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Disentangling the effect of physical and chemical factors on phytoplankton dynamics in a highly eutrophic and turbid lake

Fysiske og kjemiske faktorer som påvirker dynamikken av planteplankton i en svært eutrof og turbid innsjø

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Abstract

The fact that nutrients limit primary production in aquatic ecosystems is widely accepted, and phosphorus is demonstrated as the main limiting nutrient in freshwaters. Strict control of phosphorus supply has therefore become the primary goal in the management of eutrophic freshwaters, regardless of differences in catchment properties, lake morphometry and hydrology, and lake physical characteristics. Various abiotic and biotic factors may, however, impact the primary production of phytoplankton and blooms of potentially toxic cyanobacteria. The majority of these factors vary both seasonally and annually, especially in highly dynamic lakes that respond rapidly to changing weather conditions.

This thesis consists of three different approaches in limnology: (1) a lake survey, (2) an *in situ* experiment with the lakes natural phytoplankton community, and addition of either phosphorus, both phosphorus and nitrogen, or no nutrients, and (3) a dynamic model to better understand the effects of physical and chemical factors on the phytoplankton dynamics in eutrophic lakes.

Phosphorus was the limiting nutrient for phytoplankton growth as long as the light conditions in the lake were satisfactory. Nitrogen addition had minor effect on phytoplankton growth. The concentration of phosphorus, however, did not affect the biomass of cyanobacteria. Neither nitrogen nor the nitrogen to phosphorus ratio could explain dominance of the nitrogen fixing cyanobacteria *Aphanizomenon* cf. *klebahnii* and *Anabaena planctonica*.

Light significantly influenced phytoplankton growth during the whole growing season. Cyanobacteria development was supported by warm water, shallow euphotic depth and low water turbulence rather than by nutrients. Inter-annual variation in weather highly influenced thermal, mixing and light regimes, which further determined nutrients and phytoplankton dynamics in the lake. Risk of cyanobacteria blooms seem to be especially high if increased fluxes of suspended matter and phosphorus during mild and wet winters are followed by warm summers, increased water temperatures and stable thermal stratification. More frequent mild winters, as predicted by climate scenarios for Scandinavia, increase the risk of cyanobacteria blooms in eutrophic and turbid lakes.

Sammendrag (in Norwegian)

Det er allment akseptert at næringsstoffer begrenser primærproduksjonen i akvatiske økosystemer, og fosfor er hovedsaklig det begrensende næringsstoffet i ferskvann. Streng kontroll av fosfor har derfor blitt det primære målet i forvaltningen av eutrofe ferskvann, uavhengig av egenskapene til nedslagsfeltet og morfometriske, hydrologiske og fysiske egenskaper ved innsjøen. Flere abiotiske og biotiske faktorer kan imidlertid virke inn på primærproduksjonen av planteplankton og oppblomstring av potensielt giftige cyanobakterier. Flertallet av disse faktorene endres både sesongmessig og årlig, spesielt i svært dynamiske innsjøer som responderer raskt på skiftende værforhold.

Denne oppgaven består av tre ulike tilnærminger innen limnology: (1) en innsjø-undersøkelse, (2) et *in situ* eksperiment med innsjøens naturlige planteplankton-samfunn og tilsetting av enten fosfor, både fosfor og nitrogen eller ingen næringsstoffer, og (3) en dynamisk modell for å bedre forstå effekten av fysiske og kjemiske faktorer på planteplanktondynamikken i eutrofe innsjøer.

Fosfor var det begrensende næringsstoffet for vekst av planteplankton så lenge lysforholdene i innsjøen var tilfredsstillende. Tilsetting av nitrogen hadde ubetydelig effekt på tilvekst av planteplankton. Konsentrasjonen av fosfor påvirket derimot ikke biomassen av cyanobakterier. Hverken nitrogen eller forholdet mellom nitrogen og fosfor kunne forklare dominansen av de nitrogenfikserende cyanobakteriene *Aphanizomenon* cf. *klebahnii* og *Anabaena planctonica*.

Lys påvirket signifikant tilveksten av planteplankton gjennom hele vekstsesongen. Utviklingen av cyanobakterier ble fremmet av varmt vann, grunt eufotiske dyp og lav turbulens, snarere enn av næringsstoffer. Årlige variasjoner i værforhold påvirket i høy grad varme-, sirkulasjons- og lysregimet, noe som videre påvirker dynamikken til næringsstoffer og fytoplankton i innsjøen. Faren for oppblomstring av cyanobakterier synes å være spesielt stor dersom økt avrenning av suspendert stoff og fosfor under milde og våte vintre etterfølges av varme somre, økte vanntemperaturer og stabil termisk sjiktdannelse. Høyere frekvens av milde vintre som indikert i klimascenarier for Skandinavia økter risikoen for oppblomstring av cyanobakterier i eutrofe og turbide innsjøer.

Table of contents

List of papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

Paper I

Romarheim AT, Andersen T, Brettum P, Haande S, Riise G and Rohrlack T. Environmental factors affecting cyanobacteria dynamics in a Norwegian eutrophic and turbid lake. *Submitted to Limnologica*.

Paper II

Romarheim AT, Riise G, Brettum P, Færøvig PJ and Andersen T. The importance of light and macronutrients for phytoplankton growth in a northern temperate lake – an *in situ* bioassay study. *Submitted to J Plankton Res*.

Paper III

Romarheim AT, Tominaga K, Riise G and Andersen T. Modelling the responses of a cold temperate lake to changes in external forcing. *Submitted to Hydrobiologia.*

1. Introduction

Water accounts for more than 71% of our planet. However, only 0.009% of this is freshwater in lakes and rivers, which is essential for the maintenance and survival of life (Wetzel, 2001). Increased human population and technology growth, and enhanced water consumption and utilization have resulted in rapid degradation of water quality. A wide range of chemicals contaminants, pathogens and physical changes lead to water pollution. High supply of nutrients promotes high primary production and reduced water quality. The need for action to protect aquatic ecosystems has been recognized, and has among others resulted in the Water Framework Directive (WFD) for integrated water policy in Europe (European Union, 2000). The main objectives of the WFD are to protect all European surface and ground waters and to improve the quality of polluted waters by 2015.

High primary production in lakes is mainly caused by increased phytoplankton growth. Most phytoplankton are low motile eukaryotic algae and prokaryotic bacteria which perform photosynthesis (Wetzel, 2001). Water temperature, and availability of nutrients and light determine their growth, while other factors such as grazing by zooplankton and sedimentation may affect their total biomass (Fisher et al., 1995). Phytoplankton is the base of all aquatic food chains, and important producers of organic matter and oxygen on the Earth (Graham et al., 2009). Development of large phytoplankton biomasses in aquatic ecosystems, however, may cause problems related to water colour and transparency, decrease water aesthetic value and utility, and cause fish mortality as dissolved oxygen is depleted due to decomposition of dead phytoplankton. In addition, cyanobacteria which may form toxic blooms hazardous to wildlife and humans, are often the dominant phytoplankton in eutrophic waters.

The link between phosphorus supply and phytoplankton biomass in lakes was found by the Swiss limnologist R. A. Vollenweider in the 1960s (Schindler, 2006). Increased water productivity due to high nutrients supply caused by human activities is termed “cultural eutrophication” (Edmondson, 1991), and it has become one of today`s largest water pollution problems. Phosphorus input control has been the main issue in water management and eutrophication studies of freshwaters since it was demonstrated to limit phytoplankton growth in a whole lake manipulation experiment (Schindler and Fee, 1974). Controlled phosphorus supply has reduced phytoplankton biomass and enhanced water transparency in many lakes (Jeppesen et al., 2005), but some lakes have not responded to reduced nutrient loading and continue to suffer from high phytoplankton biomass (Jeppesen et al., 2007).

The productivity in lakes is determined by a variety of factors and complexes (Figure 1), e.g. climate affects lake physical conditions (Straile et al., 2003a; Blenckner et al., 2007; Whitehead et al., 2009), which are important in controlling the phytoplankton dynamics (Reynolds, 1994; Padisak et al., 2010; Zohary et al., 2010). More knowledge on the effect of various factors and their interaction is therefore required to reduce eutrophication in the future (Schindler, 2006). The use of dynamic models in limnology may increase the understanding of lake responses, and predict development in water ecosystems for different climatic scenarios (Mooij et al., 2010).

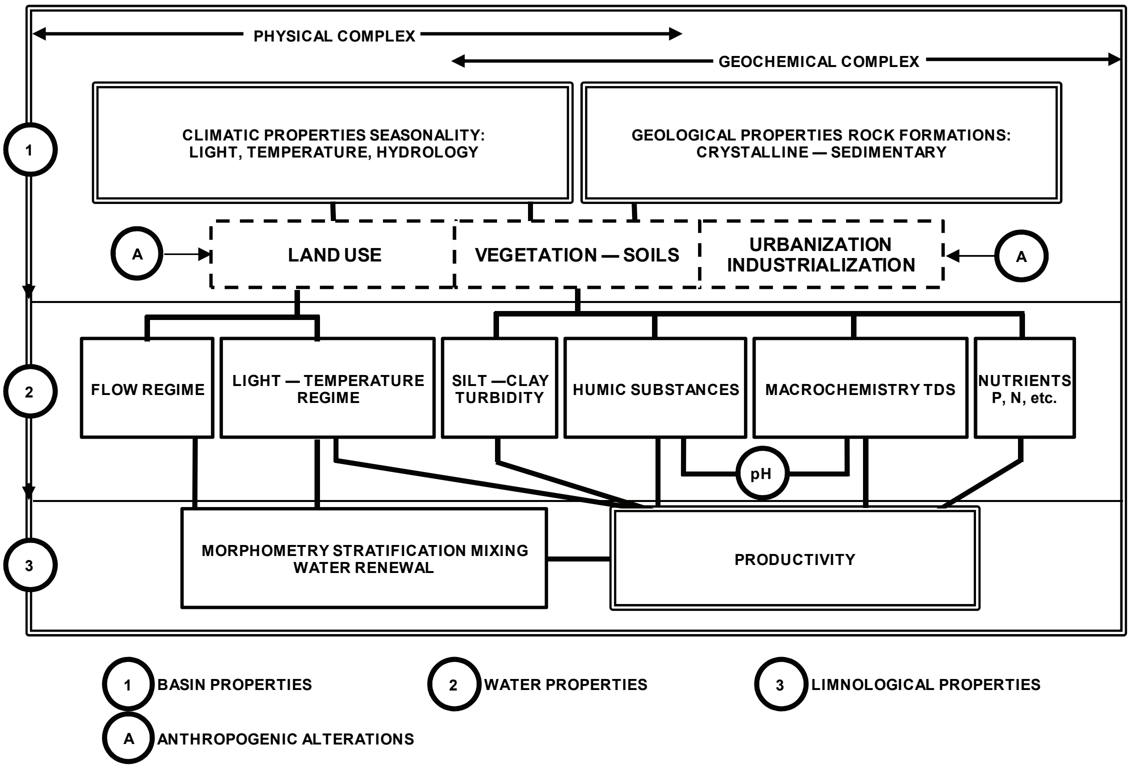

Figure 1. Hierarchical scheme of factors and complexes determining lake productivity (Vollenweider and Kerekes, 1980).

2. Background

2.1. Nutrient limitation of freshwater phytoplankton

Both phytoplankton and ambient water have a ratio among carbon, nitrogen and phosphorus of approximately 106C:16N:1P by atoms or 41:7.2:1 by weight (Redfield, 1958). Phosphorus requirements for phytoplankton is thus low compare to other macronutrients, but phosphorus appears frequently to limit phytoplankton growth in freshwaters as it often binds to metal cations and form insoluble complexes in soil and lake sediments (Wetzel, 2001; Graham et al., 2009). The importance of phosphorus for phytoplankton primary production has been confirmed in many lake studies (Schindler and Fee, 1974; Hecky and Kilham, 1988; Jeppesen et al., 2005; Håkanson et al., 2007). However, dual limitation of phytoplankton growth by both phosphorus and nitrogen in lakes has also been found in several studies (Morris and Lewis, 1988; Camacho et al., 2003; Dzialowski et al., 2005) and addressed in several reviews (Elser et al., 1990; Lewis and Wurtsbaugh, 2008; Sterner, 2008). It has therefore been suggested that the input of both phosphorus and nitrogen to freshwaters should be controlled (Conley et al., 2009).

Several methods are used to determine the limiting nutrient for phytoplankton growth (Hecky and Kilham, 1988; Elser et al., 1990; Fisher et al., 1995; Beardall et al., 2001), and some of these are listed in Table 1. Experiments with manipulation of a whole ecosystem are most reliable, but they are rarely applied due to high expenses and intensive labour effort (Fisher et al., 1995). Nutrient concentrations, their ratios and bioassay studies are more easily applicable methods and therefore widely applied in limnological studies to determine the nutrient limiting phytoplankton growth.

Table 1. Methods to study nutrient deficiency in eutrophic aquatic ecosystems (from Fisher et al., 1995).

Nutrient concentration	Activity of the alkaline phosphatase
Molar DIN:SRP ratio	NH_4-enhanced, dark $^{14}CO_2$ uptake
Molar TN:TP ratio	Carotenoids to chlorophyll a ratio
Particulate C:N:P ratio	PO_4 turnover time
Nutrient addition bioassays	Ecosystem level

Phytoplankton utilize nutrients in dissolved form, and nutrient concentrations below the requirement for phytoplankton may limit phytoplankton growth. High concentration of one nutrient relative to others eliminates this nutrient as the limiting (Fisher et al., 1995). Although the ratio between dissolved nutrient has been found to be a better indicator of phytoplankton growth limitation (Morris and Lewis, 1988; Wagner and Adrian, 2009; Wood et al., 2010), the ratio between total nitrogen and total phosphorus (TN:TP) is commonly used ratio to determine the limiting nutrient in lakes. The Redfield ratio proposes phosphorus limitation when the nitrogen to phosphorus (N:P) ratio exceeds 7 in the water. A wide range of TN:TP ratios, however, have been found as a boundary for the transition from limitation by one nutrient to limitation by another (Dillon and Rigler, 1974; Smith, 1982; Downing and McCauley, 1992; Dzialowski et al., 2005).

Bioassays with nutrient addition are spatially and temporally limited experiments with selected organisms or the whole natural phytoplankton community in combination with e.g. selected nutrient levels, light conditions and temperatures. The experiments may be conducted under controlled conditions in laboratory or *in situ*. Phytoplankton growth rate is usually used as response parameter. Bioassays are important diagnostic tool in determining the nutrient limitation (Elser et al., 1990; Lewis and Wurtsbaugh, 2008), although experiments with enclosures have shortcomings due to growth of organisms on the wall of the bottles, alteration of the natural food web and exclusion from sediment nutrient fluxes (Rudek et al., 1991; Fisher et al., 1995; Schindler, 1998). However, an incubation time between 2 and 5 days of small to intermediate size enclosures ensure low bottle effect and low number of cell divisions, but it is sufficiently long to avoid an early response to the nutrient addition (Lewis and Wurtsbaugh, 2008).

2.2. Phytoplankton and lake physical conditions

High inter-annual variation in phytoplankton dynamics within a lake and among lakes of the same trophic state indicates that other factors than nutrients are also important in regulating phytoplankton dynamics. The lake thermal and mixing regime, and light conditions may have an important role in shaping the phytoplankton community and controlling their total biomasses (Berger et al., 2007; Padisak et al., 2010; Zohary et al., 2010). As the lake physical properties are climate dependent, variable weather conditions and global climate changes may affect the lake physical environment, and consequently the chemical and biological lake responses (Straile, 2003b; George et al., 2007). Warmer climate increases water temperature, promote stable stratification in temperate lakes (Straile et al., 2003a), influence ice-lake dynamics (Weyhenmeyer et al., 2011), lead to earlier and higher phytoplankton production (Weyhenmeyer et al., 2002; Wiedner et al., 2007; Huber et al., 2008), and shift the phytoplankton community towards dominance of species adapted to warmer water (Domis et al., 2007).

Light conditions vary among lakes and within a lake due to different solar radiation, light attenuation, and changes in water mixing (Dokulil, 1994; Diehl, 2002). In the northern chemosphere, the global radiation is highest during the summer months (June to September), but decreases from June since the daily light duration is reduced. Many other factors also interfere with light available to phytoplankton in the water. Dissolved matter increase water colour and suspended matter of organic or inorganic origin increase the light attenuation, both thereby decreasing the light available for phytoplankton (Wetzel, 2001). Dokulil (1994) found that light affect phytoplankton dynamics in turbid lakes as the non-algal light attenuation influence the light regime and phytoplankton uptake of available nutrients which prevents potentially high production in the lake.

2.3. Cyanobacteria in freshwaters

The key sign of eutrophication are blooms of cyanobacteria which may produce toxins hazardous to wildlife and humans (Conley et al., 2009). Cyanobacteria are prokaryotic organisms able to perform photosynthesis. In addition to chlorophylls, they contain phycobiliproteins which enable them to utilize light of a wider spectrum than other phytoplankton, and may colour them blue-green or red which is why they are often referred to as blue-green algae. They are generally slow growing organisms and strong competitors for light (Mur et al., 1999). High concentrations of phosphorus, low N:P ratio, warm water, stabile stratification, and low turbulence are some of factors presumed to favour development of cyanobacteria in lakes (Mur et al., 1999). Additionally, some physiological and ecological cyanobacteria adaptations, such as phosphorus uptake above their requirements, positive buoyancy, formation of large and mucus colonies, and toxin production may enable their dominance over other phytoplankton. A low TN:TP ratio is often used to explain the dominance of cyanobacteria, particularly of nitrogen fixing species since low TN:TP ratio favours their development in lakes (Schindler, 1977). Many studies, however, did not find a relationship between the TN:TP ratio and cyanobacteria concentrations (Håkanson et al., 2007; Kosten et al., 2009), and Tilman et al. (1986) found that cyanobacteria developed regardless of TN:TP ratios if only the water temperature were high enough.

2.4. Lake Årungen

Lake Årungen is a lowland Norwegian lake located in an agricultural region approximately 25 km south of the capital Oslo (59°41'18"N, 10°44'38"E; Figure 2). The lake is a relatively shallow (mean depth 8 m), and dimictic lake, and thermally stratified during the summer. Six streams supply the lake with water, and the lake outputs through one stream to the Oslofjord. The lake surface is 1.2 km^2 and has a catchment area of 51 km^2 which is mostly used for agricultural purposes. The lake is 3 km long and highly exposed to wind and erosion of nutrient rich soils.

Figure 2. Lake Årungen (Photo: Lasse Tur).

The geological bedrock of the catchment is dominated by gneiss and granite covered by deposits of mainly marine clay and till. There is no paleological data on the lake natural trophic state, but geological studies suggest that the natural phosphorus concentration of lakes in this area is 7-8 μg L^{-1} (Borch et al., 2007). The lake is therefore considered as naturally mesotrophic.

Increased human population, high discharges of untreated sewage, and increased land use for agriculture and livestock after the 1950s significantly increased the input of phosphorus to the lake (Skogheim and Erlandsen, 1984). Eutrophication became a serious problem during the 1960s as the phosphorus concentration exceeded 400 μg L^{-1} (Løvstad and Krogstad, 1993) and blooms of algae developed in the lake. Measures to reduce the nutrient loading to the lake began in the late 1970s, nearly 20 years after the problem with eutrophication of the lake was recognized. The period from the 1980s should therefore be considered as a period of lake recovery. Improved sewage treatment from the nearby university (Norwegian University of Life Sciences) and a former potato and vegetables processing industry was establish during the 1970s. Improved agricultural practices and strategies to reduce phosphorus transport by soil erosion were implemented during the 1990s and four artificial wetlands were constructed in the catchment from 1997 to 2001. Removal of large size pikes to initiate a trophic cascade leading to higher zooplankton pressure on the phytoplankton was done twice, in 1990 and in the period from 2004 to 2006. Further upgrading of sewage treatment from municipal wastewater and single households started in 2002 and should be completed by 2012 (Borch et al., 2007). The lake is one of the Norwegian reference lakes for the WFD which means that it should achieve a good ecological status by 2015. Additionally, the lake hosts the national rowing stadium and it is an important sports and recreation area. The lake eutrophication problem therefore receives much public attention.

Sporadic analyses of water quality, lack of systematic monitoring data, differences in water sampling methodology and analyses, as well as the location and depth of sampled water makes it difficult to evaluate the importance of the individual abatement measures. However, the long term trend of nutrient concentrations and phytoplankton can be determined. The lake has been characterised by extremely high nutrient concentrations and high phytoplankton biomasses, particularly of the cyanobacteria *Planktothrix* spp. until the 1980s. The highest reduction in total phosphorus and chlorophyll *a* concentrations took place in the mid 1980s. In the mid

1990s, decreased total phytoplankton biomass and reduced cyanobacteria biomasses indicated partial recovery of the lake, probably as a result of reduced phosphorus loading from agricultural areas and improved waste water treatment (Løvstad and Krogstad, 1993). The improved water quality was unfortunately only temporary as high biomasses of nitrogen fixing cyanobacteria of the genera *Anabaena* and *Aphanizomenon* occurred in the late 1990s. More frequent lake monitoring data during the last years indicate a high inter-annul variation in phytoplankton biomass (Borch et al., 2007). Cyanobacterial toxin production was reported for the first time in 1992 (Løvstad and Krogstad, 1993), but the toxicity was rarely assessed in the following years as the microcystin concentrations were low. The problem of cyanotoxins in the lake regained attention in 2007 when the Norwegian Institute for Water Research measured microcystin concentration higher than 80 $\mu g \ L^{-1}$ this summer, which is eight times higher than the concentrations proposed by the World Health Organisation (WHO) for recreation waters (Kuiper-Goodman et al., 1999).

3. Objectives of the thesis

The aim of this thesis was to increase our understanding of phytoplankton ecology and to disentangle the complex array of factors that limit or promote phytoplankton growth. The study consists of a lake survey in Lake Årungen (**Paper I**), an *in situ* bioassay study (**Paper II**), and a model approach with data from the lake and its catchment (**Paper III**). The objectives of the study were:

- To find the relationship between cyanobacteria and environmental factors (**Paper I**).

- To quantify cyanobacterial toxins in the lake, and to identify their producers (**Paper I**).

- To determine the nutrient that limit phytoplankton growth (**Paper II**).

- To determine the most important factor controlling phytoplankton growth (**Paper II**).

- To evaluate the applicability of the dynamic MyLake model in a eutrophic northern temperate lake (**Paper III**).

- To model lake responses under different external forcing scenarios, and to determine the importance of weather and external loading for the lake response (**Paper III**).

4. Methods

4.1. Lake data and analyses

Water samples for chemical and biological analyses were collected at the lake's deepest location and from eight depths with a Rüttner water sampler in the period from January 2008 to September 2010, with a frequency of every two weeks during the phytoplankton growing season (May-September), and once a month through the rest of the year (n=49). Water temperature was measured by a MultiLine P4 (WTW, Weilheim, Germany) device and Secchi depth was determined with a 13×18 cm white disk. Hourly vertical water temperatures were measured by Hobo pendant temperature loggers (64K-UA-002-64, Onset Computer Corporation, Bourne, MA, USA) during the whole period at eight depths between 0.7 and 12.6 m. Water temperature data were used to estimate the mixed-layer depth (Z_m) and mixed-layer temperature. The euphotic depth (Z_{eu}) was calculated as 2.2 × Secchi depth. Water turbidity was determined by a laboratory turbidimeter, and expressed in formazin nephelometric units (FNU).

Chemical analyses were conducted according to standardised methods and the principles followed below. Dissolved inorganic nutrients were analysed after filtration through 0.45 μm pore-size membrane filters. Soluble reactive phosphorus (SRP) was determined by the ascorbic acid method where a yellow antimony-molybdate phosphorus complex in acid solution was reduced to a blue colour by ascorbic acid, and the colour absorbance was measured spectrophotometrically at 880 nm (detection limit: 1 μg P L^{-1}). Ion chromatography with an electrical conductometer as the detector was used for determination of nitrate (NO_3-N; detection limit: 0.005 mg NO_3-N L^{-1}). Ammonium (NH_4-N) was determined spectrophotometrically at 655 nm after reaction of ammonium ions with salicylate and hypochlorite ions in an alkaline solution. Samples for TP and TN analyses were added peroxidisulfate under acid conditions for TP, and alkaline conditions for TN, then heated in an autoclave at 121°C for 30 min. TP and TN were then determined spectrophotometrically at 880 nm and 540 nm, respectively.

Phytoplankton primary production was assessed by determining the chlorophyll concentration and phytoplankton biomass. Chlorophyll *a* analysis was done by filtration of pre-measured volumes of water on glass fibre filters, drying the filters for 2 h in the dark, then chlorophyll extraction by keeping the filters in methanol for 20 h and centrifugation for 10 min at 4000 rpm. The absorbance was determined spectrophotometrically at 665 and 750 nm. In the bioassay study, chlorophyll was extracted from glass fibre filters by ethanol, and then the fluorescence of the extracts was measured at 665 nm using a microplate reader. Phytoplankton composition and biomass were determined in water samples after preservation with Lugol's solution, sedimentation according to Utermöhl's method (Olrik et al., 1998), and counting of phytoplankton under an inverted phase contrast microscope (Leitz. Fluovert FS, Leica, Wetzlar, Germany). The phytoplankton biomass was calculated according to Rott (1981) and Olrik et al. (1998).

Microcystin concentrations were quantified by liquid chromatography with mass spectrometric detection (LC-MS/MS) after lyophilisation and microcystin extraction from the filters by 50% methanol according to the method described by Rohrlack et al. (2009). Genetic analysis for the presence of *mcyB*, *mcyE* and *mcyB* genes, which indicate microcystin-producing genotypes from *Microcystis*, *Anabaena* and *Planktothrix*, respectively (Kurmayer and Kutzenberger, 2003; Vaitomaa et al., 2003; Briand et al., 2008), was performed by quantitative real time polymerase chain reaction (qPCR) as described by Kurmayer and Kutzenberger (2003).

4.2. Bioassay study

Nine *in situ* bioassays were carried out with natural phytoplankton communities from Lake Årungen to study factors limiting phytoplankton growth in the period May to October 2009 (**Paper II**). Initial samples were obtained by sampling water at the lake's deepest location on the first day of each experiment. The water was collected from five depths (0.7, 1.4, 2.1, 2.8 and 3.5 m), and then mixed in a 20 L canister to obtain one composite sample for each experiment (Figure 3A). One water volume was filtered through 90 μm nylon net to remove metazoan grazers, whereas the rest was used as unfiltered water. Both unfiltered and filtered water samples were added either phosphorus alone (100 μg L^{-1} P), phosphorus in combination with nitrogen (100 μg L^{-1} P and 700 μg L^{-1} N), or no nutrients (control water). The experimental units were 75 mL tissue culture bottles which were incubated for three days at five depths (0.7, 1.4, 2.1, 2.8 and 3.5 m) to obtain different levels in the vertical light gradient. The bottles were oriented randomly within coarse-meshed nylon netting bags and placed in the lake within three hours after preparation (Figure 3B). After three days of incubation, the experimental units were taken from the lake and kept in dark until filtration for chlorophyll analysis. Each treatment was done in triplicate, giving 90 observations per experiment and a total of 810 observations for the whole study. The composite initial samples from each experiment were also analysed in triplicate. Net specific phytoplankton growth rate (μ; d^{-1}) was used as the main response parameter, and it was estimated from the changes in chlorophyll over the three days incubation by the formula: $\log(Chl_t\ Chl_0^{-1})\ t^{-1}$, where Chl_0 and Chl_t are the initial and final chlorophyll concentrations, respectively, and t is the duration of the experiment in days.

Figure 3. Bioassay study: A) Initial composite sample for each experiment, and B) randomly orientation of experimental units within coarse-meshed nylon netting bags before placement in the lake.

4.3. Model experiment

The MyLake model, a one-dimensional model that simulates daily changes in lake physical, chemical and biological variables over the depth gradient (Saloranta and Andersen, 2007), was used to evaluate the relative importance of year-to-year variation in meteorological forcing and external loading for the lake's physical, chemical and biological conditions (**Paper III**). Meteorological and loading data from 1994 to 2010 were used as the model input (Figure 4).

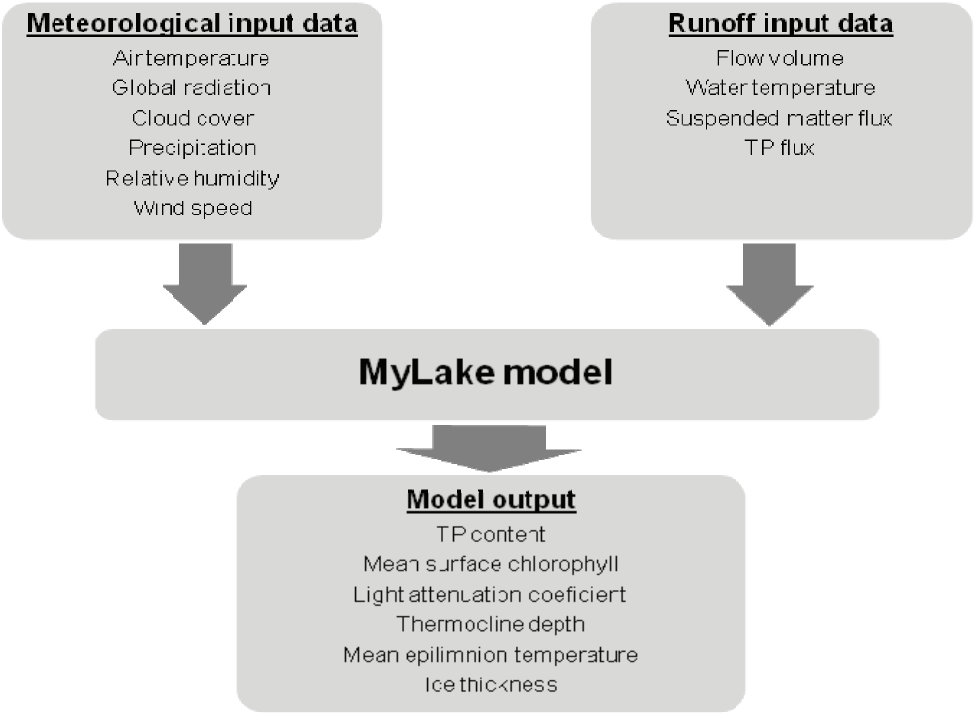

Figure 4. Flow scheme for the MyLake model experiment.

Daily air temperature, global radiation, cloud cover, precipitation, relative humidity and wind speed data were obtained from a nearby meteorological station located at the Norwegian University of Life Sciences (59°39'37"N, 10°46'54"E; Thue-Hansen and Grimenes, 2011). Daily time series of runoff volume, runoff water temperature, and fluxes of suspended inorganic particles and total phosphorus were obtained from the Skuterud monitoring station located at an inlet stream to Østensjøvann. Østensjøvann is a small lake of 0.4 km^2 which belongs to the Lake Årungen catchment (59°41′18″N, 10°49′45″E). Runoff from the other subcatchments was estimated by scaling up the Skuterud data, using previously determined flow and nutrient factors for each subcatchment based on the size of the area and land use (Askilsrud, 2010). Water temperature, TP, SRP, and chlorophyll *a* concentration from the deepest location in the lake over the period 2008-2011 were used for model calibration.

The model was run for six loading and weather scenarios to quantify their impact on the whole-lake average TP pool, mean surface chlorophyll concentration, light attenuation coefficient, thermocline depth, epilimnion temperature, and ice thickness. Principal component analysis (PCA) was used to explore the relationship between external forcing and the lake response.

5. Main results and discussion

5.1. Nutrient limitation

Nutrient concentrations varied seasonally in Lake Årungen (**Paper I**). The highest concentrations of phosphate were measured in winter and early spring, and the lowest concentrations from May to September (Figure 5). The low summer epilimnion SRP measured in **Paper I** indicated phosphorus limitation since SRP below 3 µg L^{-1} is regarded to limit phytoplankton growth (Reynolds, 1992).

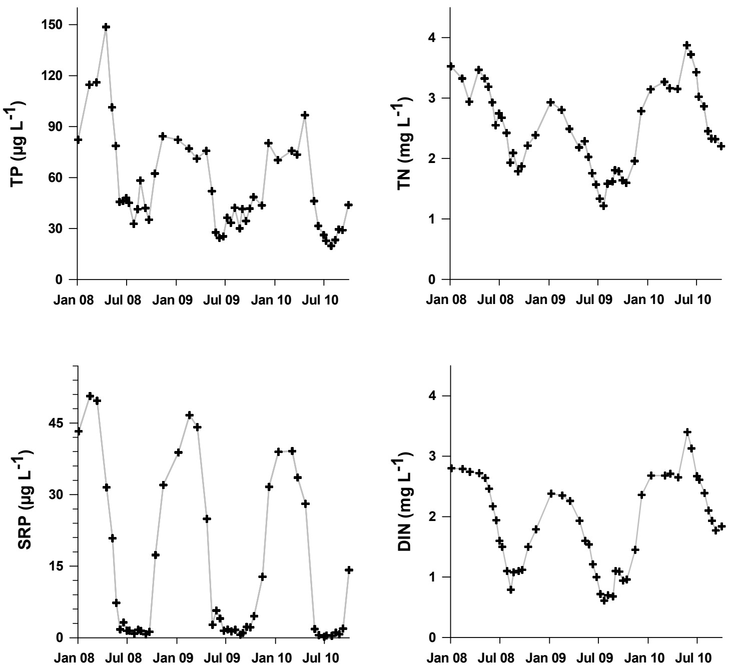

Figure 5. Total phosphorus (TP, µg L^{-1}), total nitrogen (TN, mg L^{-1}), chlorophyll *a* concentration (Chl *a*, µg L^{-1}), soluble reactive phosphorus (SRP, µg L^{-1}), and dissolved inorganic nitrogen (DIN = NO_3-N + NH_4-N, mg L^{-1}) in the epilimnion (0.5-5 m) of Lake Årungen in the period from January 2008 to September 2010.

The TN:TP ratio from 23 to 144 by weight in the period 2008-2010 (**Paper I)** were similar or higher than in other lake studies where phosphorus was found to be the limiting nutrient (Downing and McCauley, 1992; Faafeng and Hessen, 1993; Sterner, 2008), although the concentrations of both TN and TP in the present study were supportive of intensive phytoplankton production (Wetzel, 2001). The ratio of dissolved nutrients has been proposed to be a better indicator of phytoplankton growth limitation than the ratio between total nutrients (Morris and Lewis, 1988; Wagner and Adrian, 2009; Wood et al., 2010). However, this approach might be inapplicable to determine nutrient limitation if the nutrient concentrations are very high (Grobbelaar and House, 1995) such as in Lake Årungen. The finding from **Paper I** that phosphorus limit phytoplankton growth was confirmed by the *in situ* bioassay experiment (**Paper II**) where addition of phosphorus increased phytoplankton growth (Figure 6).

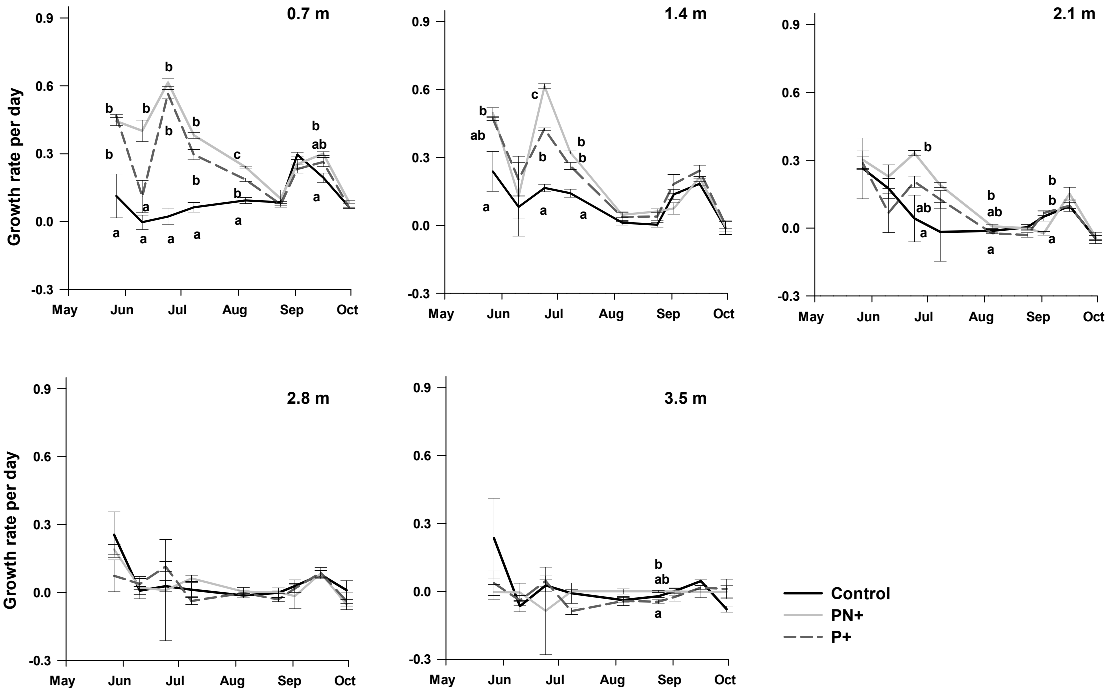

Figure 6. Net specific growth rate per day at 0.7, 1.4, 2.1, 2.8 and 3.5 m depth over three days incubation. Water with natural phytoplankton community from Lake Årungen was either added phosphorus (P+), both phosphorus and nitrogen (PN+), or no nutrients (control). Significant differences among treatments at a given depth and time are denoted by different letters, $p < 0.05$.

Limitation by phosphorus in Lake Årungen coincides with many other lake studies where phosphorus has been found to be the most important factor for phytoplankton primary production (Schindler and Fee, 1974; Hecky and Kilham, 1988; Jeppesen et al., 2005; Håkanson et al., 2007). However, the effect of phosphorus addition on phytoplankton growth was highest in surface water and during the first part of the growing season when the water transparency was relatively high (Figure 6, **Paper II**). Development of buoyant cyanobacteria species such as *Aphanizomenon* cf.

klebahnii and *Anabaena planctonica*, which are capable of reaching the surface layer (Mur et al., 1999), contributed to further reduced light for phytoplankton in deeper water.

Cyanobacteria were not significantly ($p > 0.05$) correlated to the concentration of SRP (**Paper I**). The ability of cyanobacteria to use intracellular stored phosphorus and their higher affinity for nutrients than many other phytoplankton restrict the use of nutrient concentrations approaches in determination of the limiting nutrient in waters with dominance of cyanobacteria (Fisher et al., 1995).

The high DIN concentrations in the epilimnion (> 0.6 mg L^{-1}) during all three years of the lake survey and the bioassay experiments exclude nitrogen as a limiting nutrient in the lake, although increased phytoplankton growth was observed in June and August when nitrogen was added together with phosphorus (**Paper II**). Schindler (1977) suggested that low N:P ratios favour development of cyanobacteria in freshwaters, but this was not supported by the results from Lake Årungen where high TN:TP ratios coincided with dominance of nitrogen fixing cyanobacteria such as *Aphanizomenon* cf. *klebahnii* and *Anabaena planctonica* (Figure 7, **Paper I**). The negative correlation between DIN and cyanobacteria biomasses in **Paper I** could be a result of reduced nitrogen utilization when cyanobacteria growth decline, and may indicate that cyanobacteria in the lake did not fixate nitrogen since nitrogen fixation by heterocystous cyanobacteria is promoted by DIN concentrations below 0.1 mg L^{-1} (Horne and Commins, 1987). Other lake studies also disagree with Schindler's hypothesis of high heterocystous cyanobacteria development under low N:P ratio (Håkanson et al., 2007; Kosten et al., 2009).

These findings indicate that factors related to light could be more important than nutrients for phytoplankton growth in the studied lake, and that cyanobacteria may dominate under low light conditions and low concentration of SRP.

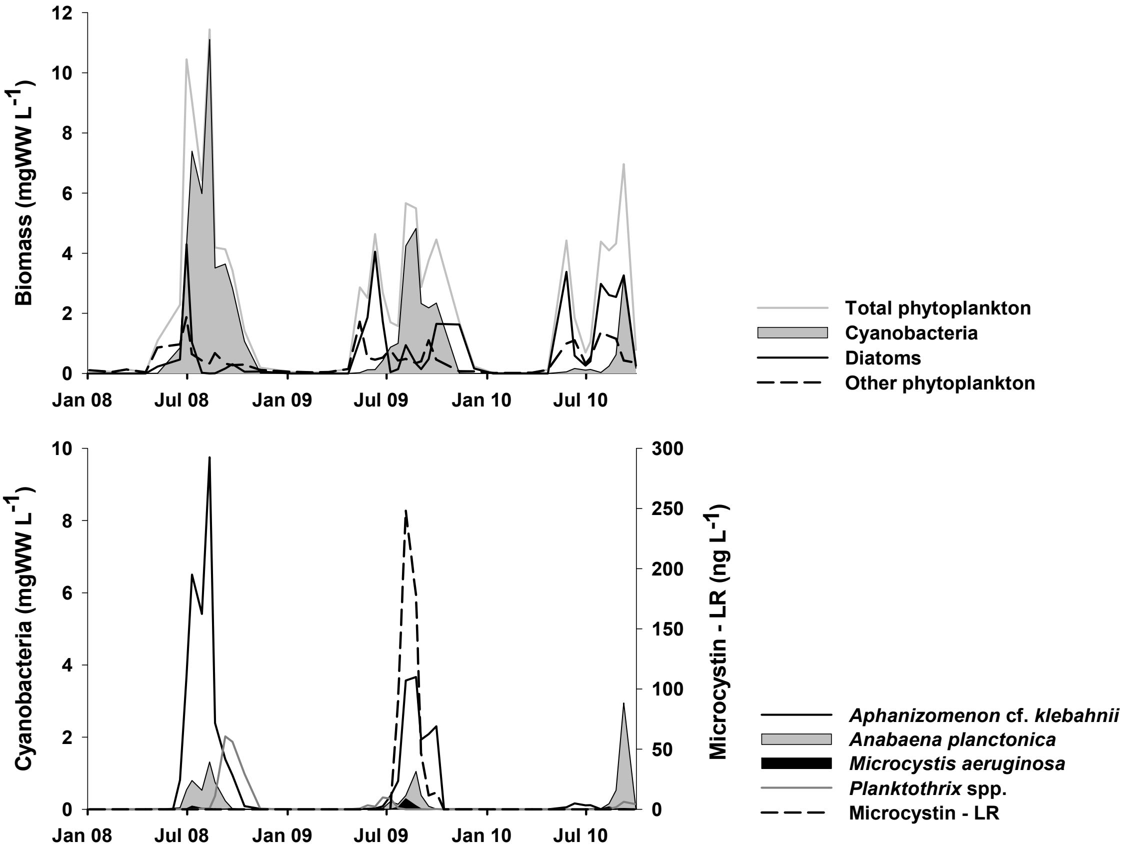

Figure 7. Phytoplankton biomass and microcystin concentration in Lake Årungen in the period from January 2008 to September 2010.

5.2. External loading

The accordance between simulated and observed lake data for the period 2008-2010 in **Paper III** implied that the MyLake model is suitable for simulation of thermal, phosphorus and biological dynamics in the winter-freezing lake Årungen. The model experiment, which simulated the lake response for a 16-years period (1994-2010) with six different weather and external loading scenarios, showed that the year-to-year variation in the lake phosphorus content and chlorophyll concentration corresponded to variations in external loading. This indicates that phosphorus sources from the catchment remains important in determining the level of phosphorus in the lake, and that input of phosphorus increased the risk for development of high phytoplankton biomass in the lake.

External loading is, however, influenced by catchment characteristics and hydrology, land use and climate. The impact of weather conditions on the lake nutrient level and primary productivity are therefore highly relevant. Heavy rainfall is shown to increase soil erosion and the transfer of particulate phosphorus from arable land (Fraser et al., 1999), and loading of dissolved nutrients and organic matter to the lake (Nõges et al., 2007). In the northern temperate region, higher winter rainfall during mild winters is expected to increase the phosphorus loading (Jeppesen et al., 2009). More than 70% of the phosphorus variation in a Norwegian lake during the summer was explained by phosphorus loading in the period March-April (Bechmann et al., 2005). Therefore, the high phosphorus concentrations during the winter and spring in 2008 compared to 2009 and 2010 may be a consequence of the intensive precipitation in January that year (**Paper I**). However, other factors than phosphorus were also contributing to high primary production of phytoplankton in 2008 compared to the following two years (Figure 8, **Papers I** and **III**). Although the phosphorus concentration in the period 2008-2010 was low compared to the 1980s when the TP exceeded 400 µg L^{-1} (Løvstad and Krogstad, 1993), TP and SRP concentrations above 100 and 50 µg L^{-1}, respectively, indicate a highly eutrophic lake (Figure 5). The lake is thus under constant risk of development of toxic cyanobacteria blooms if other lake conditions are favourable.

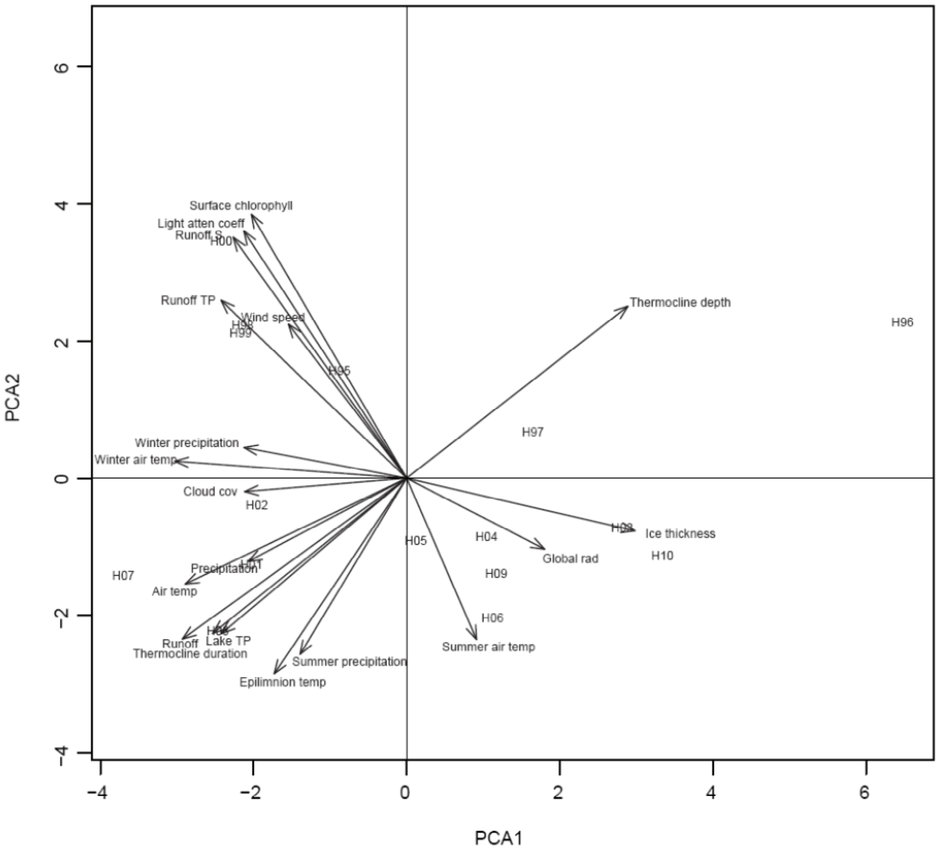

Figure 8. Principal component analysis (PCA) of calculated MyLake model input and output data for 16 water years (1994-2010). The water years are indicated by letters.

5.3. Lake thermal and mixing regime

Lake physical conditions change over time as a response to both local meteorological forcing and global climate changes (Weyhenmeyer et al., 2002; Livingstone, 2008). The effect of lake physical factors on the biological responses has recently gained more attention as phytoplankton dynamics could not only be explained by the nutrient state of the lakes (Padisak et al., 2010; Zohary et al., 2010). Variable weather conditions during the three years of study caused different thermal and mixing regimes in the lake (Figures 9 and 10), and hence variable phytoplankton dynamics (**Papers I** and **III**).

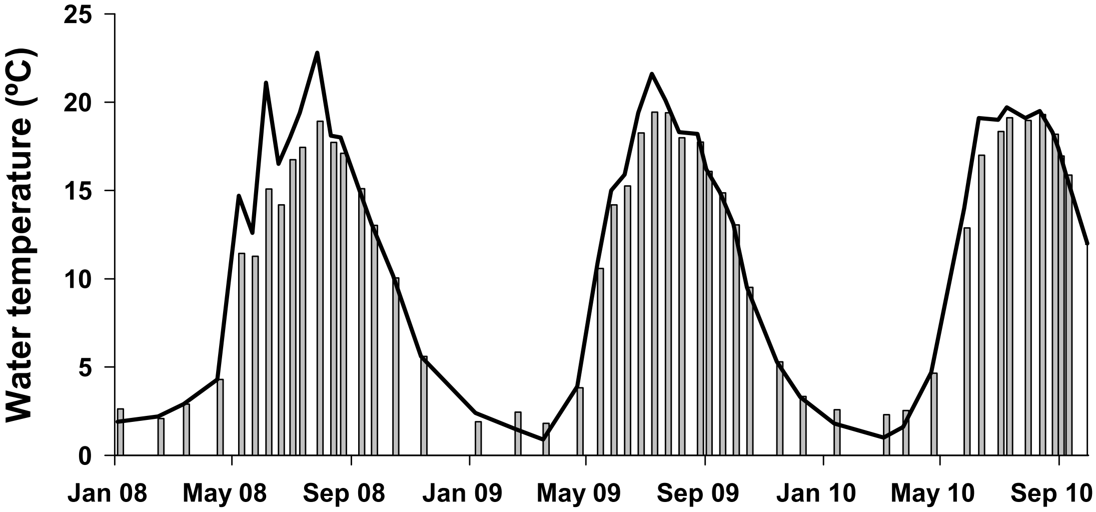

Figure 9. Epilimnion temperature (average 0-5 m; bar-type graph) and surface water temperature (line) in Lake Årungen from January 2008 to September 2010. The temperature is given in °C.

The year 2008 was relatively warm with an annual average air temperature1.8°C above normal for the period 1961-1990, whereas 2009 and 2010 were characterized by cold winters. This led to earlier ice-out, higher epilimnion temperature and shallower thermocline in 2008 compared to 2009 and 2010 (**Papers I** and **III**). Results from the PCA analysis in **Paper III** also suggested that increased air temperature led to increased epilimnion water temperature, shallow thermocline depth and long lasting thermocline.

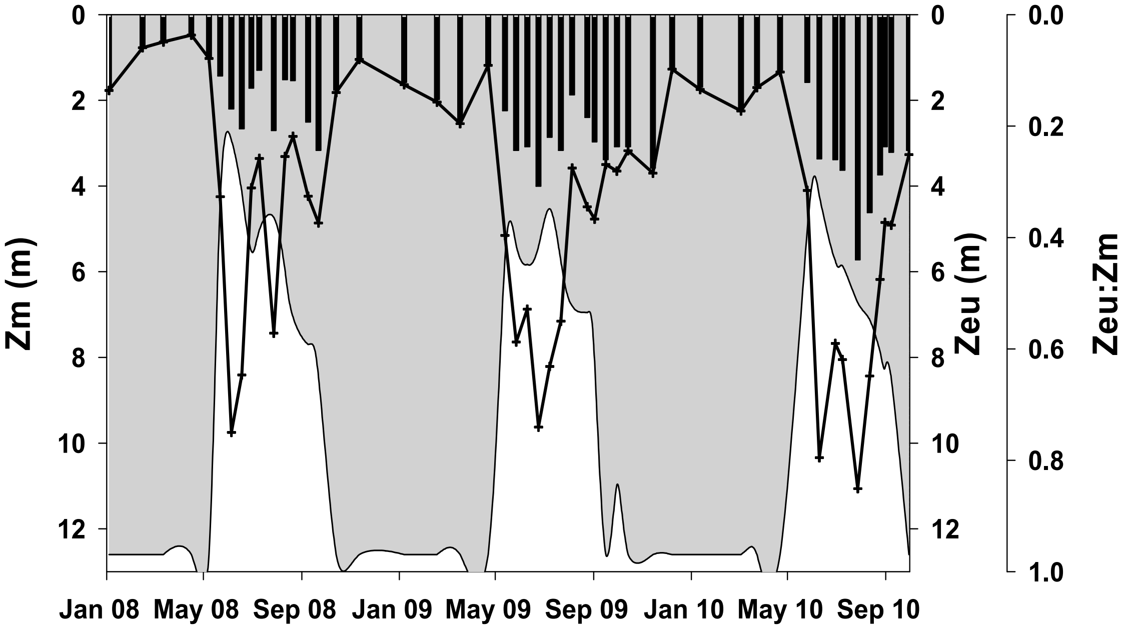

Figure 10. Mixed-layer depth (Z_m; grey area graph), euphotic zone (Z_{eu}; black bar-type graph) and Z_{eu}:Z_m ratio (black line) in Lake Årungen from January 2008 to September 2010.

The highest chlorophyll concentration and phytoplankton biomass were found in 2008 (Figures 5 and 7), which corresponds to a high biomass of cyanobacteria in the lake this year (**Paper I**). The time of cyanobacteria occurrence in the season, their total biomass and their community structure varied from year-to-year and were mostly controlled by the lake thermal and mixing regimes (**Paper I**). Water temperature significantly affected the biomasses of *Aphanizomenon* cf. *klebahnii*, *Anabaena planctonica* and *Microcystis aeruginosa* (**Paper I**). Increased water temperatures, and stable and shallow thermocline after mild winters favour development of cyanobacteria (Mur et al., 1999; Weyhenmeyer et al., 2002; Paerl and Huisman, 2008; Wagner and Adrian, 2009; Tirok and Gædke, 2007), which could cause the bloom of the buoyant cyanobacteria species *Aphanizomenon* and *Anabaena* in 2008. This was confirmed in **Paper III** where the chlorophyll concentration in the lake co-varied positively with winter air temperature (Figure 8). Early disappearance of thermocline in 2009 postponed the cyanobacteria dominance, and deep and intensive mixing in 2010 highly restricted the development of cyanobacteria (**Paper I**). The mean epilimnion water temperatures were relatively low and did not exceed 20°C during all three growing seasons (Figures 9 and 10). Water temperature of 25°C and greater is optimal for cyanobacteria growth (Robarts and Zohary, 1987), and the relatively low water temperatures in the present study probably hampered the development of cyanobacteria. Low water temperature affects particularly *Microcystis* (Robarts and Zohary, 1987) which was poorly developed during this study (Figure 7, **Paper I**). The cyanotoxins production depends on the biomass of their producers and environmental conditions that favour development of these cyanobacteria (Davis et al., 2009; Joung et al., 2011). Consequently, the concentration of microcystin-LR produced by *Microcystis aeruginosa* was low during the study period (Figure 7, **Paper I**).

5.4. Light

There were large seasonal and inter-annual variations in light condition according to the lake survey and the MyLake model simulations for the period 2008-2010 (**Papers I** and **III**). The lake was most turbid during winter, early spring, and late summer (**Paper I**). The winter of 2008 was followed by high water turbidity and low water transparency with a Secchi depth below 1 m in spring this year (**Paper I**). The year 2008 had almost 50% more precipitation than normal, and 3.5 times more than normal in January. The intensity of precipitation determines runoff, suspended matter discharge and water transparency (Arheimer et al., 2005; Nõges et al., 2007; Ulén et al., 2007) and could lead to poor light conditions in the lake in 2008. In contrary to this, water transparency drastically increased after the cold winters and long lasting ice cover in 2009 and 2010 which reduced fluxes of suspended mater, and increased the Z_{eu} and Z_{eu}:Z_m (Figure 10). The PCA analyses in **Paper III** confirmed the relationship between external loading and light conditions as the wet winters led to high runoff of suspended matter and increased light attenuation in the lake (Figure 8). Increased precipitation, and consequently higher soil erosion and particle transport into lakes, may not only affect the nutrient status, but also the light condition in waters and thus the phytoplankton production (Mallin et al., 1999).

The importance of light for phytoplankton dynamics was demonstrated by the *in situ* bioassay study in **Paper II** (Figure 11). Light significantly affected the phytoplankton production during the whole growing season (May to September), but the effect was most pronounced in the period from end of July to mid September. This period coincided with the period of high cyanobacteria biomasses which further reduced the light availability in the turbid Lake Årungen. Low response of phytoplankton to nutrient addition during that period is consistent with statement that nutrients are important in turbid waters only when favourable light prevails (Grobbelaar, 1992).

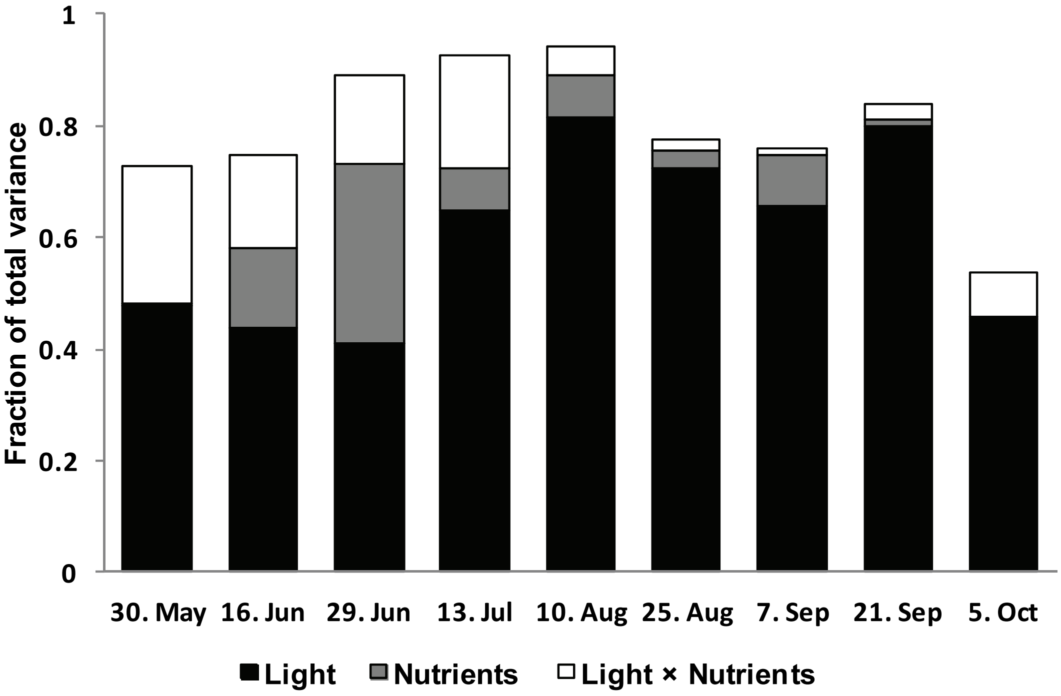

Figure 11. Effect sizes (partial R^2-values) of light, nutrient addition, and their interaction on specific growth rate.

Low Z_{eu}:Z_m ratios that might limit phytoplankton growth were found in midsummer of 2008, August 2009 and September 2010, and it coincided with the periods of cyanobacteria development in the lake (Figures 7 and 10, **Paper I**). Poor light conditions favour development of cyanobacteria (Humphries and Lyne, 1988; Mur and Schreurs, 1995; Litchman, 1998), particularly of buoyant cyanobacteria which move to water layers with favourable light conditions (Mur et al., 1999). Buoyant *Aphanizomenon* cf. *klebahnii* was negatively correlated to Z_{eu} ($p < 0.001$, **Paper I**), and the most abundant cyanobacteria under low light conditions (Figures 7 and 10). The decreased water transparency in 2008 probably contributed to the high cyanobacteria biomass this year.

As the future climate scenarios predicts warmer winters and increased winter precipitation in south-eastern Norway (Hanssen-Bauer et al., 2005), we should expect higher fluxes of suspended matter and phosphorus, reduced water transparency, and increased biomasses of cyanobacteria in lakes exposed to erosion of nutrient rich soils, such as Lake Årungen.

6. Conclusions and future perspective

- Lake Årungen remains highly eutrophic with high concentrations of both total and dissolved phosphorus and nitrogen, despite more than 40 years of applied measures to control input of nutrients.

- The phosphorus content in the lake varies in response to external loading. Increased winter temperatures and precipitation promote high runoff and fluxes of suspended matter and TP into the lake.

- Low concentrations of SRP during the phytoplankton growing season, high TN:TP ratio and increased phytoplankton growth with addition of phosphorus suggest that phosphorus is the limiting nutrient for phytoplankton growth in Lake Årungen. However, the cyanobacteria biomass was not significantly correlated to phosphorus, which could be due to the use of intracellular stored phosphorus when the lake phosphorous concentration is low.

- Available nutrients may increase total phytoplankton growth only when the light condition in the lake is satisfactory. Increased light attenuation of inorganic and organic origin, promotes light limitation in the lake. However, low light conditions favours development of buoyant cyanobacteria in the lake.

- High biomasses of the nitrogen fixing cyanobacteria *Aphanizomenon* cf. *klebahnii* and *Anabaena planctonica* were developed despite high concentrations of both TN and DIN, and high DIN:SRP ratio. This is in contradiction to the statement that the growth of nitrogen fixing cyanobacteria is promotes by nitrogen deficiency and low N:P ratio.

- Year-to-year variation in weather affects the lakes thermal, mixing and light regimes, and consequently the phytoplankton dynamics in the lake. The highest phytoplankton biomass was observed after a mild and wet winter that was followed by high water temperature, shallow and stable thermocline and low water transparency.

The results from this study suggest that further control of phosphorus input to the lake will reduce the risk of intensive phytoplankton production; nevertheless, blooms of cyanobacteria may still occur under favourable thermal and light conditions regardless of the nutrient concentrations in the lake.

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PAPER I

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Environmental factors affecting cyanobacteria dynamics in a Norwegian eutrophic and turbid lake

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Abstract

Blooms of potentially toxic cyanobacteria often develop in the eutrophic and highly turbid Norwegian Lake Årungen. The objective of the present study was to evaluate the effect of water temperature, nutrient availability, light and mixing depth on the seasonal dynamics of cyanobacteria and their toxins in the lake. The study comprises 49 sampling dates in the period from January 2008 to September 2010. The time of cyanobacteria occurrence, their total biomass, community structure and toxicity varied considerably between years although similar nutrient conditions were observed. The potentially nitrogen fixating cyanobacteria *Aphanizomenon* cf. *klebahnii* and *Anabaena planctonica* were most abundant in the lake, followed by *Planktothrix* spp. and *Microcystis aeruginosa*. Water temperature and light were the most determining factors for cyanobacteria biomass in the lake as biomasses of *Aphanizomenon, Anabaena* and *Microcystis* was positively correlated with water temperature ($p < 0.05$), and the biomass of *Aphanizomenon* was negatively correlated with euphotic depth. The epilimnion soluble reactive phosphorus (SRP) concentration decreased from 40-50 µg L^{-1} in the winters to below 4 µg L^{-1} during the growing seasons. That may indicate phosphorus limitation, but no significant ($p > 0.05$) correlation was found between SRP and cyanobacteria biomass. The high epilimnion dissolved inorganic nitrogen (DIN) concentration (> 0.6 mg L^{-1}) during the entire study excluded DIN as a limiting factor for cyanobacteria biomass and composition, although a significant negative correlation was found between DIN and cyanobacteria biomass. Genetic analyses revealed that *Microcystis aeruginosa* produced the cyanobacterial toxins in Lake Årungen. However, the concentration of toxins was low, probably as a consequence of the unstable weather conditions during the summers and poor development of *Microcystis* during the study period.

Introduction

High cyanobacterial biomass may impact the structure and function of aquatic ecosystems (Havens, 2008) and cause water quality problems (Bartram et al., 1999; Kardinaal and Visser, 2005). Additionally, cyanobacteria may produce toxins harmful to human and animal health (Codd, 1995; Bartram et al., 1999). Dominance of cyanobacteria is traditionally associated with eutrophication, and nutrient loading reduction, particularly of phosphorus, is often the first step in management of freshwater systems. However, many lakes continue to experience cyanobacterial dominance despite of reduced nutrient concentrations (Bailey-Watts and Kirika, 1999; Jeppesen et al., 1999, 2007). Low total nitrogen to total phosphorus ratios (TN: TP) has been found to favor cyanobacterial growth in some lakes (Schindler, 1977; Bulgakov and Levich, 1999; Schindler et al., 2008), whereas such relationship is not found in other lakes (Pick and Lean, 1987; Downing et al., 2001; Håkanson et al., 2007; Kosten et al., 2009). Physical factors like water temperature, light and water mixing processes can be equally, or even more important, than nutrients for cyanobacterial growth and community structure (Huisman and Weissing, 1995; Padisak et al., 2010; Zohary et al., 2010). Luxury phosphorus uptake, buoyancy regulation and nitrogen fixation are ecostrategies that enable cyanobacteria to prevail in a wide range of environmental conditions and to dominate over other phytoplankton groups (Mur et al., 1999). In addition, production of toxic substances and formation of large and often mucous colonies make them less attractive to zooplankton grazing.

Cyanobacteria toxin production can be determined by community and population structure, but environmental conditions such as water temperature, light and nutrient availability may also have an effect (Kardinaal and Visser, 2005). Microcystins are widely distributed cyanotoxins in freshwaters and include more than 90 isoforms

(Welker and von Döhren, 2006). The most common microcystin producer in lakes world-wide is *Microcystis aeruginosa* (Gobler et al., 2007), but species of other genera may also produce these compounds (Kardinaal and Visser, 2005).

The present study was carried out in Lake Årungen which is highly eutrophic and one of the nutrient richest lakes in Norway due to heavy load from soil erosion and runoff from surrounding agricultural areas. The lake hosts the national rowing stadium and is an important recreational and fishing area. Eutrophication and mass occurrence of toxic cyanobacteria has therefore gained widespread public attention. High cyanobacteria biomass has been considered to be a consequence of large nutrients input, and particularly of phosphorus. Great efforts have therefore been made to reduce phosphorus loading into the lake since the 1970's by controlling point sources and reducing agricultural runoff and erosion by improved agriculture practices (Borch et al., 2007). These measures have reduced the total phosphorus concentration from approximately 400 μg L^{-1} to below 100 μg L^{-1}, but the lake continues to suffer from high cyanobacteria biomass and cyanobacterial toxin production. Lake Årungen differs from other lakes in the area due to its high turbidity, especially in periods after snow melting and heavy rainfall. The lake is also highly exposed to wind since its longitudinal position coincides with the dominant winds. Physical processes such as light attenuation and vertical mixing seems therefore to be highly variable in the lake.

The aim of the present work was to study the seasonal dynamic of cyanobacteria and microcystin, and to relate their seasonal pattern to environmental factors. Descriptive statistics and regression models were used to study data sampled in the period over three years. The data includes 49 sampling dates with water and phytoplankton from four depths in the lake epilimnion at each sampling.

Materials and methods

Study site and water sampling

Lake Årungen is a shallow Norwegian lake located approximately 30 km south of the capital Oslo (59˚41' N, 10˚45' E). Morphometrical and catchment characteristics of the lake are given in Fig. 1. Mean and maximum depths are 8 and 13 m, respectively, and the lake is dimictic and thermally stratified during the summer. It has a surface area of 1.2 km^2 and a watershed area of 51 km^2. More than half of its catchment area is used for agricultural purposes, and the lake is thus highly exposed to input of particles and nutrients from soil erosion and agricultural runoff.

Water samples were collected with a modified Rüttner water sampler at 0.5, 1.0, 3.0 and 5.0 m depth at the deepest location in the period from January 2008 to September 2010. Water was sampled twice a month from May to September and once a month through the rest of the year (n = 49). The water samples were used for physical, chemical and phytoplankton analyses. Meteorological data were obtained from the nearby meteorological station located at the Norwegian University of Life Sciences (59˚39' N, 10˚46' E) (Thue-Hansen and Grimenes 2009−2011).

Physical and chemical analyses

Water temperature was measured *in situ* by a MultiLine P4 measuring device (WTW, Weilheim, Germany) at 0.5, 1.0, 3.0 and 5.0 m depth on each sampling date. Vertical water temperatures were also obtained from hourly temperature logging at eight depths between 0.7 and 12.6 m by Hobo pendant temperature loggers 64K-UA-002-64 (Onset Computer Corporation, Bourne, MA, USA). Water transparency was measured *in situ* as Secchi depth (SD) with a 13×18 cm white disk. Turbidity was determined according to NS-ISO 7027 with a laboratory turbidimeter model 2100AN IS

(Hach Company, Loveland, CO, USA). Water filtration, subsampling and conservation were carried out on the day of sampling. Dissolved inorganic nutrients were analysed after filtration through 0.45 µm pore-size membrane filters. TP (detection limit: 3µg L^{-1}) and soluble reactive phosphorus (SRP; detection limit: 1µg L^{-1}) were analysed spectrophotometrically (UV-VIS Spectrophotometer UV-1201, Shimadzu, Kyoto, Japan) according to Norwegian standard NS-EN 1189, where SRP was quantified after a reaction with ammoniummolybdate. TN (detection limit: 0.01 mg L^{-1}) was determined after oxidation by peroxodisulphate according to Norwegian standard NS 4743. Nitrate ($NO_{3;}$ detection limit: 0.005 mg L^{-1}) was quantified by liquid chromatography (*Zellweger Analytics Lachat IC5000 ion chromatograph,* Milwaukee, WI, USA) according to NS-EN ISO 10304-1, and ammonium (NH_4) spectrophotometrically according to NS-EN 4746.

Phytoplankton and microcystin analyses

Phytoplankton biomass and composition were analysed in water samples preserved with Lugol's solution added acetic acid. Utermöhl's sedimentation method, described in detail by Olrik et al. (1998), was applied to 10 mL of each water sample prior to counting by an inverted phase contrast microscope (*Leitz. Fluovert FS,* Leica, Wetzlar, Germany). Phytoplankton volumes were calculated according to Rott (1981) and Olrik et al. (1998).

Microcystins were extracted from filters with field samples after lyophilisation using 50% methanol (Rohrlack et al., 2003). The quantification was done by liquid chromatography with mass spectrometric detection (LC-MS/MS). The instrumental setup included a Waters Acquity UPLC System equipped with a Waters Atlantis C18 column (2.1 × 150 mm, 5 µm particle size) directly coupled to a Waters Quattro Premier

XE tandem quadrupole MS/MS detector. The UPLC system was set to deliver a linear gradient from 20% to 60% acetonitrile in water, both containing 0.1% formic acid, within 10 min at a flow rate of 0.25 mL min^{-1}. The column and auto sampler temperatures were 20 and 4 ºC, respectively. At all times, the MS/MS detector was run in positive electrospray mode (ESI+). Other general settings included a source temperature of 120 ºC, a desolvation temperature of 350 ºC, a drying gas flow rate of 800 L h^{-1}, a gas flow at the cone of 50 L h^{-1}, and standard voltages and energies suggested by the manufacturer for the ESI+ mode. Nitrogen, continuously delivered by a nitrogen generator (NG 11, Parker Balston, Haverhill, MA, USA), served as drying, nebulising and cone gas. Samples were screened for known microcystins by running the detector in total scanning mode for the mass range 500 to 1200 Da. The time for a single scan was 2 sec. For the quantification, the MS/MS detector was run in selective ion monitoring mode with a dwell time of 0.05 sec.

Genomic DNA extraction and qPCR amplification

Genetic analysis in order to determine microcystin producers in the lake were performed on four water samples from the deepest location and two samples from the outflow of the lake. 100 µl of water was frozen and thawed twice, boiled for 5 min, and genomic DNA was then extracted using a MoleStrips DNA blood kit and a DNA-Cyano protocol on GeneMole (Mole Genetics, Oslo, Norway) according to the manufacturer's instructions. Possible microcystin-producing genotypes was analysed by quantitative real time polymerase chain reaction (qPCR) as described by Kurmayer and Kutzenberger (2003). Each sample was analysed for the presence of *mcyB*, *mcyE* and *mcyB* genes which are indicative of microcystin-producing genotypes from *Microcystis*, *Anabaena* and *Planktothrix*, respectively (Kurmayer and Kutzenberger, 2003; Vaitomaa et al., 2003; Briand et al., 2008).

The primers 30F and 108R were used for *Microcystis* (Kurmayer and Kutzenberger, 2003), McyE-F2 and AnamcyE-12R were used for *Anabaena* (Vaitomaa et al., 2003) and t MAPF and MAPR were used for *Planktothrix* (Briand et al., 2008). The qPCR was performed with 5 µl of DNA, 1 µl of each primer (Eurofins MWG Operon, Ebersberg, Germany), 12.5 µl of a master mix (MESA blue qPCR Master mix for SYBR green assay with low rox, Eurogentec, Seraing, Belgium) and water in a total volume of 25 µl. Amplifications were performed as follows: an initial preheating for 2 min at 50 ºC and 10 min at 95 ºC, followed by 40 cycles with one cycle consisting of 15 sec at 95 ºC and 1 min at 60 ºC. The melting temperatures for the amplification products were determined by a melt curve stage after qPCR and the temperature was reduced from 95 ºC to 60 ºC with continuous fluorescence detection. All analyses were done in triplicate using an ABI 7500 mastercycler (ABI, Carlsbad, CA, USA).

Calculations and statistical methods

Mixed-layer depth (Z_m; m) was estimated as the depth of maximum vertical density gradient. The density gradient was estimated by analytical differentiation of a 4-parameter logistic function fitted to the vertical density profile by non-linear regression. Water density was calculated from the temperature logger time series according to Millero and Poisson (1981). The euphotic zone (Z_{eu}; m) was calculated as 2.2 × SD (Romarheim et al., unpublished). The effects of environmental parameters on biomasses of the dominant cyanobacteria *Anabaena planctonica*, *Aphanizomenon* cf. *klebahnii*, *Microcystis aeruginosa*, and *Planktothrix* spp. were studied by multiple regression models using surface temperature, dissolved inorganic nitrogen (DIN), SRP, Z_{eu}, and Z_m

as explanatory variables. All cyanobacterial biomasses were 4th root transformed to stabilize variances while none of the explanatory variables were transformed. Effect sizes were visualized as term plots where the partial effects of individual terms are shown as straight lines with the same slope as their fitted model coefficient. 95% confidence bands for the partial effects are based on the standard errors of the fitted model coefficients, such that an effect is not significantly different from zero if the confidence band can fully contain a horizontal line corresponding to zero effect size. All statistical analyses were done with the statistical computing environment R (R Development Core Team, 2010).

Results

Environmental conditions of Lake Årungen

Environmental data of Lake Årungen in the period from January 2008 to September 2010 is presented in Fig. 2. The year 2008 was characterised by a mild winter, whereas the winters in 2009 and 2010 were colder compare to 2008 (Fig. 2 a). The precipitation in January 2008 was 3.5 times higher than normal for that month, and all three years were characterised by high precipitation in July and August (Fig. 2 b). Mean epilimnion water temperatures were below 20 ºC during all three growing seasons, and the highest surface water temperatures were recorded in July to August (Fig. 2 c). The lake was ice-free from mid March in 2008, and from the end of April in 2009 and 2010 (Fig. 2 d). Z_m ranged between 3 and 6 m in June to mid August in 2008 and between 5 and 7 m during the same period in 2009. In 2010, the Z_m was 4 m in June and declined continuously throughout the growing season. The water column was entirely mixed by the end of September 2008 and 2010, while the autumnal mixing was two weeks earlier in 2009. Wind speed varied between 0.5 and 8 m s^{-1} during the studied period (Fig. 2 e). The period from mid June to October in 2008 and June to mid August in 2009 had in general wind speed below 4 m s^{-1}, whereas the wind was generally stronger in the summer 2010. The turbidity was highest in the spring and autumn in all three years, although a less pronounced increase was also observed in late summer (Fig. 2 f). Maximum water turbidity was recorded in the springs after the lake ice disappeared and was 74 NTU, 28 NTU, and 24 NTU in 2008, 2009 and 2010, respectively. SD in the period from May to September varied between 0.4 and 1.4 m in 2008, 0.9 and 1.8 m in 2009 and between 0.7 and 2.6 in 2010. Z_{eu} also varied between years, and was highest in the summer of 2010 (Fig. 2 g). The Z_{eu}: Z_m ratio was below 0.4 during the whole phytoplankton growth season in 2008, except mid-June and the end of July, and below 0.4 from August and September in 2009 and 2010, respectively.

The mean annual concentration of epilimnion SRP decreased throughout the study period and was on average 15.7, 12.7 and 12.4 µg L^{-1} in 2008, 2009 and 2010, respectively (Fig. 2 h). SRP was below 4 µg L^{-1} in the period from June to mid September all three years. Epilimnion DIN (NO3 + NH_4) exceeded 0.6 mg L^{-1} during the entire sampling period (Fig. 2 i). TN: TP by weight ranged between 23 and 144 during the study period, and was highest in 2010 (Fig. 2 j).

Phytoplankton dynamics and microcystin production

The highest total phytoplankton concentration was found in 2008 (Fig. 3 a). Cyanobacteria and diatoms were the most abundant phytoplankton during the study period. The biomass of other phytoplankton groups, e.g. Chlorophyceae, Cryptophyceae, Crysophyceae, Dinophyceae and Euglenophyceae, were low. Cyanobacteria were most abundant phytoplankton during the whole growing season in

2008 and midsummer 2009, whereas low biomass was found at the end of the growing season 2010. Fifteen cyanobacteria species were detected, with *Aphanizomenon* cf. *klebahnii*, *Anabaena planctonica, Microcystis aeruginosa* and *Planktothrix* spp. as the most abundant (Fig. 3 b).

The growing season in 2008 started with a biomass peak of *Aphanizomenon* cf. *klebahnii* that was later replaced by *Planktothrix* spp. The opposite succession pattern was observed in 2009, but the presence of both cyanobacteria were lower in 2009 than in 2008. *Anabaena planctonica* developed in July and August 2008 and 2009, and appeared as the dominant cyanobacteria in September 2010. Biomass of *Microcystis aeruginosa* was low during the entire study, and the highest concentration was found in July-August 2009.

Microcystin-LR was the only cyanotoxin detected in the lake, and it was found during the growing season of 2009. Highest mean epilimnion microcystin concentration was 248 ng L^{-1} measured in August 2009. The qPCR analyses identified *Microcystis aeruginosa* as the microcystin producer, and the highest concentrations of microcystin in the lake coincide with the appearance of *Microcystis aeruginosa* in the phytoplankton community in 2009.

Cyanobacteria and their relation to environmental factors

Regression analyses of cyanobacteria biomasses as function of water temperature, DIN, SRP, Z_{eu} and Z_m in the period from January 2008 to September 2010 are given in Fig. 4. Biomass concentrations of *Aphanizomenon* cf. *klebahnii*, *Anabaena planctonica*, and *Microcystis aeruginosa* were positively and significantly correlated to water temperature. Biomass concentration of all four studied cyanobacteria species were negatively correlated to DIN, and biomass of *Aphanizomenon* cf. *klebahnii* was negatively correlated to Z_{eu}.

Discussion

Cyanobacteria biomasses and community structure varied among years despite similar nutrient conditions during the sampling period. Factors other than nutrients, such as thermal stratification, water mixing and transparency that are mainly controlled by climate, may control cyanobacteria species composition and biomass (Pick and Lean, 1987). Increased water temperature, early and stable thermal stratification and poor light conditions favour cyanobacteria growth (Mur et al., 1999; Weyhenmeyer et al., 2002; Paerl and Huisman, 2008; Wagner and Adrian, 2009). Different weather conditions between years seem to cause interannual variation in cyanobacteria biomass and community structure in weather-sensitive lakes such as Lake Årungen. The highest cyanobacterial biomass was observed in 2008 which was the warmest and most precipitation-rich year of the study period with particularly warm and wet winter. Mild winters can cause incomplete water mixing in spring (Straile et al., 2003), and this may have led to early and shallow thermocline formation in the Lake Årungen in 2008. Mild winters also increase soil erosion and runoff (Jeppesen et al., 2009) which probably caused high water turbidity and decreased light conditions in the lake during the spring and early summer 2008. Low wind speed below 4 m s^{-1} may support accumulation of cyanobacteria on the lake surface (Serra et al., 2007; Moreno-Ostos et al., 2009), and could contribute to high cyanobacteria biomass in the epilimnion in summer 2008. The combination of early stratification, low light conditions and calm weather may favour cyanobacteria that are capable of buoyancy regulation and vertical migration towards the surface when light availability is reduced (Walsby et al. 1997; Mur et al., 1999). This may explain the dominance of *Aphanizomenon* cf. *klebahnii* over non motile diatoms in early summer 2008.

Cyanobacteria developed later in the growing seasons of 2009 and 2010, and their abundance was lower than in 2008. Cold winters probably resulted in reduced soil erosion, low turbidity and better light conditions at the beginning of the growing seasons in 2009 and 2010. Improved light conditions, early thermocline erosion in 2009, and unstable thermal stratifications with continuously increasing mixed-layer depth in 2010 may have postponed cyanobacterial dominance in these years compared to 2008 by favouring phytoplankton organisms that are better adapted to high turbulence and variable light conditions (Reynolds et al., 1983; Huisman et al., 2004). Frequent wind speed above 4 m s^{-1} from mid August 2009 and the whole summer of 2010 probably increased water turbulence and prevented accumulation of cyanobacteria on the lake surface. Dominance of cyanobacteria decline when Z_{eu} approaches Z_m (Humphries and Lyne, 1988). The cyanobacteria biomass in Lake Årungen decreased when the Z_{eu}: Z_m ratio was above 0.4, but disappeared from the lake at Z_{eu}: Z_m ratios above 0.7. This was in agreement with the critical Z_{eu}: Z_m ratio of 0.8 for cyanobacteria disappearance found by Mur and Schreurs (1995). Additionally, the relatively low water temperatures, which did not exceed 20 °C, probably contributed to poor development of cyanobacteria during our study. Light and temperature were found to be the most important factors in the regulation of cyanobacteria in several other lakes (Scheffer et al., 1997; Halstvedt et al., 2007).

The cyanobacterial community of Lake Årungen includes two groups with different adaptations: (1) cyanobacteria that often dominate during stable stratification (*Aphanizomenon, Anabaena* and *Microcystis*), and (2) those adapted to less stable stratification with fluctuating light availability (especially *Planktothrix* spp.; Reynolds et al., 1983, 1987; Reynolds, 1994; Lindenschmidt and Chorus, 1998). The shift from dominance of *Aphanizomenon* and *Anabaena* to *Planktothrix* spp. after the thermocline deepening in August 2008 underlines the importance of physical factor for controlling the cyanobacterial community of Lake Årungen. Reduced biomasses of *Aphanizomenon* in 2009 compared to 2008, and the almost absence in 2010, may also be explained with a more unstable thermal stratification and enhanced water mixing (Visser et al., 1995; Wagner and Adrian, 2009). Mur and Schreurs (1995) found that *Planktothrix* disappeared from two Dutch lakes at Z_{eu}: Z_m ratio of 0.4. In Lake Årungen, *Plankthotrix* appeared either before thermocline formation or after its erosion, i.e. at times of intensive water mixing and low water temperature. A similar behaviour was observed in German lakes, where even small differences in water mixing and light regime led to the replacement of Nostocales by Oscillatoriales (Mischke, 2003). However, our study failed to identify a particular Z_{eu}: Z_m ratio that marked the borderline between a *Planktothrix* dominated community and the dominance of other cyanobacteria.

Nutrient ratios could not explain the development of cyanobacteria since the N: P ratios were much higher than in other studies where development of cyanobacteria was associated with low N: P ratio and nitrogen limitation (Schindler, 1977; Bulgakov and Levich, 1999; Schindler et al., 2008). Furthermore, Pinto and Litchman (2010) found that cyanobacteria may develop in lakes with high N: P ratio when the light conditions are low, such as in Lake Årungen. High concentrations of DIN, which exceeded 0.6 mg L^{-1} during the entire sampling period, indicate sufficient nitrogen for phytoplankton growth. Nitrogen may therefore be excluded as both a limitation factor for phytoplankton dynamics in Lake Årungen and the rationale for dominance of cyanobacteria capable of N_2-fixation. The negative correlation between DIN and cyanobacteria biomasses could possibly be a result of reduced N utilization when cyanobacteria growth decline, and thus may indicate absence of cyanobacterial nitrogen fixation in the lake.

Phosphorus is considered to be the limiting nutrient for cyanobacteria growth in freshwaters (Schindler, 1977), and SRP concentration below 3 μg L^{-1} most probably limit phytoplankton growth (Reynolds, 1992). The SRP concentration lower than that in all three growing seasons therefore indicates that phosphorus could limit phytoplankton growth in the lake, which is in agreement with an earlier microcosm study in Lake Årungen (Romarheim et al., unpublished) where phosphorus addition was found to increase phytoplankton growth. Cyanobacteria are slow-growing phytoplankton that can take up phosphorus beyond their immediate requirements, and therefore may prevail in the phytoplankton community during periods of phosphorus deficiency. This mechanism likely allows cyanobacteria in Lake Årungen to utilize high phosphorus concentrations in the spring to form intensive and long lasting blooms in late summer. Increased phytoplankton production in lowland Norwegian lakes was also observed after an increased input of agricultural phosphorus prior to phytoplankton growing season (Bechmann et al., 2005). Nutrient input control therefore remains the most important eutrophication measures, especially in lake ecosystems that are situated in agricultural areas and are at high risk of erosion and runoff such as Lake Årungen. Intensive soil erosion and run-off after a warm and rainy winter increase phosphorus load to freshwaters (Fraser et al., 1999; Jeppesen et al., 2009) and could contribute to the high cyanobacterial biomass in spring 2008. If so, bloom-formation should be most prevalent after mild winters with intensive soil erosion that causes both the intensive phosphorus loading and higher lake turbidity. Our study supports this conjecture.

Genetic analyses suggest that *Microcystis aeruginosa* is the main microcystin producer in Lake Årungen. *Microcystis* has also been found to produce microcystin in some other lakes such as Lake Erie (Millie et al., 2009) and Lake Taihu (Ye et al., 2009). Lake Årungen was prior to our study, and particular in 2007, characterised by a bloom of *Microcystis aeruginosa* and microcystin concentrations above 100 µg L^{-1} (unpublished data). Increased water temperature supports growth of potentially toxic *Microcystis* (Davis et al., 2009; Joung et al., 2011), and the relatively low water temperatures from 2008 to 2010 may have prevented *Microcystis* blooms and high toxin concentrations during that period. However, the apparent lack of microcystin production by the current *Planktothrix* strains in Lake Årungen is worth noticing since *Planktothrix* frequently produce microcystins (Rohrlack et al., 2009). This observation emphasis the importance of microcystin producing specific strains in determining the toxicity of cyanobacterial blooms. It also suggests that microcystins are not essential for the persistence of *Planktothrix* populations.

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Figure legends

Fig. 1. Morphometrical and catchment characteristics of Lake Årungen.

Fig. 2. Environmental conditions in Lake Årungen from January 2008 to September 2010 (a) Mean monthly air temperature (bars) and average monthly air temperature in the period from 1961 to 1990 (black line). (b) Monthly precipitation (bars) and average monthly precipitation in the period from 1961 to 1990 (black line). (c) Epilimnion temperature (average 0-5 m; bar-type graph) and surface water temperature (black line). (d) Mixed-layer depth (Z_m; area graph) and ice coverage (white line). (e) Wind conditions at Lake Årungen. Gray areas represent the phytoplankton growing season from May throughout September. Wind speed above the dashed line at (4 m s^{-1}) prevents surface cyanobacteria accumulation according to Serra et al. (2007) and Moreno-Ostos et al. (2009). (f) Water turbidity (area graph) and Secchi depth (SD; bar-type graph). (g) Euphotic zone (Z_{eu}; black line) and Z_{eu}: Z_m ratio (bar-type graph). (h) Epilimnion soluble reactive phosphorus (SRP; average 0-5 m). (i) Epilimnion dissolved inorganic nitrogen (DIN; average 0-5 m). (j) Total nitrogen to total phosphorus (TN: TP) ratio by weight.

Fig. 3. Phytoplankton in Lake Årungen from January 2008 to September 2010: (a) Phytoplankton community structure and biomass. (b) Biomass of *Aphanizomenon* cf. *klebahnii, Anabaena planctonica*, *Planktothrix* spp., *Microcystis aeruginosa* and microcystin concentration.

Fig. 4. Partial term plots for linear regression models of the biomasses of *Aphanizomenon* cf. *klebahnii, Anabaena planctonica*, *Planktothrix* spp. and *Microcystis aeruginosa* as a function of water temperature, dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (SRP), euphotic zone (Z_{eu}) and mixed-layer depth (Z_m) in Lake Årungen in the period from January 2008 to September 2010. Effect size lines have slopes equal to the fitted regression coefficient, with 95% confidence bands based on their estimated standard errors. Significance probabilities for the individual terms are shown in the figure, with n.s. (not significant) representing $p > 0.05$. The gray circles are the residuals of the fitted model for each taxonomic group.

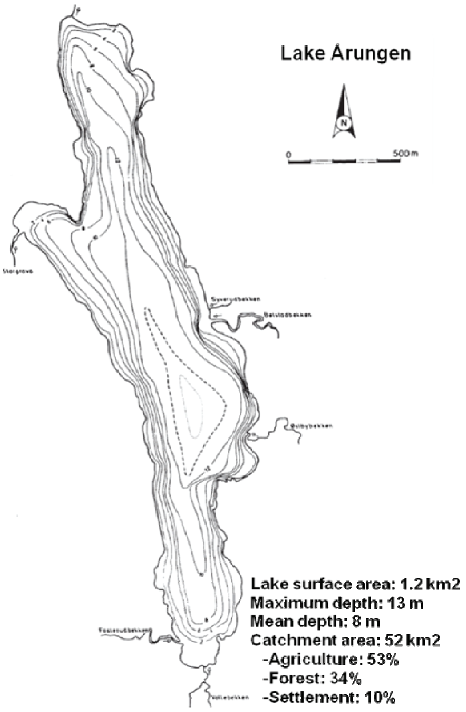
Lake Årungen
N
0
500 m
Storgrava
Syverudbekken
Bølstadbekken
Fosterudbekken
Vollebekken
Lake surface area: 1.2 km2
Maximum depth: 13 m
Mean depth: 8 m
Catchment area: 52 km2
-Agriculture: 53%
-Forest: 34%
-Settlement: 10%

Fig. 1

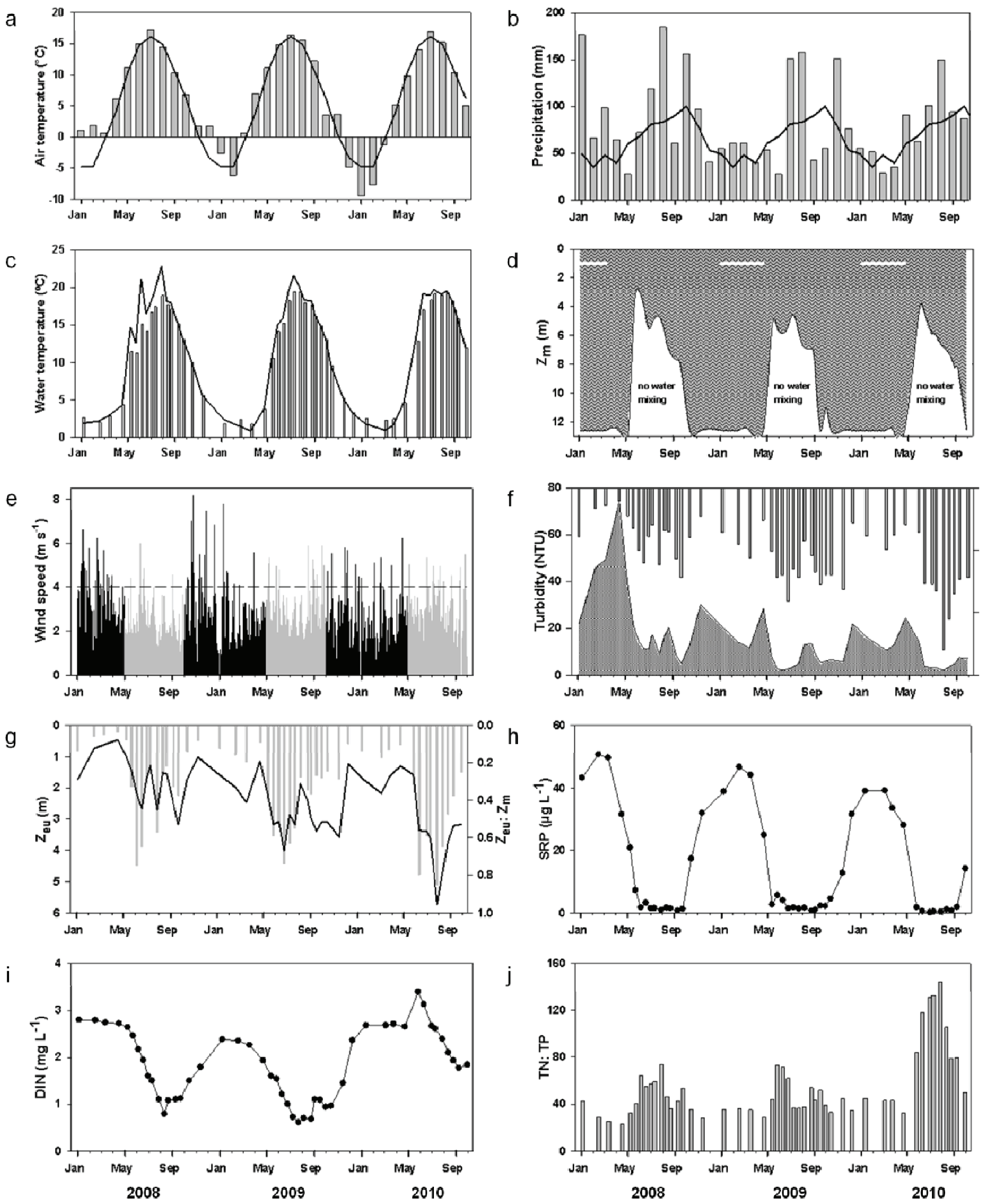
a
Air temperature (°C)
20
15
10
5
0
-5
-10
b
Precipitation (mm)
200
150
100
50
0
c
Water temperature (ºC)
25
20
15
10
5
0
d
Z_m (m)
0
2
4
6
8
10
12
no water mixing
no water mixing
no water mixing
e
Wind speed (m s^{-1})
8
6
4
2
0
f
Turbidity (NTU)
80
60
40
20
0
SD (m)
0
1
2
3
g
Z_{eu} (m)
0
1
2
3
4
5
6
Z_{eu} : Z_m
0.0
0.2
0.4
0.6
0.8
1.0
h
SRP (µg L^{-1})
60
40
20
0
i
DIN (mg L^{-1})
4
3
2
1
0
j
TN: TP
160
120
80
40
0
Jan
May
Sep
Jan
May
Sep
Jan
May
Sep
2008
2009
2010

Fig. 2.

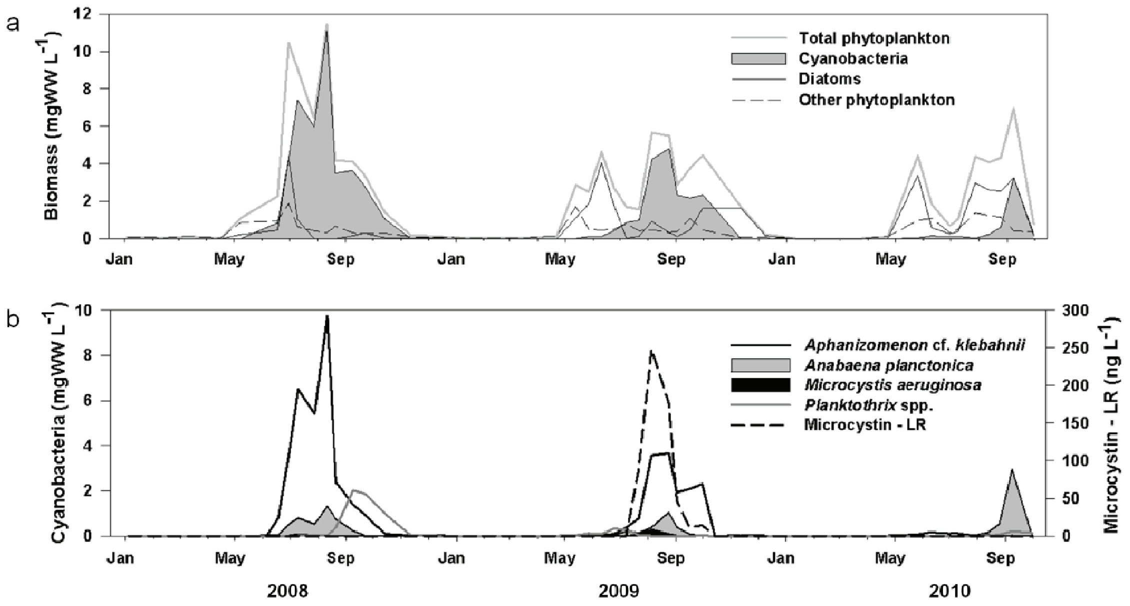
a
Biomass (mgWW L-1)
12
10
8
6
4
2
0
Total phytoplankton
Cyanobacteria
Diatoms
Other phytoplankton
Jan
May
Sep
Jan
May
Sep
Jan
May
Sep
b
Cyanobacteria (mgWW L-1)
10
8
6
4
2
0
Microcystin - LR (ng L-1)
300
250
200
150
100
50
0
Aphanizomenon cf. klebahnii
Anabaena planctonica
Microcystis aeruginosa
Planktothrix spp.
Microcystin - LR
Jan
May
Sep
Jan
May
Sep
Jan
May
Sep
2008
2009
2010

Fig. 3

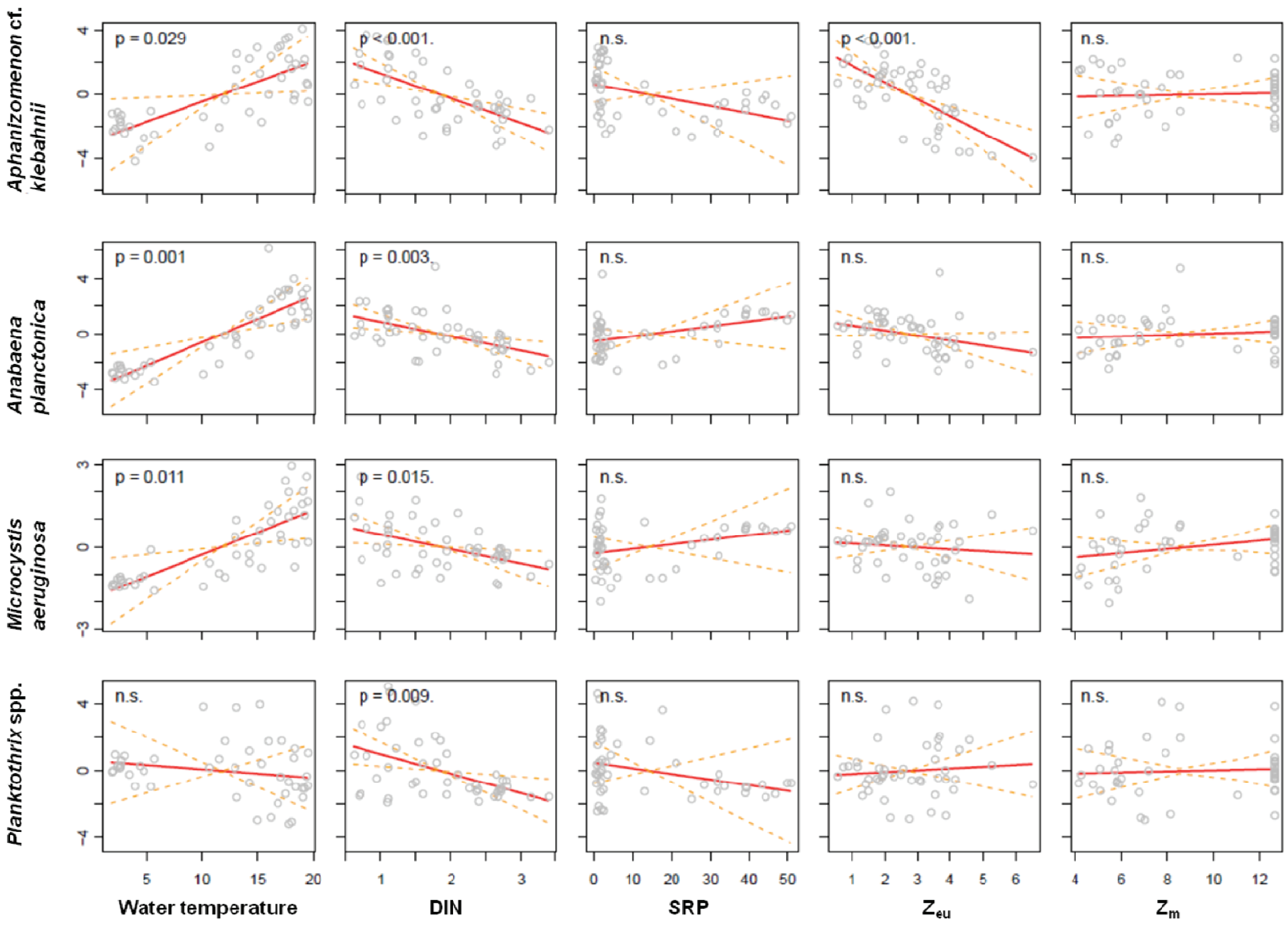
Aphanizomenon cf. klebahnii
Anabaena planctonica
Microcystis aeruginosa
Planktothrix spp.
p = 0.029
p < 0.001.
n.s.
p < 0.001.
n.s.
p = 0.001
p = 0.003
n.s.
n.s.
n.s.
p = 0.011
p = 0.015.
n.s.
n.s.
n.s.
n.s.
p = 0.009.
n.s.
n.s.
n.s.
4
0
-4
3
-3
5
10
15
20
1
2
3
0
10
20
30
40
50
1
2
3
4
5
6
4
6
8
10
12
Water temperature
DIN
SRP
Z_{eu}
Z_m

Fig. 4.

PAPER II

Romarheim AT, Riise G, Brettum P, Færøvig PJ and Andersen T. **The importance of light and macronutrients for phytoplankton growth in a northern temperate lake – an *in situ* bioassay study.** *Submitted to J Plankton Res.*

The importance of light and macronutrients for phytoplankton growth in a northern temperate lake – an *in situ* bioassay study

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KEYWORDS: Phytoplankton growth; Bioassay; Light; Nutrients; Lake Årungen

ABSTRACT

Large interannual variation in the phytoplankton biomass is observed in many lakes despite similar nutrient conditions. We therefore studied the effect of nutrients, zooplankton grazing and light on phytoplankton growth in the Norwegian eutrophic Lake Årungen. Nine *in situ* experiments were carried out with the natural phytoplankton community from the lake during the growing season from May to October 2009. Each experiment included a combination of three nutrient treatments (control, +P, +P+N), two pre-filtering treatments (control, < 90 µm) and five vertical light gradients. Each treatment was done in triplicate, giving a total of 810 samples. Net specific phytoplankton growth rate estimated from the changes in chlorophyll over a three days incubation period was used as response parameter. The effect of nutrient addition was highest in the first period of the growing season, and phosphorus was the main limiting nutrient for phytoplankton growth. Zooplankton was of minor importance for the phytoplankton biomass, probably due to high biomass of poorly edible phytoplankton or low abundance of mesozooplankton grazers. High cyanobacteria biomass at the water surface shaded out the phytoplankton in deeper water layers and increased the relative importance of light for phytoplankton growth. Light explained from 40 to 90% of the phytoplankton growth in the experiments, and was the overall most important factor for phytoplankton production in the eutrophic and turbid Lake Årungen.

INTRODUCTION

Phosphorus has been considered as the primary limiting nutrient for phytoplankton growth in freshwater ecosystems since R.A. Vollenweider found a link between the phosphorus supply and eutrophication in the 1960s (Schindler, 2006). The importance of phosphorus for growth of freshwater phytoplankton has been demonstrated in whole-lake experiments (Schindler *et al*., 1971; Schindler and Fee, 1974), and nutrient limitation studies at different spatial and temporal scales (Hecky and Kilham, 1988; Kim *et al*., 2007). The classical theory of phosphorus as the major limitation factor for freshwater phytoplankton growth has recently been challenged by studies suggesting that nitrogen is of similar or greater importance than phosphorus (Elser *et al*., 2007; Lewis and Wurtsbaugh, 2008; Sterner, 2008). However, Schindler *et al*. (2008) argued that reduced nitrogen supply alone cannot control freshwater eutrophication since low input of nitrogen may favour development of nitrogen-fixing cyanobacteria.

The importance of lake physical environment for phytoplankton dynamics was indicated by Reynolds (1984) and Reynolds and Reynolds (1985), and has lately regained attention as large year-to-year variation has been observed in lakes despite similar nutrient level between years (Padisak *et al*., 2010; Zohary *et al*., 2010). Light conditions in a lake vary due to differences in surface radiation, light attenuation, and changes in water mixing depth and regime (Diehl, 2002; Kunz and Diehl, 2003; Loiselle *et al*., 2007). In highly turbid waters, non-algal light attenuation might significantly influence the light regime and phytoplankton uptake of available nutrients (Dokulil, 1994).

Zooplankton grazing has also an important role in regulation of the phytoplankton biomass, and manipulation of the lake trophic structure can be used as a measure to remove excess algae (Hansson *et al*., 1998). Furthermore, both phytoplankton biomass and community structure show great seasonal variations as a response to variations in weather conditions, external inputs of nutrients, and food web dynamics. Experiments with controlled treatments, e.g. nutrients level, light, and temperature, are therefore necessary to untangle the complex array of factors that limit or promote phytoplankton growth.

The present study was conducted in Lake Årungen, a low latitude lake in an agricultural district of south-eastern Norway. Phosphorus is considered to be the determinant factor for phytoplankton growth in the lake as high phosphorus input from the catchment has been followed by high in-lake phosphorus concentration and intensive phytoplankton production (Ensby *et al.*, 1984). Priority has therefore been given to countermeasures with emphases to reduce the phosphorus loading. Large cannibalistic pikes (*Esox lucius*) were also removed from the lake during several periods (1978–1980, 1991–92 and 2001–2006) (Sharma, 2007) with the intention to increase the zooplankton abundance and grazing pressure on phytoplankton. Nevertheless, large interannual variability in phytoplankton biomass and composition has been observed in the lake regardless of the phosphorus concentration, and factors important in regulating the lake biological response have been questioned. The lake is exposed to soil erosion from surrounding agriculture land and experience periods with high turbidity, especially during heavy rainfall and snow melting (Borch *et al.*, 2007). Furthermore, the lake is north-south orientated and exposed to the dominant wind direction which promotes effective mixing of the water masses. The light conditions in the lake therefore seem to be highly dynamic and sensitive to meteorological changes.

The main goal of the present study was to determine the most important factor for controlling the phytoplankton growth in the northern temperate Lake Årungen by an *in situ* bioassay. The lakes natural phytoplankton community was used to measure the effect of light, grazing by zooplankton and macronutrients on phytoplankton growth. The lake physical, chemical and biological conditions were used to interpret the experimentally obtained results since the lake was the ambient environment for the experimental units. We also investigated if the limiting factors for phytoplankton growth changed seasonally.

METHODS

Study site

Lake Årungen is located south-east in Norway (59˚41' N, 10˚45' E) approximately 30 km south of Oslo, and it is an important sports, recreation, and fishing area. The lake covers 1.2 km^2, is 3 km long and has an average depth of 8 m and a maximum depth of 13 m. It is a dimictic lake with thermal stratification during the summer and two periods of water turnover, one in the spring and one in the autumn. Agricultural land covers more than 50% of the 51 km^2 catchment area. Treatment and diversion of waste water took place during the 1970s and 1980s, whereas measures have been taken to reduce phosphorus input from agriculture through improved practices and construction of artificial wetlands since the 1990s (Borch *et al*., 2007).

Physical, chemical and biological lake conditions

Water temperature was logged hourly at eight depths from 0.7 to 12.6 m by Hobo pendant temperature loggers (Onset Computer Corporation, MA, USA). Water transparency was measured as Secchi depth (SD) with a 13×18 cm white Secchi disk. Daily surface photosynthetically active radiation (PAR) irradiance and wind speed were measured on the meteorological station at the Norwegian University of Life Sciences (59˚39' N; 10˚46' E) which is located nearby the study site (Thue-Hansen and Grimenes, 2010).

Water used for chemical and biological analyses was collected in the epilimnion (0.5, 1.0, 3.0 and 5.0 m) at the deepest location in the lake with a modified Rüttner water sampler. The water samples were collected in the period from May to October 2009 (n = 9), and up to six days before or after the sampling of water for the *in situ* bioassays. Dissolved inorganic nutrients were analysed after filtration through 0.45 µm pore-size membrane filters. Soluble reactive phosphorus (SRP) was determined spectrophotometrically (UV-VIS Spectrophotometer UV-1201, Shimadzu, Kyoto, Japan) after a reaction with ammoniummolybdat according to Norwegian standard NS-EN 1189 (detection limit: 0.03 µM). Ammonium (NH_4-N) was determined spectrophotometrically according to NS-EN 4746 and nitrate (NO_3-N) by ion chromatography (Zellweger Analytics Lachat IC5000 ion chromatograph, Milwaukee, WI, USA) according to NS-EN ISO 10304-1 (detection limit: 0.4 µM). Reactive Si was determined by the molybdate method (detection limit: 1.4 µM) described by Mullin and Riley (1955). Phytoplankton biomass and composition were analysed in water samples preserved with Lugol's solution. The sedimentation method described by Utermöhl (1958) was applied to 10 mL of each water sample prior to counting by an inverted phase contrast microscope (Leitz Fluovert FS*,* Leica, Wetzlar, Germany). Phytoplankton biomasses were calculated according to Rott (1981). Chlorophyll *a* (Chl *a*) was analysed according to NS-EN 4767. Turbidity was determined according to NS-ISO 7027 by a laboratory turbidimeter (2100AN IS, Hach Company, Loveland, CO, US).

Bioassay experiment

Nine *in situ* bioassay experiments were carried out during the growing season from May to October 2009 with the natural phytoplankton communities from Lake Årungen to study factors controlling phytoplankton growth. Equal amounts of water were collected from 0.7, 1.4, 2.1, 2.8 and 3.5 m depth prior to each experiment and mixed in a 20 L canister to obtain a composite sample. The depths for water collection were based on the highest SD measurements from 2008 (1.4 m; unpublished data) multiplied by 0.5, 1.0, 1.5, 2.0 and 2.5 to obtain five vertical light gradients. The experimental units were 75 mL tissue culture bottles (Nunc, Roskilde, Denmark). The bottles were filled with unfiltered water, or water pre-filtered through 90 µm nylon net to remove metazoan grazers. Thereafter the bottles were added no nutrients (control), 3.2 µM P (+P) as $Na_2HPO_4 \times 2H_2O$, or the combination of 3.2 µM P and 50 µM N (+P+N) added as NH_4NO_3 and NH_4Cl. The final N to P ratio of approximately 7:1 by weight is equivalent to the Redfield (1958) ratio of 16:1 by atoms. The experimental units were placed in the lake within three hours after preparation, and then incubated at 0.7, 1.4, 2.1, 2.8 and 3.5 m for three days. Bottles incubated at the same depth were oriented randomly within a coarse-meshed nylon netting bag to avoid systematically biased treatment responses. Each treatment was done in triplicate, giving 90 observations per experiment and a total of 810 observations for the whole study. In addition, the composite initial samples from each experiment were analysed in triplicate. The samples were kept in dark from the end of the incubation and until analysis. Each water sample was analyzed for Chl only, and the difference between the initial and final Chl concentrations was used to estimate the phytoplankton growth. 25 mL from each water sample was filtered through a glass fibre filter that was stored frozen until analysis. Thawed filters were extracted in 2.5 mL ethanol and analysed for Chl by measuring the fluorescence at 665 nm with 430 nm excitation using a microplate reader (Synergy Mx, BioTek, Winooski, VT, USA). Calibration was performed against a commercial Chl standard (Sigma C5753).

Calculations and statistical methods

The mixed-layer depth (z_m; m) was estimated for each day as the depth of maximum vertical density gradient. The density gradient was estimated by analytical differentiation of a 4-parameter logistic function fitted to the vertical density profile. Water density was calculated from the logged temperature series according to Millero and Poisson (1981). Mixed-layer temperature was calculated by trapeze integration of daily temperature averages down to the mixing depth. Mixed-layer averaged PAR was calculated using the formula:

$$I_0\,[1 - \exp(-\varepsilon\, z_m)]\,(\varepsilon\, z_m)^{-1}$$

where I_0 is the daily surface PAR irradiance (mol m^{-2} d^{-1}) and ε is the light attenuation coefficient (m^{-1}). ε was estimated from Chl *a* as

$$\varepsilon^{\circ} + K_c\,(\mathrm{Chl}\ a)$$

where $\varepsilon^{\circ} = 0.95$ m^{-1} is the non-algal attenuation (Andersen, 1982) and $K_c = 0.016$ m^2 (mg Chl *a*)$^{-1}$ is the optical Chl specific cross section (Kirk, 1994). Independent attenuation coefficient estimates, calculated as 1.7 SD^{-1} (Scheffer, 1998), gave results comparable to ε ($R^2 = 0.82$). Total dissolved inorganic N (DIN) was calculated as NO_3-N + NH_4-N. Specific phytoplankton growth rates (μ; d^{-1}) were estimated as

$$\log(\mathrm{Chl}_t / \mathrm{Chl}_0) / t$$

where Chl_0 and Chl_t are the initial and final Chl concentrations, respectively, and t is the duration of the experiment in days. The euphotic zone depth (z_{eu}; m) was estimated as the x-axis intercept in a linear regression for specific growth rate in the treatment with full N and P addition as function of depth.

Reduced Major Axis Regression (Bohonak, 2004) revealed that the Chl concentrations of unfiltered and 90 μm pre-filtered water at the end of experiments were highly correlated ($r = 0.97$, $P < 0.001$) and with a slope not significantly different from 1. It was therefore decided to pool data from unfiltered and pre-filtered water in the further data analyses. Light decreases non-linearly, but monotonously, with depth and the analysis will therefore be the same whether light or depth is used as treatment variable. The data from each experiment was analysed by linear models to determine the effect of nutrient addition, light, and their interaction on phytoplankton specific growth rate. Diagnostic plots of model residuals, residual quantiles, and leverages were used to check for violations to the standard assumptions of linear models. Statistical analysis for specific growth in surface water was carried out using the general linear model procedure in SAS from SAS Institute Inc. Significant ($P < 0.05$) differences among means were ranked by Bonferroni multiple range test. The results are presented as means ± standard deviation.

RESULTS

Physical, chemical and biological lake conditions

Physical, chemical and biological conditions in the lake during the phytoplankton growing season are presented in Fig. 1. The thermocline was formed between 4 and 6 m depth in May, and remained until mid August when the autumn thermocline erosion started (Fig. 1A). The temperature of the mixed-layer increased from 11°C in May to approximately 20°C in the start of July, before it declined steadily to below 10°C in mid October (Fig. 1B). Mixed-layer average PAR decreased rapidly from approximately 15 mol m^{-2} d^{-1} in late June to below 9 in July, and then further to < 2 from October (Fig. 1C). Epilimnion SRP went from 0.19 µM in the end of May to below 0.05 µM from the end of June to the start of September (Fig. 1D). The mean DIN declined from 110 µM at the end of May to a steady level below 50 µM during July and August, and then increased to about 80 µM in September (Fig. 1E). The mean NH_4 ranged between 0.4 and 10.7 µM throughout the studied period. Epilimnion reactive Si decreased from 60 µM in late May to below 18 µM in June and July, and then increased again up to 80 µM in mid September (Fig. 1F). Total phytoplankton biomass had two peaks, one in early June and one higher during August (Fig. 1G). Diatoms were the most abundant phytoplankton during the first peak, whereas cyanobacteria were the most abundant phytoplankton during the biomass peak in August (Fig. 1H and I). *Planktothrix* cf. *agardhii* was the most abundant cyanobacteria until mid July, whereas the N_2-fixing cyanobacteria *Aphanizomenon* cf. *klebahni* and *Anabaena planctonica* were most abundant from July throughout August (data not presented). Chl *a* in the lake varied between 17 and 80 µg L^{-1} and followed a pattern similar to that of phytoplankton biomass, although the peak in August was twice as high compared to that in June (Fig. 1J). Turbidity was below 5 FNU until mid-July, rose to a maximum value of about 13 FNU in August, and returned to about 5 FNU in September (Fig. 1K). The lowest SD was measured in August (< 1 m) while it varied between 1.3 and 1.8 m during the rest of the growing season (Fig. 1L). The wind speed went up to approximately 5 m s^{-1} in

May and 6 m s^{-1} from mid August, whereas it did not exceed 4 m s^{-1} in the period from June to mid August (data not shown).

Bioassay experiment

The Chl concentrations in the control bottles at the end of the incubation were generally lower than the Chl *a* concentrations in the lake, but followed a similar seasonal pattern (Fig. 1J). The effect of nutrient addition and light in the nine bioassay experiments are given in Table I and Figs. 2 and 3. Eight samples (~1% of the total observations) were considered to be outliers and thus removed from the data set, and the specific growth rates in bottles with no nutrients added in the experiment from 30th May were inexplicably high and therefore not presented in Fig. 3. The models explained between 54 and 94% of the total variation in specific growth rate (Fig. 2). Light had the highest R^2 values and explained more than 50% of the observed phytoplankton growth in the period from 13th July to 21st September. The effect of nutrient addition was significant, except at the beginning (30th May) and end (5th October) of the growing season. Nutrient addition explained from 14 to 32% of the total variation in the period from 16th June to 13th July, but less than 9% during the other experiments (Fig. 2). A significant interaction between light and nutrient treatment ($P < 0.001$) was found in all experiments from 30th May to 13th July, and at 21st September ($P = 0.007$) (Table I). The R^2 values in the first four experiments ranged from 0.16 to 0.24 (Fig. 2). Net specific growth rate in water without nutrient supplement responded less to increased light (shallower depth) than when supplied with P or PN (Fig. 3).

The highest phytoplankton growth was found in the beginning of the growing season, then declined towards August, but increased slightly at the end of the growing season (Figs. 3 and 4). Phytoplankton growth at 0.7 m was used to determine the limiting nutrient since light was expected to be most available at this depth (Fig. 4). Significant higher phytoplankton growth rates were found when P was added in the end of May to mid August, except at16th June. The addition of N in combination with P had significant effect on phytoplankton growth only at16th June and 10th August when compared to P addition alone.

The z_{eu}, calculated from growth rates in bottles with +P+N treatment, decreased from 3.6 m in May to 2.8 m in August, and then increased to above 3 m in September.

The average ratio between z_{eu} and SD was 2.3 ± 0.2. z_m was deeper than the z_{eu} during the whole growing season, and the z_{eu}:z_m ratio ranged from 0.3 to 0.7.

DISCUSSION

The importance of factors controlling phytoplankton growth in the eutrophic Lake Årungen varied during the season, and the transition between them coincided with seasonal changes in surface radiation, turbidity, and water mixing depth and regime, as also shown in other lakes (Millard *et al.*, 1996). Light was the overall most important factor for phytoplankton growth in the Lake Årungen, and low water transparency caused by e.g. cyanobacteria probably masked the effect of nutrient addition. The effect of light was most pronounced in the period from mid July to mid September which coincided with high algal biomass and increased water turbidity. A mixed-layer depth below the euphotic zone depth during the whole study indicated poor light conditions in the lake, although the z_{eu}:z_m ratio never went below 0.3 which has been suggested as a threshold ratio for phytoplankton light limitation (Robertson, 1999). Changes in water mixing and thermal regime coincided with the time of changed light and nutrient conditions in the lake, and consequently with changes in phytoplankton growth and community structure. Phytoplankton response to nutrient addition was highest in the period from June to mid July, which probably was a result of stable stratification and nutrient depletion by the diatom bloom in the spring, as well as surplus nutrient uptake by cyanobacteria. The effect of nutrient addition decreased with reduced light conditions caused by the seasonal reduction in light radiation and increased cyanobacteria biomass.

Primary production of phytoplankton in freshwater ecosystems has traditionally been explained by a phosphorus surplus (Schindler, 2006). Phosphorus was also identified as the main limiting nutrient in our bioassay study as addition of both N and P gave only minor or no increased phytoplankton growth compared to P addition alone. Low SRP concentrations in the lake from late June to early September (below 0.05 µM; Fig. 1D) also indicated P limitation since SRP concentrations below 0.1 µM is found to limit phytoplankton growth (Reynolds, 1992). The high DIN concentrations above 40 µM in the lake during the whole growing season did not indicate nitrogen limitation although significantly higher growth rates were found in early June and August when N was added in addition to P. Phytoplankton differs in the competitive ability for different forms of inorganic N (Hecky and Kilham, 1988; Elser *et al*., 1990). Addition of NH_4 to a level more than two times greater than NO_3 (5:2 ratio) could therefore increase the growth of phytoplankton that prefer N in the form of NH_4 in the present study. Low phytoplankton response to nutrients addition from August to the end of the growing season suggests that other factors were more effective in controlling the phytoplankton growth in this period.

The lack of response to the 90 µm pre-filtering treatment indicated that grazing by mesozooplankton > 90 µm is of minor importance for regulation of the phytoplankton biomass in Lake Årungen. This could partly be due to low edibility of the dominant phytoplankton groups and partly to low abundance of efficient grazers. The phytoplankton of Lake Årungen is often dominated by colonial diatoms and cyanobacteria, which are known to have a predation refuge from most metazoan grazers due to their size and shape (Gliwicz and Lampert, 1990; DeMott *et al*., 2001). The dense populations of planktivorous fish, especially roach (*Rutilus rutilus*), has also been suggested to be the reason for the low biomass of large cladocera and other efficient phytoplankton grazers in Lake Årungen (Brabrand, 2001).

Sufficient nutrients, relatively high water transparency (SD > 1 m) and low effect of zooplankton grazing during the spring allowed development of diatoms in the period from May to June. The shift from diatoms to cyanobacteria in July could possibly be caused by nutrients depletion and/or diatom sedimentation caused by thermocline formation (Winder and Hunter, 2008). Therefore, during this period when nutrients were depleted and the lake stratified with no nutrient supply from the hypolimnion, experimental phytoplankton responded to nutrient addition with increased growth. The calm weather in July could have contribute to the high cyanobacteria biomass since wind speed below 4 m s^{-1} allow for accumulation of cyanobacteria on the lake surface (Serra *et al*., 2007; Moreno-Ostos *et al*., 2009). Buoyant cyanobacteria of genera *Aphanizomenon* and *Anabaena* are capable of vertical migration toward the surface when light availability is reduced (Walsby *et al*., 1997; Mur *et al*., 1999). Their high biomasses in the surface water in the period from July to August would likely reduce the light conditions for phytoplankton in deeper water layers, and may thus explain the low difference in phytoplankton growth obtained at greater depths in August regardless of nutrient treatment. It is difficult to completely untangle the effects of light and nutrients on phytoplankton dynamics, since light limitation is to a large extent caused by self-shading, and sufficient nutrients are needed to build up a biomass for this to take a place. The early thermocline erosion could result from increased wind speed (up to 6 m s^{-1} in mid August), as well as small water vertical temperature differences. Increased mixing has prevented cyanobacteria accumulation on the lake surface in other lakes with similar physical conditions (Moreno-Ostos *et al*., 2009), which further could promote higher light penetration in the epilimnion. Pronounced water mixing and surface cyanobacteria biomass disintegration were observed in Lake Årungen, and were followed by increased phytoplankton growth in late September, even at higher depths. In addition, the phytoplankton community shifted towards species such as diatoms that are better adapted to increased water mixing and altering light availability (Reynolds *et al*., 1983; Dokulil, 1994; Huisman *et al*., 2004). Increased availability of soluble P and Si transported from deeper water by turbulent mixing likely contributed to increased phytoplankton growth at the end of the growing season, even in the control water without added nutrients. Water temperature, however, became an important limiting factor at the end of the season, particularly for growth of cyanobacteria which prefer warm waters (Paerl and Huisman, 2008).

Our results suggest that light was the overall most important factor for the phytoplankton growth in the nutrient rich and highly turbid lake. It was also found that addition of phosphorus increased phytoplankton growth when light was sufficient, whereas additional nitrogen had minor effect. Weather conditions may alter the water mixing and light regime, and thereby affect the phytoplankton growth and community structure. The effect of weather should therefore be considered in management of lakes with variable environmental conditions, such as lakes exposed to wind and soil erosion.

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TABLE AND FIGURE LEGENDS

Table I: Degrees of freedom and *P*-values for the effect of light, nutrient addition, and their interaction on phytoplankton growth by experiments.

Fig. 1. Physical, chemical and biological conditions in the epilimnion of Lake Årungen in the period from May to October 2009: **A)** Mixed-layer depth (z_m; m), **B)** Mixed-layer temperature (°C), **C)** Mixed-layer average photosynthetically active radiation (PAR; mol quanta m^{-2} d^{-1}), **D)** Soluble reactive phosphorus (SRP: µM; average 0-5 m), **E)** Dissolved inorganic nitrogen (DIN: µM; average 0-5 m, dashed line is the NH_4-N fraction of DIN), **F)** Soluble reactive Si (µM; average 0-5 m), **G)** Total phytoplankton biomass (mg wet weight L^{-1}; average 0-5 m, dashed line is the phytoplankton other than diatoms and cyanobacteria), **H)** Diatoms biomass (mg wet weight L^{-1}; average 0-5 m), **I)** Cyanobacteria biomass (mg wet weight L^{-1}; average 0-5 m), **J)** Chlorophyll *a* (Chl *a*; µg L^{-1}; average 0-5 m, dashed line is the Chl response in the bioassay control), **K)** Water turbidity (FNU), **L)** Secchi depth (m).

Fig. 2. Effect sizes (partial R^2-values), by experiment, in linear models with specific growth rate as dependent variable and light, nutrient addition, and their interaction as independent variables.

Fig. 3. Trellis plot of net specific growth rate (d^{-1}) in unfiltered and 90 µm pre-filtered water (n = 6) as a function of depth, grouped by experiments and nutrient addition treatment. Trend lines are fitted as locally weighted regressions (LOESS; Cleveland, 1979).

Fig. 4. Net specific growth rate (d^{-1}) over 3 days incubation at surface water (0.7 m) with no nutrient addition (light gray line), addition of P (dark gray line), or addition of both P and N (black line), split by nutrient treatment. Significant differences among treatments at a given time are denoted by different letters, $P < 0.05$.

Table I

	df	30. May	16. Jun	29. Jun	13. Jul	10. Aug	25. Aug	7. Sep	21. Sep	5. Oct
		P-value								
Light	4	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Nutrients	2	0.73	< 0.001	< 0.001	< 0.001	< 0.001	0.016	< 0.001	0.041	0.76
Light × Nutrients	8	< 0.001	< 0.001	< 0.001	< 0.001	0.081	0.53	0.30	0.007	0.37

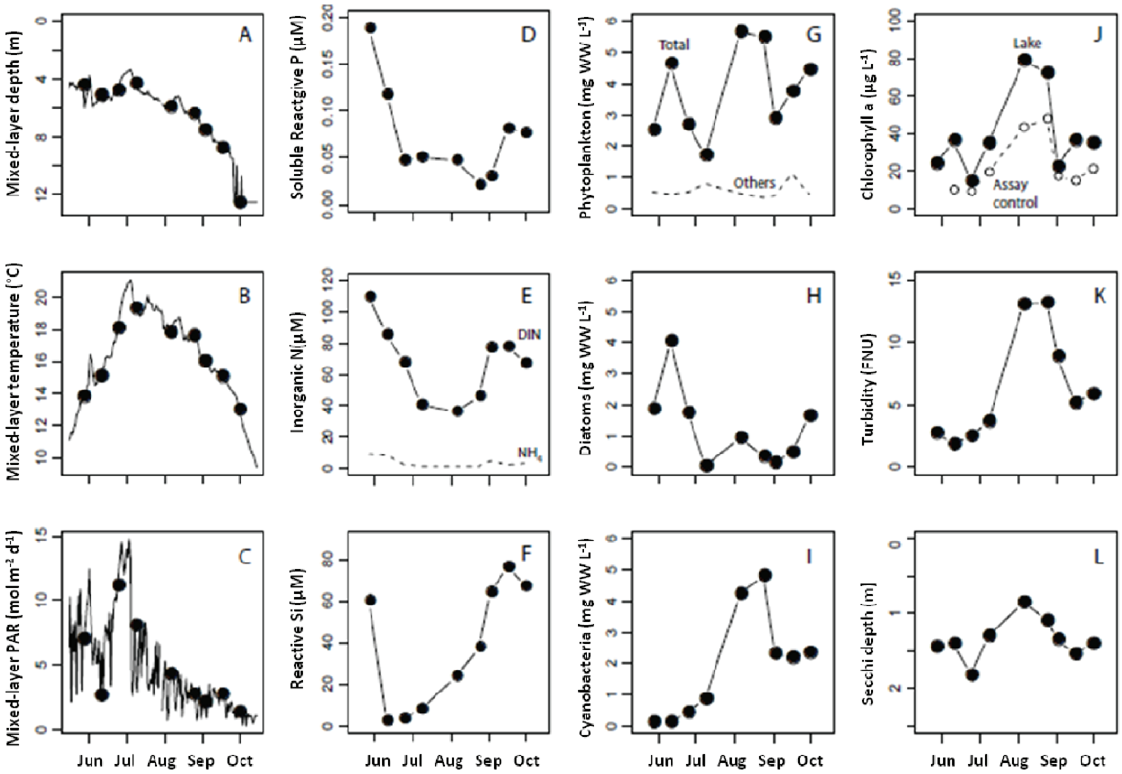
A
Mixed-layer depth (m)
0
4
8
12
B
Mixed-layer temperature (°C)
10
12
14
16
18
20
C
Mixed-layer PAR (mol m⁻² d⁻¹)
0
5
10
15
Jun Jul Aug Sep Oct
D
Soluble Reactgive P (µM)
0.00
0.05
0.10
0.15
0.20
E
Inorganic N (µM)
0
20
40
60
80
100
120
DIN
NH4
F
Reactive Si (µM)
0
20
40
60
80
Jun Jul Aug Sep Oct
G
Phytoplankton (mg WW L⁻¹)
0
1
2
3
4
5
6
Total
Others
H
Diatoms (mg WW L⁻¹)
0
1
2
3
4
5
6
I
Cyanobacteria (mg WW L⁻¹)
0
1
2
3
4
5
6
Jun Jul Aug Sep Oct
J
Chlorophyll a (µg L⁻¹)
0
20
40
60
80
100
Lake
Assay control
K
Turbidity (FNU)
0
5
10
15
L
Secchi depth (m)
0
1
2
Jun Jul Aug Sep Oct

Fig. 1

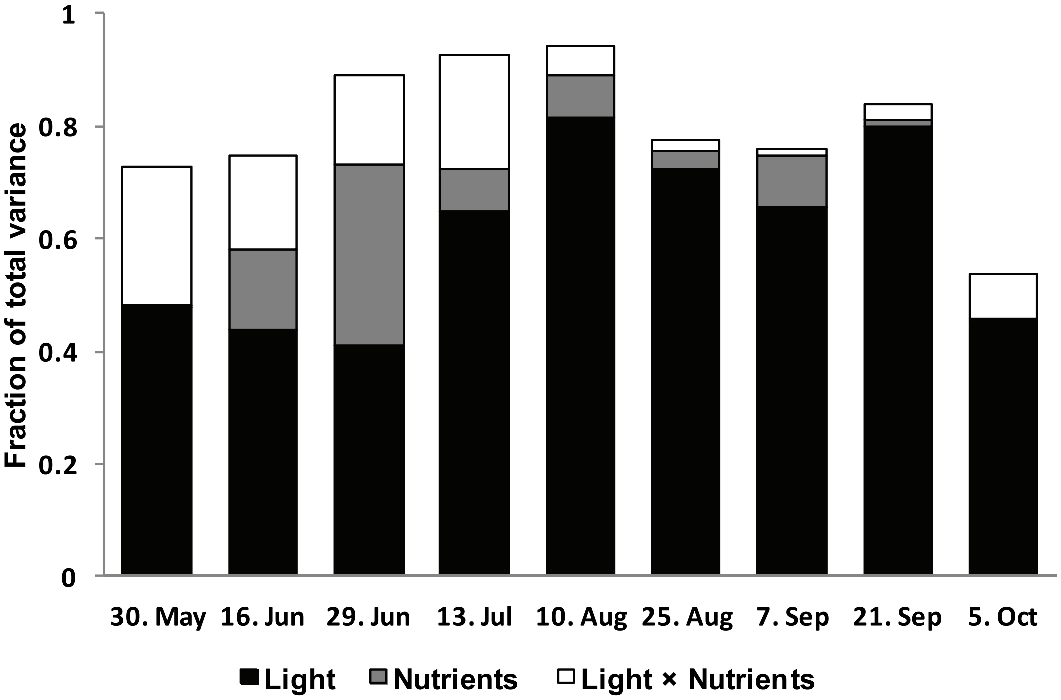
Fraction of total variance
1
0.8
0.6
0.4
0.2
0
30. May
16. Jun
29. Jun
13. Jul
10. Aug
25. Aug
7. Sep
21. Sep
5. Oct
Light
Nutrients
Light × Nutrients

Fig. 2

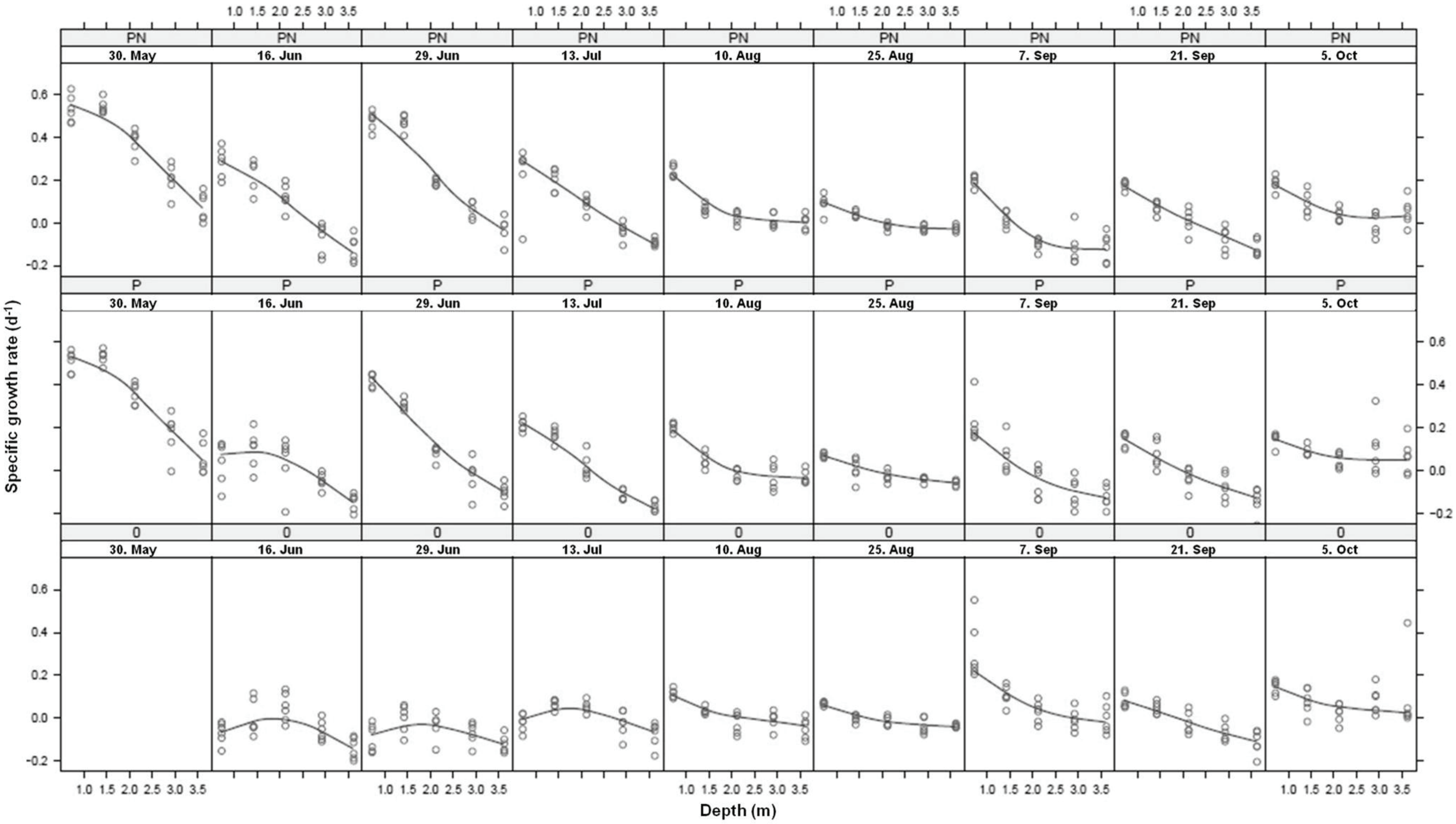
Specific growth rate (d-1)
Depth (m)
PN
P
0
30. May
16. Jun
29. Jun
13. Jul
10. Aug
25. Aug
7. Sep
21. Sep
5. Oct
0.6
0.4
0.2
0.0
-0.2
1.0 1.5 2.0 2.5 3.0 3.5

Fig. 3

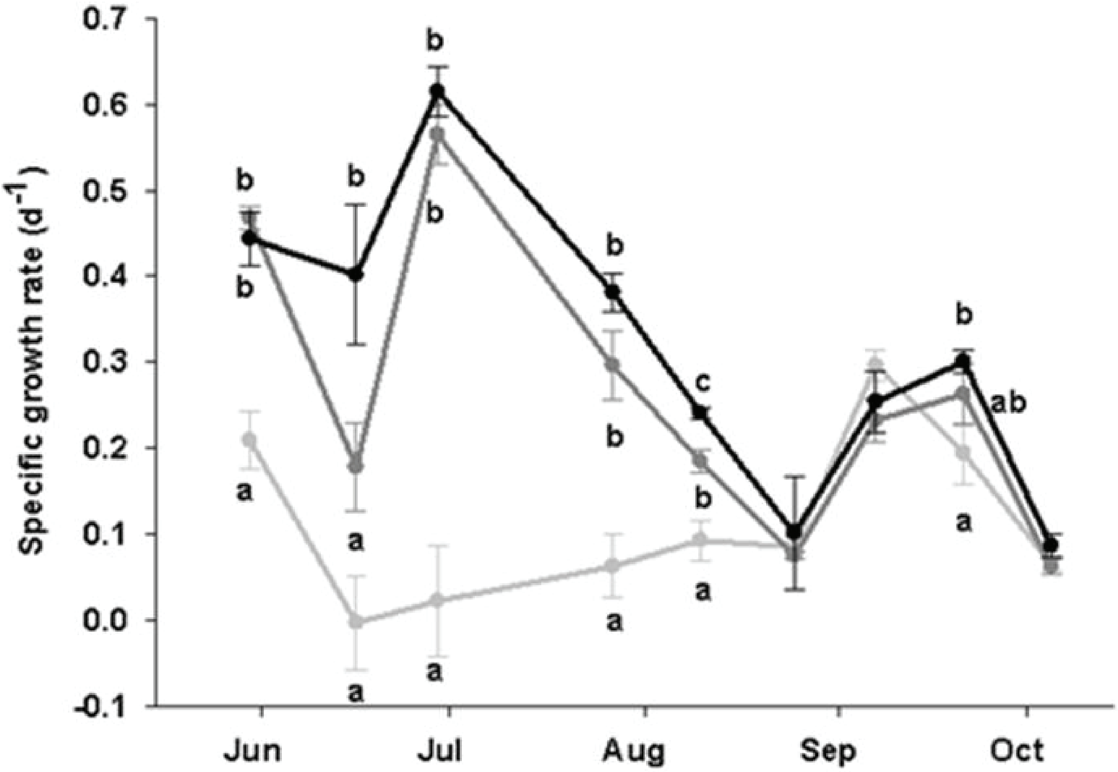
Specific growth rate (d^{-1})
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
Jun
Jul
Aug
Sep
Oct
b
b
a
b
a
a
b
b
a
b
b
a
c
b
a
b
ab
a

Fig. 4.

PAPER III

Romarheim AT, Tominaga K, Riise G and Andersen T. **Modelling the responses of a cold temperate lake to changes in external forcing.** *Submitted to Hydrobiologia.*

Modelling the responses of a cold temperate lake to changes in external forcing

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Keywords MyLake model · Cold temperate lake · Eutrophication · Weather · Loading · Lake Årungen

Abstract

The simultaneous action of multiple environmental pressures limits our understanding of the impact of individual factors on the lake processes. In this model experiment, the MyLake model was used to disentangle the importance of year-to-year variations of meteorological forcing and nutrient loading on the eutrophic and particle loaded Lake Årungen, Norway. Daily meteorological and runoff input data were used to reconstruct the lake response for the period 1994 to 2010, and to simulate the lake responses under different weather and loading scenarios for the same period. Both weather and loading exhibited year-to-year variation, and therefore affected physical, chemical and biological lake responses. Air temperature and precipitation were most variable during the winter. Variation in runoff volume was most prevalent during autumn and winter, while variation in phosphorus inflow was most extensive from late winter to early spring. Thermal related properties in the lake were highly determined by weather conditions, whereas loading was the most important factor for phytoplankton biomass and water transparency. Mild winters and increased input of suspended matter and phosphorus were followed by increased phytoplankton biomass and light attenuation. Thus, in lowland lakes surrounded by erosive soil, mild winters may promote summer blooms with low water transparency. Our study shows that future changes in the global climate may have important implications for local water management decision-making.

Introduction

Nutrient enrichment of lakes may lead to high phytoplankton mass development, low water transparency, and fish mortality due to oxygen depletion (Smith et al., 1999). Mainly two factors affect the nutrient loading to lakes: 1) the soil and land use in the lake catchment, and 2) the hydrology of the watershed. Phosphorus is generally regarded as the determining nutrient for phytoplankton production in freshwater lakes (Schindler, 1977). Much effort has therefore been given to reduce phosphorus input to aquatic ecosystems, which has demonstrably lead to reduced phytoplankton production and increased water transparency in many lakes in Europe and North America (Jeppesen et al., 2005). On the other hand, many lakes have showed delayed or negligible improvements in water quality despite reduced nutrients loading (Jeppesen et al., 2007a).

Year-to-year weather variation has also been recognised to affect physical, chemical and biological processes in lakes (Bailey-Watts & Kirika, 1999; Blenckner et al., 2007; Jeppesen et al., 2007b, 2009; Whitehead et al., 2009). Increased air temperature has been shown to increase the water temperature (George et al., 2007) and the stability of thermal stratification (Straile et al., 2003a), change the phytoplankton community towards dominance of species adapted to warmer water (Weyhenmeyer et al., 2002), and may lead to earlier and higher phytoplankton production (Weyhenmeyer et al., 2002; Huber et al., 2008). The changes in thermal conditions and mixing regime can in turn influence the light, oxygen and nutrient dynamics in the lake, and thereby impact the phytoplankton primary production and community structure (Tirok & Gaedke, 2007; Wilhelm & Adrian, 2008). Precipitation is also deemed as an important factor determining the water transparency, runoff intensity, and suspended matter discharge (Arheimer et al., 2005; Nõges et al., 2007; Ulén et al., 2007).

Norway has, generally, a low fraction of arable land (3%) and low population density (12 persons km^{-2}), so eutrophication is mainly recognizable in intensive agricultural districts at low altitudes. Lake Årungen is situated in a developed agricultural area south-east in Norway, and is one of the most nutrient rich lakes in the country. Geological studies suggest that the natural phosphorus concentration of lakes in this area is 7-8 mg m^{-3} (Borch et al., 2007). Eutrophication became a problem in the lake during the 1960s, with phosphorus concentration exceeding 400 mg m^{-3} in the 1980s (Løvstad & Krogstad, 1993). Algal blooms, low water transparency, malodorous water, reduced fish stocks, and occasional mass mortality of fish were observed in the lake in this period (Ensby et al., 1984). Heavy algal blooms still occur, despite investments in sewage treatment and extensive changes in agricultural practices since the 1970s to reduce nutrient leaching and erosion from the catchment.

Predicting eutrophication responses to nutrient loading is a complex task due to the temporal dynamics of lake's response to weather and runoff. This makes the traditional empirical approach to the problems unreliable. Thus, lake models based on system of processes have been identified as a primary tool for improving our understanding of recovery and progression of eutrophication (Mooij et al., 2010).

The main aim of the current study is to evaluate the relative importance of year-to-year variation of two major factors, namely meteorological forcing and nutrient loading, contributing to lake's physical, chemical and biological conditions. To this aim, 1) the MyLake model (Saloranta & Andersen, 2007) was calibrated against the lake data, 2) various meteorological and nutrient loading scenarios were then applied, and finally 3) the predicted lake responses were compared among different scenarios.

Material and methods

Study site

Lake Årungen is a dimictic lake with maximum and average depths of 13 and 8 m, respectively. The lake is located in south-east Norway, 25 km south of Oslo (59°41'18"N, 10°44'38"E; Fig. 1), and has a surface area of 1.2 km^2. The catchment area covers 51 km^2, where 53% is agricultural land, 34% forestry, 10% densely populated and 3% open water surfaces. The lake is highly exposed to agricultural runoff that causes high nutrient and particle loading. Runoff is mainly through 6 streams of 1.5 to 5 km length. The outlet connects the lake to the marine environment as Lake Årungen enters the Oslofjord through a 3 km long stream.

MyLake model inputs and outputs

MyLake is a one-dimensional lake model, adapted from MINLAKE (Riley & Stefan, 1988), which simulates daily changes in physical and chemical dynamics over the depth gradient (Saloranta & Andersen, 2007). The model simulates ice and snow dynamics in a mechanistic manner and it has been applied to winter-freezing lakes in Norway and Finland (Lydersen et al., 2003; Saloranta, 2006; Kankaala et al., 2006; Saloranta et al., 2009). It was therefore considered as a suitable model for Lake Årungen.

MyLake requires input of meteorological forcing, runoff volume and temperature, and fluxes of suspended inorganic particles and total phosphorus (TP) to model phosphorus and phytoplankton dynamics in the lake (Table 1). Meteorological data for daily air temperature, global radiation, cloud cover, precipitation, relative humidity and wind speed were obtained from the nearby meteorological station located at the Norwegian University of Life Sciences (59°39'37"N, 10°46'54"E). Time series of daily runoff volume, runoff water temperature, and fluxes of suspended inorganic particles and total phosphorus are available for the period 1994 to 2010 from the Skuterud monitoring station (Fig. 1) with a hydrovolumetric weir. The monitoring station is located at an inlet stream to Østensjøvann (59°41′18″N, 10°49′45″E), a small lake of 0.4 km^2 which drains into the Lake Årungen (Deelstra et al., 2007). Runoff from the other sub-catchments was estimated by scaling up the Skuterud data. The up-scaling was based on previously determined flow and nutrient scaling factors that take into account differences in area and land use between sub-catchments (Askilsrud, 2010). A separate MyLake model was set up for Lake Østensjøvann to account for the buffering effects of this lake in the largest sub-catchment of Lake Årungen. The simulated water properties of Lake Østensjøvann were combined with runoff from the other sub-catchments as an estimate of the total runoff to Lake Årungen.

Six variables including whole-lake average TP pool, mean surface chlorophyll concentration, light attenuation coefficient, thermocline depth, epilimnion temperature, and ice thickness were calculated from unprocessed model outputs (Table 1) in order to ease interpretation of the statistical analyses for scenario experiment described below.

Model calibration

Water temperature, TP, soluble reactive phosphorus (SRP), and chlorophyll *a* concentration from the deepest location in the lake were used to calibrate the model (Table 1). Vertical water temperature profile was continuously logged every hour at eight depths between 0.7 and 12.6 m by Hobo pendant temperature loggers (model 64K

- UA-002-64; Onset Computer Corporation, Bourne, MA, USA) in the period from November 2008 to August 2010. Water samples for chemical and biological analyses were collected with a modified Rüttner water sampler at seven depths twice a month or monthly (n = 49) from January 2008 to September 2010. TP, SRP and chlorophyll *a* were determined spectrophotometrically (UV-VIS Spectrophotometer UV-1201, Shimadzu, Kyoto, Japan).

We deployed the Markov chain Monte Carlo (MCMC) method (Andrieu et al., 2003) to calibrate the model. The calibration consisted of two stages. The first MCMC calibration stage involved three physical parameters (see Table 2) that only affect heat dynamics, in particular thermocline depth. This first calibration was run against daily temperature measurements, using 2,000 MCMC steps with the first 1,000 for burn-in. The second MCMC calibration stage involving eight parameters (see Table 2) that affect phosphorus and chlorophyll dynamics, but not temperature, was run against measurements of TP, SRP, and chlorophyll *a* in 30,000 MCMC steps with the first 10,000 for burn-in. For these MCMC applications, convergence was monitored visually. Linear interpolation was used to match model outputs on a 0.5 m vertical grid to the actual measurement depths. Although it was not used directly during the MCMC calibration, model goodness of fit was assessed by root mean squared prediction errors (RMSE). The medians of the posterior parameter distributions generated from both stages of the MCMC calibrations were used for the scenarios experiments described in the following.

Model scenarios

The model was run for six nutrient loading and weather scenarios to quantify the impact of weather variation and loading conditions on phosphorus and phytoplankton dynamics (Table 3). These scenarios were based on input combination of observed data (original data, 1994-2010) and synthetic data, where the synthetic data were created by taking the year-to-year mean (n = 16) of each of the day of the year. Synthetic data repeats the calculated mean year with 365 days sixteen times. The 29th of February is removed in year-to-year mean calculation, and 28th of February was repeated to account for the 29th in leap years.

Statistical methods

A two-way analysis of variance (ANOVA) was run on the 16 years of water year based simulation statistics (water year mean, see Table 1), among scenarios A, B, C and D (two weather factors by two loading factors, see Table 3). All annual averages are computed over the period from 1st October to 30th September, commonly used in Europe to refer on a hydrological year, or a water year (Otnes & Ræstad, 1978). Since treatment contrasts are nested within water years we factored out the between-year variances to gain a greater power in the statistical tests. Principal component analysis (PCA) was used to explore the relationships between meteorological and land-related forcing and their relevance for the simulated lake response. Four water years with extreme PCA scores were selected for studying contrasting lake responses in closer details.

Results

Calibration

The simulated water temperature and thermal dynamics of the lake were in agreement with the lake observed data. Observed water temperature measurements were well predicted by simulation and the RMSE was less than 2°C at all lake depths (Fig. 2). After the water temperature calibration, parameters controlling TP, SRP, and chlorophyll *a* were calibrated against observed data for the period from January 2008 to September 2010. The epilimnion TP, SRP, and chlorophyll *a* were well predicted by the model, although their prediction was less successful than the prediction of the water temperature. The TP and SRP were better predicted by the model in shallower water than in deeper water whereas the chlorophyll *a* showed the opposite pattern. In general, the model simulated well TP and SRP, although both phosphorus forms were overestimated in early spring and autumn at shallow depths, while underestimated in bottom water. Simulated SRP concentrations were also somewhat higher than observed in winters. However, the simulation succeeded in showing a decreasing trend of lake phosphorus in spring and midsummer, and in mimicking its increase during the autumn mixing of water. Although the simulated chlorophyll concentrations were lower than measured, the model was able to predict seasonal variation in phytoplankton primary production and to simulate high phytoplankton biomasses in the lake epilimnion during midsummer.

Input variability

Inter-annual variation was expressed as the standard deviation in inputs and outputs between the years. All weather inputs varied between years (Fig. 3), with air temperature and global radiation having the strongest seasonal pattern in inter-annual variation (i.e. greater 16-year variation as compared to year-to-year variation on a day-of-year basis). The inter-annual variation in air temperature was strongest in the winter period, whereas global radiation varied most during the summer months. The variation in cloud cover, precipitation and relative humidity was generally similar across seasons. The year-to-year variation for precipitation was particularly high in December and in the period from July to September, the latter reflecting extreme precipitation events. Wind speed varied most in winters.

Runoff input data on water flow and concentrations of TP and suspended matter all varied seasonally and between years. The variation in runoff volume was greatest in the period from October to May. No clear seasonal pattern in the degree of variability could be found for suspended matter and TP fluxes, although the variation of TP influx seemed to peak in February-March.

Output variability

Differences in year-to-year variation among the scenarios (Fig. 4) and the annual statistics (Table 4) illustrate the seasonal influence of the external forcing on the thermal regime and the phosphorus and phytoplankton dynamics in the lake. The lake responded differently between years; all simulated outputs, except ice thickness, showed large variation in the beginning and at the end of the phytoplankton growing season (Fig. 4).

All simulated output variables were influenced by external forcing to some extent as they varied inter-annually for all model scenarios. Ice thickness was significantly affected by weather ($P < 0.001$) as both air temperature and winter precipitation highly contributed to its variation between years (Table 4; Fig. 4). The variation in thermocline depth in May and October was well revealed by the model, and seemed to be equally dependent on weather and loading. The epilimnion temperature during the whole growing season was largely controlled by weather. The TP content in the lake was most variable in the period from November to January, and in April and July. Loading could mostly explain the inter-annual TP variation in the lake, whereas precipitation contributed to TP variation only in the spring, and air temperature only during the winter period. Loading was the overall most important factor in controlling the light attenuation coefficient ($P < 0.001$) and surface chlorophyll concentration ($P < 0.001$). Weather seemed to be important in controlling chlorophyll and light attenuation in early spring whereas loading was the most important factor controlling the both variables from June to September.

The years 1996, 2000, 2006 and 2007 were the four most extreme years determined on the basis of the PCA analysis (Fig. 5). The year 1996 was characterized by relatively low average annual air temperature, a thin cloud cover and low precipitation, which resulted in low epilimnion water temperature, short lasting thermocline, low runoff volume, and TP in the lake. The year 2007 represents an opposite to 1996, regarding weather characteristics and resulting model simulation with relatively high average annual air temperature and precipitation. Increased wind speed and low winter air temperatures and precipitation coincided with increased ice thickness and global radiation such as in 2003-2006, 2009 and 2010. These weather conditions resulted in lower suspended inorganic particles and TP in runoff which coincided with lower surface chlorophyll concentration and light attenuation. The year 2006 was identified as the extreme during this period, with a cold winter followed by a warm summer. In contrast, the year 2000 was characterized as a year with less global radiation, lower summer air temperature, and higher wind speed, but with higher winter temperature and precipitation. Such weather conditions pronounced higher TP and suspended particle in runoff compared with an average year, resulting in a high surface chlorophyll concentration and lower water transparency.

Discussion

Year-to-year variability in lake responses

Year-to-year weather variations, as well as the influence of catchment land-use and hydrology hinder our understanding of how individual stressors may affect the lake response (Blenckner, 2005). Our model experiment, which involved the input of weather and loading data for 16 years, was able to outline the importance of year-to-year variation in external forcing on physical, chemical and biological response in Lake Årungen. The combination of high forcing variability and high lake response sensitivity made the inter-annual variation most dramatically expressed in spring and autumn.

Air temperature, precipitation, and wind speed are the principal factors influencing freshwater ecosystems in a changing climate (Nickus et al., 2010). The lake thermal regime was to a large extent affected by weather conditions, particularly by air temperature. Variable winter air temperatures were an important factor influencing the heating and mixing processes during spring. A dynamic physical environment at the beginning of the growing season has considerable influence on the phytoplankton community structure and its dynamics (Weyhenmeyer et al., 2002). Increased surface water temperatures in the English Lake District (George et al., 2007) and incomplete water mixing in Lake Constance (Straile et al., 2003a) have earlier been associated with mild winters. High inter-annual variation in winter air temperatures in Lake Årungen was reflected in the simulated ice thickness and phenology of ice formation, with ice forming in December-January and disappearing in March-April. Also in other lake studies, decreased ice thickness and reduced duration of ice cover have been related to mild winters (Nickus et al., 2010). In this study, the time of thermocline formation varied among years by more than one month, from mid-April to mid-May. The large year-to-year variation in thermocline depth and duration could lead to changes in temperature, light, and nutrient condition in the lake, which further shape the phytoplankton community and determine its total biomass (Padisak et al., 2010; Zohary et al., 2010). For instance, early disappearance of diatoms and high development of cyanobacteria in European lakes has been related to winter warming and increased water temperature (Weyhenmeyer et al., 2002). Furthermore, increased water stability also favors the buoyant phytoplankton species such as bloom forming cyanobacteria (Reynolds et al., 1983; Winder & Hunter, 2008).

Year-to-year variation in phosphorus content in the lake was highly influenced by loading which indicated that the external nutrient supply remains an important source of phosphorus in the lake. Although the changes in nutrient loading are primarily linked to anthropogenic activities in the catchment, particularly to practices in agriculture, the short-term variations in weather and runoff will also influence the nutrient supply from external sources. Lake Årungen is surrounded by agricultural land, and is especially sensitive to variable weather conditions that promote nutrient loading from the catchment. Air temperature and rainfall frequency and intensity, all affect the runoff and the soil erosion pattern, particularly during the winter period. Increased winter temperatures with frequent freezing and melting events increase the risk of erosion, which again will increase the nutrient loading to the lake (Bechmann et al., 2005; Nõges et al., 2007; Jeppesen et al., 2009). Although not statistically demonstrated in the present study, the indirect impacts of weather conditions on discharge may still be important in regulating the nutrient dynamics. Variable winter weather conditions, and the time of ice out were most important cause of year-to-year variable phosphorus content in the period from November to January and in April in Lake Årungen. Enhanced phosphorus concentrations in streams during winter, and high phosphorus loading in early spring, both contribute to the total lake phosphorus concentration during the following summer in two Norwegian lakes with agricultural catchments (Bechmann et al., 2005). In addition, the variation in summer TP content could also be the result of between-year variation in rainfall, especially due to extreme precipitation events as observed for particular days in July. High inter-annual variation in TP content can consequently result in variable phytoplankton biomass between years.

Phytoplankton biomass and light were significantly affected by loading, although atmospheric forcing also contributed to their inter-annual variation in the lake. The effect of loading was pronounced during the whole algal growing season, whereas weather had the strongest effect in early spring and from mid-August to the end of the growing season. Thermal stratification is highly dependent on weather and may further influence water mixing as well as light and nutrient regimes, which are important in controlling the phytoplankton dynamics (Padisak et al., 2010; Zohary et al., 2010). Similar pattern of year-to-year variation in water temperature, chlorophyll concentration, and light attenuation indicates a close relationship between these variables. Increased air temperature promoted higher water temperatures and higher stability of the thermal stratification which enhanced phytoplankton production, particularly of bloom forming cyanobacteria (Reynolds et al., 1983; Weyhenmeyer et al., 2002). Increased runoff and soil erosion, caused by intense precipitation and frequent melting of snow and ice during mild winters, affect eutrophication and water turbidity (Bechmann et al., 2005; Jeppesen et al., 2009). Light, therefore, may limit phytoplankton growth more than nutrients in highly turbid lakes such as Lake Årungen (Dokulili, 1994). Reduced light availability may be crucial for the competitive success of cyanobacteria which are functionally adapted to low light conditions (Litchman, 1998). Particularly high dominance of cyanobacteria has been observed in the Lake Årungen after mild winters followed by low light conditions in spring (Romarheim et al., unpublished). Therefore, the additional measures to control soil erosion should be considered in water management, not only to reduce the supply of nutrients, but also to avoid low water transparency which may favour development of potentially toxic cyanobacteria.

Implications for lake management

According to our PCA analysis, most of the 1990s were categorized by winters with higher temperatures and more rainfall. The mild winters were related to a positive North Atlantic Oscillation (NAO) phase which has been shown to strongly influence physicochemical and biological responses in western European lakes (Weyhenmeyer et al., 2002; Straile et al., 2003b; George et al., 2007). The effect of climate condition on water ecosystems, however, should be considered individually as the lake response is also determined by the lake’s geographical position, landscape topography, and the lake's morphometry and mixing regime (Nickus et al., 2010). Our model experiment indicated that increased inflow of suspended matter and phosphorus to the lake Årungen may be expected after mild winters with high precipitation. Consequently, high chlorophyll concentrations and increased light attenuation were predicted after mild and wet winters such in the year of 2000. Mild winters may thus counteract measures aimed to reduce external nutrient supply and to control phytoplankton production in temperate lakes. On the contrary, cold winters were related to thicker ice layer, less inflow of suspended matter and phosphorus, and low chlorophyll and light attenuation. This was consistent with the observed increase in water transparency and reduction of phytoplankton biomass, particularly of cyanobacteria, in Lake Årungen after the cold winter in 2010 (Romarheim et al., unpublished). Special attention must therefore be given to management practices, which should minimize the use of fertilizers and reduce the risk of nutrient runoff and soil erosion, especially in areas that drains directly into the lake. Increased annual air temperature coincided with warmer epilimnion, shallower thermocline and extended summer stratification such as for the year of 2007. In addition, high annual precipitation and runoff volume, particularly in summer, coincided with warmer years. The latest Intergovernmental Panel on Climate Change Assessment reported that all years in the period from 1995 to 2006, with exception of 1996, were globally among the warmest since 1850 (Trenberth et al., 2007). Similarly, the year 1996 was characterised with low average annual air temperature in our model experiment. Lower annual air temperature and low rainfall in 1996 lead to low epilimnion temperature, and a deep and short lasting thermocline. According to best available future climate predictions for Scandinavia, warmer winters and increased winter precipitation are expected in south-eastern Norway (Hanssen-Bauer et al., 2005). If so, we should also expect more intensive soil erosion, higher phosphorus loading, reduced water transparency, and increased phytoplankton biomasses, primarily of cyanobacteira in the lakes. Global climate changes and inter-annual variations in the local weather both directly, and indirectly through an impact on the catchment, influence the physicochemical and biological processes in lakes. Therefore, the effect of climate should be considered in future decision-making concerning water management.

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Table 1 Input and output data, and observed lake data for the calibration of the MyLake model and statistics for the ANOVA and PCA analyses

MyLake inputs	**MyLake outputs (selected)**	**Observed lake data**
Meteorological[a, f]	*Calibration purpose*[a]	*Calibration purpose*
Air temperature	(every 0.5 m by depth)	(at 7 depths)
Global radiation	Water temperature	Water temperature[a]
Cloud cover	TP concentration	TP concentration[b]
Precipitation	SRP concentration	SRP concentration[b]
Relative humidity	Chlorophyll concentration	Chlorophyll *a* concentration[b]
Wind speed		
	Statistics calculated for PCA	
Runoff [a, g]	(volume weighted mean 0-3.0 m)	
Flow volume	TP content	
Water temperature	Mean surface chlorophyll	
Suspended matter flux	Light attenuation coeficient	
TP flux	Thermocline depth	
	Mean epilimnion temperature[e]	
Statistics calculated for PCA	Ice thickness	
Global radiation[c]		
Cloud cover[c]		
Air temperature[c]		
Wind speed[c]		
Precipitation[c]		
Flow volume[c]		
Winter air temperature[d,h]		
Summer air temperature[d,i]		
Winter precipitation[d,h]		
Summer precipitation[d,i]		
Suspended matter flux[d]		
TP flux[d]		

[a] Daily data

[b] Biweekly data

[c] Annual mean

[d] Water year basis (October through September)

[e] Volume weighted above thermocline depth

[f] Inferred with data from Ås meteorological station (http://www.umb.no/fagklim/artikkel/meteorologiske-data-for-as)

[g] Inferred with data from Skuterud monitoring station and land use

[h] December through March, mean

[i] June through September, mean

Table 2 Parameters involved in calibration based on two-stage Markov chain Monte Carlo (MCMC) application (first stage for three parameters using 2,000 MCMC steps with 1,000 steps for burn-in and second stage for eight parameters using 30,000 MCMC steps with 10,000 for burn-in). MyLake equation numbers refer to the original model description (Saloranta & Andersen, 2007). Median values were chosen among the posterior parameter distribution

	Value	MyLake equation	Unit
Physical parameters			
Open-water vertical diffusion coefficient	5.00E-01	eq. 10	m^2 day^{-1}
Wind sheltering coefficient	7.96E-02	eq. 13	—
Minimum possible stability frequency	9.31E-05	eq. 10	sec^{-2}
Biological and chemical parameters			
PAR saturation level for photosynthesis	2.04E-04	eq. 29	mol quanta m^{-2} sec^{-1}
Particle resuspension mass transfer coefficient	2.94E-05	§ 2.7	m day^{-1}, dry sediment
Settling velocity for suspended matter	1.38E+00	eq. 20	m day^{-1}
Settling velocity for chlorophyll	7.31E-02	eq. 20	m day^{-1}
Specific mortality rate of phytoplankton	1.86E-01	eq. 26	day^{-1}
Max specific growth rate of phytoplankton	1.76E+00	eq. 27	day^{-1}
Half saturation inorganic phosphorus concentration for Langmuir isotherm	9.99E+02	eq. 24	mg m^{-3}
Saturation level for inorganic phosphorus isotherm	4.96E+04	eq. 24	mg kg^{-1}

Table 3 Model scenarios. The scenarios comprise either original input data (denoted O), pseudo repeated average year based on 16-years of input data (denoted R), or a combination of O and R

	Model scenarios					
Model inputs	A	B	C	D	Dt	Dp
Weather						
Global radiation	**O**	R	**O**	R	R	R
Cloud cover	**O**	R	**O**	R	R	R
Relative humidity	**O**	R	**O**	R	R	R
Wind speed	**O**	R	**O**	R	R	R
Air pressure	**O**	R	**O**	R	R	R
Air temperature	**O**	R	**O**	R	**O**	R
Precipitation	**O**	R	**O**	R	R	**O**
Runoff						
Flow volume	**O**	**O**	R	R	R	R
Suspended matter flux	**O**	**O**	R	R	R	R
Inflow water temperature	**O**	**O**	R	R	R	R
TP flux	**O**	**O**	R	R	R	R

Table 4 Summary results for six two-factor within-subject ANOVA (n = 16 × 2 × 2). Significance of additive and interactive effects of weather (two levels, original O or repeated average R) and loading (two levels, original O or repeated average R) inputs on the six selected model outputs. High *P*-values for interactive effects for all six tests indicate pure additive two-factor model and test for each factor separately. *P*-values below 0.05 are highlighted (shown bold) and indicate significant differences between corresponding sample means

	***P*-value**		
Model outputs	**W**	**L**	**W × L**
Ice thickness, m	**< 0.001**	0.839	0.560
Thermocline depth, m	0.281	0.218	0.398
Epilimnion temperature, °C	**0.014**	0.135	0.771
TP content, kg	0.365	0.088	0.726
Surface chlorophyll, mg m^{-3}	0.699	**< 0.001**	0.791
Light attenuation coefficient, m^{-1}	0.360	**< 0.001**	0.836

Figure captions

Fig. 1 Map of catchment draining into (1) Lake Årungen (59°41'18"N, 10°44'38"E), with the (2) weather station at Ås, (3) Lake Østensjøvannet and (4) the Skuterud monitoring station. Runoff data from the Skuterud sub-catchment (indicated by dark shading) are scaled up according to land area and usage of the rest of the catchment to estimate the total loading to Lake Årungen

Fig. 2 Simulated (line) and observed (circles) lake state variables for water temperature, TP, SRP, and chlorophyll *a* concentrations at seven depths

Fig. 3 Input variability shown as standard deviations on a water year scale (day-by-day, year-to-year variation, n =16, curves), with the overall 16-year standard deviations indicated by horizontal lines

Fig. 4 Output variability shown as standard deviations on a water year scale (day-by-day, year-to-year variation, n =16) for scenarios A (top of the forward diagonal band), B (bottom of the forward diagonal band), C (top of the backward diagonal band), and D (bottom of the backward diagonal band). See Table 3 for scenario configurations

Fig. 5 Principal component analysis (PCA) loadings for the two greatest components, together with the scores for the two components for 16 water years (letters). The arrows are scaled and therefore only meaningful for comparison within PCA axes

Fig. 1

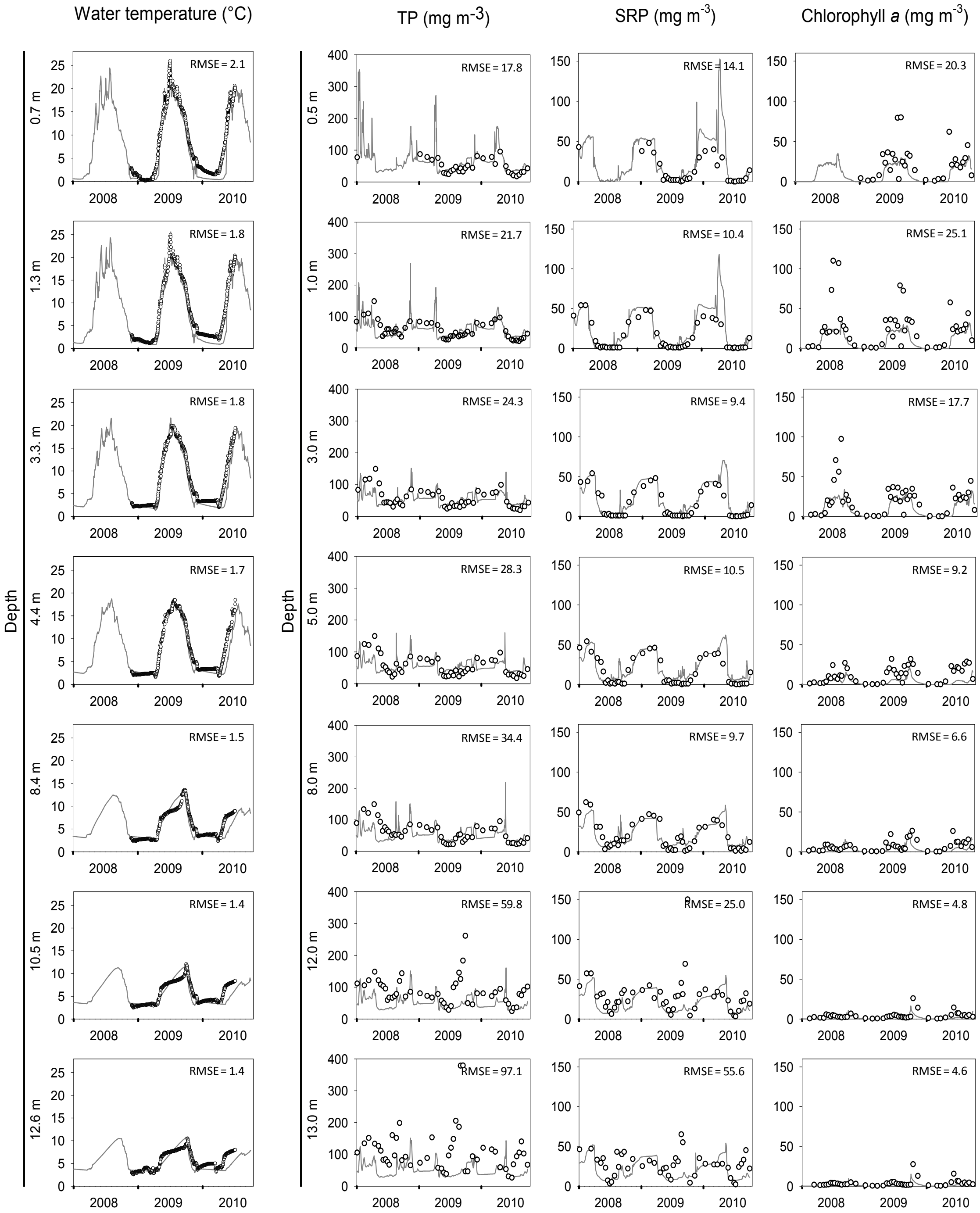
Water temperature (°C)
TP (mg m^{-3})
SRP (mg m^{-3})
Chlorophyll *a* (mg m^{-3})
Depth
0.7 m
1.3 m
3.3. m
4.4 m
8.4 m
10.5 m
12.6 m
0.5 m
1.0 m
3.0 m
5.0 m
8.0 m
12.0 m
13.0 m
RMSE = 2.1
RMSE = 1.8
RMSE = 1.8
RMSE = 1.7
RMSE = 1.5
RMSE = 1.4
RMSE = 1.4
RMSE = 17.8
RMSE = 21.7
RMSE = 24.3
RMSE = 28.3
RMSE = 34.4
RMSE = 59.8
RMSE = 97.1
RMSE = 14.1
RMSE = 10.4
RMSE = 9.4
RMSE = 10.5
RMSE = 9.7
RMSE = 25.0
RMSE = 55.6
RMSE = 20.3
RMSE = 25.1
RMSE = 17.7
RMSE = 9.2
RMSE = 6.6
RMSE = 4.8
RMSE = 4.6
0
5
10
15
20
25
0
100
200
300
400
0
50
100
150
2008
2009
2010

Fig.2

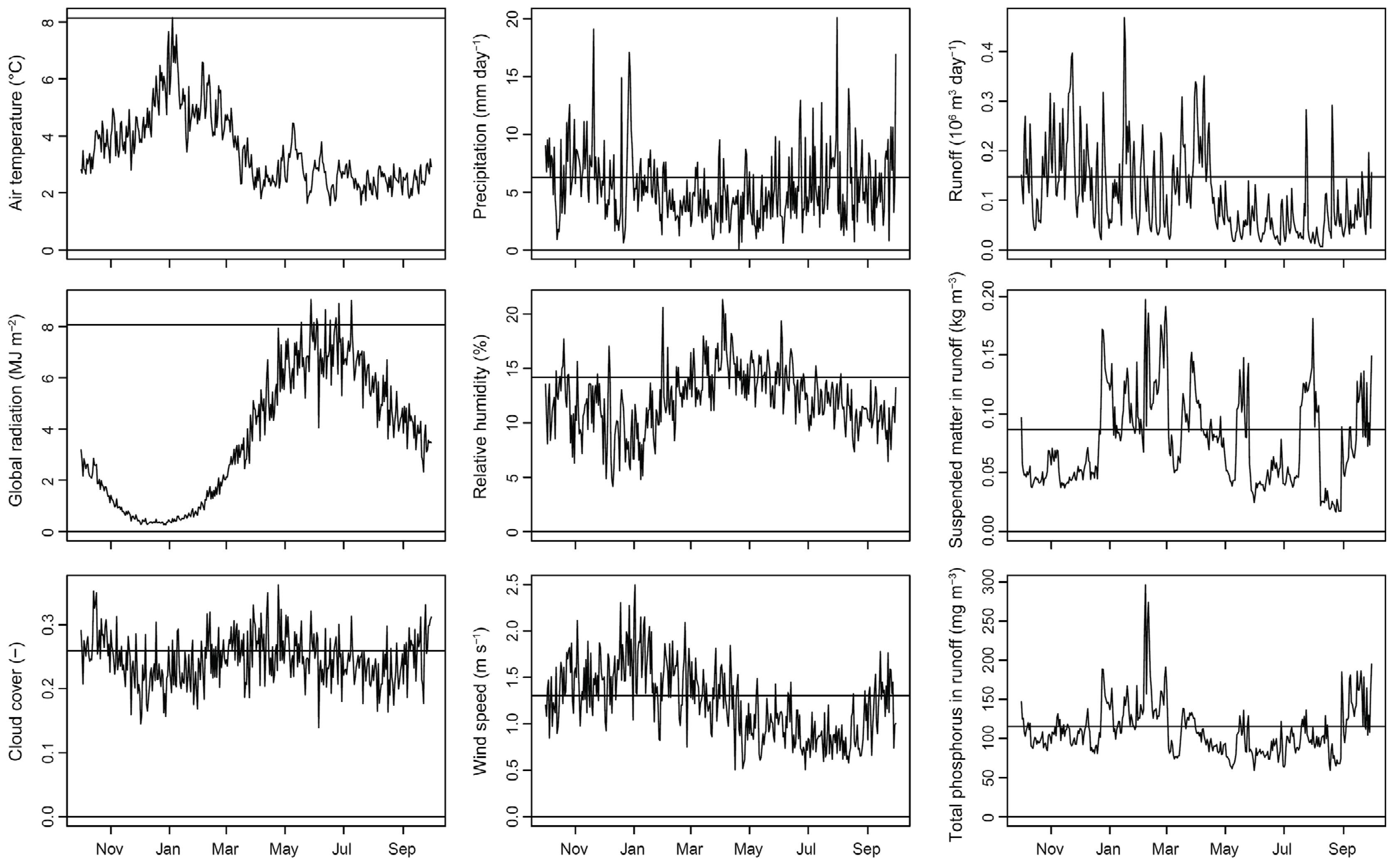
Air temperature (°C)
Precipitation (mm day^{-1})
Runoff (10^6 m^3 day^{-1})
Global radiation (MJ m^{-2})
Relative humidity (%)
Suspended matter in runoff (kg m^{-3})
Cloud cover (−)
Wind speed (m s^{-1})
Total phosphorus in runoff (mg m^{-3})
0 2 4 6 8
0 5 10 15 20
0.0 0.1 0.2 0.3 0.4
0.00 0.05 0.10 0.15 0.20
0.0 0.1 0.2 0.3
0.0 0.5 1.0 1.5 2.0 2.5
0 50 100 150 200 250 300
Nov Jan Mar May Jul Sep

Fig. 3

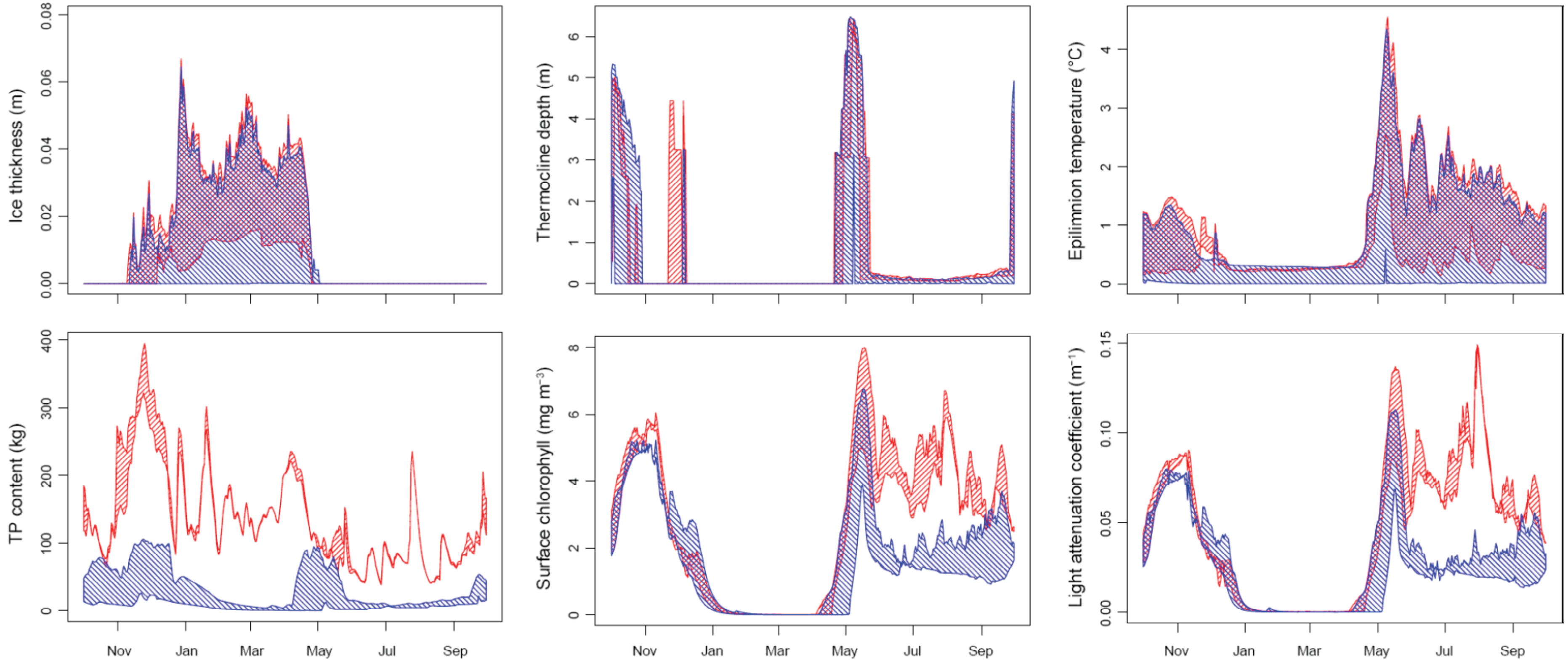
Ice thickness (m)
0.00
0.02
0.04
0.06
0.08
Thermocline depth (m)
0
1
2
3
4
5
6
Epilimnion temperature (°C)
0
1
2
3
4
TP content (kg)
0
100
200
300
400
Surface chlorophyll (mg m^{-3})
0
2
4
6
8
Light attenuation coefficient (m^{-1})
0.00
0.05
0.10
0.15
Nov
Jan
Mar
May
Jul
Sep

Fig. 4

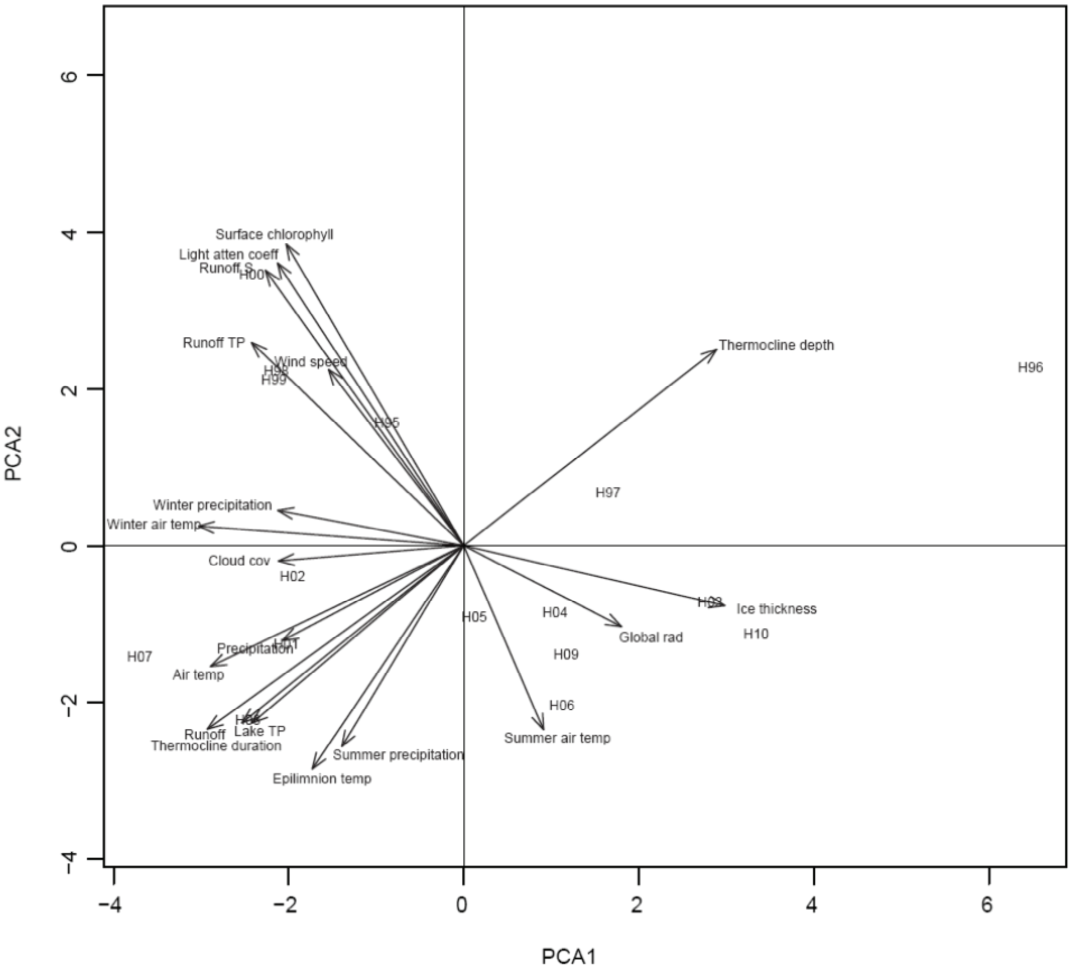
PCA2
PCA1
6
4
2
0
−2
−4
−4
−2
0
2
4
6
Surface chlorophyll
Light atten coeff
Runoff S
H00
Runoff TP
Wind speed
H98
H99
H95
Thermocline depth
H96
H97
Winter precipitation
Winter air temp
Cloud cov
H02
H05
H04
H03
Ice thickness
Global rad
H10
H01
Precipitation
H07
Air temp
H09
H06
H08
Lake TP
Runoff
Thermocline duration
Summer precipitation
Summer air temp
Epilimnion temp

Fig. 5