Phytoplanktonic life in boreal humic lakes: special emphasis on autotrophic picoplankton and microbial food webs

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Academic dissertation

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ABSTRACT

Phytoplankton constitute the autotrophic, photosynthesizing component of the plankton community in freshwaters as well as in oceans. Today, phytoplankton account for about half of Earth's primary production (PP). Carbon and energy fixed by phytoplankton are transported further in the aquatic food web to heterotrophic zooplankton and finally to fish or, alternatively, are decomposed by heterotrophic bacteria that also act as food for higher trophic-level organisms. Since phytoplankton fix inorganic carbon (IC), they are highly important in lake carbon cycling and balance.

Many of the lakes in the boreal area are characterized by heavy loadings of brown-coloured humic matter, mostly dissolved organic carbon (DOC), that diminishes light penetration in the water column. This is problematic for phytoplankton which, as photosynthetic organisms, are dependent on solar radiation. The phytoplanktonic life in boreal humic lakes is also hampered by strong thermal stratification patterns that due to nutrient uptake, lead to inorganic nutrient limitation in the illuminated epilimnion. However, nutrients are often plentiful in the dark hypolimnion.

Since phytoplankton are ubiquitous in aquatic ecosystems, they must have several adaptations to help them survive in various environments, including boreal humic lakes. The present study focused on the traits of motility and cell size, both of which affect phytoplankton capability to not only obtain nutrients and light, but also to avoid zooplankton grazing. Special attention was given to the group of autotrophic picoplankton (APP), which are nonmotile, small (cell size 0.2–2 µm) and less studied than the larger phytoplankton. The seasonal dynamics of APP and larger phytoplankton were associated with changes in the abiotic environment, especially parameters prone to the ongoing climate change. In addition, the associations between phytoplankton and their competitors and grazers in the microbial food web (MFW), as well as the possible top-down effects of fish on the MFW, phytoplankton and surface water carbon dioxide (CO_2) concentrations were studied in more detail. Four of the five studies were undertaken *in situ* in the small, strongly stratified, humic headwater Lake Valkea-Kotinen. The fifth study was a fish biomanipulation experiment conducted in enclosures in the humic Lake Pääjärvi and the clearwater Lake Vesijärvi.

The most successful phytoplankton taxa in Lake Valkea-Kotinen in terms of PP as well as biomass were flagellated. However, motility was really advantageous only when combined with large cell size (> 20 µm): *Peridinium* dinoflagellates dominated in PP and the biomass in spring and autumn, whereas in summer *Gonyostomum semen* (Ehr.) Diesing took over. This was probably because only the large cells were able to migrate long distances between the illuminated epilimnion and nutrient-rich hypolimnion. Interestingly, the most abundant phytoplankton taxa in Lake Valkea-Kotinen were the nonmotile and tiny (~ 2 µm) *Choricystis* (Skuja) Fott-like eukaryotic APP. The strength of the APP was in isopycny, i.e. the capability to remain at the boundary layer between the epi- and hypolimnion, where they obtained access to light and nutrients. Both *G. semen* and APP correlated positively with high water column stability, which also indicates that they benefitted from strong stratification patterns.

There were changes in the water quality in Lake Valkea-Kotinen during the study period of 1990–2006, most importantly, as increases in DOC and water colour, whereas phosphorus, which was the limiting nutrient, decreased. This was problematic for the large flagellates (studied in 1990–2003) and prokaryotic APP (*Merismopedia warmingiana* Lagerheim; in 2002–2006). However, the eukaryotic APP (in 2002–2006) were favoured by the increased water colour.

APP abundance correlated negatively with heterotrophic bacteria in the epilimnion of Lake Valkea-Kotinen, which indicates nutrient competition between these two groups. The bacteria correlated positively with large phytoplankton (measured as chlorophyll *a*), and probably were partly sustained by *G. semen*, which was associated with high extracellular organic carbon (EOC) release. However, both the APP and bacterial numbers were in general low in Lake Valkea-Kotinen, which was explained by the high nanoflagellate (NF) and ciliate abundance. Nevertheless, the NFs did not graze on the APP, and the APP as well as the larger phytoplankton were able to avoid ciliate grazing during the strongest stagnation by remaining in the anoxic parts of the water column, where algivorous ciliates were less abundant.

The enclosure experiment in lakes Pääjärvi and Vesijärvi showed no top-down effects of fish on APP or any other components of the MFW. This was probably due to the low abundance of cladocerans, especially the large daphnids. However, in the humic Lake Pääjärvi, fish influenced the food web via nutrient enrichment, i.e. through bottom-up effects. The total phytoplankton biomass did not change, but the PP increased and led to increments in bacterial production (BP) and ciliates, which took advantage of the enhanced phytoplankton production. Therefore, although unexpected, the higher PP did not translate into lower water CO_2 concentration, but the BP and ciliate algivory increased concurrently and produced more CO_2. Thus, the net ecosystem production (NEP) remained stable.

LIST OF ORIGINAL ARTICLES

This thesis is based on the following articles, which in the text are referred to by their Roman numerals:

I.   **Peltomaa E.** & Ojala A. 2010. Size-related photosynthesis of algae in a strongly stratified humic lake. *Journal of Plankton Research* 32: 341–355.

II.   **Peltomaa E.** & Ojala A., 2012. Meteorological drivers of autotrophic picoplankton. *Freshwater Biology* 57: 1005–1016.

III.   **Peltomaa E.**, Ojala A., Holopainen A.-L. & Salonen K. Changes in phytoplankton in a boreal lake during a 14-year period. *Boreal Environment Research*, in press.

IV.   **Peltomaa E.**, Zingel P. & Ojala A. 2013. Weak response of the microbial food web of a boreal humic lake to hypolimnetic anoxia. *Aquatic Microbial Ecology* 68: 91–105.

V.   Bręk-Laitinen G., **Peltomaa E.**, López Bellido J., Ojala A. & Huotari J. Gap filling in aquatic biomanipulation studies: effects of fish on microbial food webs and CO_2 concentrations. (Submitted manuscript in Ecology and Evolution).

THE AUTHOR'S CONTRIBUTION

I.   The original idea came from AO. EP planned the experiments and was responsible for all the field sampling and laboratory analyses. EP analysed the data, drew the figures and wrote the article together with AO. AO also supervised the work.

II. AO planned the data collection. EP carried out the field sampling and most of the laboratory analyses of the environmental samples. EP (4 years) and AO (1 year) analysed the picoplankton samples. EP planned and performed the data analyses, drew the figures and wrote the article. AO revised the article and supervised the work.

III. KS planned the monitoring programme and provided the environmental data (years 1990–2001). EP carried out 2 years of the field sampling programme and laboratory analyses (in 2002–2003). EP (6 years) and A-LH (8 years) analysed the phytoplankton samples. EP analysed the data and drew the figures. EP also wrote the article together with AO. The coauthors commented on the manuscript. AO supervised the work.

IV. AO planned the data collection. EP was responsible for the sampling and laboratory analyses. PZ analysed the ciliate samples. EP planned and performed data analyses, drew the figures and wrote the article. The co-authors commented on the manuscript. AO also supervised the work.

V.   AO and JH planned the data collection. EP participated in the experimental work (field sampling and microscopy) together with GB-L, JH and JLB. GB-L planned and wrote the article with contributions by EP and JH. AO revised the article and supervised the work.

In addition to the results of the original papers, the thesis also includes unpublished material analysed by the author.

ABBREVIATIONS

|			
ANOVA	analysis of variance	O_2	oxygen
APP	autotrophic picoplankton	OC	organic carbon
BP	bacterial production	OM	organic matter
C	carbon	P	phosphorus
CCA	canonical correspondence analysis	pCO_2	partial pressure of CO_2
		PEG	Plankton Ecology Group
CH_4	methane	PER	percentage of extracellular release
Chl a	chlorophyll a		
CO_2	carbon dioxide	PLS	partial least square regression analysis
CO_3^{2-}	carbonate ion		
DIC	dissolved inorganic carbon	PO_4	phosphate
DIN	dissolved inorganic nitrogen	PP	(phytoplankton) primary production
DIP	dissolved inorganic phosphorus	PP: Chl a	(phytoplankton) primary production to chlorophyll a -ratio
DOC	dissolved organic carbon		
EC	eddy covariance	Pt	platinum
EOC	extracellular organic carbon	P_{tot}	total phosphorus
		SA:V	surface area to volume - ratio
EU	European Union		
Fe	iron	TOC	total organic carbon
GC	gas chromatograph	WA	time-weighted average
HCO_3^-	bicarbonate ion		
IC	inorganic carbon		
ICP IM	International Cooperative Programme on Integrated Monitoring of Air Pollution Effects on Ecosystems		
IPCC	Intergovernmental Panel on Climate Change		
LTER	Long-Term Ecological Research		
MANOVA	multivariate analysis of variance		
MFW	microbial food web		
MK	Mann-Kendall test		
N	nitrogen		
NEP	net ecosystem production		
NF	nanoflagellate		
NH_4	ammonium		
NO_2&NO_3	nitrite and nitrate		
N_{tot}	total nitrogen		
N_s	Brunt-Väisälä stability frequency (water column stability)		

1. INTRODUCTION

1.1 Lacustrine food webs

The description of the food web of an ecosystem is essential for understanding how the ecosystem works (Pimm et al. 1991). The food webs are comprised of trophic levels that describe the number of steps an organism is from the start of the chain. Classically, it is believed that carbon (C) and energy in lake pelagic areas are transported from the autotrophic level, i.e. photosynthetic phytoplankton (size > 2 µm), to heterotrophic zooplankton (size > 200 µm) and further to planktivorous fish (Fig. 1; Sieburth et al. 1978; Carpenter et al. 1987; Hansson et al. 1993). However, this classical grazer food chain theory was challenged in the 1980s when Azam et al. (1983) published the concept of 'microbial loop', first introduced by Pomeroy already in 1974. In the microbial loop - or more precisely - the microbial food web (MFW), the first trophic level is comprised of tiny autotrophic and heterotrophic picoplankton (APP and bacteria, respectively; size 0.2–2 µm) that are grazed by microzooplankton (2–200 µm), i.e. heterotrophic nanoflagellates (NFs) and ciliates, which are further grazed by meso- and macrozooplankton (> 200 µm) and finally by planktivorous fish (Fig. 1). Since fish are the top grazers in the classical food chain as well as in the MFW, it has been postulated that there is a trophic cascade from fish to the lowest trophic level, i.e. to phytoplankton and bacteria (Jürgens et al. 1994).

One of the greatest differences between the classical food chain and the MFW is that the initial source of C in the classical chain is inorganic, but in the MFW it can be either inorganic or organic. Furthermore, the organic carbon (OC) can be either of autochthonous or allochthonous origin, i.e. derived from the phytoplankton primary production (PP) or

from the surrounding catchment area (Tranvik 1992). The MFWs currently are widely studied in marine habitats as well as lakes of varying trophic status and geographical areas (e.g. Arndt 1993; Amblard et al. 1995; Hadas & Berman 1998; Zingel et al. 2006; Gobler et al. 2008; Pestova et al. 2008). However, MFWs in lakes with deep thermal stratification and hypolimnetic oxygen (O_2) depletion are still poorly defined. One fairly large group of lakes undergoing seasonal hypolimnetic hypoxia and finally anoxia are the humic lakes in the Boreal Zone. The special characteristic of humic lakes is the high content of allochthonous OC deriving from the forests and peatlands of the catchment. Thus, MFW studies in boreal humic lakes have focused on bacterial utilization of OC. Since they do not comprehensively describe the trophic structure, the role of APP in the MFWs of these lakes is still largely unknown.

1.2 Phytoplankton

Phytoplankton constitute the autotrophic, photosynthesizing component of the plankton community in freshwaters as well as in oceans. Today they account for about half of Earth's PP (Litchman & Klausmeier 2008 and references therein), and since they fix inorganic carbon (IC), they are key players in carbon dioxide (CO_2) exchange between the lake/ocean and the atmosphere (Schindler et al. 1997). Phytoplankton are comprised of unicellular (cell size 0.2–200 µm) prokaryotic and eukaryotic taxa and, depending on the species, are either solitary or colonial. The various phytoplankton species have different environmental requirements, but primarily they are bottom-up-controlled by nutrients, mainly nitrogen (N) and phosphorus (P), and light (Reynolds 2006; Callieri 2007). The photosynthetic characteristics, light requirements and C fixation patterns of phytoplankton differ
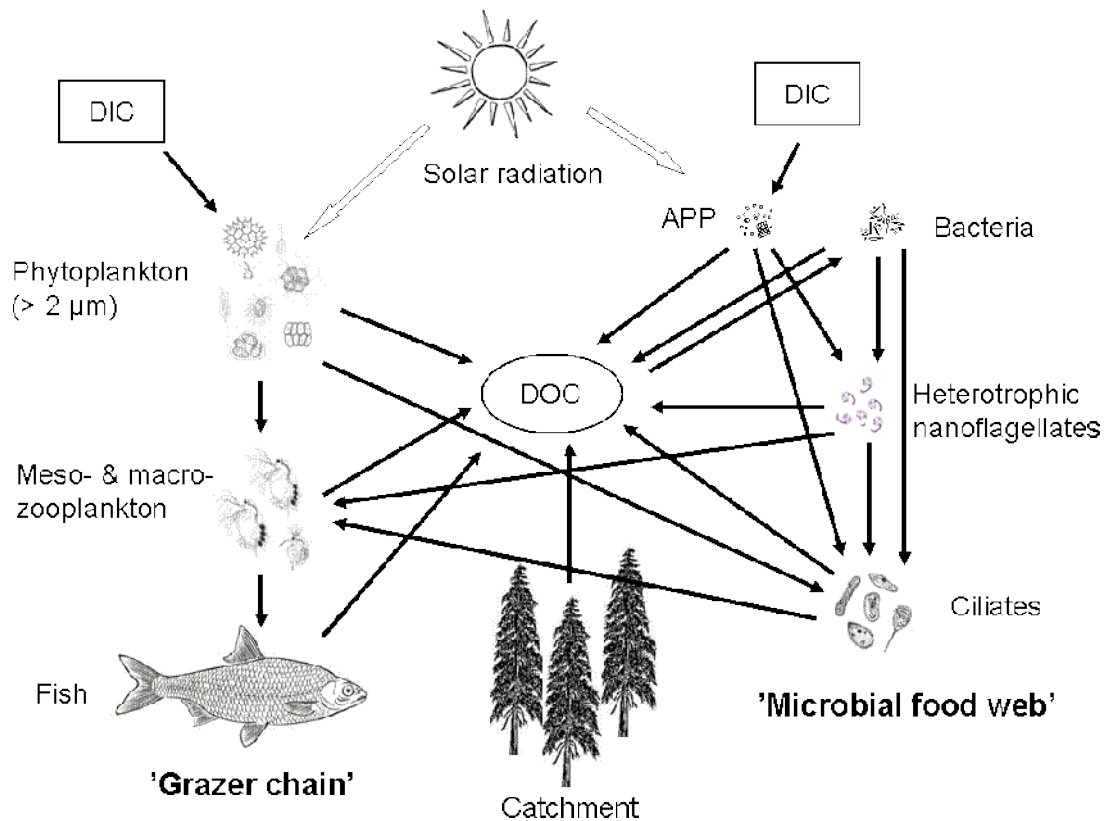
Figure 1. A simplified schema of carbon and energy transfer in lakes. In the 'classical grazer chain', phytoplankton fix dissolved inorganic carbon (DIC) into organic compounds through photosynthesis; the energy comes from the sun. In the 'microbial food web' (MFW), the carbon and energy are obtained either from DIC and solar radiation by autotrophic picoplankton (APP) or from organic compounds (dissolved organic carbon, DOC) by heterotrophic prokaryotes (bacteria). The DOC derives from the catchment or is released by aquatic organisms. In both grazer chain and MFW, the carbon and energy are transported to meso- and macrozooplankton; however, in the MFW the route is via protozoa, i.e. heterotrophic nanoflagellates and ciliates. Finally, zooplankton are grazed by planktivorous fish. The black arrows show carbon flow in the food web.

according to cell size, and the responses of small phytoplankton species to the physical and chemical conditions of the water column are different from those of larger species (Malone 1980; Glover et al. 1985; Frenette et al. 1996; Mei et al. 2003). Small cells are considered to have shorter cell cycles and higher growth rates than the large cells (Raven 1998; Reynolds 2006). Furthermore, small phytoplankton species with higher surface area-to-volume (SA:V) ratios are more effective in nutrient uptake and light acquisition than larger species. However, it is not all about the cell size, since

several phytoplankton species are flagellated and therefore able to actively ameliorate nutrient and light acquisition by swimming towards these resources (Sommer 1988; Reynolds 2006). Flagella and the potential for independent movements are actually considered to be among the most important adaptations of phytoplankton against ecological stress, because the flagellated phytoplankton can enhance their growth by up to 50% and attain growth rates close to the maximal through vertical migrations into deep nutrient-rich water layers (Raven & Richardson 1984; Ilmavirta 1988; Ojala

et al. 1996). Large phytoplankton species can, in general, resist zooplankton grazing better than the smaller species (Reynolds 2006), but for flagellates the large cell size is also advantageous for other reasons. Large flagellates swim faster than the small flagellates and they also have wider maximal migration amplitudes than their small counterparts: large flagellates such as *Peridinium* dinoflagellates and the raphidophyte *Gonyostomum semen* (Ehr.) Diesing have 5–10-m migration amplitudes, whereas flagellates smaller than 5 µm can migrate vertically only about 2 m (Sommer 1988 and references; Salonen & Rosenberg 2000).

Phytoplankton species composition in boreal lakes varies during the open-water season in accordance with light and nutrient availability as well as losses caused by grazers. This variation is called seasonal succession, and it starts all over again every spring after ice-out. The PEG (Plankton Ecology Group) model by Sommer et al. (1986), which originally depicted the seasonal succession of phytoplankton in temperate lakes but has often also been applied also to boreal lakes, states that the first species appearing in spring are small and fast-growing, e.g. cryptophytes and diatoms, and are rapidly grazed by herbivorous zooplankton, which then decline in numbers through fish predation. As a consequence of decreased grazing pressure, a diverse phytoplankton community develops. Later in summer nutrients become depleted, and the abundance of phytoplankton species varies in relation to their biological needs for these nutrients (Reynolds 2006). The components of the phytoplankton community may be exposed to exploitative competition, in which consumption of a limiting resource by one species makes that resource unavailable for another (Jasser 1997; Litchman & Klausmeier 2008). The PEG

model states that the phytoplankton summer crop is comprised of some edible species as well as inedible colonies, and later cyanobacteria, large diatoms and dinophytes become abundant (Sommer et al. 1986). However, in boreal humic lakes the end of the seasonal succession differs from that in clearwater lakes, since diatoms and cyanobacteria are not as numerous as in clearwater lakes and the phytoplankton flora are comprised mainly of flagellated species (Burns & Rosa 1980; Riebesell 1989; Lepistö & Rosenström 1998).

Autotrophic picoplankton

APP are the smallest (size 0.2–2 µm) phytoplanktonic primary producers. They are present in all types of aquatic habitats (Sieburth et al. 1978; Callieri 2007) and include both solitary cells and colonial forms of 'nonblooming' species (Callieri & Stockner 2002). Probably due to their small cell size, the APP are less studied than the larger phytoplankton and are also excluded from the PEG model (Sommer et al. 1986). However, despite their small size and thus comparatively low total biomass, the contribution of the APP to total PP is far from negligible: APP are responsible for 5–90% of the IC uptake in freshwater lakes (Callieri & Stockner 2002; Belykh et al. 2006; Greisberger et al. 2008). Furthermore, the photoautotrophic CO_2 fixation in the world's oligotrophic oceans is dominated by two pico-sized cyanobacterial genera, i.e. *Prochlorococcus and Synechococcus*, which together contribute 30–80% of marine PP (Liu et al 1997; Price 2011). The lacustrine APP communities vary along trophic gradients as well as seasonally (Callieri & Stockner 2002). Generally, the APP communities in oligotrophic and mesotrophic lakes are dominated by solitary prokaryotes (picocyanobacteria), whereas their colonial forms are common in eutrophic lakes. Eukaryotic APP thrive at low pH

and in low light and thus are often more abundant in acidic humic and eutrophic lakes (Callieri & Stockner 2002; Callieri 2007). However, various APP taxa can coexist due to nonoverlapping ecological niches (Winder 2009). Most APP studies in boreal lakes have been carried out for single open-water periods, and thus the existence of interannual variations and drivers behind the variations remain so far unclear.

Competition with heterotrophic bacteria

Prokaryotic, heterotrophic bacteria are the main organisms responsible for organic matter (OM) decomposition in lakes and in aquatic ecosystems in general (Tranvik 1992; Cotner & Biddanda 2002). They are important for fluxes of O_2 and C, and bacterial respiration is the main cause of net heterotrophy (photosynthesis to respiration ratio < 1) in oligo- and mesotrophic lakes (del Giorgio & Peters 1994; Cotner & Biddanda 2002). It is well known that, despite their heterotrophy, bacteria account for large proportions of inorganic nutrient uptake: on average 60% of phosphate (PO_4) and 30% of ammonium (NH_4) are taken up by heterotrophic bacteria (Kirchman 1994). This creates competition between bacteria and phytoplankton for growth-limiting nutrients (Caron 1994). Due to their small cell size, bacteria are often considered more effective in nutrient uptake than phytoplankton (Currie & Kalff 1984; Thingstad et al. 1993; Kirchman 1994), but the tiny APP may be able to challenge bacteria (Cotner & Biddanda 2002). However, the laboratory experiment of Drakare (2002) suggests that APP can fare in competition with heterotrophic bacteria only if the bacteria are under OC limitation.

Food source in the MFW

The abundance as well as vertical distribution of grazers in MFWs is largely controlled by food and/or O_2 availability (Guhl et al. 1996). Many studies have reported NF grazing on bacterial-sized particles (Sherr & Sherr 2002 and references therein). However, not all NFs are bacterivorous, but feed on phytoplankton or are omnivorous (Mischke 1994; Simek et al. 1997; Bręk-Laitinen & Ojala 2011). The small size of NFs makes them incapable of ingesting large cells, which may restrict their grazing efficiency (Amblard et al. 1995). However, prey size is not an obstacle for ciliates; some algivorous ciliates can consume phytoplankton larger than 200 µm, either by engulfing them or via extracellular digestion (Sherr & Sherr 1994). Some of the ciliates have specialized diets and are thus dependent on the location of their food resources (Amblard et al. 1995; Guhl et al. 1996; Verni & Gualtieri 1997; Gobler et al. 2008; Bręk-Laitinen et al. 2012). In addition to the specialists, the ciliate community is composed of omnivorous species that have mixed diets of phytoplankton, bacteria and NFs (Posh & Arndt 1996; Pernthaler 2005). NFs do not respond to anoxia (Gobler et al. 2008; Bręk-Laitinen et al. 2012), whereas the abundance and community structure of the ciliated protozoa differ considerably between oxic and anoxic waters (Guhl et al. 1996; Gobler et al. 2008; Bręk-Laitinen et al. 2012).

In addition to being prey items for the upper trophic levels, phytoplankton can play an important role in the food webs of humic as well as clearwater lakes through release of OC compounds (extracellular organic carbon, EOC), which act as sources of C for heterotrophic bacteria (Sundh & Bell 1992). The average proportion of EOC release, i.e. the percentage of extracellular release (PER), varies between 3% and 55% of the PP (Søndergaard et al. 1985; Baines & Pace 1991; Sundh & Bell 1992). The EOC is released by healthy phytoplankton cells,

but EOC production may also result from cell lysis after the collapse of an algal bloom. The seasonal succession of phytoplankton leads to large seasonal variations in PER within lakes, since EOC release is dependent on cell size, phytoplankton taxa and growth phase (Berman & Holm-Hansen 1974; Lancelot & Billen 1984; Sundh & Bell 1992; Malinsky-Rushansky & Legrand 1996).

1.3 Boreal humic lakes

Humic lakes are typically found in the boreal landscape of the Northern Hemisphere. These lakes are often small and located in the middle of forests and peatlands, and are thus characterized by heavy loadings of OC (mostly in the form of dissolved organic carbon, DOC) from the catchment. High inputs of C in association with bacterial activity lead to supersaturation of CO_2, causing a net diffusion of CO_2 from the surface water to the atmosphere and making these lakes conduits of terrestrial C to the atmosphere (Cole et al. 1994; del Giorgio et al. 1999; Huotari et al. 2011). This is significant in areas such as Finland, where as many as 93% of the lakes are humic and the number of lakes and ponds larger in area than 0.05 ha is around 190 000, i.e. the lakes cover on average 10% (locally up to 20%) of the land area (Raatikainen & Kuusisto 1990; Kortelainen 1993).

Mixing periods and stratification

Boreal lakes are in general dimictic, which means that each year the water column mixes from top to bottom during two mixing periods: in spring and autumn. The lakes are ice-covered in winter and stably stratified in summer (Lewis 1983). The stable stratification in summer derives from the temperature dependence of the water density, which divides the water column into warm surface waters (epilimnion) and colder bottom waters (hypolimnion). These two layers are separated by a thermocline, where temperature decreases rapidly. In winter the thermal stratification is weak, and the warmest water layers are near the bottom sediment. Despite the rather low water temperature, the bacterial decomposition of OC releases nutrients and C gases (CO_2 and methane, CH_4) into the hypolimnion in winter (Tulonen 1993; Liikanen et al. 2002).

The brownish water absorbs solar radiation and in spring, when the amount of irradiance increases, surface temperatures in humic lakes rise rapidly. This shortens the duration of the spring overturn to a few days, or the spring mixing may even be incomplete and does not reach the bottom. In the latter case, neither nutrients nor C gases are mixed in the upper water column but remain in the deep-water layers (Huotari et al. 2009). The effective absorption of solar radiation also results in shallow thermocline depths and very stable thermal stratification. Since the epilimnion and the hypolimnion are not mixed during stratification, the metabolic activity of phytoplankton as well as bacteria leads to inorganic nutrient depletion in the epilimnion during the course of summer. In contrast, active OM decomposition releases nutrients and C gases into the hypolimnion and finally results in hypolimnetic anoxia (Salonen et al. 1984). The thermal stratification breaks down in autumn and the nutrients and gases are again spread over the water column.

Effects of humic substances on microorganisms

Lakes with high humic matter content are traditionally termed as dystrophic, and in terms of productivity they resemble oligotrophic rather than eutrophic conditions (Wetzel 2001). Humic lakes are indeed harsh environments for growth and reproduction of photosynthetic

organisms, such as phytoplankton. Since the brownish humic substances diminish light penetration, the photosynthetic production is restricted to the uppermost few metres of the water column (Ilmavirta 1988; Karlsson et al. 2009). Humic acids lower the water pH, which is disadvantageous for many phytoplankton species (Findlay 2003; Jasser et al. 2009). However, due to the low pH (< 6.5), IC in humic lakes is in the form of CO_2. This is important for phytoplankton, since all freshwater species can use CO_2 as their source of C, but only a few species are able to employ the bicarbonate (HCO_3^-) or carbonate (CO_3^{2-}) which predominate at higher pH and are available for many marine phytoplankton species (Wetzel 2001, Cassar et al. 2004, Reinfelder 2011). In addition to acids, the humic substances are comprised of neutral and alkaline compounds that bind inorganic and organic nutrients and make them unavailable for autotrophic phytoplankton (Peuravuori & Pihlaja 1999). However, inorganic nutrients are released in decomposition of OM, especially in the hypolimnion during the thermal stratification, and since most of the phytoplankton species in humic lakes are motile, they are capable of vertical migrations between the nutrient-rich hypolimnion and the illuminated epilimnion (Jones 1988; Lepistö & Rosenström 1998). Therefore, detailed investigations have shown that the productivity in humic lakes may not be as low as first thought (Wetzel 2001). Although the high amount of brownish DOC acts against the photosynthetic organisms, the growth of heterotrophic bacteria is favoured (Jones 1992). Nevertheless, the easily available (labile) part of the allochthonous OC is often already degraded in the catchment, and therefore bacteria in humic lakes are at least partly dependent on phytoplanktonic EOC (Kuuppo-Leinikki & Salonen 1992; Tranvik 1992; Tulonen 1993; Arvola et al. 1996).

1.4 Predicted effects of climate change

The physical and chemical properties of lakes are modified by local weather conditions, and therefore the phytoplankton community composition, biomass and the timing of the phytoplankton blooms are mediated by climatic forces (Moss et al. 2003). Climate change, with resulting global warming, affects the hydrology, and thus changes in precipitation as well as in evaporation are expected in the Northern Hemisphere (Intergovernmental Panel on Climate Change, IPCC, 2007). In Finland, the annual mean temperature and precipitation have been increasing, compared with the baseline period of 1961–1990, and the projected increases by 2020 are 1–3 °C and up to 15%, respectively (Jylhä et al. 2004). The increase in precipitation will probably increase lake DOC and nutrient concentrations, because they are flushed into the lake along with the runoff from the catchment (Vuorenmaa et al. 2006; Bärlund et al. 2009; Einola et al. 2011). The increment in the brown-coloured DOC in lakes will lead to stronger light absorption, and thus shallower thermocline depth and more stable thermal stratification (Péres-Fuentetaja et al. 1999).

In phytoplankton communities, the effects of climate change have most often been linked with the development of the spring phytoplankton crop: an earlier ice-out induces an earlier phytoplankton spring bloom (Adrian et al. 2006). However, warming in general and changes in nutrient concentrations through alterations in hydrology and thermal stratification can affect phytoplankton. For example, De Senerpont Domis et al. (2007) suggested that cyanophyte densities will increase following the temperature rise, whereas chlorophytes and diatoms will not benefit

from warming. Moss et al. (2003), for their part, reported that the abundance of cyanophytes would not change, whereas certain chlorophytes would increase and some, together with cryptophytes, may decrease. Furthermore, Winder (2009) postulated that when the thermal stratification in lakes becomes stronger, the sinking losses of nonmotile phytoplankton species, especially large diatoms, will increase. This will shift the phytoplankton communities towards smaller species, and it may also favour flagellates, which are not as dependent on the water column instability as the nonmotile species (Huisman et al. 2002).

2. OBJECTIVES OF THE PRESENT STUDY

In this thesis, I try to gain a deeper knowledge and understanding of phytoplanktonic life in boreal humic lakes. My special emphasis is on the traits of cell size and motility, and all my studies were performed *in situ*. I focus on the consequences of dark water colour, strong thermal summertime stratification and epilimnetic nutrient depletion, all of which are limnological parameters prone to change as a result of the ongoing climate change. I also try to couple the phytoplankton with the unicellular grazers in the MFW and determine if there is a trophic cascade in the food web from planktivorous fish to large phytoplankton and the MFW, including APP.

The thesis begins with a study (I) of the importance of various phytoplankton size-classes in autotrophic PP (IC uptake) and EOC release in the small, humic and deeply stratified Lake Valkea-Kotinen. The following two studies (II and III), also conducted in Lake Valkea-Kotinen, emphasize the abiotic drivers behind the dynamics of the annual and interannual changes in APP (II) and three dominant

(in biomass) phytoplankton taxa (III). These three taxa belong to the nanoplankton (2–20 µm) and microplankton (> 20 µm) size-classes and, in contrast to APP, are flagellated and thus have survival strategies different from those of APP. To consider the effects of the climate change-related processes on phytoplankton and further on pelagic food webs, the phytoplankton dynamics in II and III are coupled with meteorological and climatic drivers. These include precipitation, water temperature and stability of the water column. The fourth study (IV) associates phytoplankton with their competitors and grazers in the MFW of Lake Valkea-Kotinen. This study focuses on the possible differences between the epilimnion and hypolimnion, i.e. the oxic and anoxic parts of the water column. Finally, the perspective is expanded to food web interactions in general and the trophic cascade from planktivorous fish to phytoplankton and the MFW, and further to surface water CO_2 concentration (IV). The last study was conducted as an enclosure experiment in two large boreal lakes, i.e. in the humic Lake Pääjärvi and the clearwater Lake Vesijärvi, and thus, there was an opprtunity to compare a brown-water and clearwater lake.

The main questions in this thesis are:

- Do cell size and motility matter; which phytoplankton taxa are dominant in production, abundance and biomass in a strongly stratified humic lake with epilimnetic nutrient depletion? (I–III)
- Are there climatic or climate-mediated drivers behind the dynamics of phytoplankton? (II–IV)
- What is the role of phytoplankton, including APP, in the MFW in a strongly stratified humic lake with an anoxic hypolimnion? (I, IV)

- Do fish regulate phytoplankton, including APP, and furthermore the surface water CO_2 concentrations via trophic cascades in boreal lakes? (V)

3. MATERIAL AND METHODS

3.1 Study sites

Lake Valkea-Kotinen – a small and sheltered humic lake

Lake Valkea-Kotinen, the main study area of this thesis (I–IV), is a small (area 3.6 ha; maximum depth 6.5 m; mean depth 2.5 m), humic, acidic headwater lake located in the Evo forest area in southern Finland (61°14´N, 25°04´E; Fig. 2). As a result of the brownish colour, light penetration is poor (Secchi depth 1.4–1.6 m), and thus the photoautotrophic production is restricted to the uppermost 1.5–2.5 m, which also is the depth of the epilimnion (Table 1).

Lake Valkea-Kotinen is ice-covered for 5.5–6 months each year from November to late April or early May. Due to the dark water colour and sheltering by the surrounding forest, the thermal stratification develops shortly after thawing. Even though the lake is regarded as dimictic, the spring overturns are often short or incomplete and the hypolimnion remains anoxic until the autumnal overturn (Fig. 3; Salonen et al. 1984; Huotari et al. 2009). In addition to temperature, the lake is also chemically stratified during summer. The differences in the physical and chemical properties between the epi- and hypolimnion are large; e.g. the summer concentration of dissolved inorganic carbon (DIC) is low
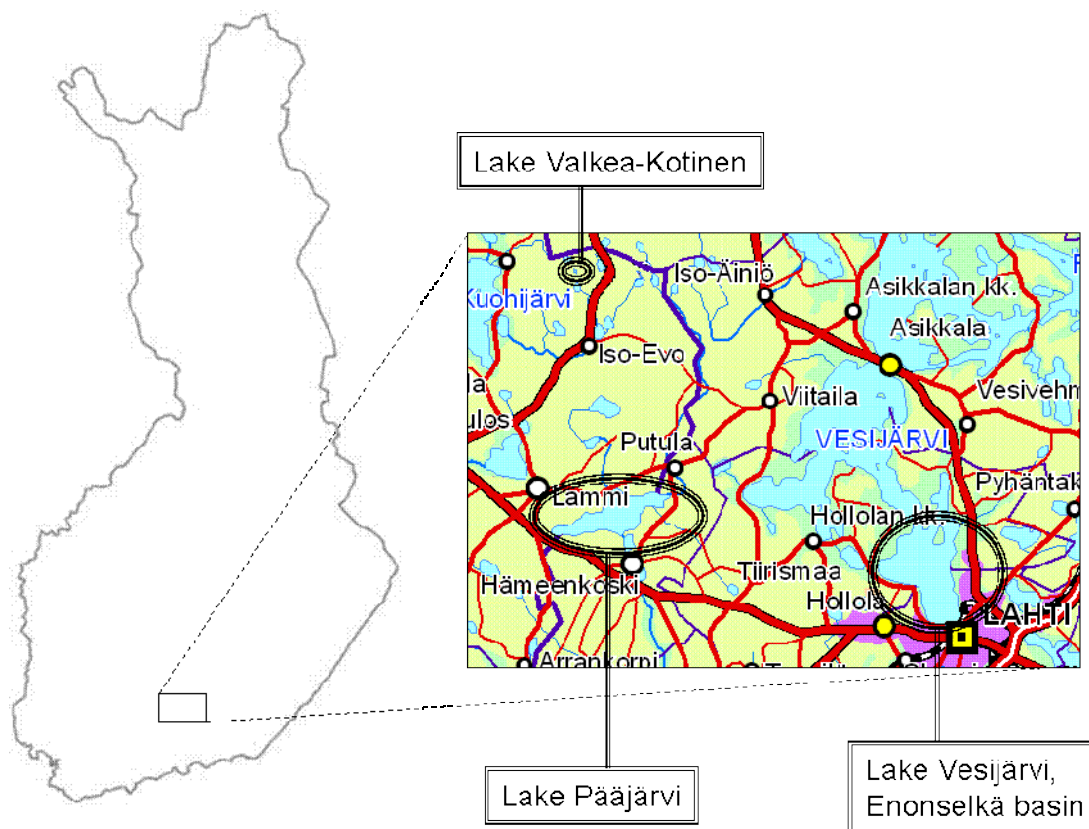

Figure 2. A map of Finland showing the locations of the study lakes, i.e. Lake Valkea-Kotinen, Lake Pääjärvi and Lake Vesijärvi and the Enonselkä basin.

Table 1. Average chemical properties of the epi- and hypolimnion during the open-water periods (May–September) in 1990–1996 in Lake Valkea-Kotinen (data from Keskitalo et al. 1998). ND: not determined.

	pH	Alkalinity eq m^{-3}	Conductivity mS m^{-1}	Colour g Pt m^{-3}	N_{tot} mg m^{-3}	NH_4 mg m^{-3}	NO_3 mg m^{-3}	P_{tot} mg m^{-3}	PO_4 mg m^{-3}	DIC g m^{-3}	DOC g m^{-3}
Epilimnion	5.2	0.005	3.0	137	487	12.1	7.5	18.6	< 2	0.6	11.3
Hypolimnion	5.3	0.053	3.3	162	640	137	12.5	22.9	< 2	5.2	ND
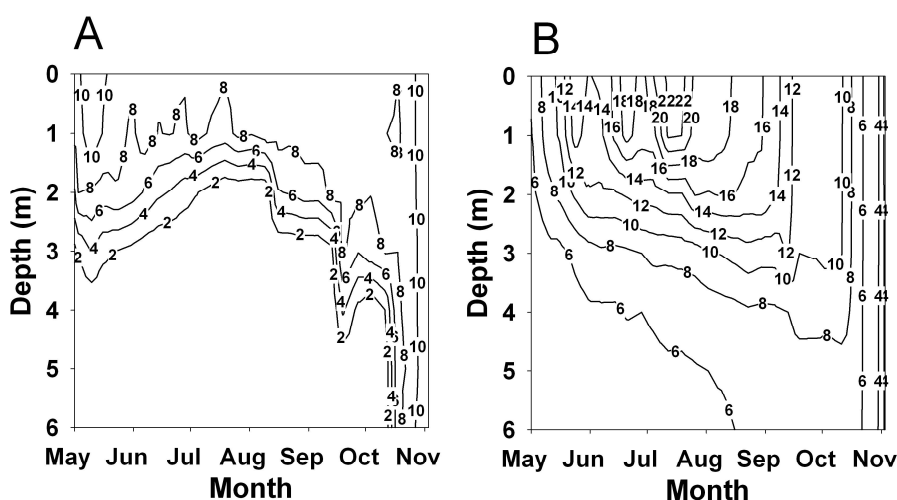
Figure 3. Typical A) oxygen (mg l^{-1}) and B) temperature (°C) profiles in Lake Valkea-Kotinen. The spring overturn is incomplete and thus the hypolimnion remains anoxic until the autumnal overturn. The thermocline is located approximately at 2 m depth throughout the summer. © Oxford University Press.

in the epilimnion but substantially higher in the hypolimnion, and PO$_4$, nitrite and nitrate (NO$_2$&NO$_3$) and NH$_4$ are typically depleted in the epilimnion in early summer (Table 1).

Lake Valkea-Kotinen is surrounded by old-growth forest and located in a nature reserve area (Fig. 4). Therefore, it is regarded as a true reference site (Huotari et al. 2009). However, the area has been exposed to atmospheric acid deposition (Ruoho-Airola et al. 1998), but is now recovering (Moldan et al. 2001). The area belongs to the network of Natura 2000 of the European Union (EU) and the Finnish Long-Term Ecological Research (LTER) network. Lake Valkea-Kotinen is also the first lake in the world from which accurate long-term CO$_2$ flux measurements (eddy covariance (EC) technique) were reported (Huotari et al. 2011). The energy flux and energy balance of the lake were determined with EC as well (Nordbo et al. 2011). This is a great advantage to ecological studies, since many biological processes are either directly or indirectly temperature dependent, and also affects the water column CO$_2$ concentration.

Figure 4. Lake Valkea-Kotinen in the Evo forest area is sheltered by old-growth forest. The red circle illustrates the sampling point. © Ilpo Hakala

Lake Pääjärvi and Lake Vesijärvi – large lakes with contrasting humic matter content

Lake Pääjärvi (61°04´N, 25°08´E; Fig. 2) is a large (area 13.5 km^2), deep (maximum depth 87 m, mean depth 14 m), mesohumic lake (DOC 10 mg l^{-1}; colour 97 mg platinum (Pt) l^{-1}) characterized by fairly low production (Arvola et al. 1996). Light penetration is poor, with Secchi depth of 1.3–3 m (Arst & Reinart 2009). The euphotic zone (~ 4 m) remains thinner than the depth of the epilimnion during the summer stratification. Lake Pääjärvi has shown signs of eutrophication during recent decades, due to agricultural loading from the catchment (Hakala & Arvola 1994). Over 50% of the catchment area around the lake consists of coniferous forests, whereas nearly 20% is in agricultural use (Ruuhijärvi 1974). The rest of the catchment area consists of deciduous forests, peatlands and lakes.

Lake Vesijärvi (61°05´N, 25°32´E; Fig. 2) is larger (area 110 km^2), shallower (maximum depth 40 m, mean depth 6 m) and more eutrophic than Lake Pääjärvi. Lake Vesijärvi is a clearwater lake (DOC 7.3 mg l^{-1}; colour 30 mg Pt l^{-1}), in which the high DOC concentration in contrast to the low colour value indicates that autochthonous colourless DOC is abundant. The depth of the euphotic zone in Lake Vesijärvi equals the depth of the epilimnion (~ 10 m) and the Secchi depth varies between 1.2 m and 3.7 m (Horppila 1997; Arst & Reinart 2009). The lake is almost open, with a low number of islands; thus, it is sensitive to wind-driven mixing. The catchment area consists mainly of forests, agricultural land, waters and peatlands (Rantakari & Kortelainen 2005). The lake is divided into four main basins and the city of Lahti with its approx. 100 000 inhabitants is situated at the southern end of the lake near the Enonselkä basin (surface area 26 km^2, maximum depth 33 m, mean depth 6.8 m); as a whole, urban areas cover 13% of the basin's catchment area (Kairesalo & Vakkilainen 2004). The Enonselkä basin was heavily eutrophicated by sewage waters in the 1960s and 1970s, but was restored to a mesotrophic state in the 1990s by large-

scale biomanipulation through coarse fish removal (Kairesalo & Vakkilainen 2004). However, the lake still suffers from internal nutrient loading and lately the relative proportion of cyanobacteria has increased and late summer blooms have returned.

Both of the lakes are normally frozen from late November until late April or early May, while the maximum water temperature and stable thermal stratification occur between late June and mid-August. Both lakes are also dimictic and complete mixing occurs in May/mid-June and early September. The entire water column of Lake Pääjärvi is well oxygenated throughout the year, while in Lake Vesijärvi, the hypolimnetic hypoxia/anoxia is an annual phenomenon (Bręk-Laitinen et al. 2012).

3.2 Field studies

Plankton analyses

The field studies were focused on Lake Valkea-Kotinen. The samples were in most cases collected weekly during the open-water periods (II–IV). However, for study I, every month one week was chosen for sampling and the samples were collected twice during that week. The sampling point was located in the middle the lake, where a 6-m depth could be reached (Fig. 4). The samples were taken with a 2.1-l or 2.3-l Limnos tube sampler (length 30 cm) (I, II, IV) or 7-l Sormunen tube sampler (length 1 m) (II, III). The sampling depths varied, depending on the scientific question.

The size-factionated phytoplankton PP (I) was measured using the ^{14}C method (Steemann-Nielsen 1952). The 6-h incubations were done *in situ* in the epilimnion, depths 0 m, 0.5 m and 1.5 m. The PP was size-fractioned after incubations sequentially through a net and polycarbonate membrane filters to

obtain the proportions of picoplankton (0.2–2 µm), nanoplankton (2–20 µm), small microplankton (20–50 µm) and large microplankton (> 50 µm). The total amount of phytoplankton exudates (EOC) was measured from the final filtrate. Chlorophyll *a* (Chl *a*) was size-fractionated similarly to PP, and hot extracted with ethanol (Keskitalo & Salonen 1994). Further details on size-fractionated PP and Chl *a* as well as EOC measurements can be found elsewhere (I).

Samples for APP (II, IV) and ciliate (IV) enumerations were collected from throughout the water column (0–6 m) at 1-m intervals, whereas the bacterial abundance and NFs (IV) were counted from 0 m, 2 m, 3 m and 5 m. The phytoplankton biomass samples (III) were collected from the surface (0–1 m) from two sampling points. Total Chl *a* was collected simultaneously with the biomass samples, but from every metre between 0 m and 5 m (II). All the community samples, except APP, were preserved immediately in the field with acid Lugol's solution (III, IV). The APP samples were kept in darkness in crushed ice until determined within 4 h (II, IV). The APP, bacteria and NFs were enumerated under an epifluorescence microscope (II, IV). For counting, subsamples were filtered onto black polycarbonate membrane filters with a pore size of 0.2 µm (APP and bacteria) or 1.0 µm (NFs). APP detection was based on Chl *a* autofluorescence (Davis & Sieburth 1982), whereas acriflavine staining was used for bacteria and NFs (Bergström et al. 1986). The phytoplankton community composition (I, III) and ciliates (IV) were determined with inverted microscopy, using the settling chamber technique (Utermöhl 1958). The ciliates were grouped according to their feeding preferences into algivorous, bacterivorous, omnivorous and predatory ciliates (see IV for further details). The phytoplankton

biomass was calculated as wet weight, using unpublished values of the Finnish Environment Institute for phytoplankton volumes. In addition to the total phytoplankton biomass, three phytoplankton taxa, i.e. *Cryptomonas* spp. (size 12–30 µm), *Peridinium* spp. (20–40 µm) and *Gonyostomum semen* (> 50 µm) were examined in further detail (III). The samples for total Chl *a* were filtered onto GF/C fibreglass filters and hot-extracted with ethanol (Keskitalo & Salonen 1994). Further details on microscopy and the Chl *a* determinations are found in the original articles (I-IV).

Environmental data

Most of the environmental data were collected as a part of the International Cooperative Programme on Integrated Monitoring of Air Pollution Effects on Ecosystems (ICP IM), which has been carried out in Lake Valkea-Kotinen since 1990 (Keskitalo et al. 1998). The water temperature and dissolved O_2 concentration were measured in the field at 1-m intervals from 0 m to 6 m with a portable temperature-O_2 meter. The samples for chemical water analyses, i.e. water colour, pH, conductivity, NH_4, NO_2&NO_3, total nitrogen (N_{tot}), PO_4, total phosphorus (P_{tot}) and DIC concentrations were taken with a Limnos tube sampler from 0 m, 1 m, 2 m, 3 m and 5 m. DOC was detected from the pooled surface sample (0 m and 1 m).

Conductivity and pH were measured in the laboratory on the day of sampling. The samples for NH_4, NO_2&NO_3, PO_4, DOC and water colour were filtered through GF/C filters. The colour was determined spectrophotometrically at 420 nm immediately after filtration, and all the other samples were stored frozen before the analyses, as were the unfiltered samples for N_{tot} and P_{tot}. The nutrient determinations were based on

colorimetric assays (D'Elia et al. 1977; Grasshoff 1983; Koroleff 1983), whereas DOC and DIC were determined with infrared C analysers (Salonen 1981). For further details, see I–IV.

The precipitation data (II, III) were obtained from Lammi Biological Station of the University of Helsinki, app. 30 km from the study lake, where they were measured by the Finnish Meteorological Institute. The timing of ice-in and ice-out was observed *in situ* with an accuracy of 1 day. Lake Valkea-Kotinen was considered ice-free when there was only some ice near the shoreline, and frozen when it was completely covered by ice. The completeness of the spring overturn as well as the length of the stratified period was interpreted, based on temperature and O_2 measurements. The intensity of thermal stratification was estimated as the Brunt-Väisälä thermal stability, i.e. N_s (II), or by subtracting the bottom temperature from the surface temperature (III).

3.3 Mesocosm experiment

Experimental design and sample analyses

The enclosure experiments with fish (V) were carried out in 2005 in Lake Vesijärvi and in 2006 in Lake Pääjärvi and lasted 3 weeks and 4 weeks, respectively. The transparent enclosures were constructed in three blocks of two, i.e. the enclosures with fish and without fish were run in triplicate. The top hoop was suspended above the water surface from a floating plastic framework that offered protection from high waves, but the water in the enclosures was exposed to the atmosphere. The bottom hoop was weighted to sink, but had no contact with the sediment at the bottom. In Lake Vesijärvi, the framework with the enclosures was anchored in the Enonselkä basin 200 m from the nearest shore.
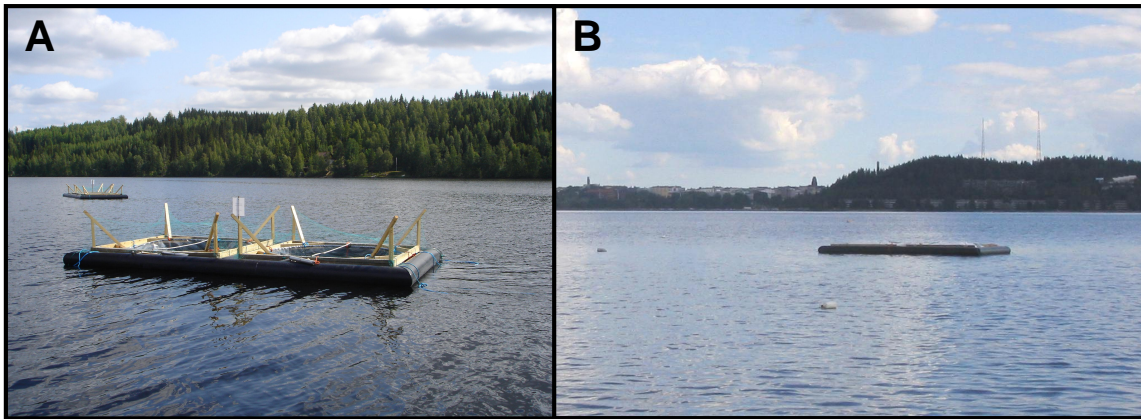
Figure 5. A) The enclosure drafts in Lake Pääjärvi were located in the forest-surrounded western end of the lake. B) In Lake Vesijärvi the drafts were lacated in the Enonselkä basin. The city of Lahti is shown in the background. © Jussi Huotari

In Lake Pääjärvi, the experimental site was about 100 m from the shore at the western end of the lake (Fig. 5). The fish introduced into the enclosures were small (8–11 cm) 1-y-old zooplanktivorous European perch (*Perca fluvialitilis* L.) (Horppila et al. 2000), and the fish density represented the fish stock in the lakes investigated. Enclosures were installed 1 week before adding the fish and filled manually with the ambient surface lake water.

Sampling was done twice per week. The water temperature and dissolved O_2 concentration were measured with a temperature-compensated dissolved O_2 meter at 50-cm intervals throughout the water column in every enclosure. The secchi disc transparency was also measured. Samples for the CO_2 determinations were taken into polypropylene syringes from the water surface (0 m). After removing any gas bubbles, the syringes were closed and kept in crushed ice until analysis. The CO_2 samples were analysed in the laboratory using the headspace technique and gas chromatograph (GC) equipped with a flame ionization detector and methanizer. Samples for water chemistry, i.e. P_{tot}, PO_4, N_{tot}, NO_2&NO_3, NH_4, DOC, as well as primary (^{14}C method; 24-h

incubations; Steemann-Nielsen 1952) and bacterial productivity (^{14}C-leucine method; 1-h incubations; Tulonen 1993) and microbial abundance were taken with a 1-m long Limnos tube sampler (volume 7 l) at 1-m intervals extending throughout the depth of the enclosures. The samples for community composition of phytoplankton and zooplankton were only taken at the onset and at the end of the experiment. The fish were removed at the end of the experiment and dissected to confirm their feeding on zooplankton. The water and plankton samples were in general analysed as in the field studies in Lake Valkea-Kotinen. Further details on the experimental design and the sample analyses can be found elsewhere (V).

3.4 Statistical analyses

Pearson's correlation analysis was used in four cases: 1) to determine the relationships between the APP dynamics and the abiotic factors, as well as the larger phytoplankton in Lake Valkea-Kotinen (II); 2) to determine the relationships between the ice-out, spring overturn, length of the stratified period, intensity of thermal stratification and the environmental parameters measured and the timing and height of the maximal phytoplankton biomass as well as the

biomasses of the three flagellated phytoplankton taxa in Lake Valkea-Kotinen (III); 3) to explore in Lake Valkea-Kotinen the relationships between the possible prey organisms of the MFW and the abiotic environmental factors as well as the relationships between the prey and the grazers/predators (IV); 4) to determine in the mesocosm experiment the relationships between the chemical variables (C, N, P) and fish and the abundance or biomass of the microbial community and dominant groups of phytoplankton (V).

Linear regression analysis was used to identify the importance of water column stability to APP abundance in Lake Valkea-Kotinen (II). A *t*-test was used for zooplankton and phytoplankton results (V), otherwise analysis of variance (ANOVA) with its several modifications, i.e. 1) multivariate analysis of variance (MANOVA) (I); 2) one-way ANOVA (II–IV); 3) repeated measurements ANOVA (V) was used for detecting the statistical significance of the results obtained. The homogeneity of the variances was tested with Levene's test and the normality of the experimental data was tested with the Kolmogorov–Smirnov goodness-of-fit test. When needed, log or square-root transformations were used to normalize the distribution of the data before the statistical analyses. However, since some variables did not meet the requirements of ANOVA even after transformations (V), time-weighted averages (WAs) were used instead. When the hypotheses were tested, *p*-values below 0.05 were considered as significant.

The long-term changes in the total phytoplankton biomass as well as in the biomass of three flagellated phytoplankton taxa were analysed with time series analysis (Census II method) and the Mann-Kendall (MK) test, and the relative importance of the various

environmental parameters on these taxa as well as the total phytoplankton was examined with partial least square (PLS) regression analysis. In the MK test *p*-values below 0.05 were considered as significant (III). Canonical correspondence analysis (CCA) was applied to link the ciliate abundance data and the environmental parameters, including APP and Chl *a* (IV).

Software used for the analysis included SPSS for Windows, versions 14.0, 15.0 and 18.0 (SPSS Inc., Chicago, IL, USA) and PAST program, version 2.12 (Hammer et al. 2001).

4. RESULTS AND DISCUSSION

4.1 Abiotic conditions in Lake Valkea-Kotinen in 1990–2006

Ice-out in Lake Valkea-Kotinen occurred in late April or early May during the study years. There was an increasing frequency of incomplete overturns from 1990 to 2006; the spring overturn was incomplete in 1993, 1998, 1999 and 2002–2006. The incomplete mixing prevented nutrient supply from the nutrient-rich bottom to the surface, and there was a clear gradient between the epilimnion and hypolimnion, especially in the NH_4 and DIC concentrations (Fig. 3 in I; Table 1 in II). Similarly, the hypolimnetic anoxia that develops during winter was not completely removed and thus at least part of the hypolimnion was already anoxic at the onset of summer (Fig. 1 in I; Table 1 in II; Fig. 1 in IV). However, the metalimnetic nutrient and O_2 gradient in Lake Valkea-Kotinen was regularly disturbed diurnally by convective mixing that resulted in variations of up to 0.5 m in the thermocline depth and showed greatest amplitude during the strongest thermal stratification (Nordbo et al. 2011). It is

Table 2. Average phytoplankton primary production (PP), chlorophyll a (Chl a) concentration and extracellular organic carbon (EOC) release in the epilimnion, i.e. 0 m, 0.5 m and 1.5 m, in Lake Valkea-Kotinen in 2005. Standard errors of means are given in parentheses. * indicates significantly different values compared with the other depths (ANOVA $p < 0.01$).

| depth m	PP mg C m^{-3} h^{-1}	Chl a mg m^{-3}	EOC mg C m^{-3} h^{-1}
0	24.1* (5.03)	20.4 (3.30)	2.27* (0.17)
0.5	14.3* (4.38)	18.2 (2.63)	1.16* (0.40)
1.5	0.89* (0.37)	13.8* (0.80)	0.24* (0.07)

also worth noting that the PO_4 concentration in Lake Valkea-Kotinen was low not only in the epilimnion, but throughout the water column (Fig. 3 in I; Table 1 in II; Table 1 in IV).

4.2 Phytoplankton PP, Chl *a* and EOC release

Phytoplankton production in Lake Valkea-Kotinen was substantially higher at the surface than deeper down in the epilimnion ($p < 0.01$; Table 2), and the epilimnetic values resembled those of eutrophic rather than oligotrophic lakes (Lande 1973; Schmitt & Nixdorf 1999). Furthermore, Huotari et al. (2011) showed that the vigorous PP in the epilimnion resulted occasionally in water CO_2 concentrations under atmospheric equilibrium, i.e. the lake was a sink of CO_2, which is not often reported for boreal humic lakes. There was a clear seasonal pattern in PP with a maximum in June and a gradual decrease towards autumn (Fig. 4 in I). These dynamics were mainly due to the two largest size fractions: the small and large microphytoplankton (size 20–50 µm and > 50 µm, respectively). In May and October, most PP was from small microphytoplankton, and more precisely *Peridinium* dinoflagellates (37–73% and 79–96% of the total PP, respectively). The large microphytoplankton, mainly *G. semen*, were the dominant primary producers (46–72% of the total PP) from late June until late September; at that time the bulk of the phytoplankton biovolume-based biomass also consisted of *G. semen* (Fig. 6). The nanophytoplankton, whose cell numbers were highest among the phytoplankton (> 2 µm), had productivity only one-third of that of the large or small microphytoplankton. The percentage of APP in production was the lowest among the different size-classes. Nevertheless, in September the APP replaced the large microphytoplankton as the most important producers at 1.5 m and 57–64% of the PP was of APP origin (Fig. 6). This was not because of expansion in APP production, but because the production of the other size-classes decreased. However, it demonstrates the APP's capability to survive at low light levels (Callieri & Stockner 2002; Callieri 2007).

Phytoplankton, regardless of their size, should to a certain extent be able to compensate for poor light conditions by increasing the chlorophyll content of the cell and thus to maintain a constant C fixation rate at low photon flux density (Reynolds 2006). In Lake Valkea-Kotinen, the total amount of Chl *a* did not differ between 0 m and 0.5 m, but was lower at 1.5 m ($p < 0.01$; Table 2). Chl *a* at 0 m and 0.5 m decreased to the same level as at 1.5 m after the maxima of phytoplankton production and biovolume-based biomass (Fig. 6 in I). On average, the Chl *a* values were similar to those in eutrophic lakes (Wetzel 2001).
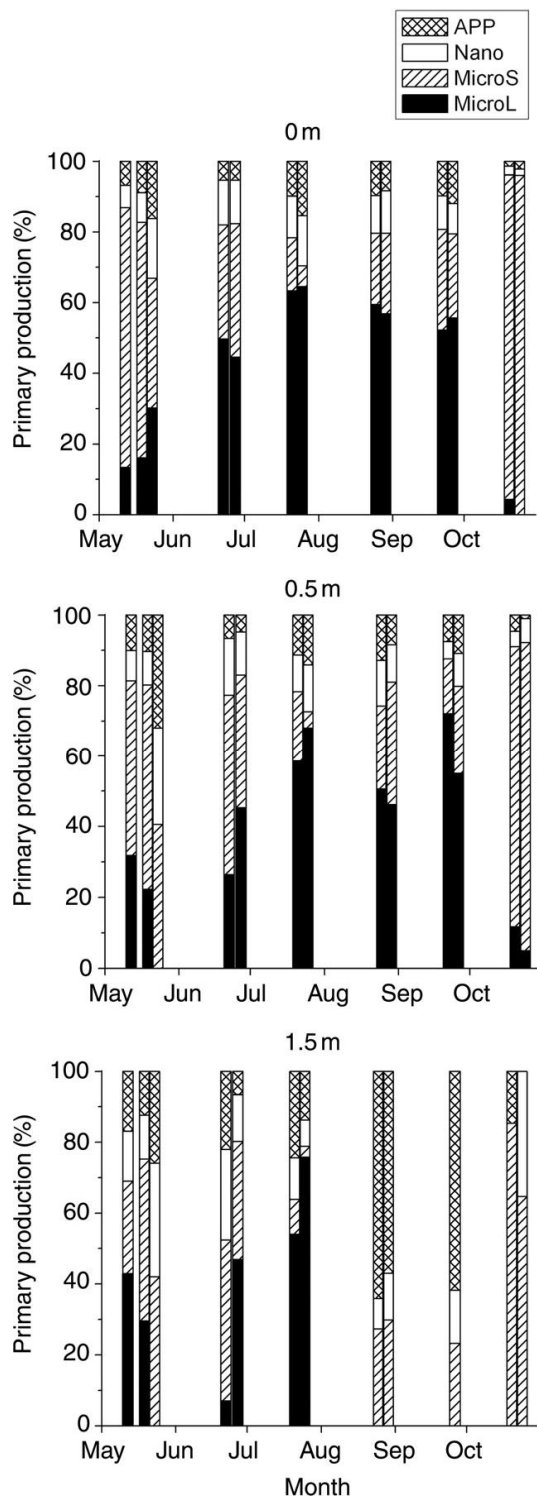
Figure 6. Relative contribution (%) of various phytoplankton size-classes to primary production in Lake Valkea-Kotinen at 0 m, 0.5 m and 1.5 m. The size-classes are: APP 0.2–2 µm, Nano 2–20 µm, MicroS 20–50 µm and MicroL > 50 µm. © Oxford University Press.

However, the rather constant Chl *a* concentration in the epilimnion implies that phytoplankton in Lake Valkea-Kotinen were already at the upper limit of adaptation and no longer able to increase their cellular content of Chl *a*. Furthermore, the volumetric PP values were high in the upper epilimnion, but the phototrophic layer was shallow, and thus the phytoplankton were most probably under light limitation. This agrees with Karlsson et al. (2009), who showed that due to high DOC concentrations, small boreal lakes are mainly light-limited. In general, small microplankton were the most important contributors of Chl *a*, with a mean proportion of 36%. Nanoplankton and APP had mean proportions of 28% and 23%, respectively, whereas large microplankton, i.e. *G. semen*, had the lowest proportion (14%; Fig. 7 in I). There were some seasonal changes in the distribution of Chl *a* between the size-classes, i.e. occasionally from May to July up to 72% of the Chl *a* originated from the small microplankton, but otherwise the differences among the size fractions were minor and considerable only in APP after the summer solstice in July (Fig. 6; Fig. 7 in I). The increase in Chl *a* in the APP did not result in enhanced PP of this size-class, but the proportion of APP increased later in summer and autumn, especially at 1.5 m, i.e. close to the oxic-anoxic boundary, where there is little light available for photosynthesis.

The chlorophyll-specific photosynthetic rate, i.e. PP:Chl *a* ratio, varied widely in every size-class and at all three depths, but was in general highest near the surface and lowest at 1.5 m (Fig. 8 in I). The dominant primary producer, *G. semen*, was also superior in terms of the PP:Chl *a* ratio (Fig. 8 in I), i.e. it had a high level of production with a low amount of Chl *a*. The chlorophyll-specific photosynthetic rates of *G. semen* were so

high that they alone equalled the total production of all phytoplankton in some eutrophic lakes (Schmitt & Nixdorf 1999). This excellence probably stems from the ingenious morphology of the species; the chloroplasts of *G. semen* are arranged in a tight layer immediately under the cell membrane, which enables maximal light harvesting (Coleman & Heywood 1981). A bit surprisingly, APP were not as effective as *G. semen* in terms of PP:Chl *a*. However, occasionally in August and September at depth of 1.5 m, the APP had PP:Chl *a* ratios higher than the other phytoplankton size fractions (Fig. 8 in I).

The amount of EOC varied temporally as well as spatially, but in general there was more EOC in the surface than deeper down in the epilimnion (Table 2; Fig. 9 in I). The highest EOC production rate coincided with the highest total PP, and also the highest PP of *G. semen*, in July and the lowest with the lowest PP in October (Fig. 6; Figs. 4 and 9 in I). The proportion of excreted PP (PER) was low in June, when *G. semen* began to dominate the phytoplankton biomass, but increased considerably (up to 34% at 0 m, to 27% at 0.5 m and to 40% at 1.5 m) in July–August when the production of *G. semen* as well as the total PP began to slowly decrease (Figs. 4 and 9 in I). This is in accordance with Berman & Holm-Hansen (1974) who observed the lowest PER values when the phytoplankton population increased exponentially, and Lancelot & Billen (1984) who detected high PER values at the end of the phytoplankton blooms. In Lake Valkea-Kotinen, the PER was on average 1.5–2 times higher near the light-limited zone than at the surface ($p < 0.01$), which was also observed by Berman & Holm-Hansen (1974) as well as Watanabe (1980) at low light intensities. Even though APP are known to excrete several times more EOC than larger phytoplankton (Malinsky-Rushansky &

Legrand 1996), the timing of high EOC or PER values could not be coupled with APP in Lake Valkea-Kotinen.

4.3 Seasonal and interannual dynamics of APP and larger phytoplankton

Different phytoplankton size-classes may compete with each other for inorganic nutrients and light (Jasser 1997). In Lake Valkea-Kotinen, the APP showed a bimodal pattern during the study of five open-water periods, but there was no clear seasonality in the dynamics (Fig. 7). However, the APP maxima usually occurred either before or after the blooms of larger phytoplankton (measured as Chl *a*), suggesting that the seasonal pattern of APP was different from that of larger phytoplankton (Fig. 5 in II). There were two groups of APP in Lake Valkea-Kotinen: the solitary APP consisting of morphologically identical, ellipsoidal, red-fluorescing *Choricystis* (Skuja) Fott (Trebouxiophyceae Friedl)-type eukaryotic cells and the cyanobacterium *Merismopedia warmingiana* Lagerheim, which is colonial, but according to its cell size fits into the group of APP. The mean cell numbers of these two APP groups varied remarkably on annual basis (Table 2 in II). In general, *M. warmingiana* dominated the APP in 2002 with the average cell numbers of 22.9 x 10^3 cells ml^{-1}, but decreased suddenly, and the solitary eukaryotic APP then increased from 5.8 x 10^3 cells ml^{-1} to 14.7 x 10^3 cells ml^{-1}. Even after the increase, the mean abundance of solitary APP was 10- to 100-fold lower than the APP abundance reported from the lakes in this region, but the cell numbers observed were in accordance with a previous study from Lake Valkea-Kotinen (Jasser & Arvola 2003). On average, 82% of the APP in Lake Valkea-Kotinen were in the uppermost 4-m layer ($p < 0.02$; Fig. 7), which can be explained through isopycny that prevents these nonmotile organisms
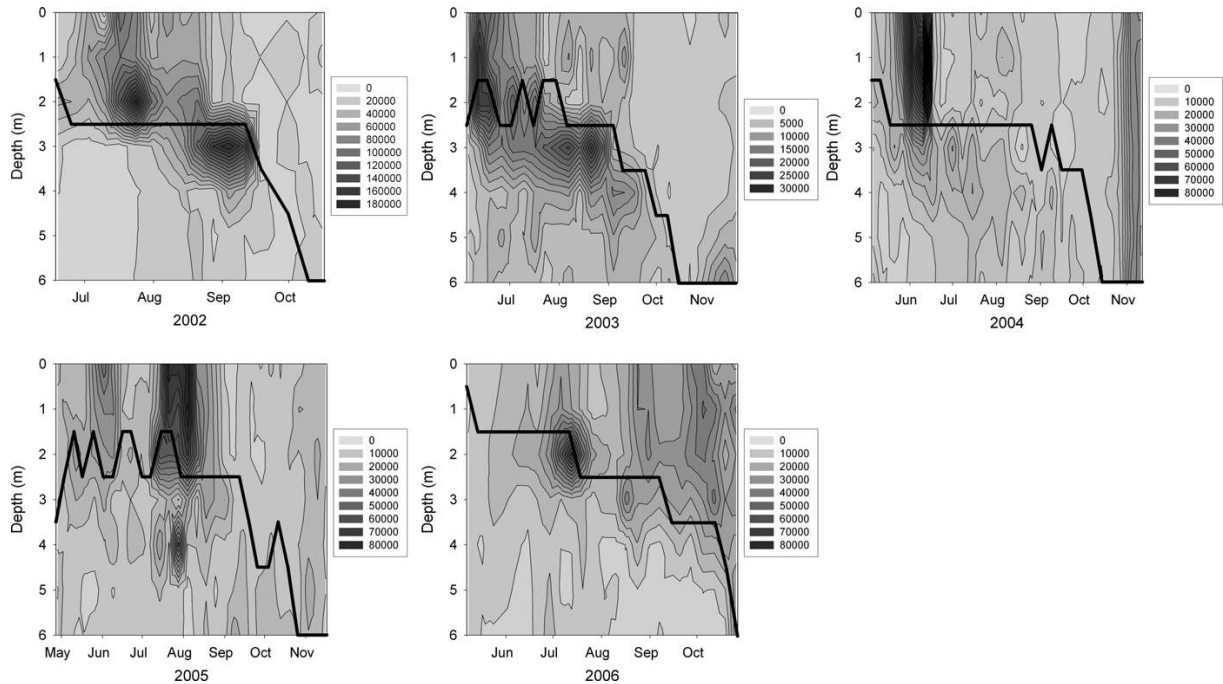
Figure 7. Total autotrophic picoplankton (APP; cells ml^{-1}) in Lake Valkea-Kotinen in 2002–06. The black lines demonstrate the thermoclines. Note the different scales on the z-axis. © Blackwell Publishing Ltd.

from sinking below the metalimnion. The isopycnic or near-isopycnic state can be achieved either by being very small or with the aid of special structures such as mucilage (Reynolds 2006). In Lake Valkea-Kotinen the solitary APP fulfil the first requirement, whereas the colonies of *M. warmingiana* are held together by a mucilaginous matrix.

In contrast to APP, the bulk of the larger phytoplankton (> 2 µm) in Lake Valkea-Kotinen were flagellated and thus capable of retrieving nutrients from the hypolimnion and returning to the epilimnion to photosynthesize (I, III). The phytoplankton community consisted of app. 180 taxa (III), all of which are typical of acidic boreal humic lakes (Lepistö & Rosenström 1998), i.e. Chrysophyceae (especially the genera *Dinobryon*, *Monochrysis*, *Pedinella*, *Uroglena*), Dinophyceae (*Gymnodinium*, *Peridinium*), Diatomophyceae (*Asterionella*, *Rhizosolenia/Urosolenia*),

Cryptophyceae (*Cryptomonas*), Chlorophyceae (*Chlorogonium*, *Chrysocapsa, Oocystis*), Cyanophyceae (*Cyanodictyon, Merismopedia, Snowella*) and Raphidophyceae (*G. semen*). However, most of the taxa appeared only occasionally and were low in number. The most abundant taxa > 2 µm in size were *Pedinella* sp. with 12.3 x 10^2 cells ml^{-1} (32% of the total abundance) and *Monochrysis parva* Skuja with 8.8 x 10^2 cells ml^{-1} (23% of the total abundance) (I, Peltomaa unpubl.). These numbers are app. 10 times lower than the average abundance of eukaryotic APP in Lake Valkea-Kotinen (II).

The seasonal succession of the biomass of phytoplankton > 2 µm in size in Lake Valkea-Kotinen showed a pattern of two maxima; the first, sharper maximum occurred in May and the second, wider and higher maximum during the strongest stratification in July–August (Fig. 1A in III). It is noteworthy that there was no

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distinct clearwater phase, but the decline after the spring peak was followed by a sinusoidal biomass succession. On average, the total phytoplankton biomass in the surface (0–1 m) in 1990–2003 was 2.09 g m^{-3} (range 1.41–2.87 g m^{-3}), which indicates a mesoeutrophic or eutrophic state (Wetzel 2001). However, there was a trend toward decrease in the total biomass, with a slope of -0.014 g m^{-3} yr^{-1} ($p = 0.05$; Fig. 8A). On average 48% of the biomass was comprised of *G. semen* and - as normal in lakes dominated by *G. semen* (Cronberg et al. 1988; Lepistö et al. 1994; Willen 2003) - the bulk of the biomass consisted mainly of very few taxa. The *Peridinium* dinoflagellates comprised most of the biomass in May and in September–October (average 0.37 g m^{-3}; 18% of the total biomass) and *G. semen* in July–August (average 1.00 g m^{-3}) (III). Similar to the total biomass, the biomass of *Peridinium* spp. and *G. semen* decreased (slopes -0.006 g m^{-3} yr^{-1}, $p < 0.001$ and -0.024 g m^{-3} yr^{-1}, $p = 0.02$, respectively; Fig. 8). However, the biomass of the third most closely studied taxa, i.e. *Cryptomonas* spp., increased slowly, but in a statistically nonsignificant manner (slope = 0.0006 g m^{-3} yr^{-1}, $p = 0.29$; Fig. 8D). *Cryptomonas* spp. showed seasonal abundance patterns very similar to those of *Peridinium* dinoflagellates (Fig. 1 in III). However, the biomass amount of *Cryptomonas* spp. was only 0.02 g m^{-3} and they were responsible for ~ 1% of the total phytoplankton biomass. None of these three more closely studied taxa had proportions higher than 1.3% of the total phytoplankton cell numbers (I).

4.4 Meteorological drivers of phytoplankton and climate change

In temperate and boreal lakes, the effects of climate change have most often been linked with the development of the spring phytoplankton crop: an earlier ice-out induces an earlier phytoplankton spring bloom (Adrian et al. 2006). Warming in general can also affect the phytoplankton composition (Moss et al. 2003; De Senerpont Domis et al. 2007). In Lake Valkea-Kotinen, the timing of ice-out or the completeness or duration of the spring overturn did not affect the build-up of total phytoplankton biomass or the biomass of *G. semen*, *Peridinium* spp. or *Cryptomonas* spp. (Table 1 in III). However, weekly sampling was probably insufficient for detecting the rapid changes in the environment and in the biota in spring. The surface water temperature in Lake Valkea-Kotinen showed a statistically nonsignificant trend towards slight increase (0.029 °C yr^{-1}, $p = 0.48$) during the 14-yr study period in 1990–2003. The total phytoplankton biomass and *G. semen* correlated positively with temperature ($r = 0.35$, $p < 0.001$ and $r = 0.31$, $p < 0.001$, respectively; III), and there was also a positive correlation ($r = 0.20$, $p < 0.001$) between the colonial APP, i.e. *M. warmingiana*, and temperature in 2002–2006 (IV). However, the correlation between temperature and *Peridinium* spp. biomass was negative ($r = -0.25$, $p < 0.001$; III). The PLS analysis supported the results from the correlation analysis, i.e. the total phytoplankton biomass and the biomasses of *G. semen* and *Peridinium* spp. were related to temperature (Fig. 5 in III). These results suggest that the future increase in temperature could cause decline in *Peridinium* dinoflagellates, some of which thrive at low temperature (Grigorsky et al. 2003). However, the positive correlation with temperature and the other taxa probably reflects the annual increase in solar radiation, which enhances phytoplankton growth, but also increases surface water temperature and thus strengthens the thermal stratification. The latter may also have had a positive effect on total phytoplankton, *G. semen* and *M. warmingiana* (see below).
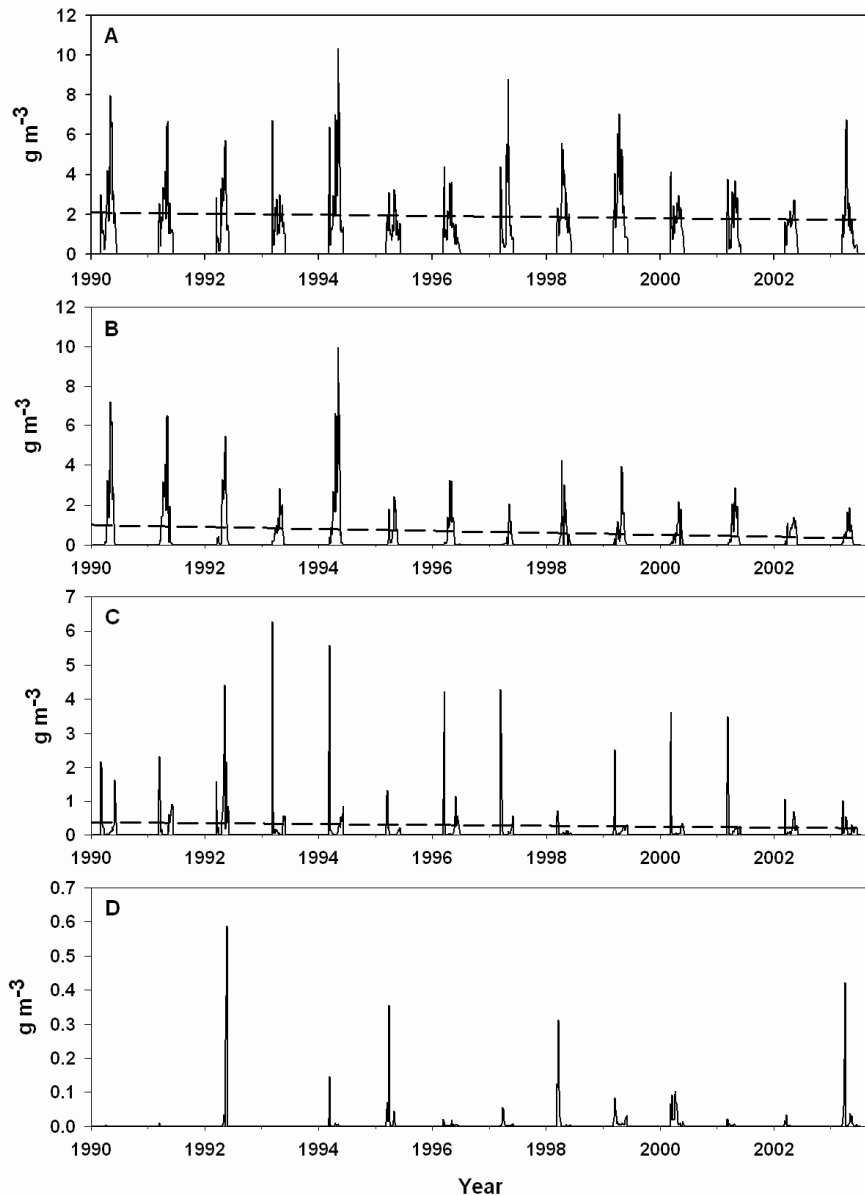
Figure 8. Phytoplankton biomass (solid lines) and the deseasonalized trends (dashed lines) during1990–2003 in Lake Valkea-Kotinen ($n = 728$). A) Total phytoplankton (trend -0.014 g m^{-3} yr^{-1}, $p = 0.05$), B) *Gonyostomum semen* (trend -0.024 g m^{-3} yr^{-1}, $p < 0.001$), C) *Peridinium* spp. (trend -0.006 g m^{-3} yr^{-1}, $p = 0.02$) and D) *Cryptomonas* spp. (no trend, $p = 0.29$). Note the different scales on the Y-axis. © Boreal Environment Research.

As a consequence of the climate change, precipitation is probably increasing in Finland (Jylhä et al. 2004). Indeed, the summer precipitation in the study area increased (0.055 mm yr^{-1}, $p < 0.001$; Table 2 in III) in 1990–2003 (III). However, neither the larger nor the pico-sized phytoplankton could be directly associated with precipitation (II, III). Despite the increased summer precipitation, the annual precipitation decreased by a rate of -0.066 mm yr^{-1} ($p < 0.001$; Table 2 in III). This indicates decreasing snowfall and suggests that the spring load from the catchment is declining. When the spring mixing of the water column is incomplete, as now seen more frequently in Lake Valkea-Kotinen, the importance of spring runoff as the way to replenish nutrient reserves is

emphasized. The combination of incomplete mixing and decline in spring loading will result in lower amounts of available P and N. In Lake Valkea-Kotinen, the N_{tot} and P_{tot} concentrations declined in 1990–2003 at the rates of -0.149 µg l^{-1} yr^{-1} ($p < 0.001$) and -0.071 µg l^{-1} yr^{-1} ($p < 0.001$), respectively. The average N:P -mass ratio in Lake Valkea-Kotinen was 31:1, which according to the Redfield ratio means that the phytoplankton production is P- rather than N-limited (Redfield 1958). The microphytoplankton, i.e. *G. semen* and *Peridinium* dinoflagellates, were strongly associated with P_{tot} in the PLS models (Fig. 5 in III), and the correlation analysis supported their dependence on P (with P_{tot} $r = 0.69$, $p = 0.01$ and $r = 0.54$, $p = 0.05$, respectively, III). Unfortunately, the dependence of these taxa - or any other phytoplankton species - on PO_4 could not be studied, because in Lake Valkea-Kotinen the PO_4 concentrations were almost undetectable throughout the water column. This, of course, also indicates severe P limitation. However, since PO_4 is released under the anoxic conditions in the hypolimnion, the results imply that the newly released PO_4 is taken up efficiently by phytoplankton or bacteria. On the other hand, it is also possible that some or even a majority of the phytoplankton species in Lake Valkea-Kotinen are capable of obtaining P in organic form, a life strategy called mixotrophy that combines both photoautotrophy and heterotrophy (Raven 1997; Jones 2000). In fact, Rengefors et al. (2008) showed that *G. semen* may ease nutrient depletion via osmotrophy. The eukaryotic APP did not correlate with the nutrients (Table 3 in II), and despite their being nonmotile, they probably obtained enough nutrients, due to very effective nutrient uptake (Currie & Kalff 1984; Thingstad et al. 1993; Kirchman 1994), under conditions of convective mixing (Nordbo et al. 2011). In turn, the colonial non-N-fixing *M. warmingiana* was dependent on the inorganic N (Table 3 in II). *Merismopedia warmingiana* prefers NH_4 over NO_2&NO_3 (Blomqvist et al. 1994), and thus it probably was disfavoured when the dominance of NH_4 shifted to that of NO_2&NO_3 after some heavy rains in 2004 (Table 1 in II).

Arvola et al. (2006) showed that high summer precipitation increases DOC loading from the catchment and results in increased riverine DOC concentrations. In Lake Valkea-Kotinen, the increase in summer precipitation during the study period of 1990–2003 (III) could not be associated with increased loading of OC, probably in part because Lake Valkea-Kotinen is the uppermost lake in a lake chain and thus without visible inflow. A trend towards increase was still detected in the surface water DOC concentration (0.046 mg l^{-1} yr^{-1}, $p = 0.02$) and in water colour (1.22 mg Pt l^{-1} yr^{-1}, $p < 0.001$) in 1990–2003 (III). Similarly, the heavy rains in summer 2004 resulted in higher epilimnetic DOC concentrations and water colour ($p < 0.02$; Table 1 in II). These observations are similar to those reported in Lake Valkea-Kotinen by Vuorenmaa et al. (2006) for the total organic carbon (TOC) concentration in 1987–2003 and by Arvola et al. (2010) for the autumnal water colour in 1990–2007, and may stem from the recovery of the landscape from anthropogenic acidification rather than climate change (Nickus et al. 2010). It has also been suggested that the increase in water colour does not only stem from DOC but also from iron (Fe) (Kritzberg & Ekström 2012), which could also explain the larger increase in water colour than in DOC. Unfortunately, Fe was not included in the ICP IM programme, and thus this cannot be confirmed. Since nutrients can be bound in DOC (Peuravuori & Pihlaja 1999), the elevated soil DOC concentration could have already affected nutrients in the catchment and thus assisted the N_{tot} and P_{tot} decrease in Lake

Valkea-Kotinen. Despite the increment in DOC, the water pH remained stable in 1990–2003 (Table 2 and Fig. 4 in III), but decreased in 2004 ($p < 0.02$; Table 1 in II). The eukaryotic APP and *G. semen* were the only phytoplankton groups studied, which could have been associated with pH ($r = -0.178$, $p < 0.02$ (II) and $r = 0.24$, $p < 0.001$ (III), respectively). The eukaryotic APP were favoured by lowering the pH during 2002–2006, but, since there was no clear trend in pH during the decline of *G. semen* in 1990–2003, the results of *G. semen* were inconclusive.

In general, the increment in DOC and water colour leads to more effective absorption of solar radiation and thus to stronger thermal stratification as well as to light limitation (Salonen et al. 1984; Keller et al. 2006; Karlsson et al. 2009). Stronger stratification is predicted to increase the sinking losses of nonmotile phytoplankton species and thus shift phytoplankton communities towards small and flagellated species (Winder 2009). In Lake Valkea-Kotinen, the biomass of the large flagellated *G. semen* ($r = 0.26$, $p < 0.001$) as well as total phytoplankton biomass ($r = 0.22$, $p < 0.001$) was positively related to the strong thermal stratification (III). However, at the same time the increase in water colour had a negative effect on *G. semen* ($r = -0.29$, $p < 0.001$; Table 2 and Fig. 5 in III) and on total phytoplankton biomass ($r = -0.21$, $p < 0.001$; Table 2 and Fig. 5 in III). As predicted, the high water column stability (N_s) was also an important abiotic factor controlling the nonmotile but tiny APP in 2002–2006 (total APP $r = 0.413$, $p < 0.001$; Table 3 in II). Under low-light conditions, the eukaryotic APP are favoured over the prokaryotic APP (Callieri & Stockner 2002; Callieri 2007), and the eukaryotic APP were also more numerous in Lake Valkea-Kotinen when the water colour increased ($r = 0.112$, $p < 0.02$), whereas the contrasting situation

held for *M. warmingiana* ($r = -0.124$, $p < 0.02$). Despite the increase in water colour after the heavy rains in 2004, the location of the thermocline did not change in Lake Valkea-Kotinen (Fig. 1 in II). However, the Chl *a* maximum, resulting from the larger phytoplankton, was located in the upper water column (Fig. 4 in II), thus indicating that the phytoplankton changed their location in response to deteriorated light conditions. The nonmotile *M. warmingiana* had access to the hypolimnetic NH_4 only in the epilimnion-hypolimnion interface, and thus it probably was under light limitation when the water colour increased. This means that the nonmotile phytoplankton in humic lakes must have their pycnoclines quite close to the lake surface, or otherwise photosynthesis can be severely light-limited (Karlsson et al. 2009; I).

4.5 Role of phytoplankton in the MFW

Competition with bacteria

Due to their better SA:V ratio, bacteria are regarded as more effective in nutrient uptake than phytoplankton (Thingstad et al. 1993; Kirchman 1994). However, the small-sized APP may challenge bacteria in nutrient competition (Cotner & Biddanda 2002). The average abundance of the heterotrophic bacteria in Lake Valkea-Kotinen in 2003 was 2.4×10^6 cells ml^{-1}, i.e. $\sim 10^3$-fold higher than the abundance of APP, and within the range of bacterial abundance in eutrophic lakes (Wetzel 2001). The bacteria and the eukaryotic APP correlated negatively in the epilimnion ($r = -0.493$, $p < 0.001$), indicating competition for nutrients during the epilimnetic nutrient depletion. However, the APP and bacteria correlated positively ($r = 0.359$, $p < 0.05$) in the hypolimnion, and were both most abundant at a depth of 3 m, i.e. in the upper hypolimnion, where nutrients were

available but where light began to limit photosynthesis (Fig. 2 in IV). The bacterial abundance reached a small maximum in the hypolimnion in August during the Chl *a* maximum ($r = 0.752$, $p < 0.01$). More importantly, the bacterial abundance followed the maximum of phytoplankton EOC production, which in Lake Valkea-Kotinen occurred in July–August (I). Thus, the bacteria were probably dependent on the phytoplankton and their exudates (Kritzberg et al. 2005; Guenet et al. 2010).

Prey for protozoa

The heterotrophic NFs are known to control bacterial abundance in humic lakes (Kankaala et al. 1996), but in addition to bacteria, NFs graze on small phytoplankton (Sherr & Sherr 2002; Bręk-Laitinen & Ojala 2011). However, in Lake Valkea-Kotinen the high APP and NF abundances did not overlap (Figs. 7 [year 2003] and 9A), and there was no correlation between the NFs and APP or the larger phytoplankton (measured as Chl *a*; Table 3 in IV). However, NFs with surprisingly high abundance (average 6.6 x 10^3 cells ml^{-1}), i.e. similar to eutrophic rather than humic lakes (Amblard et al. 1995; Kalinowska 2004; Bręk-Laitinen et al. 2012; V), correlated negatively with small bacteria in the epi- and hypolimnion ($r = -0.265$, $p < 0.05$ and $r = -0.576$, p < 0.01, respectively). This indicates that the main NF prey in Lake Valkea-Kotinen were bacteria. The eukaryotic APP (cell size ~ 2 μm) were probably too large for efficient grazing by NFs (Amblard et al. 1995) or that the NFs simply preferred bacteria, which were much more abundant in Lake Valkea-Kotinen than were the APP.

Similar to the NFs, the average ciliate abundance (7.1 cells ml^{-1}) in Lake Valkea-Kotinen was reminiscent of the abundances in eutrophic lakes (Zingel et al. 2002; Bręk-Laitinen et al. 2012). The algivorous ciliates were the most abundant group (51% of the total ciliate abundance) and were present in the epilimnion throughout the summer (Fig. 9B; Table 4 in IV). Their numbers were highest in June during the epilimnetic APP maximum and they correlated positively with the APP in the epilimnion ($r = 0.401$, $p < 0.001$), but could not be associated with the larger phytoplankton (Table 3 in IV). The CCA showed that, the algivorous ciliates did not demonstrate clear responses to changes in the environment (Fig. 4 in IV). This was probably because they were, together with the APP, prone to convective mixing. However, during the strongest stratification in August the algivorous ciliates were less abundant in the anoxic hypolimnion ($p < 0.001$), which then acted as a refugee for the APP and larger phytoplankton (Fig. 9B). Another group most likely feeding on the APP ($r = -0.454$, $p < 0.05$) were the omnivorous ciliates in early summer. The omnivores, which made up 6.7% of the total ciliate numbers, were the only feeding group that formed a clear cluster in the CCA analysis, showing specialization for hypoxic/anoxic ($O_2 < 2$ mg l^{-1}) conditions in the upper hypolimnion in early summer (Fig. 9D; Fig. 4 in IV).

4.6 Planktivorous fish and the trophic cascade

Fish manipulation affected the zooplankton in the humic Lake Pääjärvi but not in the clearwater Lake Vesijärvi (Table 2 in V). In Lake Pääjärvi, the zooplankton community shifted towards small-bodied species, which is consistent with the concepts of the food chain theory (Fig. S2 in V; Carpenter et al. 1985) and size-selective fish predation (Brooks & Dodson 1965). According the food chain theory, the strength of the trophic cascade in dependent on the abundance of the keystone herbivorous zooplankton, namely *Daphnia* (Carpenter et al. 1985).
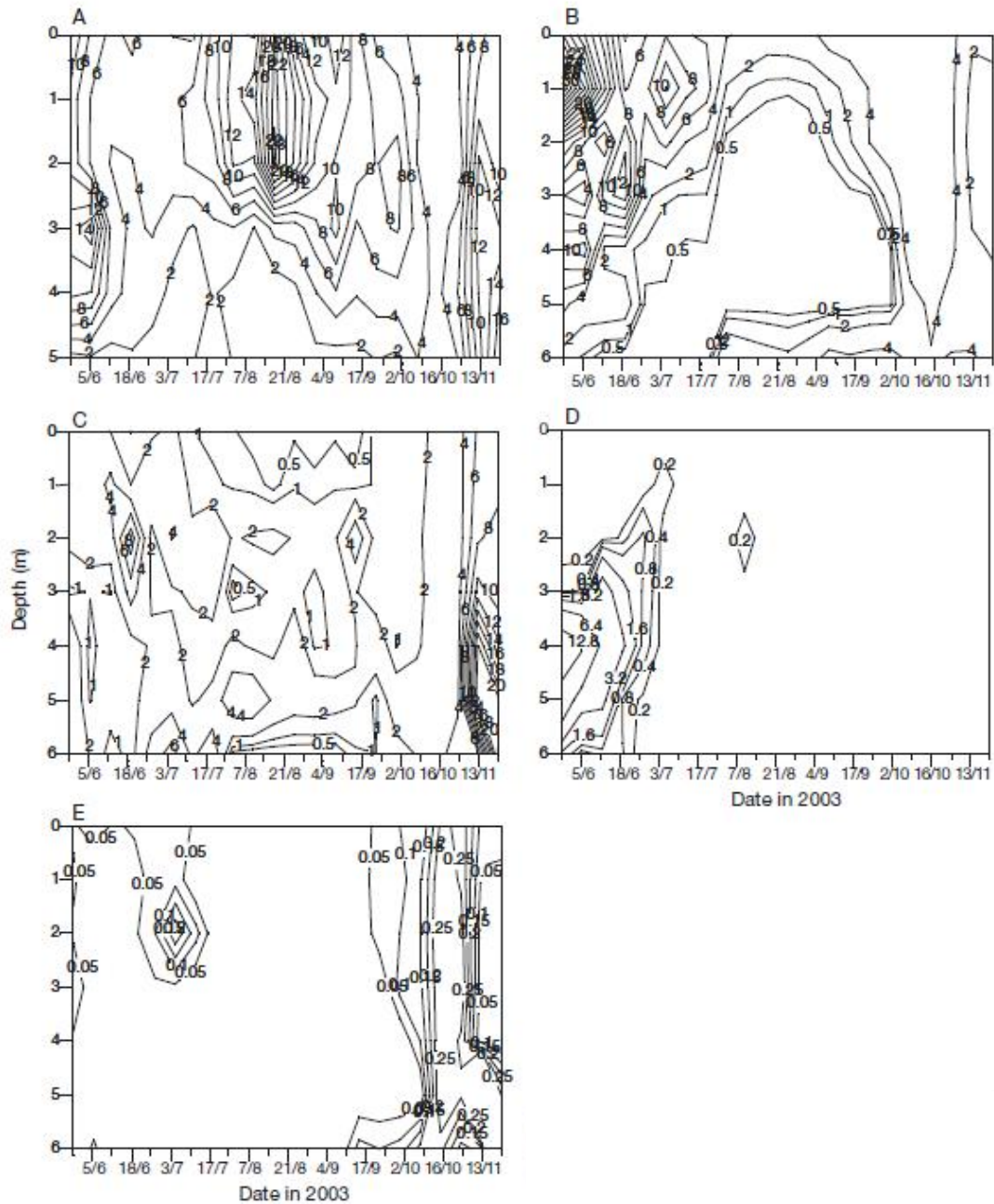
Figure 9. Abundances of A) heterotrophic nanoflagellates (NF; 10^3 cells ml^{-1}) and B) algivorous, C) bacterivorous, D) omnivorous and E) predatory ciliates (cells ml^{-1}) in Lake Valkea-Kotinen during the open-water period in 2003. Note the different scales on the y-axis. © Inter Research.

However, the *Daphni*a numbers were in general low, and even though the inspection of gut contents ensured that the fish were really feeding on zooplankton, changes in the community composition of the phytoplankton were only seen in the cryptophytes, which had higher biomass when fish were present (Table 2 and Fig. S3 in V). Although the effect of fish was not strong enough to produce the conventional trophic cascade down to the total phytoplankton biomass or APP abundance (Table 2 in V), the fish affected the PP and Chl *a* concentration:

PP   BP   Chl *a*

Lake Pääjärvi
Fish

150
100
50
0

PP and BP [mg C m^{-3} d^{-1}]

12 Jul 19 Jul 26 Jul 2 Aug 9 Aug

15
10
5
0

Chl *a* [µg L^{-1}]

No fish

150
100
50
0

12 Jul 19 Jul 26 Jul 2 Aug 9 Aug

15
10
5
0

Lake Vesijärvi
Fish

150
100
50
0

PP and BP [mg C m^{-3} d^{-1}]

27 Jul 2 Aug 8 Aug 14 Aug 20 Aug

15
10
5
0

Chl *a* [µg L^{-1}]

No fish

150
100
50
0

27 Jul 2 Aug 8 Aug 14 Aug 20 Aug

15
10
5
0
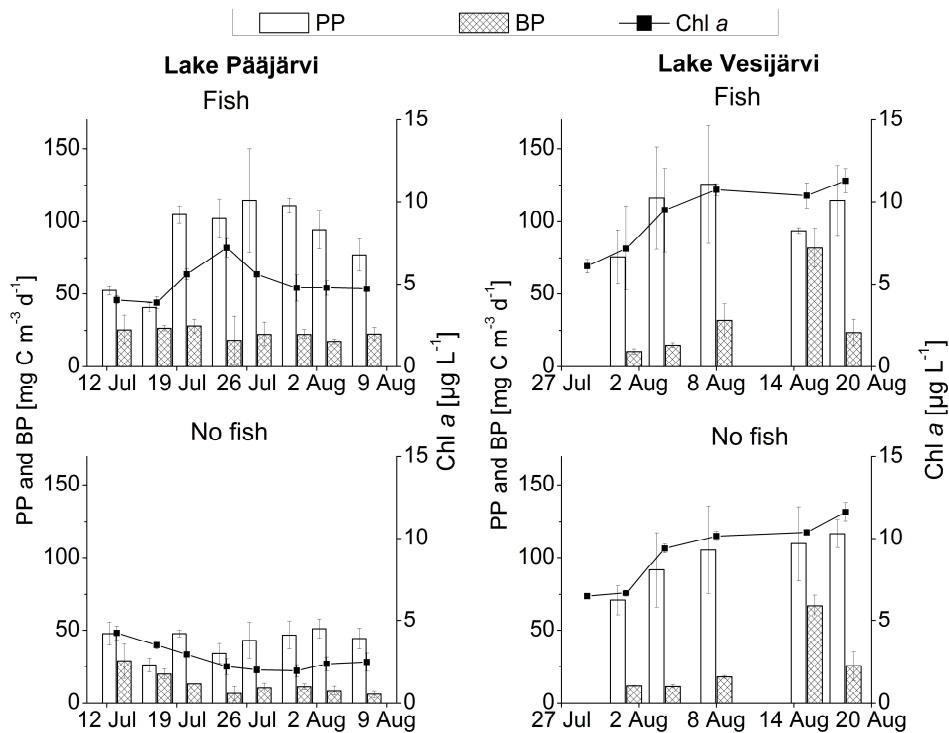
Figure 10. Primary production (PP, mg C m^{-3} d^{-1}), chlorophyll *a* concentration (Chl *a*, µg l^{-1}) and bacterial production (BP, mg C m^{-3} d^{-1}) in L. Pääjärvi (humic lake) and L. Vesijärvi (clearwater lake) in enclosures with and without fish.

the initial PP and Chl *a* doubled in the fish enclosures in Lake Pääjärvi (Fig. 10; Table 2 in V).

The changes observed in the phytoplankton of the humic, P-limited Lake Pääjärvi were probably due to the increase in PO$_4$, which is released by small planktivorous fish (Vanni & Layne 1997; Attayde & Hansson 1999, 2001; Romo et al. 2004). In Pääjärvi, fish increased the NH$_4$, P$_{tot}$ and PO$_4$ concentrations (Table 2 and Fig. S1 in V), and also the dissolved inorganic nitrogen:dissolved inorganic phosphorus (DIN:DIP) ratio was better with than without fish, i.e. 472 and 1013, respectively. Under P-limited conditions, bacteria should rapidly increase in response to fish addition (Vanni & Layne 1997; Pace et al. 1999; Findlay et al. 2005). Bacterial production (BP) indeed

responded strongly to manipulation of resources, especially P (*p* < 0.001); however, the bacterial abundance did not change. The NF numbers declined in the presence of fish, but the ciliates increased in numbers, and the ciliate community shifted towards dominance of algivorous oligotrichs and prostomatids (Fig. 11; Table 2 and Fig. S5 in V). Thus, probably the algal food resources rather than metazoan grazing were responsible for the changes observed in the protozoan community. In the clearwater Lake Vesijärvi, fish had a significant positive effect on PO$_4$ concentration (p < 0.01), but otherwise the fish did not affect the nutrient concentrations (Table 2 and Fig. S1 in V). The DIN:DIP ratio was ~ 4.7 in all enclosures throughout the experiment, indicating N limitation (Redfield 1958). The diatoms and cyanophytes responded to fish manipulation: their biomasses
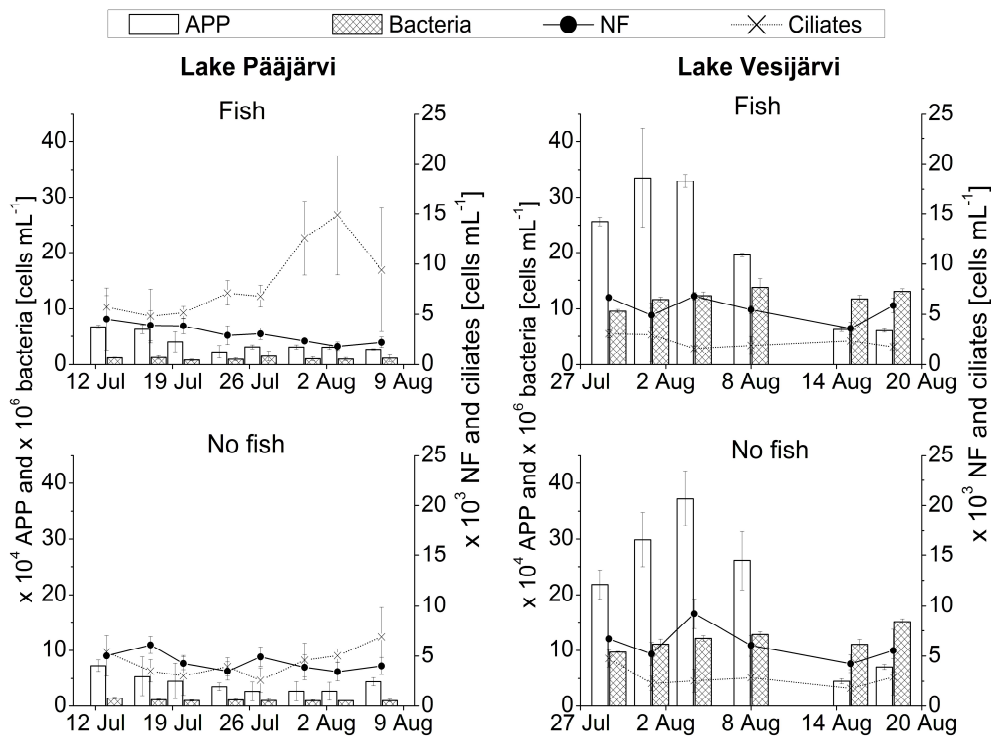
Figure 11. Components of microbial food web (cells ml^{-1}) (autotrophic picoplankton [APP], bacteria, nanoflagellates [NF] and ciliates) in L. Pääjärvi (humic lake) and L. Vesijärvi (clearwater lake) in enclosures with and without fish.

were two times higher in the fishfree enclosures than in the enclosures with fish (Table 2 in V). However, there were no effects of fish-induced P addition on total phytoplankton biomass, PP, Chl a or microbial community dynamics (Table 2 in V). A previous study also showed lack of positive influence of P on the microbial community in Lake Vesijärvi (Bręk-Laitinen et al. 2012).

CO_2 exchange between the lakes and the atmosphere designate the ecosystem metabolism (Schindler et al. 1997), i.e. lakes strongly CO_2-supersaturated are considered as net heterotrophic, whereas subsaturation is a sign of autotrophy. We did not measure the CO_2 flux between the enclosures and the atmosphere directly, but since it is known that the CO_2 flux is well explained by the partial pressure of CO_2 (pCO$_2$) (e.g. Huotari et al. 2011), we determined the pCO$_2$. The pCO$_2$

exceeded the atmospheric equilibrium value nearly every time in both lakes, and there was no effect of fish (Table 2 and Fig. S7 in V). This is not surprising in the clearwater lake, where the plankton community did not respond to fish manipulation. However, the observed bottom-up effect of fish on PP and Chl a in the humic lake implies that utilization of CO_2 by phytoplankton must also have increased, which should have shifted the community to a more autotrophic state, but it did not show in the CO_2 results. This could have been due to simultaneous increase in respiration: BP and the algivorous ciliates increased in the humic lake in tandem with PP and Chl a and resulted in unaltered net ecosystem production (NEP). This observation is in contrast to the study by Cole et al. (2000), who detected positive NEP, i.e. autotrophy, under high nutrient loading and low abundance of large zooplankton.

5. CONCLUSIONS

The aim here was to increase our understanding of the phytoplanktonic life in boreal humic lakes. Humic lakes are very numerous in Finland, but still most phytoplankton studies focus on the species composition of nano- and microplankton or total PP, whereas studies including APP or the MFW are rather scarce. In addition, most of the previous studies described short time periods, i.e. covered at most a single open-water season. Thus, I felt very fortunate and privileged that, in addition to my own measurements, I had access to the long-term ICP IM data collected in Lake Valkea-Kotinen since 1990. To my surprise, the changes in the phytoplankton could not be directly coupled with the climatic variables in spring (e.g. ice-off, spring circulation). In summer the changes were also related to nutrient and light availability as well as thermal stratification, all of which are, of course, prone to weather events, rather than directly to parameters such as precipitation and temperature. However, my studies were performed *in situ*, and therefore the effects of recovery from acidification or any other ongoing environmental changes in the lake and its catchment area could not be ruled out. The original idea of the ICP IM programme was to collect data appropriate to the organism's life cycle and metabolic activity, and then relate the changes in the environment and the biota, as well as in the food web (Keskitalo et al. 1998). Thus, when the programme was initiated it was considered as an extremely high-frequency monitoring programme with weekly vertical sampling. Such intensive programmes are rare even today! However, in hindsight, the sampling frequency perhaps followed the phytoplankton life cycles in midsummer, but definitely not in spring, when the environment as well as phytoplankton change most rapidly. In addition, the vertical frequency of 1 m was inadequate for detection of the delicate changes in the biota and the environment in the thermocline, which actually was one of the most important regions of study in strongly stratified lakes. Unluckily, there were also parameters, such as the Fe concentration, that were not included in the monitoring programme, but which today are known to be behind the changes observed in surface water quality (Kritzberg & Ekström 2012). Thus, one of my major conclusions is that it is very difficult to design a long-term monitoring programme that is simultaneously extensive, detailed, flexible and cost-effective.

One of my main themes was to determine whether cell size and/or motility are among the traits that make certain phytoplankton taxa more competitive than others in strongly stratified lakes with epilimnetic nutrient depletion. In theory, the shallow epilimnion and strong thermal and chemical summertime stratification should favour flagellated species. In Lake Valkea-Kotinen, most of the phytoplankton biomass was indeed composed of flagellates, especially the raphidophyte *G. semen* and *Peridinium* dinoflagellates. Furthermore, these two taxa were responsible of most for the IC uptake (i.e. PP), and *G. semen* was also strongly coupled with the high EOC and PER values. Due to of their large size, *G. semen* and *Peridinium* spp. can go through steep temperature gradients and migrate fairly long distances, which explains their superiority over the smaller flagellates in strongly stratified lakes. Compared with other species and taxa, such as *Cryptomonas* flagellates, these two are also large as prey items. Despite their importance in biomass and IC uptake, *G. semen* and *Peridinium* dinoflagellates were not among the most abundant phytoplankton species in Lake Valkea-Kotinen. Actually, none of the

flagellated species surpassed the nonmotile, solitary, *Choricystis*-like APP, which probably have more effective nutrient uptake and intracellular transportation due to small (< 2 µm) cell size and high SA:V ratio. The solitary APP do not spend energy on movements, but apply isopycny for remaining in the thermocline and thus benefit from the diurnal convective mixing when obtaining nutrients (Nordbo et al. 2011). By residing in the thermocline the APP can, in addition to easing nutrient competition with bacteria, escape ciliate grazers, which in Lake Valkea-Kotinen were very abundant, but avoided the anoxic waters during the strongest thermal stratification. However, as was observed in *M. warmingiana*, the isopycny was not the only key to success, but the isopycnic species had to be able to balance between nutrient and light limitation.

Except for nutrient depletion, the autotrophic life in humic lakes is hampered by the high concentration of brown-coloured DOC and thus rapid attenuation of light. In Lake Valkea-Kotinen, the dark water colour restricts phytoplankton photosynthesis to the uppermost ~ 2-m layer. Dark water also promotes the development of stratification and high water column stability, which favour both the large flagellates and the tiny APP. My studies showed that the phytoplankton production in Lake Valkea-Kotinen was negligible below 1.5 m. However, the surface PP was so high that the lake rather resembled eutrophic conditions; this also held for Chl *a*, the PP:Chl *a* ratio and the phytoplankton biomass, and was further reflected in the protozoan abundance and surface water CO_2 concentration (Huotari et al. 2011). This indicates that the present phytoplankton taxa are very well adapted to their environment. Nevertheless, the rather stable Chl *a* concentrations imply that phytoplankton

are at the upper limit of their photoadaptation, and the increase in DOC concentration and water colour that were both occurring will cause problems, i.e. result in light limitation. Indeed, despite the effective light-harvesting capacity of *G. semen*, the biomass of this dominant primary producer was decreasing and, probably due to its large proportion in the biomass also caused a decline in the total phytoplankton biomass. In contrast, the eukaryotic APP, which did not have as high PP:Chl *a* ratios as *G. semen*, but were located near the bottom of the euphotic zone, were effective enough at light harvesting and increased. Therefore, the future increases in DOC concentration, water colour and water column stability will all favour the eukaryotic APP.

The classical grazer food chain in Lake Valkea-Kotinen is not based on *G. semen* (Jones et al. 1999), and although the algivorous ciliates were coupled with APP, the MFW was not driven solely by APP but also by bacteria. Therefore, at first glance it appears that when the biomass of *G. semen* decreases and the eukaryotic APP increase, the ciliates feeding on APP become more abundant, but otherwise there are no major changes in the MFW. However, the decrease in *G. semen* can result in decrease of bacterial abundance. This is because the labile part of the allochthonous OC is often already degraded in the catchment and thus phytoplankton EOC is of great importance as a source of OC; in Lake Valkea-Kotinen EOC was coupled with *G. semen*. Furthermore, EOC can act as a primer for allochthonous DOC decomposition (Guenet et al. 2010), and thus the decrease in EOC may change the total OC decomposition in the lake. The subsequent possible decrease in bacterial abundance may then lower the numbers of NFs and bacterivorous ciliates. This can be more important for the lake ecosystem than the increase in APP

alone. EOC reduction can also make APP more competitive against bacteria (Drakare 2002), and thus shift the community towards autotrophy by favouring the IC-fixing APP over the heterotrophic bacteria. However, the results from the enclosure experiment in Lake Pääjärvi show that the increase in IC uptake may not necessarily make a humic lake more autotrophic (see below).

The purpose of the enclosure experiment in the humic Lake Pääjärvi and the clearwater Lake Vesijärvi was to detect the possible trophic cascade from planktivorous fish to the microbial community, including phytoplankton, and further to surface water CO_2 concentrations. Unfortunately, the effect of fish biomanipulation was not strong enough to produce changes in total phytoplankton biomass or APP abundance. Nevertheless, in the P-limited humic Lake Pääjärvi, the fish-induced nutrient addition increased PP and Chl *a*.

Thus, the IC uptake probably increased, which should have made the community more autotrophic. However, the changes in nutrients and phytoplankton promoted BP and growth of algivorous ciliates, which increased respiration, i.e. CO_2 release. As a consequence, there was no change in the NEP, and the lake remained net heterotrophic.

Clearly the phytoplankton in Lake Valkea-Kotinen, as in boreal humic lakes in general, are adapted to exceptional environmental stresses. However, yearly events are not always similar and even though certain traits make some species more competitive than others, there is no guarantee that the same trait combination would be the most beneficial year after year. In conclusion, diverse aspects of adaptation need to be considered when making future predictions of phytoplankton communities and lake food webs under various environmental changes.

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