in Lake Kutjern through nutrient limitation. Measurements of epilimnetic NH_4-N and NO_3-N are both below detection limit throughout most of the period. NH_4^+ is preferred over NO_3^- as an N source for alga, so the lack of NO_3-N is a clear indication of N-limitation (Wetzel, 2001). Furthermore, if bioavailable N were present at the surface, phosphate would likely be exhausted below detection, as P tends to be the least abundant and thus the limiting nutrient for phytoplankton growth (Wetzel, 2001). Its presence therefore further suggests N-limitation at the surface, while NH_4^+ accumulates at the bottom. This scenario commonly leads to a selection for motile algae

(Cronberg et al., 1988; Deininger et al., 2017; Rengefors et al., 2012; Rohrlack, 2020b; Salonen & Rosenberg, 2000).

The results indicate that salt stratification and its effect on nutrient distribution is shaping the algal community by selecting for flagellated algae, and specifically for *G. semen*. Firstly, ammonium is only reachable for algae that can assimilate it from below the chemocline. The pigment analysis shows that most of the phytoplankton in Lake Kutjern are flagellated and motile, with insignificant concentrations of other groups present. Phytoplankton with flagella, such as *Synura*, dinoflagellates, and *G. semen*, can swim to obtain nutrients and avoid grazers (Hagman et al., 2020; Kristiansen, 2009; Salonen et al., 1984). In 2017, the majority of the biomass consisted of cyanobacteria, and no Raphidophycea (*G. semen*) were present (Saunes & Værøy, 2017). These results suggest a potential shift in the community towards motile algae.

In Lake Kutjern, decreases in monimolimnic NH_4^+ at the start of *G. semen* blooms indicate uptake through DVM at 6 m. The results indicate small variations in NH_4^+ in relation to *G. semen* blooms, and a 0.56 mg/L decrease in NH_4^+ at 6 m from the beginning until August 15. Studies have shown that *G. semen* can regulate ammonium and phosphate concentrations in lakes through hypolimnetic assimilation during DVM (Cronberg et al., 1988; Rohrlack, 2020a, 2020b; Salonen & Rosenberg, 2000). However, there is not a clear decreasing trend in NH_4^+ or Tot-N. The increases in bottom NH_4^+ at and after maximum blooms could originate from breakdown of sinking organic matter. Green sulfur bacteria could also be taking up ammonium at this level, possibly “cancelling” out any large variations that would have corresponded with *G. semen* uptake. Ammonium accumulation in the monimolimnion and an overrepresentation of *G. semen*, suggest that meromixis due to road salt has an effect on the phytoplankton composition in Lake Kutjern.

Another likely consequence of salt stratification in Kutjern is the presence of green sulfur bacteria (GSB) - a competitor to *G. semen* and other alga. Bacteriochlorophyll measurements indicate a presence of GSB at 2-4 meters, and below. These are autotroph bacteria that perform anoxygenic photosynthesis, oxidizing sulfide in the process (Hanada, 2016). They are able to grow at low sulfide and light availability (Chapin et al., 2004). Due to the specific requirements of GSB, they tend to appear in abundance at the boundary between oxic and anoxic conditions (Wetzel, 2001). This is where they are found in Lake Kutjern. Stratified lakes often provide conditions optimal for the growth of GSB (low redox potential and somewhat alkaline pH)

(Wetzel, 2001). GSB are sensitive to water column stability and have been found to produce deep maxima in meromictic lakes around the world (Chapin et al., 2004; Croome & Tyler, 1984; Tuomi et al., 1997; Vila et al., 2002). The steep and long-lasting salt gradient in Lake Kutjern may provide the necessary stability for the GSB to thrive here.

In addition to promoting the dominance of motile algae, meromixis may favor mixotrophy in Kutjern. *Synura* and dinoflagellates were both found in Lake Kutjern, and many algae in these groups are mixotrophic (eg. *Peridinium* and *Chrysophytes*) (Kristiansen, 2009; Rogers, 2017). Mixotrophy is the combination of photoautotrophy and heterotrophy for energy acquisition (Weithoff & Wacker, 2007). Mixotrophic alga can obtain energy both from grazing on other organisms and from sunlight in photosynthesis. Some can also obtain dissolved organic compounds through osmosis (osmotrophy). Mixotrophy is a trait that is beneficial and common in humic and stratified lakes (Jansson et al., 1996; Pålsson & Granéli, 2004; Salonen et al., 1984; Wetzel, 2001). It is uncertain whether the dinoflagellates and *Synura* found in Lake Kutjern are mixotrophs, but feeding on bacteria could be an advantage there, where there is little nutrient availability above the chemocline (Wetzel, 2001). N-limitation can also be a sign of mixotrophy (Jansson, 1996 #325). Bacteria have a high affinity for P and are also better at taking up P than N (Wetzel, 2001). They may occur at the interface between the oxic and anoxic zone, taking up P from the hypolimnion. Being in reach for alga in the euphotic zone, mixotrophs have access to bacteria as a P source. This can lead to N-limitation (Jansson, 1996 #325). In Lake Kutjern, GSB are potentially an additional food source for mixotrophs.

The results indicate that also *G. semen* may be mixotrophic. There was a much greater density of the pigment diadinoxanthin, the proxy for *G. semen*, than of pigments of other algae in Lake Kutjern. This suggests that *G. semen* must have similar traits and a competitive advantage over *Synura* and dinoflagellates. It may be consuming its own competitor as a result of nutrient limitation and competition (Thingstad et al., 1996). When *G. semen* blooms, bacteriochlorophyll decreases in extent and in intensity. After the second bloom of GSB, the *G. semen* pigments increase, during the last month of the study period. Although GSB may be competing for the same nutrients, the second bloom of GSB does not seem to negatively affect the blooming of *G. semen*. Since the bacteria are present within reach of *G. semen,* they may therefore be its prey. Rengefors et al. (2008) showed through laboratory experiments that providing *G. semen* with the flagellated phytoplankton *Rhodomonas lacustris,* enhanced its growth rate. *R. lacustris* was negatively affected by *G. semen* presence during culturing. The study did not find evidence of phagotrophy, but of cell lysis. It suggests that cell lysis through trichocyst expulsion (slime thread ejection), and osmotrophy are two possible reasons for *G. semen* dominance in humic lakes (Rengefors et al., 2008). In Kutjern, *G. semen* could potentially be obtaining nutrients from cell lysis of its competitor, the GSB. Future studies should look at pigments unique to GSB to examine *G. semen*'s consumption of these bacteria.

Given that both dinoflagellates and *Synura* are motile and potentially mixotrophic, *G. semen* must have other adaptations that makes it dominate the phytoplankton community of Lake Kutjern. *G. semen* is known to be especially good at diurnal vertical migration (DVM), making it competitive in humic and stratified lakes (Johansson, 2013; Salonen & Rosenberg, 2000). One reason could be that it is better at swimming. However, dinoflagellates and *Synura* may also be able to swim (Wetzel, 2001). On the other hand, *G. semen* may be better at swimming than these as it has two flagella, one that acts as a motor, the other to steer it. *Synura* have many cells and may have difficulties coordinating its swimming, while dinoflagellates tend to have only one flagellum and thus less motility. In a lake like Kutjern, where DOC causes light limitation and meromixis nutrient limitation, it would be an advantage to be able to swim fast and determined towards food (bacteria) and light. Furthermore, *G. semen*'s competitiveness may be due to other traits, which should be studied further. *G. semen*'s mucus production could potentially be a defense mechanism against grazers (Lebret et al., 2012; Rengefors et al., 2012). Rengefors et al. (2008) also argue that *G. semen* may become dominant as a result of DOC utilization. DOC content in Lake Kutjern is high, providing the conditions for this to be beneficial. These are theoretical reasons for its dominance over other algae, and should be further examined to confirm such assumptions.

If *G. semen* is not eating its competitor, the stability of its dominance may be restricted by competition with GSB. The results indicate that immediately after the maximum production of the first *G. semen* bloom, GSB start increasing in density. At the same time, the *G. semen* bloom breaks down. It is uncertain whether *G. semen* can take advantage of GSB by eating them, especially if GSB make colonies too big to consume. Additionally, autotrophy is less energy-intensive than heterotrophy, which leaves GSB with a competitive advantage, if *G. semen* depends on heterotrophy to sustain its growth (Thingstad et al., 1996; Wetzel, 2001). The nutrient levels are low in the areas occupied by both *G. semen* and GSB. PO_4^{3-} concentration is low between 4 and 6 meters, and especially at 6 m, which is likely the limit to where *G. semen* swims. This is also where GSB are found ahead of thermal stratification.

Nitrogen is low between 2 and 4 meters, where the largest GSB bloom was found. GSB have previously been found to be nitrogen limited (Chapin et al., 2004). A study also found phosphatase activity in GSB, indicating a potential to exploit tot-P (Baneras et al., 1999). In Lake Kutjern they may therefore be competing with *G. semen*, especially for nitrogen. GSB do not require as much light as algae do, and they stay outside the oxic zone (Wetzel, 2001). Therefore, they can access nutrients from below the thermocline and chemocline, similarly to *G. semen.* It is difficult to determine the extent of competition between GSB and *G. semen* without having biomass measurements for GSB. Either way, if stability makes GSB more competitive, road salt-induced stratification could put a restriction on *G. semen* through nutrient competition in the long term.

4.1.5. Road salt and G. semen dominance in the future

Climate change may cause an additional impact on processes affecting *G. semen*. In Kutjern, both salt and humus create conditions that are conducive for *G. semen* growth (Hagman, Skjelbred, et al., 2019; Johansson, 2013; Lepisto et al., 1994). Climate change may lead to *G. semen* dominance in more lakes, if it causes perturbations to lake mixing regimes and increased salt application on roads (Kraemer et al., 2015; Woolway et al., 2020; Woolway & Merchant, 2019). For example, reduced ice cover due to warming could lead to more stable and longer periods of thermal stratification (Woolway & Merchant, 2019; Woolway et al., 2020). Furthermore, a wetter climate will lead to browning, through erosion and input of humic substances (Meyer-Jacob et al., 2019; Woolway et al., 2020; Woolway & Merchant, 2019). This would create a shallower and steeper thermocline leading to dominance of *G. semen* and other algae or cyanobacteria with similar competitive advantages (Johansson, 2013). An indirect implication of climate change is the increase in road salt use due to increased periods with temperatures around freezing point in Norway (Norwegian Public Roads Administration (NPRA), 2019). This could also lead to the expansion in *G. semen* dominance to more roadside, having negative implications for water management and recreation.

Knowledge on factors affecting freshwater algal communities is especially important in Norway, where most drinking water sources are surface waters. *G. semen*'s slime may clog water filters and may therefore cause increased costs for drinking water management in the future (Cronberg et al., 1988; Hongve et al., 1988; Rengefors et al., 2008). With this study, road salt is added to the suggested drivers for *G. semen*'s success in Norwegian lakes, which has previously been shown to be to some degree caused by temperatures, TOC, watercolor, and phosphorus (Hagman et al., 2015; Hagman et al., 2020).

4.2. LIMITATIONS

While the study has pointed out the consequences of salt stratification on algal community composition in Lake Kutjern, lakes are complex systems with unique processes, such that what influences the dynamics in one lake may not result in the same in other lakes. Although there are some processes that are made possible by the special conditions in meromictic lakes, they have varying chemical compositions depending on local conditions and lake history (Wetzel, 2001). Hence, it is difficult to apply general theories to specific lakes.

This study has several limitations with regards to data availability and variables studied. For instance, measurements were only taken every other week and not at every meter of depth. Algae are not necessarily evenly distributed, so taking samples at only one location may not be representative for the whole lake system. While this project has identified effects of salt stratification and it being a potential driver of *G. semen* dominance, other factors that have not been considered here, such as metal content, could play an important role. Predators may have a significant effect on algal community composition, and these were not studied (Rohrlack). Dominance of *G. semen* could also be due to humus (Hagman et al., 2015; Hagman, Skjelbred, et al., 2019). Thus, the confounding effects of DOC and temperature on algal community dynamics should be accounted for. The lack of extensive, year-round, historic, and continuous data restricts the understanding of processes occurring in Lake Kutjern.

5. CONCLUSIONS

By studying parameters such as temperature, oxygen, conductivity, and chloride in Lake Kutjern through the summer of 2021, this study shows that salt stratification is present throughout the study period. Bottom accumulations of iron, manganese, and ammonium, as well as a lack of nitrate and sulfate suggest meromixis. Furthermore, the results indicate that nutrients “trapped” in the monimolimnion have encouraged a dominance of flagellated algae in the lake’s algal community. The findings of this study suggest that prolonged salt stratification could lead to a dominance of the flagellated nuisance alga *G. semen*. This is due to its competitive advantage in such conditions, namely its ability to perform vertical migration and obtaining nutrients separated from the euphotic zone, in this case ammonium. Potentially, mixotrophy adds to its competitiveness and leads to further access to nutrients from bacterial uptake. The detection of green sulfur bacteria suggests competition for resources or a potential food source to *G. semen*. Further research is necessary to understand the mixotrophic abilities of *G. semen* and the role of GSB as potential prey, or as a competitor restricting its dominance in meromictic lakes. Future studies should also look at the interactions between Mn and Fe and effect on PO_4^{3-} release together with biological controls on monimolimnic PO_4^{3-} concentrations.

This study's findings suggest that salt application on roads has a potential negative effect on lake biodiversity and may cause future trouble for water management if climate change indeed leads to changed mixing regimes and increased periods of stratification. With climate change and increased salt use in mind, future road planning should take into consideration the proximity of roads to lakes and the impacts of road salt application on lake ecosystems and water management.

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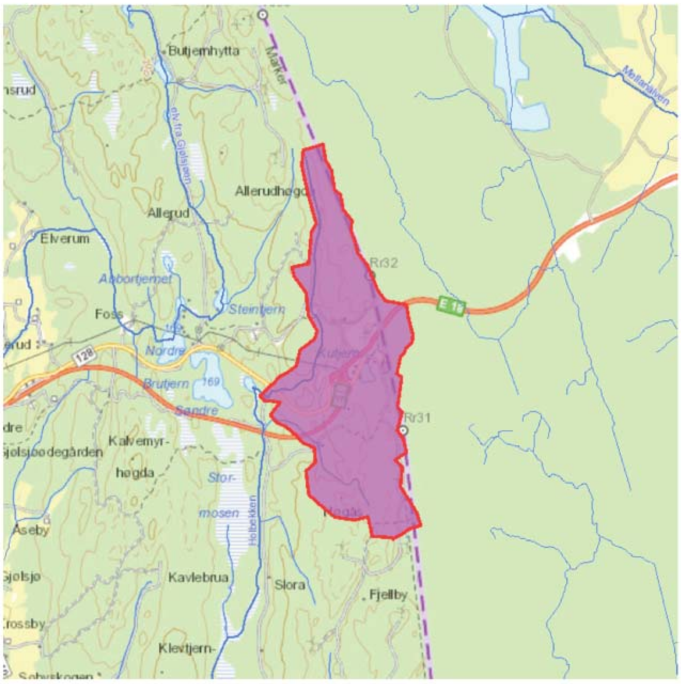
Supplemental material



Norges vassdrags- og energidirektorat

NVE

Kartbakgrunn:	Statens Kartverk
Kartdatum:	EUREF89 WGS84
Projeksjon:	UTM 33N
Beregn.punkt:	314920 E 6598229 N

Nedbørfeltgrenser og feltparametere er automatisk generert og kan inneholde feil. Resultatene må kvalitetssikres.

Nedbørfeltparametere

Vassdragsnr.:	**314.A4Z**
Kommune.:	**Marker**
Fylke.:	**Viken**
Vassdrag.:	**Holbekken**

Feltparametere		
Areal (A)	2.3	km²
Effektiv sjø (A_{SE})	-999	%
Elvleengde (E_L)	2.5	km
Elvegradient (E_G)	25.1	m/km
Elvegradient$_{1085}$ ($E_{G,1085}$)	27.3	m/km
Helning	4.9	°
Dreneringstetthet (D_T)	1.7	km^{-1}
Feltlengde (F_L)	2.1	km

Arealklasse		
Bre (A_{BRE})	0	%
Dyrket mark (A_{JORD})	0	%
Myr (A_{MYR})	0	%
Leire (A_{LEIRE})	0	%
Skog (A_{SKOG})	98.5	%
Sjø (A_{SJO})	0.7	%
Snaufjell (A_{SF})	0	%
Urban (A_U)	0	%
Uklassifisert areal (A_{REST})	0.6	%

Figure S 1. Map and statistics of the catchment area of Lake Kutjern. The catchment area is automatically generated based on river networks and natural boundaries in the field. It may be erroneous. The effective sea area is incorrect due to the modification done while adding together two catchment areas. Map report generation was only available in Norwegian. Source: Statens Kartverk. (2022). Nedbørfeltparametere. Available at: https://nevina.nve.no/. Access date: 7 March, 2022.

Figure S 2. Map of superficial deposits in the area where lake Kutjern is located. Map generation was only available in Norwegian. Source: Geological Survey of Norway. (n.d). *Contains data under the Norwegian License for Open Government Data (NLOD) made available by Geological Survey of Norway (NGU). Available at: https://geo.ngu.no/kart/losmasse_mobil/. Access date: 7 March, 2022.*

Table S1. Raw in situ data measured in Lake Kutjern.

Date	Depth	Temperature	O2	O2	Conductivity
d.m.yyyy	m	°C	mg/L	%	µS/cm
20.5.2021	0.50	12.3	10.07	97.0	109.5
20.5.2021	1	10.6	9.55	82.0	89.1
20.5.2021	2	7.4	7.47	66.5	127.7
20.5.2021	3	6.5	6.85	59.1	131.6
20.5.2021	4	5.8	4.83	40.1	135.5
20.5.2021	5	5	0.07	0.7	1167.0
20.5.2021	6	4.5	0.03	0.2	1368
20.5.2021	7	4.3	0.02	0.1	1419
20.5.2021	8	4.2	0.01	0.1	1429
6.6.2021	0.50	20.2	10.23	115.8	78.3
6.6.2021	1	15.8	6.69	67.7	74.4
6.6.2021	2	8.3	4.35	37.8	131.5
6.6.2021	3	6.4	4.1	33.6	136.4
6.6.2021	4	5.7	0.2	1.1	149.7
6.6.2021	5	5.1	0.02	0.2	1116.0
6.6.2021	6	4.6	0.01	0.1	1381.0
6.6.2021	7	4.4	0.01	0.1	1418.0
6.6.2021	8	4.3	0.01	0.1	1427.0
20.6.2021	0.50	19.4	8.29	94.6	82.3
20.6.2021	1	19.10	6.72	75.1	85.2
20.6.2021	2	10.8	2.03	17.2	132.3
20.6.2021	3	7.1	2.73	23.3	135.5
20.6.2021	4	6	0.14	1.2	145
20.6.2021	5	5.2	0.04	0.3	1115
20.6.2021	6	4.7	0.01	0.1	1357
20.6.2021	7	4.5	0.01	0.1	1414
20.6.2021	8	4.4	0.01	0.1	1425
3.7.2021	0.50	20.1	9.34	104.1	88.2
3.7.2021	1	18.4	5.32	67.1	90.1
3.7.2021	2	11.2	0.01	0.2	137.4
3.7.2021	3	7.4	1.33	11.3	136.3
3.7.2021	4	6	0.02	0.2	176.3
3.7.2021	5	5.2	0.01	0.1	1104
3.7.2021	6	4.7	0.01	0	1373
3.7.2021	7	4.5	0	0	1413
3.7.2021	8	4.4	0	0	1427
18.7.2021	0.50	22.4	8.24	97.5	92.2
18.7.2021	1	21.5	3.08	45.6	94.7
18.7.2021	2	13.2	0.02	0.1	135.3
18.7.2021	3	8.5	0	0	136.6
18.7.2021	4	6.2	0	0	162.3
18.7.2021	5	5.2	0	0	1123
18.7.2021	6	4.8	0	0	1354
18.7.2021	7	4.7	0	0	1394
18.7.2021	8	4.6	0	0	1423

Date	Depth	Temperature	O2	O2	Conductivity
d.m.yyyy	m	°C	mg/L	%	µS/cm
31.7.2021	0.50	18.30	6	64.65	79.3
31.7.2021	1	17.3	3.9	43	81
31.7.2021	2	15.4	0	0	113.1
31.7.2021	3	9.2	0	0	138.7
31.7.2021	4	6.7	0	0	145.6
31.7.2021	5	5.5	0	0	1004
31.7.2021	6	5.1	0	0	1328
31.7.2021	7	4.8	0	0	1402
31.7.2021	8	4.8	0	0	1416
15.8.2021	0.50	18.3	7.75	87.7	68.5
15.8.2021	1	17.3	6.66	72.2	69
15.8.2021	2	13.8	0.33	3.1	119.4
15.8.2021	3	8.9	0.05	0.6	139.9
15.8.2021	4	6.7	0.01	0.1	172.5
15.8.2021	5	5.5	0.01	0.1	1037
15.8.2021	6	5	0.01	0.1	1350
15.8.2021	7	4.8	0	0	1405
15.8.2021	8	4.7	0	0	1420
28.8.2021	0.50	14.6	7.08	73	89.2
28.8.2021	1	14.2	6.93	69	88.4
28.8.2021	2	13.7	6.41	63.1	88.3
28.8.2021	3	9.4	0.03	0.2	127
28.8.2021	4	6.9	0.01	0.1	144.1
28.8.2021	5	5.8	0.01	0.1	507
28.8.2021	6	5.1	0.01	0.1	1047
28.8.2021	7	4.8	0.01	0	1320
28.8.2021	8	4.8	0	0	1358
12.9.2021	0.50	16.9	7.49	82.8	86.6
12.9.2021	1	15.7	6.37	66.9	86.3
12.9.2021	2	13.3	0.35	2.8	89.1
12.9.2021	3	10	0.04	0.3	104.7
12.9.2021	4	6.9	0	0	139.9
12.9.2021	5	5.7	0	0	447
12.9.2021	6	5.2	0	0	1280
12.9.2021	7	4.9	0	0	1330
12.9.2021	8	4.8	0	0	1385

Table S2. Raw data of chemicals measured in water samples obtained in Lake Kutjern.

	Date	**Depth**	**Tot-P**	**Tot-N**	**PO4-P**	**NO3-N**	**NH4-N**	**DOC**	**Cl**	**SO4**	**Fe**	**Mn**
Unit	d.m.yyyy	m	µg/L	mg/L	µg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Sample												
1	20.5.2021	0.50	26.7	0.48	2.89	0.03	0.02	16	20	4.2	0.30	0.041
2	20.5.2021	1	27.5	0.58	3.62	0.03	<0.02	17	20	3.5	0.32	0.042
3	20.5.2021	2	15.6	0.44	2.47	0.04	<0.02	17	21	3.6	0.35	0.051
4	20.5.2021	4	14.2	0.44	2.51	0.05	0.05	16	33	3.7	0.43	0.074
5	20.5.2021	6	48.4	2.9	1.83	<0.02	2.63	12	264	0.37	8.80	0.530
6	20.5.2021	8	107.7	4.9	4.41	<0.02	5.12	11	281	<0.12	8.60	0.480
7	6.6.2021	0.50	26.5	0.51	3.78	<0.02	<0.02	19	12	2.9	0.42	0.038
8	6.6.2021	1	31.1	0.49	3.07	<0.02	<0.02	19	12	2.9	0.34	0.040
9	6.6.2021	2	21.6	0.4	3.22	<0.02	<0.02	18	15	3.5	0.40	0.050
10	6.6.2021	4	16.9	0.43	2.92	0.03	<0.02	16	24	3.8	0.44	0.070
11	6.6.2021	6	46.6	3.2	2.53	<0.02	3.13	11	278	0.20	8.80	0.500
12	6.6.2021	8	112.9	5.1	3.94	<0.02	5.37	11	339	<0.12	8.90	0.480
13	20.6.2021	0.50	25.4	0.49	3.19	<0.02	0.03	18	13	2.6	0.41	0.057
14	20.6.2021	1	34.4	0.60	4.83	<0.02	<0.02	18	13	2.6	0.36	0.055
15	20.6.2021	2	29.2	0.49	3.22	<0.02	<0.02	18	14	2.8	0.41	0.076
16	20.6.2021	4	16.1	0.43	2.74	<0.02	<0.02	16	25	3.8	0.48	0.120
17	20.6.2021	6	42.3	2.9	1.73	<0.02	2.80	11	268	0.19	9.00	0.520
18	20.6.2021	8	76.7	5.2	2.78	<0.02	5.19	11	348	<0.12	8.90	0.480
19	3.7.2021	0.50	27.7	0.58	3.63	<0.02	0.03	18	14	2.5	0.40	0.044
20	3.7.2021	1	122.5	1.16	9.26	<0.02	0.02	20	14	2.4	0.42	0.060
21	3.7.2021	2	26.9	0.51	3.98	<0.02	<0.02	18	19	3.7	0.63	0.180
22	3.7.2021	4	16.1	0.42	2.84	<0.02	<0.02	16	25	3.8	0.51	0.120
23	3.7.2021	6	41.4	3	1.99	<0.02	2.84	12	275	0.20	8.90	0.520
24	3.7.2021	8	65.9	5.2	2.53	<0.02	5.34	12	354	<0.12	8.80	0.470
25	18.7.2021	0.50	49.1	0.69	5.37	<0.02	0.02	19	15	2.2	0.50	0.051
26	18.7.2021	1	55.0	0.76	6.17	<0.02	0.03	18	14	2.1	0.42	0.046
27	18.7.2021	2	33.7	0.49	3.50	<0.02	<0.02	17	20	3.6	0.69	0.220
28	18.7.2021	4	16.2	0.41	2.22	<0.02	<0.02	15	25	3.7	0.56	0.130
29	18.7.2021	6	44.2	3.4	1.63	<0.02	3.07	11	289	<0.12	9.00	0.520
30	18.7.2021	8	56.5	5.3	2.49	<0.02	5.28	11	327	<0.12	8.80	0.480
31	31.7.2021	0.50	29.1	0.66	3.49	0.03	<0.02	21	11	3.2	0.51	0.087
32	31.7.2021	1	27.8	0.66	2.73	0.03	<0.02	21	11	3.2	0.45	0.083
33	31.7.2021	2	27.8	0.59	2.67	0.06	<0.02	23	10	4.0	0.51	0.120
34	31.7.2021	4	15.8	0.42	2.50	<0.02	<0.02	16	25	3.7	0.65	0.130
35	31.7.2021	6	44.8	2.8	2.29	<0.02	2.43	11	269	0.17	9.10	0.540
36	31.7.2021	8	57.2	4.8	2.00	<0.02	4.84	11	326	0.14	8.60	0.480

	Date	Depth	Tot-P	Tot-N	PO4-P	NO3-N	NH4-N	DOC	Cl	SO4	Fe	Mn
Unit	d.m.yyyy	m	µg/L	mg/L	µg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Sample												
37	15.8.2021	0.50	34.0	0.6	4.68	<0.02	<0.02	24	9	2.6	0.56	0.068
38	15.8.2021	1	34.6	0.61	4.65	<0.02	<0.02	24	9	2.7	0.51	0.066
39	15.8.2021	2	25.0	0.56	3.79	<0.02	<0.02	24	9	3.2	0.55	0.110
40	15.8.2021	4	16.5	0.39	2.20	<0.02	<0.02	15	25	3.4	0.78	0.140
41	15.8.2021	6	37.9	2.4	1.98	<0.02	2.07	12	240	0.48	7.90	0.520
42	15.8.2021	8	58.1	5.4	2.04	<0.02	4.99	11	314	0.22	9.20	0.500
43	28.8.2021	0.50	40.8	0.6	4.91	<0.02	<0.02	23	13	3.1	0.66	0.090
44	28.8.2021	1	35.8	0.56	5.33	<0.02	<0.02	22	13	3.0	0.58	0.087
45	28.8.2021	2	30.9	0.61	4.59	<0.02	<0.02	22	13	3.0	0.58	0.088
46	28.8.2021	4	19.4	0.39	2.44	<0.02	<0.02	15	27	2.8	1.10	0.200
47	28.8.2021	6	58.4	2.5	2.57	<0.02	2.26	12	258	0.24	8.80	0.550
48	28.8.2021	8	61.5	5.2	2.03	<0.02	5.43	12	339	0.14	9.20	0.490
49	12.9.2021	0.50	42.7	0.78	5.81	0.04	0.02	22	14	3.5	0.45	0.060
50	12.9.2021	1	67.2	0.97	9.01	<0.02	<0.02	23	13	2.5	0.44	0.059
51	12.9.2021	2	22.1	0.42	3.82	<0.02	<0.02	22	14	2.8	0.43	0.064
52	12.9.2021	4	18.7	0.36	2.37	<0.02	<0.02	15	25	2.7	1.10	0.160
53	12.9.2021	6	41.5	3.2	2.33	<0.02	3.21	12	307	0.17	8.90	0.510
54	12.9.2021	8	52.0	5.4	2.26	<0.02	5.01	13	331	0.23	9.20	0.500

Table S3. Raw data of phytoplankton pigments measured in water samples obtained in Lake Kutjern.

	Date	Depth	Chlorophyll-a	Peridinin	Heteroxanthin	Fucoxanthin	Violaxanthin	Diadinoxanthin	Alloxanthin
Unit	d.m.yyyy	m	µg/L	µg/L	mAU/L	µg/L	µg/L	µg/L	µg/L
Sample									
1	20.5.2021	0.50	7.1	1.0	0.0	1.1	0.4	0.7	0.5
2	20.5.2021	1	9.4	1.1	0.0	1.0	0.4	1.1	0.7
3	20.5.2021	2	1.8	0.3	0.0	0.1	0.0	0.1	0.3
4	20.5.2021	4	0.9	0.1	0.0	0.0	0.0	0.0	0.2
5	20.5.2021	6	0.0	0.0	0.0	0.0	0.0	0.0	0.2
6	20.5.2021	8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	6.6.2021	0.50	4.7	0.4	0.0	0.4	0.1	0.6	0.5
8	6.6.2021	1	12.2	1.1	0.0	1.5	0.5	1.5	1.3
9	6.6.2021	2	10.8	0.2	0.0	1.1	0.0	0.4	1.7
10	6.6.2021	4	1.3	0.0	0.0	0.0	0.0	0.0	0.2
11	6.6.2021	6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	6.6.2021	8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13	20.6.2021	0.50	17.4	0.4	27.1	0.5	0.3	1.5	2.1
14	20.6.2021	1	27.1	0.6	56.2	0.4	0.4	3.0	2.5
15	20.6.2021	2	19.5	0.3	26.1	0.3	0.0	1.5	2.6
16	20.6.2021	4	0.0	0.0	0.0	0.0	0.0	0.1	0.2
17	20.6.2021	6	0.0	0.0	0.0	0.0	0.0	0.0	0.1
18	20.6.2021	8	0.0	0.0	0.0	0.0	0.0	0.0	0.1
19	3.7.2021	0.50	10.0	0.2	24.9	0.4	0.3	1.2	0.7
20	3.7.2021	1	131.2	0.0	540.2	0.3	2.1	29.8	8.6
21	3.7.2021	2	24.1	0.0	0.0	0.1	0.0	1.1	4.9
22	3.7.2021	4	6.1	0.0	0.0	0.0	0.0	0.6	0.6
23	3.7.2021	6	3.8	0.0	0.0	0.0	0.0	0.4	0.1
24	3.7.2021	8	3.9	0.0	0.0	0.0	0.0	0.3	0.2
25	18.7.2021	0.50	55.3	0.2	138.2	1.0	1.0	8.9	2.2
26	18.7.2021	1	78.1	0.1	198.6	0.9	1.2	12.5	3.4
27	18.7.2021	2	27.2	0.0	0.0	0.2	0.0	1.8	5.2
28	18.7.2021	4	7.6	0.0	16.5	0.0	0.0	0.9	0.4
29	18.7.2021	6	7.5	0.0	16.5	0.0	0.0	0.7	0.1
30	18.7.2021	8	3.9	0.0	8.2	0.0	0.0	0.5	0.0
31	31.7.2021	0.50	19.2	0.0	23.0	0.6	0.5	1.7	1.7
32	31.7.2021	1	15.3	0.0	29.3	0.6	0.3	1.2	1.4
33	31.7.2021	2	9.3	0.0	19.4	0.1	0.1	1.2	0.6
34	31.7.2021	4	4.5	0.0	16.9	0.0	0.0	0.7	0.2
35	31.7.2021	6	11.6	0.0	24.6	0.1	0.1	1.3	0.5
36	31.7.2021	8	4.2	0.0	0.0	0.0	0.0	0.5	0.0
37	15.8.2021	0.50	52.0	0.1	91.1	2.4	1.3	6.2	2.0
38	15.8.2021	1	37.6	0.1	81.6	1.6	1.0	4.8	1.5
39	15.8.2021	2	13.8	0.0	18.7	0.6	0.3	1.4	1.1
40	15.8.2021	4	5.1	0.0	12.4	0.0	0.0	0.8	0.3
41	15.8.2021	6	4.5	0.0	13.3	0.1	0.0	0.5	0.1
42	15.8.2021	8	5.5	0.0	13.1	0.0	0.0	0.7	0.0
43	28.8.2021	0.50	53.5	0.2	123.1	1.5	1.2	7.7	2.5
44	28.8.2021	1	50.4	0.2	111.9	1.4	0.9	7.1	2.3
45	28.8.2021	2	34.4	0.0	92.9	0.5	0.5	5.0	1.5
46	28.8.2021	4	10.4	0.0	19.8	0.0	0.1	1.4	0.4
47	28.8.2021	6	12.7	0.0	26.4	0.0	0.1	1.3	0.4
48	28.8.2021	8	8.7	0.0	14.4	0.0	0.0	0.8	0.2
49	12.9.2021	0.50	74.7	1.0	211.1	0.8	1.1	13.5	3.8
50	12.9.2021	1	168.3	0.5	462.7	0.8	2.2	29.7	8.2
51	12.9.2021	2	25.5	0.1	70.9	0.1	0.3	3.5	1.4

52	12.9.2021	4	13.6	0.0	35.7	0.0	0.1	2.1	0.6
53	12.9.2021	6	9.9	0.0	20.5	0.0	0.1	1.0	0.3
54	12.9.2021	8	2.6	0.0	0.0	0.0	0.0	0.4	0.0

Table S4. Raw data of bacteriochlorophyll pigments measured in water samples obtained in Lake Kutjern.

	Date	Depth	Bacteriochlorophyll 1	Bacteriochlorophyll 2	Bacteriochlorophyll 3
Unit	d.m.yyyy	m	mAU/L	mAU/L	mAU/L
Sample					
1	20.5.2021	0.50	0.0	0.0	0.0
2	20.5.2021	1	0.0	0.0	0.0
3	20.5.2021	2	0.0	0.0	0.0
4	20.5.2021	4	0.0	0.0	0.0
5	20.5.2021	6	135.6	68.9	45.1
6	20.5.2021	8	234.8	156.4	60.5
7	6.6.2021	0.50	0.0	0.0	0.0
8	6.6.2021	1	0.0	0.0	0.0
9	6.6.2021	2	0.0	0.0	0.0
10	6.6.2021	4	0.0	0.0	0.0
11	6.6.2021	6	265.6	170.2	93.3
12	6.6.2021	8	196.8	122.4	55.0
13	20.6.2021	0.50	0.0	0.0	0.0
14	20.6.2021	1	0.0	0.0	0.0
15	20.6.2021	2	0.0	0.0	0.0
16	20.6.2021	4	0.0	0.0	0.0
17	20.6.2021	6	237.8	215.3	87.3
18	20.6.2021	8	162.2	162.8	63.8
19	3.7.2021	0.50	0.0	0.0	0.0
20	3.7.2021	1	0.0	0.0	0.0
21	3.7.2021	2	59.0	0.0	0.0
22	3.7.2021	4	0.0	0.0	0.0
23	3.7.2021	6	122.1	118.2	52.8
24	3.7.2021	8	402.9	354.8	152.9
25	18.7.2021	0.50	0.0	0.0	0.0
26	18.7.2021	1	0.0	0.0	0.0
27	18.7.2021	2	1017.5	477.4	115.4
28	18.7.2021	4	136.2	71.3	17.3
29	18.7.2021	6	109.0	118.9	30.9
30	18.7.2021	8	316.3	287.9	111.1
31	31.7.2021	0.50	0.0	0.0	0.0
32	31.7.2021	1	0.0	0.0	0.0
33	31.7.2021	2	65.6	0.0	0.0
34	31.7.2021	4	243.8	141.5	25.5
35	31.7.2021	6	96.5	93.7	29.0
36	31.7.2021	8	368.6	328.4	134.5
37	15.8.2021	0.50	0.0	0.0	0.0
38	15.8.2021	1	0.0	0.0	0.0
39	15.8.2021	2	30.7	0.0	0.0
40	15.8.2021	4	462.8	243.7	78.7
41	15.8.2021	6	149.4	96.2	35.6
42	15.8.2021	8	348.6	304.4	149.4
43	28.8.2021	0.50	68.8	0.0	0.0
44	28.8.2021	1	81.6	42.5	0.0
45	28.8.2021	2	227.2	88.2	26.3
46	28.8.2021	4	371.8	233.3	95.3
47	28.8.2021	6	224.0	231.7	80.7
48	28.8.2021	8	386.7	349.9	133.3
49	12.9.2021	0.50	0.0	0.0	0.0
50	12.9.2021	1	0.0	0.0	0.0

51	12.9.2021	2	0.0	0.0	0.0
52	12.9.2021	4	382.3	227.6	103.0
53	12.9.2021	6	234.0	220.1	92.5
54	12.9.2021	8	170.2	130.6	49.2

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