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Development of picoplankton during natural and enhanced mixing under late-winter ice

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We studied the development of autotrophic picophytoplankton and heterotrophic bacterioplankton during the transition from winter ice cover to open water under natural and manipulated mixing conditions in eutrophic Lake Vesijärvi. During the melting of the snow and ice cover, a convection layer developed which eventually met the chemocline at the interface between the oxic and anoxic water masses. However, in the years with mechanically enhanced mixing, the whole water column remained well oxygenated and the deepening of penetrative convection was facilitated. Stochastic variations in weather, primarily the thickness of the snow cover, likely determined the timing of picophytoplankton growth. Mechanical mixing slightly increased the biomass of picophytoplankton, especially the eukaryotic fraction, probably because eukaryotic picophytoplankton coped well with changing light conditions. Bacterial biomass was not notably affected, although their mean cell size was significantly smaller in the oxic deep water than in anoxic conditions during the natural mixing regime. Our results show that, when snow melts and solar radiation increasingly penetrates into lake water, picophytoplankton biomass and numbers already start to increase weeks before ice-break and may reach abundances typical of early summer under the ice. A similar development of bacterioplankton suggests a trophic relationship between heterotrophic and autotrophic producers.

KEYWORDS: picophytoplankton; bacterioplankton; ice covered lake; mixing; vesijärvi

INTRODUCTION

Although qualitatively and quantitatively important biological processes occur in lakes during winter under ice, the last couple of weeks of ice cover are one of the least studied periods due to rapidly weakening ice that prevents sampling (Salonen *et al.*, 2009). During winter, growth of phytoplankton in ice-covered lakes is typically limited by light (Bayliss *et al.*, 1997; Eloranta, 1982; Jewson *et al.*, 2009). Only after the melting of snow in spring does more solar radiation penetrate into the water column and allow phytoplankton growth (Kiili *et al.*, 2009). However, this also creates a convective mixing layer with practically uniform temperature below the shallow under-ice boundary layer (Bengtsson, 1996; Forrest *et al.*, 2008; Matthews and Heaney, 1987). When this convective layer gets thicker, it reaches depths with higher concentrations of nutrients thus making them available for phytoplankton. On the other hand, increased depth of mixing decreases the mean amount of light available below the optimum for suspended algae (Peeters *et al.*, 2007). Convective mixing also effectively reduces the sedimentation of organisms from the productive layer (Kelley, 1997) and hence favours diatoms which are typically abundant in spring (Reynolds, 1984). Under-ice phytoplankton also frequently contain motile species which may be partly able to control their vertical distribution irrespective of convection (Vehmaa and Salonen, 2009). However, the smallest organisms, picoplankton (<2 μm, including autotrophic phytoplankton and heterotrophic bacterioplankton), cannot regulate their vertical position in a turbulent water column and their sinking rate is negligible. Thus their vertical distribution is dependent on variable mixing regimes.

Picophytoplankton comprises both prokaryotic and eukaryotic species. Eukaryotic picophytoplankton can be particularly well adapted to low light and temperature conditions (Somogyi *et al.*, 2009). Limited results are available from lakes covered by ice, but several studies have illustrated the higher proportion of eukaryotic picophytoplankton during open water spring overturn in temperate and boreal lakes (Callieri, 2008; Peltomaa and Ojala, 2010; Vörös *et al.*, 2009). In contrast to that of picophytoplankton, growth of heterotrophic bacterioplankton does not directly depend on the intensity of light penetrating through the ice, but spring mixing regimes might still be important in modifying bacterial communities (Shade *et al.*, 2007). Temperature affects the growth of bacteria, but because temperature variation under ice is only a couple degrees, this effect may be secondary; grazing, availability of nutrients and particularly dissolved organic carbon probably control bacterial abundance to a greater extent. During phytoplankton spring blooms, the amount of dissolved organic carbon in the water column typically increases with a concomitant increase in bacterioplankton (Bell and Kuparinen, 1984; Cole *et al.*, 1988; Weisse *et al.*, 1990). However, as with picophytoplankton, bacterioplankton studies have been focused on the open water season and bacterioplankton development during the evolution of under-ice spring mixing is largely unknown (Bertilsson *et al.*, 2013).

We studied the effects of mixing on under-ice abundance, biomass and vertical distribution of picophytoplankton and heterotrophic bacteria in a eutrophic boreal lake. We undertook a basin-scale experiment, whereby we compared effects of natural under-ice mixing in late winter with a situation in which the lake basin was mechanically mixed through the winter. By using a hydrocopter, we were able to take samples throughout the period of ice melting. We hypothesized that mechanically strengthened mixing should worsen the light climate for picophytoplankton and consequently its growth, and that this should also be reflected in reduced growth of heterotrophic bacterioplankton due to reduced availability of suitable organic substrates derived from autotrophic phytoplankton.

METHOD

This study was done in the Enonselkä basin (Table I) of Lake Vesijärvi, Southern Finland, in winters 2009–2011. The Enonselkä basin is connected to the rest of the lake by a narrow strait with insignificant exchange flow of water against the low outflow from the Enonselkä basin. In winters 2010 and 2011, the basin was mechanically mixed by nine mixing stations (each 1.5–2.5 kW) located at the deepest sites (Salmi *et al.*, in press). The operational principle of the mixing stations has been modelled and

Table I: General characteristics of the Enonselkä basin of Lake Vesijärvi (source: database of Finnish Environment Institute)

	2000–2009		2010–2013	
Parameter	Mean	Range	Mean	Range
Area (km^2)	26			
Depth (m)	6.8	0–32		
Tot-P (μmol L^{-1}) winter	1.0	0.7–1.6	0.9	0.8–1.0
Tot-N (μmol L^{-1}) winter	44	39–52	39	35–43
Tot-P (μmol L^{-1}) summer	1.2	0.9–1.8	1.2	1.0–1.3
Tot-N (μmol L^{-1}) summer	38	28–49	34	32–37
Chl *a* (μg L^{-1})	11	3–76	10	4–24

Total phosphorus (P) and nitrogen (N) in winter are volume weighted concentrations calculated for March–April 2000–2009 (no mechanical mixing) and March 2010–2013 (the mixing years). Corresponding nutrient results from summer are from August. Chlorophyll *a* concentrations (Chl *a*) were measured from the epilimnion at 1–5 weeks intervals in May–August.

tested using rhodamine marker in a semi-enclosed bay on the south western coast of Finland (Bendtsen *et al.*, 2013) with similar depth as our study site. Each mixing station pumped water from 3 m depth downwards through a canvas tube with a constant speed of about 1 m^3 s^{-1}. The pumped water with relatively low density ascended as a buoyant plume and entrained surrounding water (Salmi *et al.*, in press). In spring 2010, the mixing stations were shut down after ice-break and in 2011 they were switched off 12 days before ice-break. Sampling and *in situ* measurements were made at a 31 m deep site (WGS84 coordinates: 61°01.089′N and 25°36.211′E) ~50 m from a 2.5 kW mixing station off Lankiluoto islet.

Temperature profiles were measured every 30 min with Starmon Mini recorders (Star-Oddi, Iceland, accuracy 0.05°C) attached to a rope with an underwater buoy from 1 to 31 m depth at 2 m depth intervals. Heat content (Θ) for the Enonselkä basin was calculated from these measurements as:

$$\Theta = \frac{1}{A_0}\int_0^{Z_{max}} c\rho AT dz \quad (1)$$

where A_0 (km^2) is the lake surface area, c (J g^{-1} $°C^{-1}$) the specific heat capacity of water, ρ (kg m^{-3}) the density of the water layer, A (km^2) the lake area at depth z (m) and T the temperature (°C) (Johnson *et al.*, 1978).

Oxygen concentrations in late winter 2009 were determined by Winkler titration from samples taken at 2–5 m depth intervals. In late winters, 2010–2011 oxygen profiles were measured at 1–2 m depth intervals with an optical sensor (Pro-ODO, Yellow Springs Instruments, USA, accuracy 0.1 g m^{-3}) and periodically calibrated with Winkler titration. Snow and ice thickness were measured with a wooden gauge. On the two last sampling times in 2009, temperature profiles were also measured with a Micro CTD-3 (Falmouth Scientific, USA, accuracy 0.005°C) and in late winter 2011 with CastAway CTD probe (Yellow Springs Instruments, USA, accuracy 0.05°C). The interface between the convection and quiescent water layers was estimated on the depth interval where temperature and oxygen concentration changed the most.

In winter 2009, picophytoplankton and bacteria samples were taken with a tube sampler (volume 2.1 L, Limnos, Finland) from the depths of 2, 4, 6, 8, 10, 15, 20, 25 and 30 m, and in winter 2010 (picophytoplankton and bacteria) and in 2011 (only picophytoplankton) with 5 m increments from the surface to the bottom. Secchi-depths were estimated against the white upper lid of the tube sampler (diameter 11 cm). Samples immediately below the ice (denoted 0 m) were taken with a rectangular plastic tube (50 × 50 × 750 mm, see Supplementary Material online) to reduce the disturbance caused by drilling of the ice. The rectangular tube, with plastic foam sides for floatation, and a rolling brush at its front against the ice to facilitate collection of organisms possibly adhering to the underside of the ice, was placed below the ice and moved horizontally along the lower surface of the ice until its rear end was at the margin of the hole in the ice. Its opening was then closed by hand and the sample was rapidly lifted into a bucket. Subsamples were poured into 100 mL dark glass bottles and no preservatives were added. Bottles covered with slush were transported to the laboratory, where picophytoplankton and bacterioplankton samples were filtered separately on black polycarbonate filters (diameter 25 mm, pore size 0.2 μm, Millipore, USA). Bacteria were stained with acriflavine for 1 min (Bergström *et al.*, 1986). Because the abundances of bacteria were two orders of magnitude greater than those of picophytoplankton, possible difficulties in differentiating between them did not significantly affect the results. Both filters were stored in fluorescence-free immersion oil between objective and cover glasses at −20°C until counting (Booth, 1993; Turley and Hughes, 1992).

Picoplankton were counted with an epifluorescence microscope (Olympus IX50, Olympus Optical Co. Ltd, Tokyo, Japan). Unstained picophytoplankton samples were counted at ×1000 magnification using blue (Olympus U-MWB; excitation filter 450–480 nm, dichroid mirror 500 nm, barrier filter 515 nm) and green (Olympus U-MWG; excitation filter 510–550 nm, dichroid mirror 570 nm, barrier filter 590 nm) filter sets. With blue excitation, autofluorescence of phycocyanin-rich picocyanobacteria was negligible, but phycoerythrin-rich picocyanobacteria emitted orange and eukaryotic picophytoplankton emitted red light. With green excitation, phycoerythrin-rich cells emitted bright orange while phycocyanin-rich cells emitted deep red light and eukaryotic picophytoplankton showed only weak red autofluorescence (MacIsaac and Stockner, 1993). Acriflavine-stained bacteria were counted using the blue excitation filter set.

In spring 2009, part of the picophytoplankton samples were counted within 3–4 days after sampling. The longest storage time (1.5 years) was for samples from spring 2011. The preservation of fluorescent pigments in darkness and −20°C temperature was confirmed by comparing the results of the samples taken on 25 May 2009 and counted with 3–4 days storage with the same samples counted again after 2.5 years of storage (Table II). The average ratio between picophytoplankton abundances after short and long storage time was 1.00 (SD = 0.26). Thus the effect of storage was insignificant (paired *t*-test: df = 5, $P = 0.8$).

Table II: Picophytoplankton abundances in samples taken on 25 May 2009 and counted after 3–4 days and again after 2.5 years of storage

Abundances ($\times 10^4$ cells mL^{-1}) after storage time of		
Sampling depth (m)	2.5 years	3–4 days
0	10.7	12.2
6	14.1	12.4
10	9.9	8.6
20	3.1	2.3
25	2.2	2.6
30	2.3	3.5

The dimensions of each counted cell were estimated using an eye-piece graticule with a series of circles of different diameters so that the measurements were made by comparison. Due to the characteristics of the available graticule, cells with diameter equal to or smaller than 2.2 μm diameter were considered picoplankton. Necessary dimensions of cells (sphere, ellipsoid, rod, double cone) were measured to calculate approximate biovolumes which were converted to biomass assuming equivalent density to water. The target for counting was 95% confidence limits ≤30% for the mean of total biomass. Directional confidence limits (Cfl 95%) for total biomass were calculated from the sum of the variances of individual classes weighted by their proportion in total biomass. The number of replicate microscopic fields to reach this was estimated in real time according to confidence limits provided by a proprietary counting programme (VersaCount for Windows). For bacterioplankton samples, 10 random fields of view were counted and this was also the minimum number for picophytoplankton samples irrespective of confidence limits. Volume-weighted biomass and abundance for picophytoplankton and bacteria in the water column were calculated by assuming the form of a frustum for the Enonselkä basin. The water column was divided into layers so that each sampling depth was located at the middle of a layer, except 0 m depth, which corresponded to the layer of 0–1 or 0–2.5 m depending on the next sampling depth. Biomass and abundance in each layer were multiplied by the respective water volume and the products were summed and finally divided by the total volume of the basin to obtain mean values for the whole basin. Volume-weighted oxygen concentrations were calculated in a similar manner, but the vertical sampling interval varied between 1 and 5 m as described above. In 2009, bacterioplankton samples taken 2 weeks before ice-break from 25 and 30 m depths were lost. However, as the water layers below 22.5 m contributed only 1% of the total volume of the Enonselkä basin, this loss is negligible.

Statistical analyses were done with SPSS Statistics 21 (IBM, USA). Paired *t*-test was carried out to test the preservation of autofluorescence in the stored samples. Independent samples *t*-test was chosen to test differences in bacterial abundance in deep and upper water column and Mann–Whitney *U*-test to test differences in bacterial cell sizes. Prior to all tests, Shapiro–Wilk's test was applied to test normality and Levene's test was used to test equality of variances of the samples.

RESULTS

Physical and chemical properties of the water column

Four to 6 weeks before ice-break, the Enonselkä basin was covered by 24–42 cm of clear ice, 4–37 cm of snow ice and 0.5–12 cm of snow (Fig. 1). In late winter 2009, water temperature was inversely distributed with highest temperature of 3.7°C at 30 m (Fig. 2) and there was no oxygen at 25–31 m depths (Fig. 3). In 2010–2011, mechanical mixing reduced deep water temperatures to 2.6–2.7°C (Fig. 2), and oxygen concentration at 30–31 m remained high (>6 g m^{-3}, Fig. 3). During the last month of ice cover, increasing solar radiation gradually melted the snow and ice cover and increased the heat content of the basin from 38–54 to 91–110 MJ m^{-3} before ice-break (Fig. 1). Each year, the warming of the uppermost water layers by solar radiation created a vertical convection layer, in which both temperature and oxygen concentrations were practically uniform (Figs 1–3). In late winter 2009, about 2 weeks before ice-break the convective mixing reached 10 m depth and a week later this was doubled (Figs 1 and 2). Thereafter, the deepening of the convection layer was slowed by a chemocline at 20–21 m depth. The salt concentration of the deepest water increased its density so much that it could compensate for a rise in water temperature of about 0.5°C before mixing occurred (Figs 1 and 2). Thus, overturn and oxygen replenishment reached the deepest water only after ice-break. In contrast, mechanical mixing prevented the development of a significant chemocline during winter and facilitated the progress of convective mixing. Consequently, in winter 2010, the water column experienced overturn already 10 days before ice-break. In winter 2011, 1 day after switching off the mixing stations (11 days before ice-break), the convective layer was observed to reach 12 m depth (Figs 1 and 2) and the overturn of the basin started only after ice-break.

Each year, decreasing Secchi-depth towards ice-break indicated growth of phytoplankton (Fig. 1) and oxygen concentration was increased in the convection layer by primary production (Figs 3 and 4).

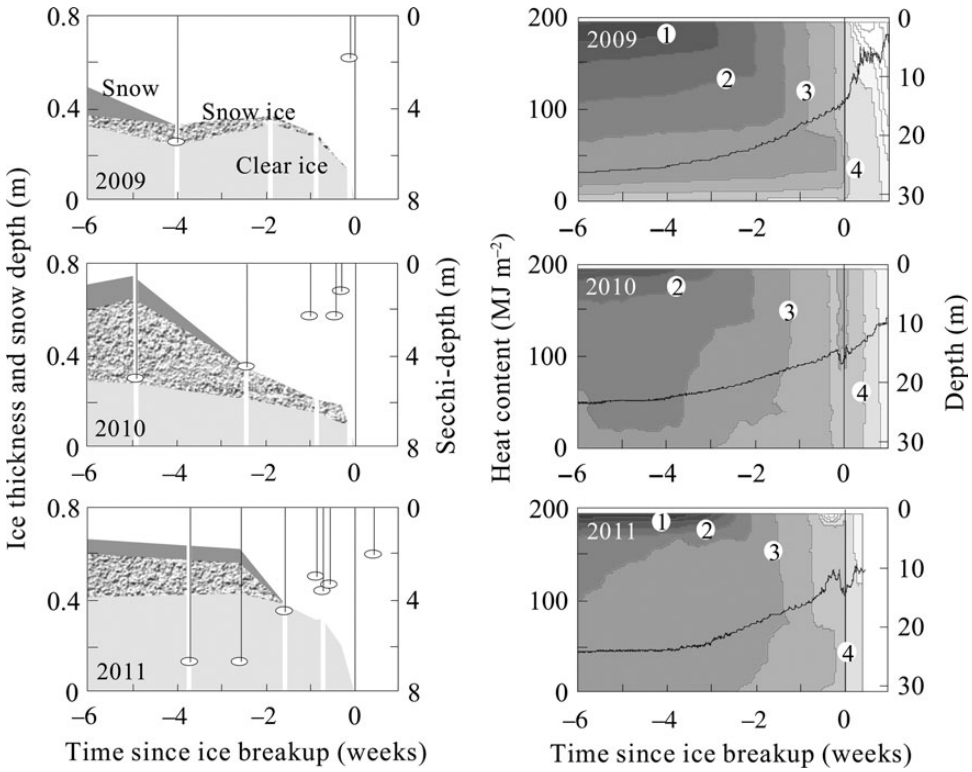

Fig. 1. Snow depth, ice thickness and Secchi-depth (white plates) in late spring (left panels), and heat volume (regular curves) and isotherms as 24 h moving average (right panels). White vertical lines (left panels) mark the sampling dates for picoplankton and black vertical lines at time 0 mark the ice breakups.

Picophytoplankton

Picophytoplankton were always present throughout the water column, even at the virtually dark greatest depths (Fig. 2). Between the melting of snow and ice-break in 2009, their biomass for the whole water volume of the basin increased by ca. 140% (Fig. 4). As a consequence of mechanical mixing, in 2010 the vertical distribution of biomass was already uniform in the whole water column before increased penetration of light into the water. Biomass of picophytoplankton increased throughout the water column and their vertical distribution was even except on the sampling date just preceding ice-break, when their biomass was reduced in the upper water column (Fig. 2). During the last 5 weeks of ice cover, the volume-weighted biomass of picophytoplankton increased by up to 13 times (Fig. 4). In 2011, roughly 4 weeks before ice-break (i.e. before shutting down the mixing), biomass just below the ice was three times higher than in the rest of the water column (Fig. 2). It was located within the under-ice boundary layer, which remained above the mixing zone, and coincided with the beginning of the increase in light intensity (Fig. 1), and shows that picophytoplankton was immediately able to utilize increased light very near to the ice. Later the surface maximum in the boundary layer was reduced and vertical distribution stayed even, probably by an increasing amount of dilute melt water from ice. In contrast to the other years, in the following weeks biomass and its distribution remained relatively constant after the mixing stations were switched off 12 days before ice-break (Fig. 4). In the study years, the development of vertical distribution of picophytoplankton abundance corresponded to that of their biomass (Supplemental Material online).

In late winters 2009–2010, picocyanobacteria and eukaryotic picophytoplankton were almost equally abundant throughout the period of convective mixing (Fig. 5). However, due to their average cell size being up to 250% larger, eukaryotic picophytoplankton were dominant in the biomass. In late winter 2011, the dominance of eukaryotic picophytoplankton over picocyanobacteria was greatest during the study in terms of both abundance and biomass.

Bacterioplankton

In late winter 2009, bacterioplankton biomass was rather uniform in the upper oxic water layers, but increased dramatically in anoxic deep water (Fig. 3). In the 25–30 m

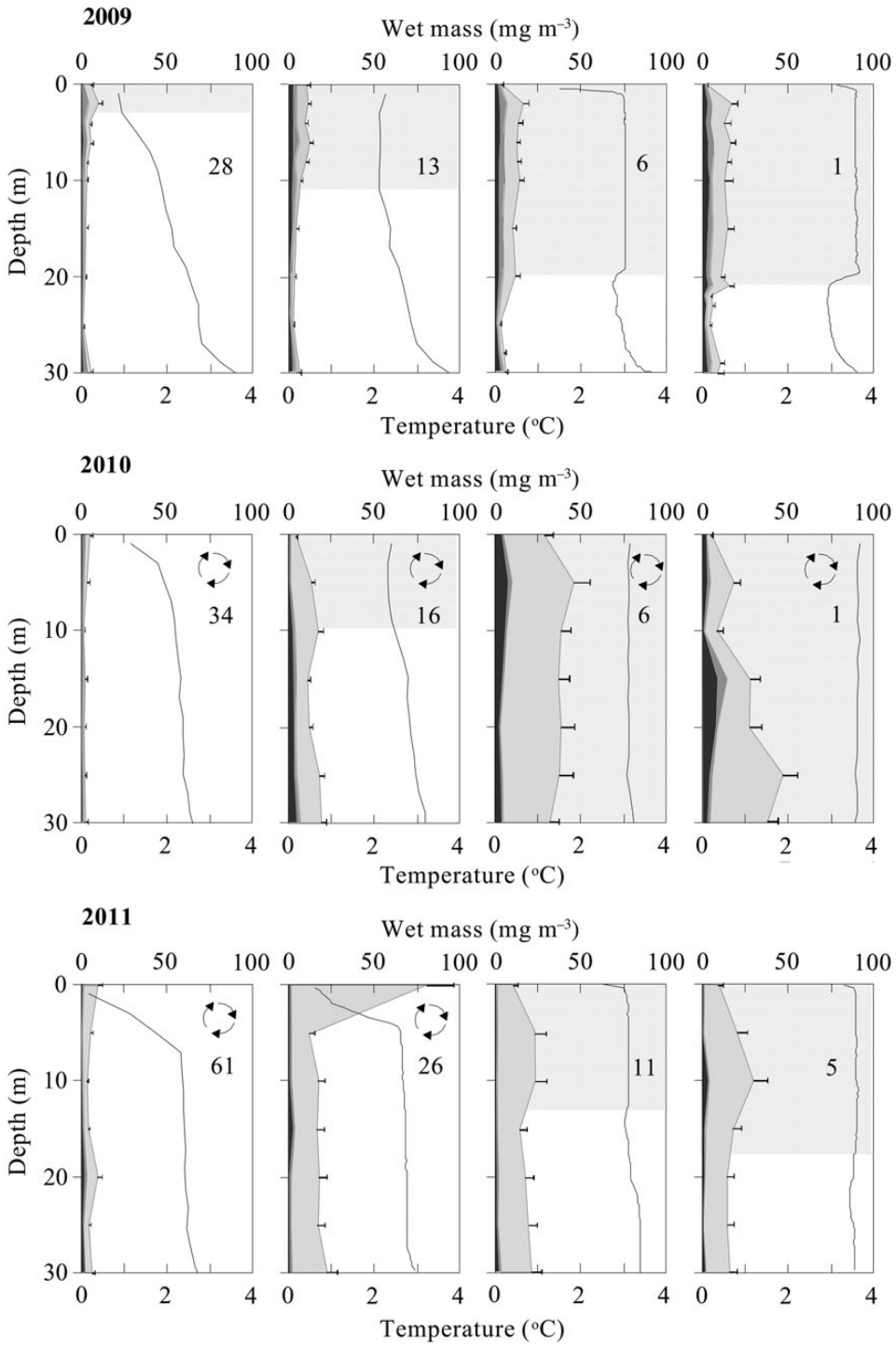

Fig. 2. Vertical distribution of picophytoplankton wet biomass and temperature (black lines) under the ice cover off Lankiluoto islet. Black: phycocyanin-rich picocyanobacteria; dark grey: phycoerythrin-rich picocyanobacteria; light grey: eukaryotic picophytoplankton. Shaded background highlights the depth of convective layer. The error bars are 95% confidence limits for the mean total biomass counted from parallel microscopic fields. The numbers inside the panels denote the number of days before ice breakup. Circular arrows in the upper right corner of the panels indicate that mechanical mixing was on.

deep water layer, the values were from three to seven times higher than in the oxic water layers, due to significantly higher abundances (*t*-test, $P < 0.001$, $df_1 = 11$, $df_2 = 26$, vertical distributions of bacterial abundances included in Supplementary Material online) and larger cell size (Mann–Whitney *U*-test, $U = 298$, $P < 0.001$,

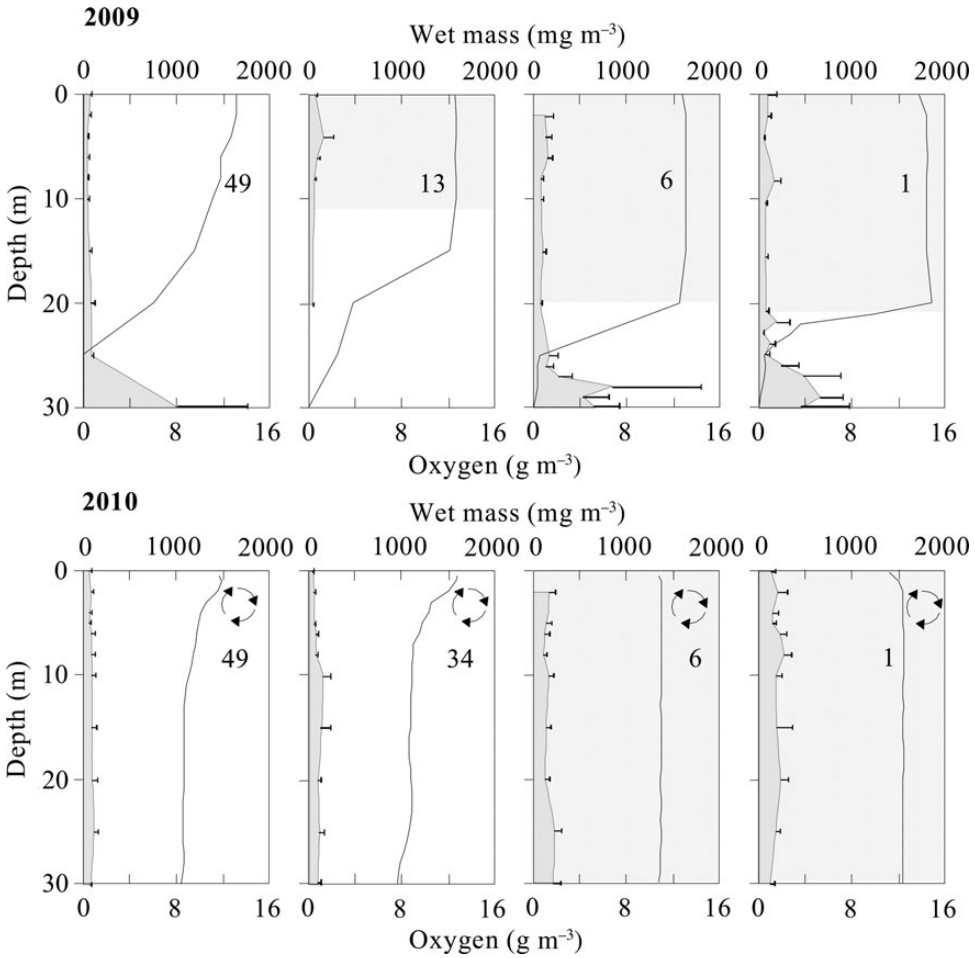

Fig. 3. Vertical distribution of wet biomass of bacteria (grey areas) and concentration of dissolved oxygen (black lines). Shaded background highlights the depth of convection layer. Vertical bars are 95% confidence limits for the mean biomass counted from parallel microscopic fields. The numbers inside the panels denote the number of days before ice breakup. In 2009, data were missing below 20 m depth 13 days before ice-break. Circular arrows in the upper right corner of the panels indicate that mechanical mixing was on.

$N_1 = 12$, $N_2 = 27$, Fig. 6). In the samples taken from 25 to 30 m depth, the mean size was 0.08 μm^3 (SD = 0.05), whereas in samples taken from 0 to 24 m depth it was 0.03 μm^3 (SD = 0.01). In 2010, mechanical mixing kept oxygen concentration and biomass of bacteria uniform throughout the whole water column (Fig. 3). In the convective layer, bacterioplankton biomass increased in parallel with that of picophytoplankton (Fig. 7). During the 6 weeks before the ice-break, volume-weighted bacterial biomass increased by 150 and 160% (increase of 70 and 110 mg m^{-3}, Fig. 4) in 2009 and 2010, respectively.

DISCUSSION

Mechanical mixing of the Enonselkä basin worked technically as anticipated. It smoothened the water column temperature, oxygen and nutrient gradients both vertically and horizontally (Salmi *et al.*, in press). Total phosphorus and total nitrogen concentrations at 29 m depth were reduced by 40–50%, but because of the small relative proportion of deep water in the total water volume of the basin, no change in volume-weighted nutrient concentrations were detected (Table I). Thus, mechanical mixing probably had no great influence on nutrient availability to picophytoplankton, although this conclusion remains tentative in view of the limited chemical determinations from only three depths.

The comparison between natural and mixing years in the Enonselkä basin is complicated by interannual differences in snow and ice conditions which are linked to the stochasticity of weather. Particularly, snow on the top of ice plays a big role, because it controls the availability of light for phytoplankton (e.g. Jewson *et al.*, 2009). The cumulative amount of solar radiation was directly reflected in the deepening of the convection layer. In late winter 2011, convection developed later than in the other years (Figs 1 and 2) and this delayed the development of all autotrophs as indicated by the deepest

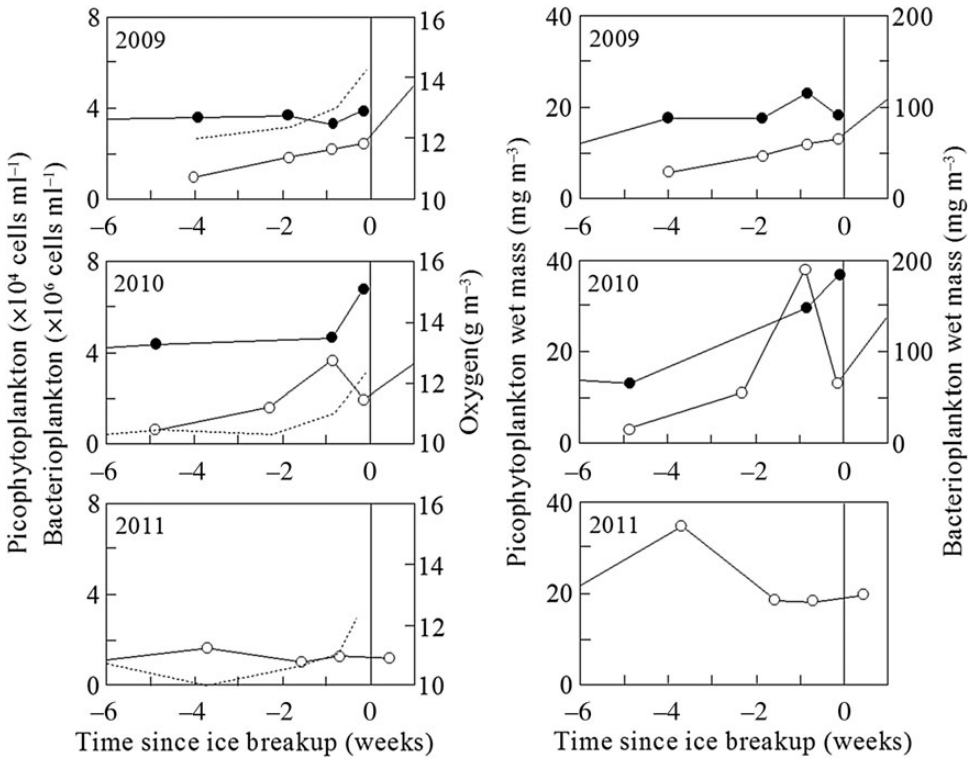

Fig. 4. Volume-weighted concentration of dissolved oxygen (grey-dashed line in left panels) and volume-weighted abundances (left panels) and wet biomass (right panels) of picophytoplankton (open circles) and bacterioplankton (black dots). Vertical lines at time 0 mark ice-break times.

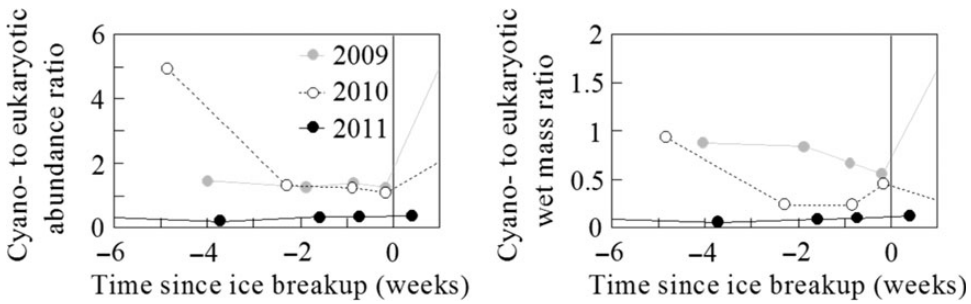

Fig. 5. The relationship between picocyanobacteria and eukaryotic picophytoplankton abundance and wet biomass. Black vertical lines at time 0 mark ice-break times.

Secchi-depths (Fig. 1). In the early phase of increased light penetration into water in the years 2010 and 2011, convection initially outweighed mixing by pumps so that a convective layer could be distinguished in the upper part of the water column (Fig. 3). The earlier switching off of the mixing stations in spring 2011 (Fig. 4) showed that, although mechanical mixing during winter broke down the chemical stratification and also smoothened vertical temperature differences, natural convection was not necessarily strong enough to mix the basin to the bottom before ice-break.

The presence of picophytoplankton in anoxic deep water in 2009 was surprising, because no light can penetrate so deep to support photosynthesis. Experiments on marine picophytoplankton strains have shown that they are able to lower their metabolism and hence to cope over months of darkness (Antia, 1976; Stockner, 1988). Therefore, deep-water picophytoplankton had likely survived from the open water of the previous year. The ability to survive for 4–5 months means picophytoplankton are ready to grow whenever appropriate light conditions may occur. In spite of low turbulence under ice, the sedimentation rate of picoplankton was evidently negligible.

Our results demonstrate that after the melting of snow light availability initially determined vertical distribution of picophytoplankton biomass and limited it to the

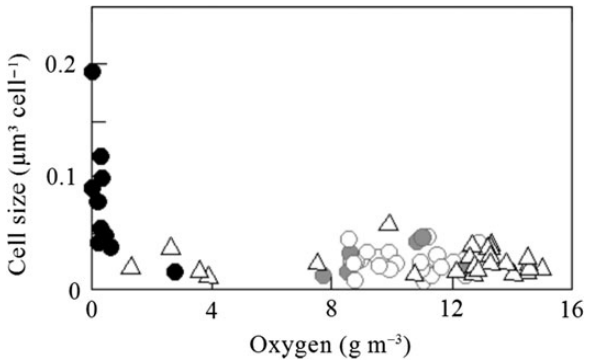

Fig. 6. Relationship between dissolved oxygen concentration and average size of bacteria in samples taken throughout the water column. Triangles represent samples from 0 to 24 m depth and black dot samples from 25 to 30 m in 2009. Grey circles and grey dots represent the same for 2010.

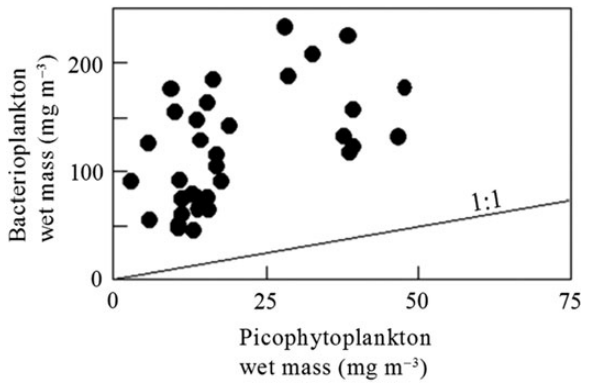

Fig. 7. Relationship between picophytoplankton and bacterioplankton wet biomass in samples taken from the convective layer in late winters 2009–2010.

uppermost water layers (Fig. 2). However, increasing penetration of solar radiation into the water soon led to convective mixing which then controlled the biomass distribution. With increasing biomass in the mixed layer (including larger phytoplankton), nutrients probably also became important. In 2010–2011, convection strengthened by mechanical mixing kept the vertical distribution of picophytoplankton homogenous in the whole water column (Fig. 2) and may then have reduced average light availability to picophytoplankton cells. However, higher biomass throughout the water column in 2010–2011 compared with 2009 (Figs 2 and 4) suggests that their growth was possibly limited by the availability of nutrients. Thus in the Enonselkä basin, mechanical mixing did not seem to keep phytoplankton below the compensation depth of photosynthesis for long enough to inhibit growth. Late winter, total picophytoplankton abundances in the Enonselkä basin correspond to abundances at the time of ice-break in a boreal lake with slightly higher total phosphorus content (Ventelä *et al.*, 1998) and to those during early summer in less eutrophic lakes (Jasser and Arvola, 2003; Peltomaa and Ojala, 2010), but were an order of magnitude lower than abundances in summer in the Enonselkä basin (Brek-Laitinen *et al.*, 2012).

In all study years, the higher proportion of eukaryotic picophytoplankton observed in the picoplankton total biomass along with increasing penetration of light through ice is consistent with observations made in many temperate and boreal lakes (Callieri, 2008; Vörös *et al.*, 2009; Winder, 2009). In the Enonselkä basin, summer picophytoplankton has been reported to consist only of phycoerythrin-rich picocyanobacteria (Brek-Laitinen *et al.*, 2012), which were also numerically dominant in our study in samples taken 1–4 weeks after ice-break in 2009–2010 (data not shown). Eukaryotic picophytoplankton strains from Hungarian soda pans had higher photosynthetic activity at lower light intensities than picocyanobacteria especially at temperatures down to 7°C (Somogyi *et al.*, 2009). We did not measure photosynthetic activity, but the increase of the biomass of eukaryotic picophytoplankton under the ice confirm those experiments, even though under-ice temperatures were notably lower.

The stronger eukaryotic picophytoplankton dominance in the years of mechanical mixing than in 2009 led to the overall higher total picophytoplankton biomass. This was unlikely to be due to temperature differences, since during the convective period the mechanical mixing did not notably affect the temperature (Figs 1 and 2). Instead, a combination of enhanced mixing of nutrients and reduced average light conditions might have been the main reasons providing a competitive advantage to eukaryotic picophytoplankton. This view is supported by the stronger picoeucaryotic dominance in late winter 2011 when the ice was thicker (Fig. 1).

The most important reasons for the different vertical distributions of bacterioplankton from those of picophytoplankton were anoxia in deep water and solar radiation in the uppermost water layers. Bacterioplankton in anoxic water are generally more abundant and show higher biomass than in oxic water (e.g. Cole *et al.*, 1993; Kuuppo-Leinikki and Salonen, 1992). Cole *et al.* (Cole *et al.*, 1993) showed that in 20 stratified lakes in USA, bacteria were 2–10 times larger (size range 0.01–0.2 μm^3) in anoxic hypolimnia than in oxic epilimnetic water, which agrees with our observations from the Enonselkä basin of 2–6 times higher sizes (size range 0.01–0.19 μm^3). The larger cell size in anoxic waters may be due to many factors such as taxonomic composition (Cole *et al.*, 1993; Hernández-Avilés *et al.*, 2012; Shade *et al.*, 2007) and size-selective or less active grazing (Cole *et al.*, 1993; Hernández-Avilés *et al.*, 2012;

Kuuppo-Leinikki and Salonen, 1992). Thus in the Enonselkä basin, the change observed in the cell size of deep water bacterioplankton after mechanical mixing indicates changes in the bacterial community. However, changes in taxonomic units of bacteria could not be evaluated microscopically. Molecular microbiological methods would be needed to reveal the changes in bacterial community along with different mixing regimes. As with picophytoplankton, under-ice abundances of bacterioplankton were an order of magnitude lower than those in summer in the Enonselkä basin (Brek-Laitinen *et al.*, 2012). The parallel increase in volume-weighted bacterial biomass and respective picophytoplankton biomass (Figs 4 and 7) suggests an interaction. However, since the increase in bacterial biomass was three to six times greater than the increase in late winter picophytoplankton biomass, dissolved excreted organic carbon originating from picophytoplankton alone may not have been enough to support bacterial growth. A comparison between the molar increase in picophytoplankton carbon biomass (assuming 200 fg C μm^{-3}, Weisse, 1993) and oxygen in the water column during the last 4 weeks of ice cover suggests that picophytoplankton contributed only 0.2–0.6% of phytoplankton primary production. Thus larger phytoplankton made up the bulk of the under-ice primary production maximum and also explained the increase of bacterioplankton biomass.

Both our picophytoplankton and bacterioplankton results demonstrate that abundances alone may be insufficient to interpret results. If a standard biovolume for bacterioplankton would have been applied, which is not unusual, the difference between total biomass in the oxic and anoxic/hypoxic zones would have been much less conspicuous. Similarly, in picophytoplankton, the common usage of only abundance (or with assumed standard cell volume) may lead to incorrect interpretation of results. Eukaryotic cells are often larger than picocyanobacteria (Callieri, 2008; Calvo-Diaz *et al.*, 2004) and this significantly changes the interpretation of our results. Although determination of biomass is much more tedious than simple counting of samples, the higher ecological significance of the results makes it preferable. Furthermore, confidence limits for abundances as well as biomass should also be evaluated, to provide the necessary perspective for the interpretation of results. This is a simple task with modern computers and therefore it is no longer any excuse to neglect confidence limits calculated from the original counts. Because we took no replicate samples, our confidence limits represent only part of the total variation, but this shortcoming was compensated for by the rather frequent vertical coverage of samples so that the general degree of the consistency of the results gives a good idea about variation between individual samples, albeit without numerical values. In fact, in terms of the total amount of information obtained per unit effort, we suggest that this approach is more economical than if vertical resolution would have been dramatically reduced in favour of counting parallel samples from fewer depths. We still lack information on horizontal distribution of picoplankton in the Enonselkä basin. However, the horizontal distribution of under-ice oxygen concentration indirectly indicates rather uniform distribution in the mechanical mixing years. Thus picoplankton cells are likely to spread accordingly. We have no similar data for years without the mechanical mixing, but the rise in the temperature of the water column during winter 2009 in the whole water column suggests respective mixing (Salmi *et al.*, in press).

Contrary to our hypothesis, deeper mixing enhanced by pumping did not decrease the abundance or biomass of picophytoplankton. Instead, picophytoplankton biomass rather increased, since eukaryotic picophytoplankton seemed to be able to cope with a changing light environment at low temperature, and were possibly further facilitated by nutrient increase due to mechanical mixing. Decrease in bacterial abundance or biomass due to deeper mixing was not observed either, possibly because it may have largely relied on organic matter produced by phytoplankton. Mechanical mixing may also have increased leaching of elements from the sediment irrespective of improved oxygen conditions in the water column. In general, our results show that the development of the spring picoplankton assemblage and biomass often assumed to occur after ice-break, probably largely occurs under ice. This influences the microbial community and its metabolism during and after the important spring primary production maximum.

SUPPLEMENTARY DATA

Supplementary data can be found online at http://plankt.oxfordjournals.org.

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