

Weak response of the microbial food web of a boreal humic lake to hypolimnetic anoxia

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ABSTRACT: As a consequence of climate change, thermal stratification in lakes strengthens and increases the tendency towards hypolimnetic hypoxia or even anoxia. In the boreal zone, numerous lakes already undergo seasonal hypolimnetic anoxia, but its consequences for the microbial food web (MFW) are still largely unknown. However, the abundance as well as vertical distribution of predators, especially ciliates, in the MFW is generally assumed to be controlled by food and oxygen availability. To determine whether oxygen regulates the MFW, we studied the autotrophic picoplankton (APP), larger phytoplankton, bacteria, nanoflagellates (NFs) and ciliates present throughout the growing season in a small, humic and seasonally strongly stratified lake (Valkea-Kotinen) with an anoxic hypolimnion. The prey numbers in Lake Valkea-Kotinen were low, whereas the predators were numerous. The abundance of NFs, which was coupled with that of bacteria, was especially high. However, our results indicated no clear response to oxygen conditions, although the abundances of the predators were generally higher in the oxic than in the anoxic water layers. The only ciliate feeding group that showed a clear response was the omnivores, which thrived in the hypoxic hypolimnion in early summer. The APP and bacterial abundances were in general high in the upper hypolimnion near the thermocline, which in Lake Valkea-Kotinen is prone to convective mixing. This diurnal disturbance nurtures the autotrophic and heterotrophic community around the thermocline and may also explain the rather high predator abundance in the MFW of Lake Valkea-Kotinen.

KEY WORDS: Autotrophic picoplankton · Bacteria · Heterotrophic nanoflagellates · Ciliates · Thermocline · Convection

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INTRODUCTION

Since the introduction of the concept of the 'microbial loop' by Azam et al. (1983), autotrophic picoplankton (APP), heterotrophic picoplankton and protozoans have become increasingly important in aquatic food web studies. Microbial loops, or, more precisely, microbial food webs (MFWs), have been widely studied in marine habitats as well as in lakes of various trophic states and over wide geographical areas (e.g. Arndt 1993, Amblard et al. 1995, Hadas & Berman 1998, Zingel et al. 2007, Gobler et al. 2008, Peštová et al. 2008). However, MFWs in lakes with

strong thermal stratification and resulting hypolimnetic oxygen depletion are still poorly defined (Eby et al. 2005, Brøk-Laitinen et al. 2012). In addition, few studies have described seasonality in the vertical structure of MFWs (Massana et al. 1996, Kalinowska 2004, Brøk-Laitinen et al. 2012, Colombet & Sime-Ngando 2012, Rasconi & Sime-Ngando 2012). This is somewhat perturbing since anoxia — which, due to eutrophication and climate change, is among the most severe threats that aquatic ecosystems are facing currently — can lead to major regime shifts in food webs. For instance, protozoans, e.g. heterotrophic nanoflagellates (NFs) and ciliates, can re-

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place macrozooplankton as the main grazers (Masson & Pinel-Alloul 1998).

In the boreal zone, numerous lakes regularly undergo seasonal hypolimnetic hypoxia and finally anoxia. The special characteristic of these usually small lakes is the high concentration of coloured organic matter (OM) deriving from the catchment, and thus MFW studies in these types of lakes typically focus on bacterial utilization of allochthonous carbon and ignore comprehensive description of the trophic structure of MFWs. Usually boreal lakes are dimictic, but as small and sheltered bodies of water they often undergo incomplete spring turnover and thus their hypolimnia remain anoxic after winter (Huotari et al. 2009). Due to rapid light attenuation, phytoplankton production in humic lakes is restricted to the upper few metres (Arvola 1984, Karlsson et al. 2009, Peltomaa & Ojala 2010). This results in depletion of epilimnetic inorganic nutrients—most often phosphorus—during summer. However, the nutrients are often abundant in the hypolimnia (Arvola 1984). Phytoplankton communities in humic lakes are dominated by flagellated species capable of migrating vertically between the epilimnion and the hypolimnion (Lepistö & Rosenström 1998). Furthermore, some of the immobile species, including the APP, have some control of their position in the water column through isopycny and thus are located at the boundary layer between light and nutrient depletion (Peltomaa & Ojala 2012). However, the importance of APP in the MFWs of humic lakes is thus far unclear. In contrast to phytoplankton, heterotrophic prokaryotes, i.e. bacteria, are more evenly distributed throughout the water column, probably due to the availability of OM (Amblard et al. 1995, Tulonen et al. 2000). The high amount of OM sustains the high biomass of heterotrophic bacteria, which further support the growth of microzooplankton and macrozooplankton (Jones 1992, Daniel et al. 2005).

In general, the abundance and vertical distribution of the grazers in MFWs is controlled by food and/or oxygen availability (Guhl et al. 1996). Many studies have reported NF grazing on bacteria-sized particles (Sherr & Sherr 2002 and references therein), and since bacteria are abundant in humic lakes, NF growth is supposedly not limited by the availability of bacterial food resources. However, not all NFs are bacterivorous, but are herbivorous or omnivorous (Mischke 1994, Šimek et al. 1997, Bręk-Laitinen & Ojala 2011). Due to their small size, NFs cannot ingest large cells, which restricts their grazing efficiency (Amblard et al. 1995). NFs do not respond to

seasonal anoxia (Gobler et al. 2008, Bręk-Laitinen et al. 2012), whereas the abundance and community structure of ciliated protozoa differ considerably in oxic versus anoxic waters (Guhl et al. 1996, Gobler et al. 2008, Bręk-Laitinen et al. 2012). Some 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).

Since hypolimnetic oxygen depletion divides the water column into 2 separate parts, and since there are few studies of MFWs in humic lakes, we examined the vertical structure of the MFW in a strongly stratified boreal humic lake with an anoxic hypolimnion. In this lake, the anoxia has strengthened as a consequence of climate change (E. Peltomaa et al. unpubl.). We explored the dynamics of potential 'prey' organisms, e.g. APP, larger phytoplankton and bacteria, and 'predators', e.g. NFs and ciliates, in the small (surface area 4 ha) Lake Valkea-Kotinen in southern Finland. Our aim was to determine the relationships between the potential prey and predators and explain the changes observed in their seasonal dynamics. We also compared the roles of NFs and ciliates as grazers of APP and bacteria in the oxic and anoxic parts of the water column. We hypothesize that NFs are coupled with the presence of bacteria and possibly with APP, but ciliates follow both the dynamics of their particular food source and the dividing line of anoxia. We compared our results with (1) those of Bręk-Laitinen et al. (2012) from the clearwater boreal Lake Vesijärvi, which has a hypoxic/anoxic hypolimnion and is situated only 50 km from our study lake, and (2) the results of Amblard et al. (1995) from the temperate Lake Vassivière in France. Lake Vassivière is a humic lake, but in terms of oxygen does not stratify and thus does not suffer from anoxia. Both of these lakes are considerably larger (surface area of Lake Vesijärvi 108 km² and Lake Vassivière 10 km²) than Lake Valkea-Kotinen, but were chosen for comparison because so far there are no comprehensive studies of the MFWs in boreal humic lakes with hypolimnetic oxygen depletion.

MATERIALS AND METHODS

Study site

Lake Valkea-Kotinen (61° 14' N, 25° 04' E) is a shallow (mean depth 2.5 m, maximum depth 6.5 m) headwater lake with brown water (colour value 160 mg Pt l⁻¹). The intense water colour and high concentra-

tions of dissolved organic carbon (DOC; 9.6 to 12.2 g m⁻³) are due to allochthonous humic substances originating from the surrounding old-growth forest and peatland (Keskitalo et al. 1998). As a result, light penetration is poor and photoautotrophic production is restricted to the uppermost 2 m of the epilimnion (Peltomaa & Ojala 2010). Lake Valkea-Kotinen is steeply stratified both thermally and chemically during summer and used to undergo mixing in spring as well as in autumn; currently the spring turnover is usually short and incomplete (Peltomaa & Ojala 2012), which was also the case in 2003, when the MFW was studied.

Sample collection and laboratory protocols

The samples were taken during the open-water period 2003 from the deepest point (6 m) of the lake once a week between 09:00 and 10:00 h (GMT +2), i.e. from the middle of May till the end of November. The chemical data were collected as a part of the International Cooperative Programme on Integrated Monitoring of Air Pollution Effects on Ecosystems monitoring programme (Niinioja et al. 1995). The water temperature and dissolved oxygen concentration were measured at 1 m intervals from 0 to 6 m with a portable YSI 55 combined oxygen meter (Yellow Springs Instruments). The concentrations of inorganic and organic nitrogen and phosphorus, i.e. nitrite and nitrate (NO₂ + NO₃), ammonium (NH₄), total nitrogen (N_{tot}), phosphate (PO₄) and total phosphorus (P_{tot}), as well as dissolved inorganic carbon (DIC), were determined from the samples taken with a Limnos tube sampler (length 30 cm) from 0, 1, 2, 3 and 5 m. The samples for inorganic nutrients were filtered through GF/C filters (Whatman International), stored frozen, and filtered after thawing through 0.45 µm MCE-filters (Millipore). The unfiltered samples for N_{tot} and P_{tot} were also stored frozen before analysis. The nutrient analyses were based on colorimetric assays (D'Elia et al. 1977, Grasshoff 1983, Koroleff 1983) carried out with a Lachat Quikchem 8000 automatic flow injection analysis system (Zellweger Analytics). DIC was determined from acidified samples with an infrared carbon analyser (Uras 3G; Hartmann & Braun), as described in Salonen (1981). The samples for enumeration of APP and ciliates were taken with the Limnos at 1 m intervals between 0 and 6 m. Samples for bacteria and heterotrophic NF enumeration were taken simultaneously with APP and ciliates from depths of 0, 2, 3 and 5 m. The APP samples were stored in crushed ice and the cells

were counted within 4 h, and all the other community samples were preserved with acid Lugol's solution.

The total phytoplankton biomass was estimated as chlorophyll *a* (chl *a*). Chl *a* was used as a proxy for the biomass of nanophytoplankton and microphytoplankton, which in Lake Valkea-Kotinen form on average 80% of the total chl *a* (Peltomaa & Ojala 2010). For these determinations, samples were drawn into black plastic bottles with a 1 m tube sampler (volume 7 l) from every metre between 0 and 5 m. From each depth, 400–500 ml of water was filtered through a Whatman GF/C fibreglass filter. The chl *a* was hot-extracted (+75°C, 5 min) from the filters with 94% ethanol and the absorption of the extract was measured with a Shimadzu UV-2100 spectrophotometer at 665 and 750 nm (Keskitalo & Salonen 1994). The concentration of chl *a* was calculated using an absorption coefficient of 83.4 l g⁻¹ cm⁻¹ (Wintermans & De Mots 1965).

Community composition

APP, bacteria and NFs were enumerated with epifluorescence microscopy (Olympus IX50; total magnification of 1000×; blue filter set U-MWB: excitation filter 450 to 480 nm, dichroic mirror 500 nm, barrier filter 515 nm). The numbers of observed fields of view were adapted to APP, bacteria and NFs to obtain an accuracy of 10% (Lund et al. 1958). The APP samples were kept in darkness in crushed ice until observed under the microscope within 4 h. For counting, the APP samples (2 to 5 ml) were filtered onto black polycarbonate membrane filters with a pore size of 0.2 µm (Osmonics), and the APP cells were detected from 40 to 50 fields of view, based on chl *a* autofluorescence (Peltomaa & Ojala 2012). The filtration for bacteria (subsamples of 0.5 to 3 ml) was otherwise similar to that of APP, but the bacteria were stained with acriflavine (Bergström et al. 1986) and at least 30 fields were counted. A video camera and the analysis 3.0 image analysing program (Soft Imaging System) were used to determine the cell volumes of the bacteria. The NF total numbers (i.e. the pigmented but possibly mixotrophic flagellates were not distinguished from the non-pigmented ones) were determined from subsamples of 5 to 10 ml, which were stained and counted as bacteria, using filters with the pore size of 1.0 µm (Sanders et al. 1989). The ciliate community composition (subsamples of 50 ml) was determined with a Nikon Diaphot-TMD inverted microscope, using a total magnification of 200 to 1000× and the Utermöhl settling

chamber technique (Utermöhl 1958). The entire content of each counting chamber was surveyed. Ciliates were usually identified to genus level consulting several works (Kahl 1930, 1931, 1932, 1935, Foissner & Berger 1996, Foissner et al. 1999). They were divided into 4 ecological groups (algivorous, bacterivorous, omnivorous and predatory ciliates) using data from several feeding experiments (e.g. Kisand & Zingel 2000, Agasild et al. 2007, Zingel et al. 2007, Zingel & Nõges 2008) and consulting corresponding literature (Foissner et al. 1991, 1992, 1994, 1995).

Statistical analyses

The relationships between the stocks of prey organisms and the abiotic environmental factors as well as grazers/predators were determined with bivariate correlation analysis (Pearson). Differences in abundance between the oxic epilimnion and the anoxic hypolimnion were analysed with one-way ANOVA. The homogeneity of variances was tested with Levene's test and the normality of the experimental data was tested with the Kolmogorov-Smirnov goodness-of-fit test. \log_{10} transformation was used to normalise the distribution of the data before the statistical analyses. The level of significance was set at $p < 0.05$, and the analyses were carried out with SPSS 15.0 for Windows. Canonical correspondence analysis (CCA) was applied to link the ciliate abundance with water temperature, oxygen concentration, abundances of APP, bacteria and NFs, as well as chl *a* concentration. Since the bacteria and NFs were counted only from depths of 0, 2, 3 and 5 m, the values for the missing depths were interpolated linearly for CCA. The randomization tests (Monte Carlo) in CCA were run with 999 permutations. The CCAs were carried out using the Palaeontological Statistics (PAST) program, version 2.12 (Hammer et al. 2001).

RESULTS

Physical and chemical conditions

The ice-out in 2003 occurred in late April and the thermal stratification developed shortly afterwards and continued into the summer (Fig. 1A). Hypolimnetic anoxia, developed during winter, was not completely resolved, and thus the lower part of the hypolimnion was already anoxic and the upper part hypoxic at the onset of summer (Fig. 1B). The incomplete spring mixing also prevented nutrient

supply from the nutrient-rich bottom to the surface, which led to large differences in the concentrations of $\text{NO}_2 + \text{NO}_3$, N_{tot} and P_{tot} between the epilimnion (0 to 2 m) and the hypolimnion (3 to 6 m) (Table 1).

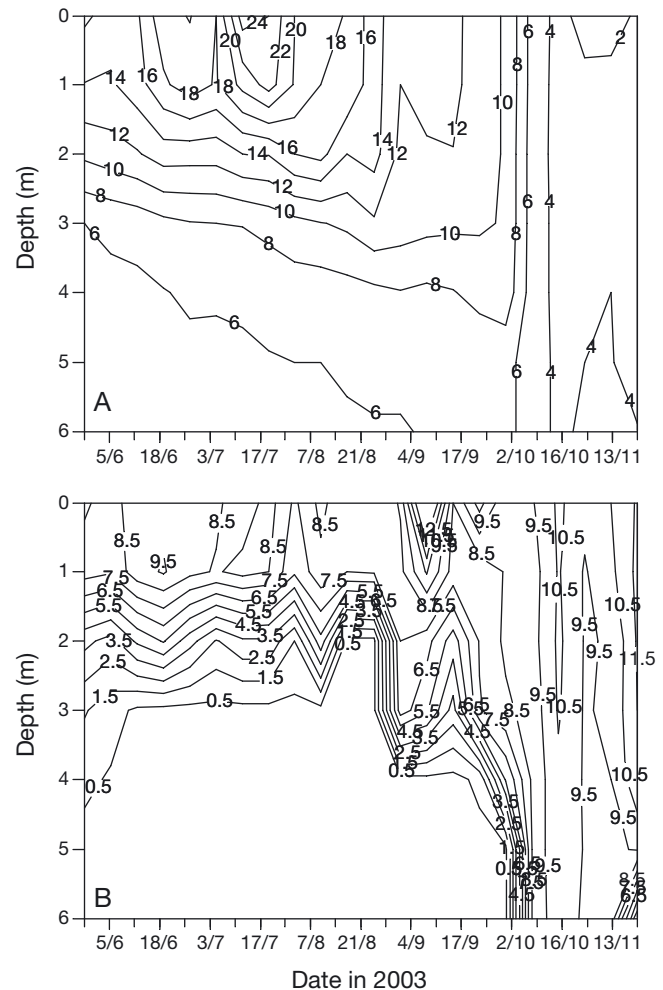


Fig. 1. (A) Temperature ($^{\circ}\text{C}$) and (B) oxygen (mg l^{-1}) in Lake Valkea-Kotinen during the open-water period in 2003. Dates are given as d/mo

Table 1. Mean concentrations of nutrients (mg m^{-3}) and dissolved inorganic carbon (DIC; g m^{-3}) during stratification in the epilimnion and the hypolimnion in Lake Valkea-Kotinen in 2003. Standard errors of means are given in parentheses

	Epilimnion	Hypolimnion
$\text{NO}_2 + \text{NO}_3$	13.3 (0.08)	138.6 (0.05)
NH_4	11.6 (0.53)	11.4 (1.83)
N_{tot}	389 (1.2)	590 (2.5)
PO_4	1.0 (0.00)	1.2 (0.01)
P_{tot}	11.9 (0.04)	20.0 (0.07)
$\text{N}_{\text{tot}}:\text{P}_{\text{tot}}$	33 (0.08)	30 (0.06)
DIC	1.1 (0.02)	6.2 (0.03)

The NH_4 and PO_4 concentrations were quite constant throughout the water column. However, the amount of inorganic phosphorus was very low and phosphorus was probably the limiting inorganic nutrient in Lake Valkea-Kotinen (Table 1). In addition, the $N_{\text{tot}}:P_{\text{tot}}$ mass ratios of 33 (SE = 0.08) and 30 (SE = 0.06) indicated phosphorus depletion in the epilimnion and hypolimnion, respectively (Table 1). In contrast to spring, the autumn turnover was complete: the breakdown in stratification began in mid-September and the entire water column was mixed in mid-October (Fig. 1). The lake froze over in late November.

Prey organisms

The prey components studied showed wide temporal as well as seasonal variation in abundance. The solitary APP consisted of eukaryotic *Choricystis*-type cells, and there was some APP present throughout the sampling period (average 6.4×10^3 cells ml^{-1} , range 1.0 to 25×10^3 cells ml^{-1} ; Fig. 2A). The first APP maximum occurred in June throughout the epilimnion (23×10^3 cells ml^{-1}), but the second peak in August (20×10^3 cells ml^{-1}) was observed at approximately 3 m, i.e. in the upper hypolimnion near the thermocline (Fig. 2A). The average concentration of

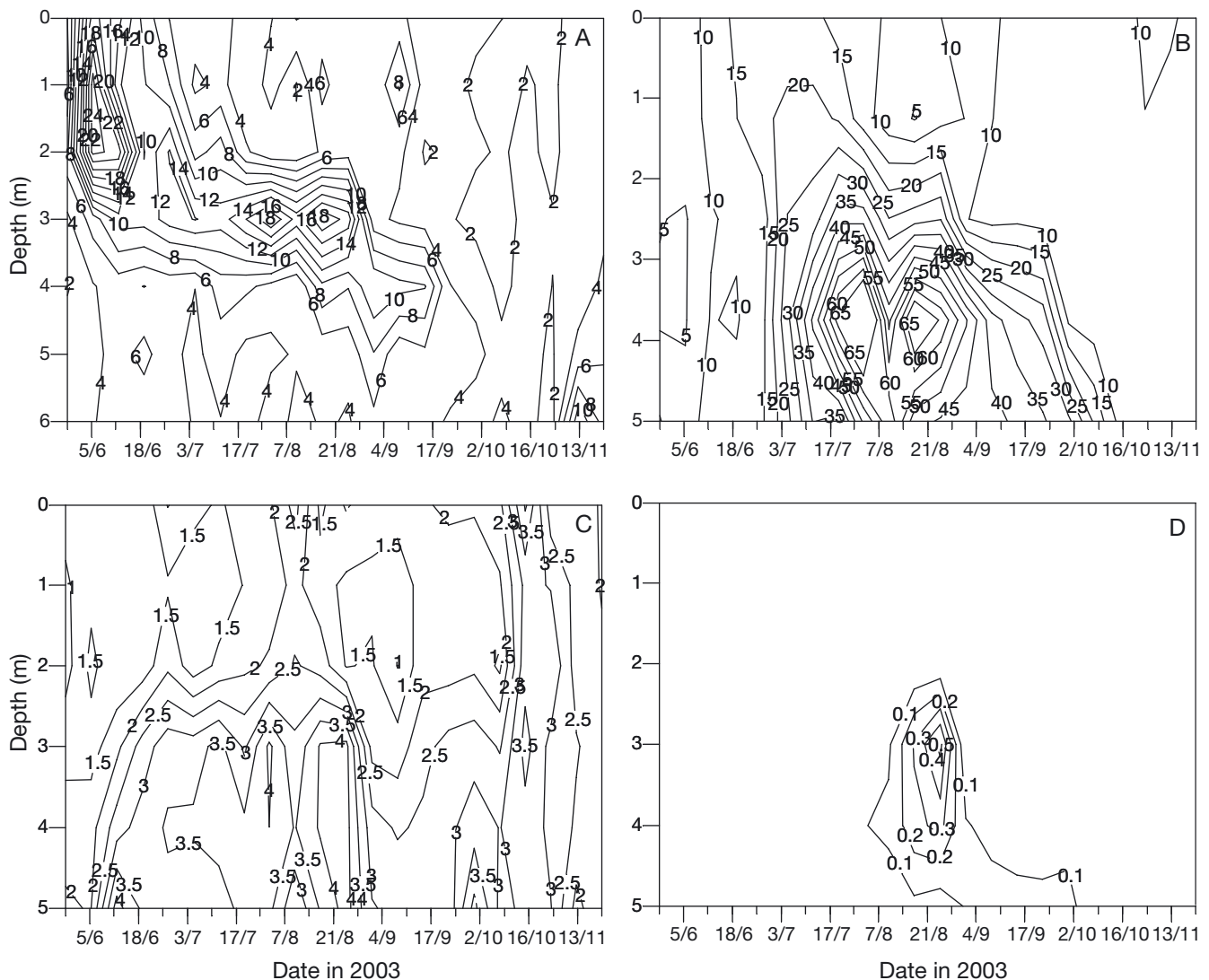


Fig. 2. Vertical distribution of (A) autotrophic picoplankton (10^3 cells ml^{-1}), (B) chlorophyll *a* (mg m^{-3}), (C) small bacteria (10^6 cells ml^{-1}) and (D) large bacteria (10^6 cells ml^{-1}) in Lake Valkea-Kotinen during the open-water period in 2003. Note different scales on y-axes. Dates are given as d/mo

chl *a* was 19 mg m⁻³ (range 4.4 to 83 mg chl *a* m⁻³), with only one clear seasonal maximum in July–August, which (instead of in the epilimnion) was recorded in the hypolimnion (Fig. 2B). The maximum mainly comprised the raphidophyte *Gonyostomum semen*. Besides *G. semen*, most of the phytoplankton biomass in Lake Valkea-Kotinen consisted of dinoflagellates (*Peridinium* and *Gymnodinium*) and flagellated chrysophytes (*Pedinella* and *Syncrypta*) and cryptophytes (*Cryptomonas*) (data not shown). APP as well as the larger phytoplankton, measured as chl *a*, correlated positively with the PO₄ concentrations, which is consistent with the assumption of the lake being phosphorus-limited. There was a negative correlation between NO₂ + NO₃, NH₄ and N_{tot} concentrations and APP, as well as between NO₂ + NO₃ and NH₄ concentrations and chl *a* (Table 2).

Although bacteria were naturally present continually throughout the water column (average 2.4 × 10⁶ cells ml⁻¹, range 0.81 to 4.2 × 10⁶ cells ml⁻¹; Fig. 2C), during thermal stratification their numbers were up to 2 times higher in the anoxic hypolimnion than in the epilimnion (ANOVA, *p* < 0.001, *n* = 88). There was a group of prokaryotes with a cell volume 50 times larger than most of the cells in the group of bacteria, which in summer during anoxia resided in the hypolimnion (ANOVA, *p* < 0.001, *n* = 88; Fig. 2D). These cells were treated separately in the statistical tests, i.e. the tests were run for the 'small' (mean ± SE volume 0.02 ± 0.001 μm³) and 'large' (mean ± SE volume 1.06 ± 0.08 μm³) bacteria. The numbers of the small bacteria increased slowly during summer and attained a modest maximum in July–August at the time of the hypolimnetic chl *a* maximum (*r* = 0.752, *p* < 0.01; Table 3). The small bacteria attained their highest numbers, 3.5 × 10⁶ cells ml⁻¹, in October after the autumn turnover, when they were also evenly distributed throughout the water column (Fig. 2C). The large bacteria attained a maximum of 0.53 × 10⁶

cells ml⁻¹ in the hypolimnion in August simultaneously with the smaller bacteria, but even then they contributed no more than 11 % of the total number of bacteria (Fig. 2D).

The numbers of small bacteria correlated positively with the concentrations of N_{tot} and P_{tot}, as well as with NH₄ and DIC (Table 2). The small bacteria and the APP correlated negatively in the oxic epilimnion (*r* = -0.493, *p* < 0.001), but the correlation was positive in the anoxic hypolimnion (*r* = 0.359, *p* < 0.05; Table 3). Similarly, the large bacteria correlated positively with the concentrations of N_{tot} and P_{tot}, as well as NH₄ and DIC (Table 2). There was also a positive correlation between the large prokaryotes and APP, chl *a* and small bacteria (*r* = 0.426, *p* < 0.05, *r* = 0.565, *p* < 0.05 and *r* = 0.495, *p* < 0.01, respectively; Table 3).

Grazers and predators

The NFs were present throughout the water column during the summer (average 6.6 × 10³ cells ml⁻¹, range 1.0 to 23 × 10³ cells ml⁻¹), but were most numerous in the oxic water layers (ANOVA, *p* < 0.01, *n* = 88; Fig. 3A). The NFs showed 3 clear maxima, 2 of which occurred in the epilimnion: the first with 12 × 10³ cells ml⁻¹ in May–June and the second with 23 × 10³ cells ml⁻¹ in August. The third maximum of 12 × 10³ cells ml⁻¹ was visible throughout the water column during the autumn turnover. We found no correlation between the NFs and APP, but the NFs correlated negatively with the small bacteria in the epilimnion as well as in the hypolimnion (*r* = -0.265, *p* < 0.05 and *r* = -0.576, *p* < 0.01, respectively; Table 3); thus, it is possible that the NFs were grazing on bacteria.

In all, 27 ciliate taxa were identified in Lake Valkea-Kotinen (Table 4). Five of the taxa were found only in the epilimnion, whereas there were only 2 taxa that were present solely in the anoxic hypolimnion. However, all of these 7 taxa were few in numbers (Table 4). The mean ciliate abundance was 7.1 cells ml⁻¹ (range 0.58 to 36 cells ml⁻¹), but during stratification the ciliates were on average 1.5 times more abundant in the epilimnion than in the hypolimnion. The dynamics of the various ciliate feeding groups varied remarkably. The algivorous ciliates, capable of feeding on APP, were the most abundant group (51 % of the total ciliate number) and were present in the epilimnion throughout the

Table 2. Pearson correlation coefficients (*r*) for environmental parameters and autotrophic picoplankton (APP), chlorophyll *a* (chl *a*) and small and large bacteria in Lake Valkea-Kotinen during the open-water period in 2003.

p* < 0.05; *p* < 0.01; ****p* < 0.001; ns: not statistically significant

	APP	n	Chl <i>a</i>	n	Small bacteria	n	Large bacteria	n
NO ₂ + NO ₃	-0.207*	110	-0.270*	88	ns	88	-0.240*	88
NH ₄	-0.202*	110	-0.305**	88	0.431***	88	0.275*	88
N _{tot}	-0.239*	109	ns	88	0.528***	87	0.345**	87
PO ₄	0.241*	110	0.334**	88	ns	87	ns	87
P _{tot}	ns	110	ns	88	0.577***	87	0.408***	87
DIC	ns	110	0.606***	110	0.571***	88	0.557***	88

Table 3. Pearson correlation coefficients (r) for autotrophic picoplankton (APP), chlorophyll *a* (chl *a*), small bacteria, large bacteria, nanoflagellates (NF), and bacterivorous, algivorous, omnivorous and predatory ciliates in Lake Valkea-Kotinen during the open-water period in 2003. Large bacteria were not detected (nd) in the epilimnion, and thus the correlations were not calculated (-). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns: not statistically significant

	APP	Chl <i>a</i>	Bacteria		NF	Ciliates		
			Small	Large		Bacterivorous	Algivorous	Omnivorous
Epilimnion								
Chl <i>a</i>	ns							
n	79							
Small bacteria	-0.493***	ns						
n	56	51						
Large bacteria	-	-						
n	nd	nd	nd					
NF	ns	ns	-0.265*	-				
n	56	51	56	nd				
Bacterivorous ciliates	ns	ns	ns	-	ns			
n	89	79	56	nd	56			
Algivorous ciliates	0.401***	ns	-0.309*	-	ns	ns		
n	89	79	56	nd	56	89		
Omnivorous ciliates	0.240*	ns	ns	-	ns	ns	ns	
n	89	79	56	nd	56	89	89	
Predatory ciliates	ns	ns	0.588***	-	-0.514***	0.583***	ns	ns
n	89	79	56	nd	56	89	89	89
Hypolimnion								
Chl <i>a</i>	0.616***							
n	31							
Small bacteria	0.359*	0.752**						
n	32	15						
Large bacteria	0.426*	0.565*	0.495*					
n	32	15	32					
NF	ns	ns	-0.576**	ns				
n	32	15	32	32				
Bacterivorous ciliates	ns	ns	ns	ns	-0.477**			
n	65	31	32	32	32			
Algivorous ciliates	ns	-0.583**	-0.369*	ns	ns	ns		
n	65	31	32	32	32	65		
Omnivorous ciliates	-0.454*	ns	ns	ns	ns	ns	0.716***	
n	65	31	32	32	32	65	65	
Predatory ciliates	ns	-0.365*	ns	ns	ns	ns	ns	ns
n	65	31	32	32	32	65	65	65

summer (average in epilimnion 5.5 cells ml⁻¹, range 0.6 to 34 cells ml⁻¹). Their numbers were highest in June during the epilimnetic APP maximum, and they correlated positively with the APP in the epilimnion ($r = 0.401$, $p < 0.001$; Table 3, Fig. 3B). The most abundant algivorous species in the epilimnion were the prostomatid *Urotricha* sp. and the oligotrich *Rimostrombidium* sp. in the size class 20 to 40 μm ; in the hypolimnion, *Urotricha* sp. and another unidentified prostomatid were the most abundant algivorous species (Table 4). In August during the strongest stratification, the algivorous ciliates were less abundant in the anoxic hypolimnion (ANOVA, $p < 0.001$, $n = 154$). Since the anoxic waters were almost void of algivores, there was a negative correlation between the algivorous ciliates and chl *a* in the

hypolimnion ($r = -0.583$, $p < 0.01$; Table 3). There was also a negative correlation between the ciliates classified as algivorous and small bacteria ($r = -0.319$, $p < 0.05$ and $r = -0.369$, $p < 0.05$ in the epilimnion and hypolimnion, respectively; Table 3).

The bacterivorous ciliates were the second largest group, contributing 42% of the total ciliate numbers. They were present throughout the water column almost continually (average 3.0 cells ml⁻¹, range 0.1 to 22 cells ml⁻¹), but showed a clear maximum after autumn mixing in November (12 cells ml⁻¹; Fig. 3C). The bacterivorous ciliates were low in numbers in the epilimnion during the NF maximum (Fig. 3), and in the hypolimnion their numbers correlated negatively with the NFs ($r = -0.477$, $p < 0.01$; Table 3), which may have indicated food competition between

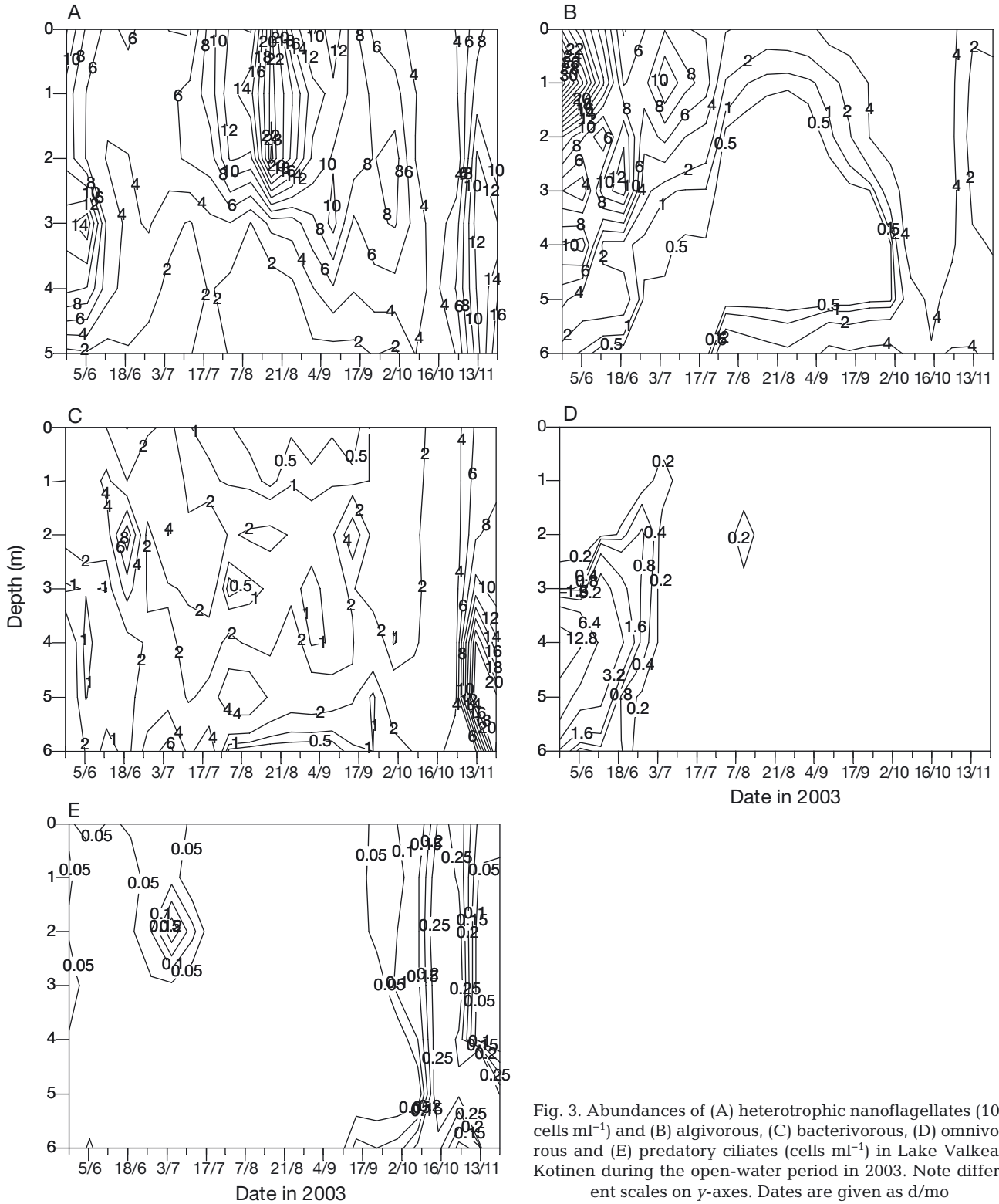


Fig. 3. Abundances of (A) heterotrophic nanoflagellates (10^3 cells ml^{-1}) and (B) algivorous, (C) bacterivorous, (D) omnivorous and (E) predatory ciliates (cells ml^{-1}) in Lake Valkea-Kotinen during the open-water period in 2003. Note different scales on y-axes. Dates are given as d/mo

these 2 groups. However, no correlation between the bacterivorous ciliates and small bacteria was found. The most abundant bacterivores in the epilimnion were the oligotrich *Rimostrombidium* sp. in the size

class $<20 \mu m$ and the scuticociliate *Cyclidium* sp. In the hypolimnion, the most numerous taxa were *Cyclidium* sp. and an unidentified scuticociliate (Table 4).

Table 4. Ciliate taxa observed and their feeding preference (A: algivorous; B: bacterivorous; O: omnivorous; P: predatory) and abundance (cells ml⁻¹) in the epilimnion and the hypolimnion. nd: not detected

Taxon	Taxonomy	Feeding preference	Abundance (cells ml ⁻¹)	
			Epilimnion	Hypolimnion
<i>Limnostrombidium</i> sp.	Oligotrichia	A	0.331	0.061
<i>Pelagostrombidium</i> sp.	Oligotrichia	A	0.118	0.031
<i>Rimostrombidium</i> sp. <20 µm	Oligotrichia	B	1.462	0.185
<i>Rimostrombidium</i> sp. >40 µm	Oligotrichia	A	0.197	0.074
<i>Rimostrombidium</i> sp. 20–40 µm	Oligotrichia	A	0.994	0.127
<i>Halteria</i> sp.	Stichotrichia	B	0.160	0.020
<i>Codonella cratera</i>	Polyhymenophora	A	0.001	nd
<i>Askenasia volvox</i>	Haptoria	A	0.240	0.041
<i>Didinium</i> sp.	Haptoria	P	0.001	nd
<i>Dileptus</i> sp.	Haptoria	P	0.038	0.006
<i>Mesodinium</i> sp.	Haptoria	O	0.001	nd
Gymnostomatida sp.	Gymnostomatida	P	0.002	0.002
<i>Cyclidium</i> sp.	Scuticociliatida	B	1.025	0.938
Scuticociliatida sp.	Scuticociliatida	B	0.061	0.915
<i>Uronema</i> sp.	Scuticociliatida	B	0.037	0.003
<i>Lembadion magnum</i>	Oligohymenophora	O	0.002	0.084
Hymenostomata sp.	Hymenostomata	O	0.006	0.802
<i>Balanion planktonicum</i>	Prostomatida	A	0.303	0.039
<i>Coleps</i> sp.	Prostomatida	O	0.007	0.044
Prostomatida sp.	Prostomatida	A	0.023	0.463
<i>Urotricha</i> sp.	Prostomatida	A	2.904	0.939
<i>Vorticella</i> sp.	Peritrichia	B	0.020	nd
Hypotrichida sp.	Hypotrichida	O	0.014	0.138
<i>Stentor</i> sp.	Heterotrichida	O	nd	0.029
<i>Caenomorpha</i> sp.	Amphorida	B	nd	0.005
<i>Lagynophora</i> sp.	Lagynophora	P	0.004	0.002
<i>Loxophyllum</i> sp.	Pleurostomatida	P	0.002	nd

The omnivores made up 6.7% of the total ciliate numbers and were abundant only in early summer in the hypoxic/anoxic ($O_2 < 2 \text{ mg l}^{-1}$; Fig. 1B) part of the upper hypolimnion (ANOVA, $p < 0.01$, $n = 154$; Table 4, Fig. 3D). At that time the average cell number was 6.7 ml^{-1} (range $0.43\text{--}14 \text{ cells ml}^{-1}$), and the population was made up almost entirely of a single hymenostomate species (Table 4). The correlation between APP and the omnivorous ciliates was negative in the hypolimnion ($r = -0.454$, $p < 0.05$; Table 3), indicating that the omnivores were grazing on APP. The omnivores also correlated positively with algivorous ciliates in the hypolimnion ($r = 0.716$, $p < 0.001$; Table 3), suggesting that they shared the same food resource.

The predatory ciliates were very low in number (on average $0.10 \text{ cells ml}^{-1}$, range 0.0 to $1.5 \text{ cells ml}^{-1}$; Table 4) and made up only 1.0% of the total ciliate community. The predators showed a small maximum of $0.24 \text{ cells ml}^{-1}$ in the epilimnion in July, but mainly they were present in autumn after the turnover with a maximum of $1.5 \text{ cells ml}^{-1}$ near the bottom (Fig. 3E). Thus, they seemed to prefer cool and oxic waters (ANOVA, $p < 0.001$, $n = 154$). The seasonal dynamics

was in contrast to that of the NFs, and the predators correlated negatively with the NFs ($r = -0.514$, $p < 0.001$; Table 3). However, in the late autumn mixing the numbers of NFs and predatory ciliates increased simultaneously (Fig. 3). The predatory ciliates correlated positively with bacterivorous ciliates and small bacteria ($r = 0.583$, $p < 0.001$ and $r = 0.588$, $p < 0.001$, respectively; Table 3) in the oxic water layers, which indicates that the predators were feeding on bacterivores.

The first 3 eigenvalues of the CCA for ciliates in the epilimnion and the hypolimnion explained approximately 90% of the variation in the data. The first eigenvalue, related to oxygen concentration and abundance of small bacteria, accounted for 42.9% ($p < 0.01$) of the overall variation. The second eigenvalue explained 25.0% ($p < 0.01$) of the variation and was related to chl *a* and large bacteria (Fig. 4). The third eigenvalue for NF and chl *a* explained 21.9% ($p < 0.01$) of the variation (data not shown). The hypolimnetic samples in June formed a clear cluster of a distinct community under hypoxic/anoxic conditions ($O_2 < 2 \text{ mg l}^{-1}$, Figs. 1B & 4). The species in this cluster were the algivorous Prostomatida, the bac-

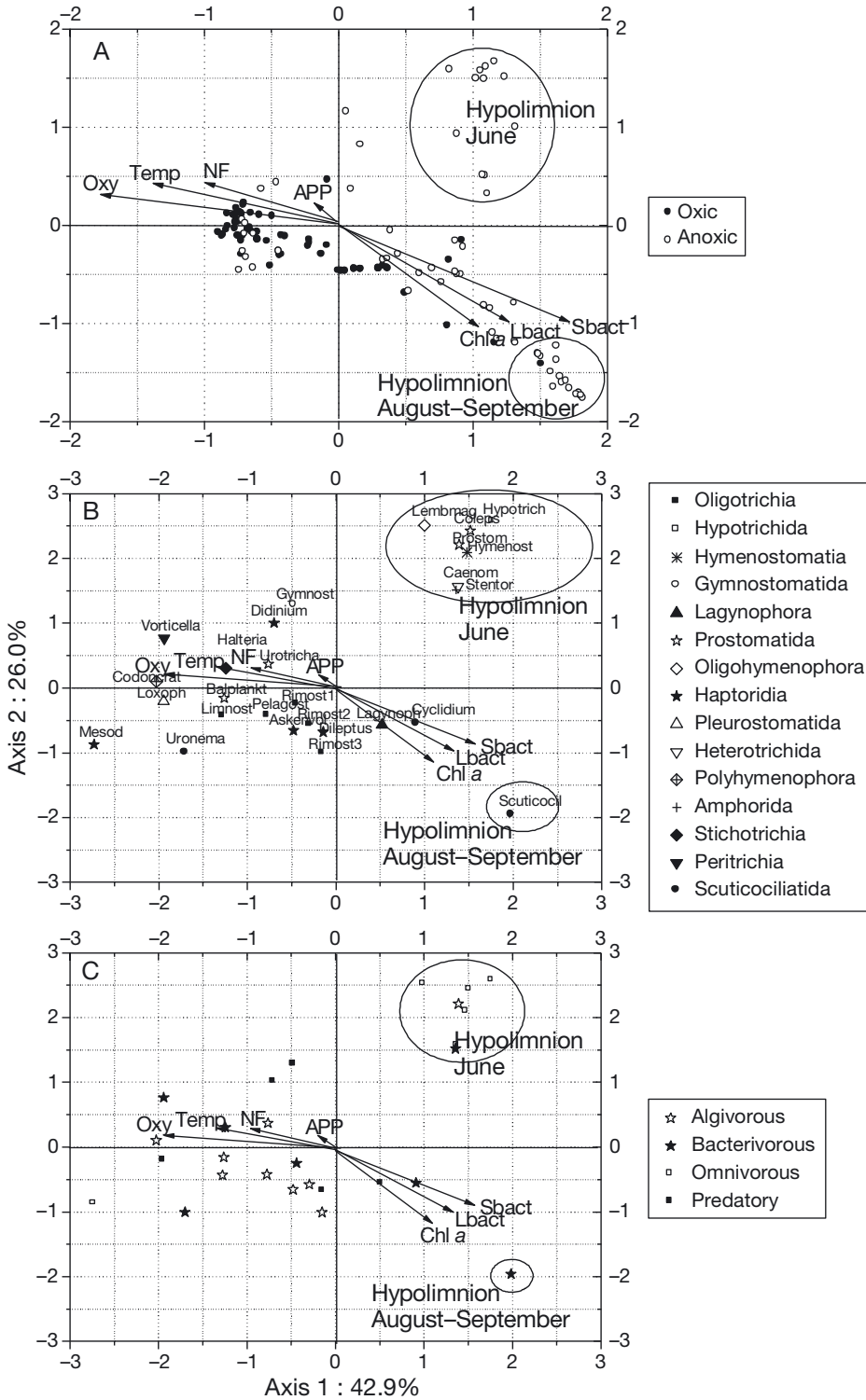


Fig. 4. Canonical correspondence analysis based on the abundance data showing the relationships between (A) environmental variables, (B) ciliate species composition and (C) ciliate feeding preferences in Lake Valkea-Kotinen. Lengths of all arrows were multiplied by 3 for clarity. Eigenvalues of the axes are shown in the ordination. APP: autotrophic picoplankton; Chl a: chlorophyll a; Lbact: large bacteria; Sbact: small bacteria; Temp: temperature. In panel B: Askenvol: *Askenasia volvox*; Balplank: *Balanion planktonicum*; Caenom: *Caenomorpha* sp.; Coleps: *Coleps* sp.; Cycloidium: *Cycloidium* sp.; Didinium: *Didinium* sp.; Dileptus: *Dileptus* sp.; Gymnost: Gymnostomatida; Halteria: *Halteria* sp.; Hymenost: Hymenostomata; Hypotrich: Hypotrichida; Lagynoph: *Lagynophora* sp.; Lembmag: *Lembadion magnum*; Limnost: *Limnostrombidium* sp.; Loxoph: *Loxophyllum* sp.; Mesod: *Mesodinium* sp.; Pelagost: *Pelagotrombidium* sp.; Prostom: Prostomatida; Rimost1: *Rimostrombidium* sp. <20 µm; Rimost2: *Rimostrombidium* sp. 20–40 µm; Rimost3: *Rimostrombidium* sp. >40 µm

terivorous *Caenomorpha* sp. and the omnivores *Lembadion magnum*, Hymenostomata, *Coleps* sp., Hypotrichida and *Stentor* sp. (Fig. 4, Table 4). Another cluster, made up only of the bacterivorous scuticociliatid, was found in the anoxic hypolimnion in August–

September (Figs. 1B & 4, Table 4). Except for these hypolimnetic groups, there were no clear signs of differentiation in ciliate community composition in relation to oxygen concentration, ciliate taxonomy or feeding preferences.

DISCUSSION

Light plays a predominant role in controlling phytoplankton in humic brown-water lakes (Karlsson et al. 2009, Peltomaa & Ojala 2010). Therefore, it was not surprising that the APP density in Lake Valkea-Kotinen was 2 orders of magnitude lower than in the clear-water Lake Vesijärvi (Bræk-Laitinen et al. 2012) and similar to that of eukaryotic APP in the humic Lake Vassiviere (Amblard et al. 1995). However, during the strongest stratification, most of the APP and chl *a* in Lake Valkea-Kotinen was not found in the illuminated epilimnion but in the dark hypolimnion. This was already recorded by Peltomaa & Ojala (2012) in their study of APP in Lake Valkea-Kotinen and can be explained by the attempts of autotrophic organisms to obtain both light, which is available only in the uppermost 2 to 3 m water layer, and nutrients, which are plentiful in the hypolimnion. In the epilimnion, the nonmotile APP must compete with larger phytoplankton and bacteria for inorganic nutrients, which was also indicated in the correlations between the APP and small bacteria. The bulk of the larger phytoplankton in Lake Valkea-Kotinen is flagellates, which are capable of retrieving nutrients from the hypolimnion (Peltomaa & Ojala 2010), whereas via isopycny, the APP can remain close to the thermocline, i.e. at the interface between the illuminated and nutrient-rich layers (Peltomaa & Ojala 2012).

While the lack of light and depletion of inorganic nutrients makes deeply stratified humic lakes harsh environments for photosynthetic organisms, the high amount of OM should favour heterotrophic bacteria (Jones 1992). We expected that bacteria would be abundant in Lake Valkea-Kotinen, but in fact their numbers were one order of magnitude lower than in the clearwater Lake Vesijärvi (Bræk-Laitinen et al. 2012). However, they were similar to those in the humic Lake Vassiviere (Amblard et al. 1995). The results on bacterial abundance were also in accordance with some previous findings on boreal humic lakes (Kuuppo-Leinikki & Salonen 1992, Tulonen et al. 2000). We did not study viruses, and thus it is possible that the bacterial abundance was low due to the viral lytic activity, which is important for example in the humic Lake Vassiviere (Pradeep Ram et al. 2011). We expected the bacteria to be equally distributed in the water column rich in OM, but they were actually more abundant in the hypolimnion than in the epilimnion. In the epilimnion, bacteria are exposed to nutrient competition with phytoplankton, but simultaneously benefit from the extracellular organic compounds (EOC) excreted by phytoplankton (Kritzberg

et al. 2005, Guenet et al. 2010). These compounds are more labile than allochthonous OM of terrestrial origin, which is poor in quality, since the easily degradable compounds are decomposed before the OM enters the lake (Lennon & Pfaff 2005, Guenet et al. 2010). However, in Lake Valkea-Kotinen, most of the EOC production is related to *Gonyostomum semen* (Peltomaa & Ojala 2010) that migrates diurnally between the epilimnion and the hypolimnion (Salonen & Rosenberg 2000), and possibly provides EOC to bacteria in both layers. Thus, in Lake Valkea-Kotinen, the results on bacteria in the epilimnion reflected the abundance of bacterivorous NFs. The importance of NFs as controllers of bacterial abundance in humic lakes has been shown previously (e.g. Kankaala et al. 1996).

In addition to small bacteria, we found a group of large prokaryotes residing in the anoxic hypolimnion. These were most likely either phototrophic and/or methanotrophic bacteria (Arvola 1984, Kankaala et al. 2006) or even Archaea (Jürgens et al. 2000). They were large enough to be oversized for NFs (Amblard et al. 1995), and thus could not be related to NFs in Lake Valkea-Kotinen, nor could they be associated with any feeding groups of ciliates. However, they could have been utilized by larger zooplankton, since Kankaala et al. (2010) showed in Lake Valkea-Kotinen that large methanotrophic bacteria contribute to crustacean zooplankton diets.

NFs were surprisingly abundant in Lake Valkea-Kotinen, e.g. on average the densities were twice as high as in Lake Vesijärvi (Bræk-Laitinen et al. 2012) and 6 times higher than in Lake Vassiviere (Amblard et al. 1995). We expected that NFs would be associated with bacteria and possibly with APP, since they graze on bacteria as well as on phytoplankton (Sherr & Sherr 2002, Bræk-Laitinen & Ojala 2011). We found no correlation between the NFs and APP or larger phytoplankton, but the NFs presumably grazed on bacteria. The eukaryotic APP in Lake Valkea-Kotinen appeared fairly large in size, which could have affected the NF grazing efficiency (Amblard et al. 1995). Alternatively the NFs could have simply preferred the small bacteria, which clearly outnumbered the APP. As for the NFs, they outnumbered the bacterivorous ciliates by a factor of 100 to 1000 and thrived in the epilimnion, but were less abundant in the anoxic hypolimnion, where they faced competition with bacterivorous ciliates. The observations from the hypolimnion of Lake Valkea-Kotinen are in contrast with those recorded in the clearwater Lake Vesijärvi (Bræk-Laitinen et al. 2012). However, in all

studies where the acriflavine staining method has been used the results may have been affected by the fact that acriflavine also stains the non-phagotrophic propagules of zoosporic organisms, which then resemble NFs under the microscope (Rasconi et al. 2011, Jobard et al. 2012).

The most common ciliates in Lake Valkea-Kotinen were the oligotrichs, prostomatids and scuticociliates, i.e. groups commonly found in lakes of various trophic states and over wide geographical areas (Hadas & Berman 1998, Zingel 1999, Peřtová et al. 2008, and references therein). The mean ciliate abundance was slightly higher than in Lake Vesijärvi (Bręk-Laitinen et al. 2012) and twice as high as in Lake Vassiviere (Amblard et al. 1995). We expected the ciliates to be selective feeders and follow both the dynamics of their particular food source as well as the dividing line of anoxia. As expected, the algivores in Lake Valkea-Kotinen were more abundant in the epilimnion and the omnivores in the hypolimnion. Furthermore, the predatory ciliates could be associated with the bacterivorous taxa and NFs after the autumn turnover. The only group of ciliates not associated with these assumed prey organisms was the bacterivores. This lack of association may have been due to selective feeding, i.e. these ciliates prefer active bacteria over dormant or dead ones, which however are often more abundant than metabolically active bacteria (del Giorgio et al. 1997; Tadonlęké et al. 2005). As a result, bacterivorous scuticociliates tend to concentrate in water layers with the highest bacterial productivity, usually near the oxycline (Müller et al. 1991). In Lake Valkea-Kotinen, the scuticociliates were indeed the dominant bacterivores in the hypolimnion, whereas in the epilimnion the small-sized oligotrichs (*Rimostrombidium* sp. and *Halteria* sp.) were most numerous. Scuticociliates and oligotrichs can compete with each other (e.g. Zingel 2005), which also emphasizes their vertical niche separation.

As is typical of ciliate communities (Amblard et al. 1995, Zingel 1999, Peřtová et al. 2008), the abundance of species in Lake Valkea-Kotinen varied with time and depth. However, regarding anoxia the results were somewhat surprising and most of the taxa observed were present in both oxic and anoxic waters, and only 5 taxa were found solely in the oxic and 2 in the anoxic layers. CCA showed that the only feeding group of ciliates clearly responding to environmental conditions was the omnivores in June, when the hypolimnion was not yet completely anoxic, but hypoxic. At that time, 5 of the 6 omnivores recorded in Lake Valkea-Kotinen were present.

Another cluster standing out in the CCA consisted of a single scuticociliatid species. This bacterivore was abundant in the anoxic hypolimnion in August–September. At lower temperatures, scuticociliates may be more effective grazers than small oligotrichs (Schönberger 1994), which could explain the dominance of scuticociliates in the deeper layers of Lake Valkea-Kotinen.

Except for the omnivores and scuticociliatids, no other feeding group or taxa responded to the oxygen concentrations or any other parameters included in the CCA. This is in contrast to the observations of Bręk-Laitinen et al. (2012), who found distinct ciliate communities in the epilimnion and hypolimnion of Lake Vesijärvi, and Guhl et al. (1996), who reported less diverse ciliate communities in the oxygen-depleted hypolimnion of Priest Pot and Esthwaite Water in the English Lake District and Lake Arcas in Spain. These authors suggested that, in addition to oxygen, the clear diversity patterns stem from monotonous food sources in the anoxic waters. However, this was not the case in Lake Valkea-Kotinen, since the APP, larger phytoplankton and bacteria were regularly available both in the oxic epilimnion and the anoxic hypolimnion. Furthermore, the dividing line between the oxic epilimnion and the anoxic hypolimnion in Lake Valkea-Kotinen is diurnally disturbed through convective mixing (Nordbo et al. 2011), supplying the boundary layer with oxygen from the epilimnion above and nutrients from the hypolimnion below and nurturing the autotrophic and heterotrophic community around the thermocline. The amplitude of the mixing is highest during the strongest stratification, i.e. when the nutrient and oxygen limitation is most severe, and therefore extremely important for the organisms. The convective mixing is highly unpredictable, since it is driven by temperature differences, and thus ultimately by weather events. Hence, to study MFWs in lakes such as Valkea-Kotinen in more detail, we should employ continuous *in situ* measurements of both abiotic and biotic variables and extend them throughout the water column, since in shallow lakes the real nature of MFWs may be masked by temporary migrations of both prey and predators.

As a consequence of human activities, the habitats of MFWs in the boreal lakes are changing; e.g. temperature and DOC concentrations are increasing due to climate change and reduction in atmospheric sulphur deposition (IPCC 2007, Nickus et al. 2010). These changes will strengthen the thermal stratification, and thus eventually lead to hypolimnetic anoxia (Pérez-Fuentetaja et al. 1999). However, most of the

boreal lakes are small and shallow (Downing et al. 2006), and our results from Lake Valkea-Kotinen indicate that in these lakes the existence of prey and predators in MFWs is not limited by strong thermal stratification and hypolimnetic anoxia. In fact, the predators were more abundant in Lake Valkea-Kotinen than in the large, boreal clear-water Lake Vesijärvi (Bræk-Laitinen et al. 2012) or in the humic, nonstratifying French Lake Vassivière (Amblard et al. 1995). In contrast, the prey numbers were considerably lower in Lake Valkea-Kotinen than in the other 2 lakes, which is not surprising since grazing pressure was probably high in Lake Valkea-Kotinen. The increase in DOC concentration may not enhance bacterial growth in phosphorus-limited lakes (Smith & Prairie 2004), but it instead leads to stronger thermal stratification and decreased light penetration, both of which favour eukaryotic APP (Peltomaa & Ojala 2012). When APP become more abundant, the algivorous and omnivorous ciliates probably increase in numbers too. This is then reflected further up in the aquatic food web (Gilbert & Jack 1993, Jürgens et al. 1999, Kalinowska 2004).

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