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The role and composition of winter picoeukaryotic assemblages in shallow Central European great lakes

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Abstract

Studies on autotrophic picoplankton (APP; <3 µm) in shallow lakes are mainly confined to the spring-fall seasons, when sampling efforts are not complicated by adverse and unsafe conditions that occur during winter. The aim of the present work was to study the role and diversity of winter APP communities in temperate shallow lakes by means of analysis of measures of size-fractionated photosynthesis and culture-based molecular taxonomic identification. Our results show that APP comprised a substantial part of planktonic primary production in shallow Central European great lakes (13-46% in Lake Balaton and 11-42% in Lake Fertő). Better acclimation of APP than that of the larger phytoplankton (>3 µm) to low-temperature and low-light winter environment was confirmed by their higher maximum photosynthetic rate and light utilization parameter. Maximum photosynthetic rate and light saturation parameter increased significantly with both temperature and available light, but with different impact on the two size groups. Twenty-two picoeukaryotic strains were isolated and identified based on 18S rRNA gene sequence analysis. Taxonomic composition of the

picoeukaryotic community in the studied shallow lakes was similar to other freshwater lakes in the temperate zone: members of genera *Choricystis* and *Mychonastes* were dominant, however, in Lake Balaton, common freshwater taxa such as *Stichococcus bacillaris* and *Nannochloris bacillaris* were also found.
Keywords: picoeukaryotes, primary production, fractionated photosynthesis, winter, ice cover

Introduction

Autotrophic picoplankton (APP) includes the smallest photosynthetic organisms, consisting of prokaryotic and eukaryotic taxa within the size range of 0.2-2 or 3 µm. The term was originally defined by Sieburth et al. (1978) to the 0.2-2 µm size range, but was later expanded to 3 µm because of the larger cell size of picoeukaryotes (Vaulot et al., 2008). APP is a major component of the photosynthetic biomass in many aquatic ecosystems, particularly in oligotrophic lakes and oceans (Agawin et al., 2000; Callieri et al., 2007; Stockner, 1991; Weisse, 1993). Thus, it constitutes an important source of energy in aquatic food webs as an integral part of the microbial loop (Azam et al., 1983; Callieri 2008 and references therein). APP cells are more effective in nutrient and light acquisition than larger phytoplankton owing to their high surface area (Irwin et al., 2006) and their reduced chromophore self-shading (Raven, 1998). Accordingly, these tiny cells are assumed to be good competitors in resource-poor habitats (Callieri, 2008) and under low light conditions (Callieri, 2016). Besides nutrient supply and light availability, other factors such as water temperature, salinity, grazing or viral infection also influence the occurrence and dynamics of APP in aquatic ecosystems (Callieri, 2008; Stockner, 1991).

The contribution of APP to total phytoplankton biomass and primary production can be significant. In the tropical Pacific Ocean, for example, Li et al. (1983) attributed 25-80% of the inorganic carbon fixation to APP, while in the South East Pacific Ocean, APP constituted 36–57% of the total primary production (Rii et al., 2016). APP was also found to constitute 60-80% of the total primary production in the Sargasso Sea (Glover et al., 1985), an average of 44% in the subtropical and tropical regions of the North Atlantic Ocean (Jardillier et al., 2010) and 12-83% in the south Atlantic and Atlantic sectors of the Southern Ocean (Froneman et al., 2001). In a temperate coastal ecosystem APP accounted for a mean annual value of 51% (4 to 76%) of total carbon fixation (Morán, 2007). Global estimates indicate that these small cells provide 39% of the planktonic primary production in the world's ocean (Agawin et al., 2000). These results show that APP dominate phytoplankton biomass and production in nutrient-poor, warm (> 26°C) waters, but represent only a minor part of the autotrophic biomass and production in nutrient-rich and cold (< 3°C) waters (Agawin et al., 2000).

Less information is available for freshwater lakes, despite the fact that the contribution of APP could be as high as in marine waters (Bell and Kalff, 2001). In North American deep lakes, for example, APP production comprised 25-60% (Lake Huron) or 10-70% (Lake Michigan) of carbon fixation (Fahnenstiel and Carrick, 1992). Higher contribution values were described in a small meromictic lake (Little Round Lake, USA), where APP composed 22-97% of the planktonic primary production. In a mesotrophic deep lake in Austria (Lake Mondsee) APP constituted 16-58% of the total, area-integrated primary production (Greisberger et al., 2008). In eutrophic, humic shallow lakes from the floodplain of the Lower Parana River (Argentina) picoplankton production represented 36-93% of the total carbon fixation (Izaguirre et al., 2010; Rodriguez et al., 2012). In a meso-eutrophic humic shallow lake in Finland, APP constituted 5-57% of the primary production (Peltomaa and Ojala, 2010), while in a eutrophic, river-dominated estuary, picoplankton accounted for 42-55% of total primary productivity (Gaulke et al., 2010).

Size-fractionated primary production measurements were also conducted in Hungarian shallow lakes. In Lake Balaton, the contribution of APP varied between 40 and 50% in the less productive Eastern basin and between 1 and 57% in the Western basin during the productive period of the year (Vörös et al., 1991). More than a decade later in summer 2005, it ranged between 23 and 54% along the longitudinal axis of the lake (Somogyi and Vörös, 2006). In a hypertrophic reservoir in Hungary, APP accounted for 10-55% of the total primary

production (Vörös et al., 1991). These results confirmed the essential role of these small cells in the carbon cycling of Hungarian shallow lakes.

Most of the fractionated photosynthesis measurements in freshwater lakes were confined to a relatively short period, usually between spring and autumn at the peak of APP development (e.g. Callieri et al., 2007; Malinsky-Rushansky et al., 1997; Steitz and Velimirov, 1999; Vörös et al., 1991). Little is known about the role of winter APP assemblages in ecosystem processes, despite the fact that studies on size-fractionated phytoplankton are essential to understand food web dynamics in aquatic ecosystems (Callieri, 2008). Undersampling is particularly obvious in the freshwater lakes of the temperate zone, where sampling in winter is difficult and sometimes virtually impossible because of variable and unsafe ice conditions (Dokulil and Herzig, 2009; Felföldi et al., 2016). There have been some size-fractionated primary production measurements performed in the winter season, but these studies were done mainly in deep lakes. In the case of Lake Kinneret (Israel), for example, the photosynthetic activity of APP and its contribution to total photosynthesis was lower in winter than during the productive period (Malinsky-Rushansky et al., 1997). In an alkaline, deep lake (Mono Lake, California), APP accounts for nearly 25% of the primary production during the winter bloom and more than 50% at other times of the year (Roesler et al., 2002). Similar results were found in the case of Lake Simcoe (Canada), where winter APP contribution was 40% on average at nearshore sites and 30% at offshore sites (Kim et al., 2015), and showed a higher share (up to 90%) from spring to autumn. In contrast, in Lake Mondsee, APP contribution to total primary production was highest (as much as 58%) in the autumn- winter period (Greisberger et al., 2008). However, it should be noted that in the case of Lake Kinneret, the minimum temperature in winter does not drop below 10 °C (Malinsky-Rushansky et al., 1997), as opposed to the abovementioned lakes, which usually possess ice cover.

Although there is growing information on the role of summer APP assemblages in ecosystem processes, there is still a gap concerning the role of APP communities in winter. This is also true for the taxonomic composition of picoplankton (Callieri, 2008). Thus, a comprehensive study is needed in order to have a better understanding of winter picoplankton. The objectives of this study were to characterize and compare the photosynthetic activity of the winter picoplankton and larger-sized phytoplankton in Central European great lakes. Our aim was to estimate the contribution of the picoplankton to total phytoplankton biomass and primary production. Additionally, we also wanted to characterize the taxonomic composition of winter picoeukaryotic assemblages by molecular phylogenetic tools.

Methods

Study site and sampling

Lake Balaton (Hungary) is the largest lake in Central Europe with a surface area of 596 km^2 and an average depth of 3.2 m (Fig. 1). Specific conductivity (SC, 700–800 µS/cm) and pH (8.3-8.6) are relatively high and constant. In winter, the lake is usually frozen for 42 ± 27 days, but years without ice cover can also occur (Vörös et al., 2009). There is a depth and trophic gradient along the longitudinal axis of the lake, which results in higher inorganic turbidity and higher algal biomass in the western parts of the lake (Felföldi et al., 2011a; Vörös et al., 2009). Two stations were chosen for photosynthesis measurements, to represent different areas of the lake: Station 1 in the deeper Eastern basin (46°55.327' N, 17°55.649' E, 4 m at the sampling station) with annual maximum chlorophyll *a* concentration between 10-20 µg/L and Station 2 (46°44.095' N, 17°16.583' E) in the shallower (3.1 m) western part of the lake with annual maximum chlorophyll *a* concentration between 30-40 µg/L (Vörös et al,

2009). For strain isolation, an additional sampling station was chosen with an average depth of 3.7 m (Station 3; 46°44.552' N, 17°26.308' E) close to Station 2.

Lake Fertő/Neusiedlersee is a wind-exposed, extremely shallow (~ 1.3 m), eutrophic (annual maximum chlorophyll *a*: 30-40 µg/L) steppe lake (SC between 1300 and 3200 µS/cm, pH between 7.8 and 9.3) straddling the Austrian/Hungarian border (Fig. 1; Löffler, 1979; Somogyi et al., 2010). The total surface area of the lake is 309 km^2, of which ~55% is covered by reed (Löffler, 1979). There are numerous reedless brown-water ponds (inner ponds) of variable size within the reed belt, which is intersected with artificial canals connecting the inner ponds with the open water areas. The open water area and the inner ponds have similar ionic composition, pH and SC, but there are big differences in their transparency (Dinka et al., 2004). As a result of wind-induced sediment resuspension, the open water of the lake is characterized by high inorganic turbidity and usually low Secchi-disk transparency (Löffler, 1979). During winter, however, when the lake is ice covered, the amount of suspended solids declines due to the absence of turbulence (Dokulil and Herzig, 2009). In winter, the lake is usually frozen for 56 days, but there is a declining tendency of ice cover duration at a rate of ca. 1 day per year, and as in Lake Balaton, ice development was not observed in some years (Dokulil and Herzig, 2009). Two sampling stations were chosen, representing different water bodies within the lake: an open water sampling station (Station 1; 47°45.424' N, 16°43.389' E) and an inner lake, Ruster Poschen (Station 2; 47°46.597' N, 16°45.260' E).

For photosynthesis measurements, water samples were taken with a 4.5 m long tube sampler from Lake Balaton at Station 1 and Station 2 on five winter sampling dates between 2009 and 2014 and from Lake Fertő/Neusiedlersee (Station 1 and Station 2) in winter 2010, 2012 and 2014 (Table 1). For strain isolation, water samples were taken from Lake Balaton at Station 2 in winter 2007 and at Station 1 and 3 in winter 2009. Additional water samples were taken from Lake Fertő at Station 2 in October 2008. When the lakes were covered by ice, sampling and measurements were done through a sawed, 30 cm wide hole.

Physicochemical measurements

Photosynthetically active radiation (PAR, 400-700 nm) in the water column was measured at 0.25 m increments with a Li-COR underwater radiometer, using a flat (2π) quantum sensor. The sensor was placed directly under the ice on a metal frame 50 cm away from the 30 cm sawed hole and slowly lowered into the water column. The hole was covered again with the ice block and the snow cover (if present) was undisturbed to acquire realistic values for PAR transmission. Diminution of light within the ice cover (and snow if present) – as a result of light reflection and attenuation – was calculated as the difference between PAR measured above and just below the ice cover. Mean irradiance (I_{mean}) in the water column was calculated according to Ferrero et al. (2006) and Allende et al. (2009) adapted to shallow waters. Assuming a fully mixed water column, I_{mean} was calculated as follows:

$$I_{mean} = I_0 \times (1 - \exp(-K_d \times z)) / K_d \times z$$

where I_0 is the average irradiance measured between 12 and 14 h, K_d is the downwelling diffuse attenuation coefficient for PAR, and z is the depth at the sampling site (Allende et al., 2009). I_0 irradiance values were calculated from the hourly averages of global radiation between 12 and 14 h for the day of sampling provided by the Hungarian Meteorological Service, assuming that 1 W/m^2 = 4.6 µmol/m^2/sec (Wetzel and Likens, 2000). PAR was considered to be 47% of global radiation according to Wetzel and Likens (2000). Light attenuation of the ice and snow cover, which was measured in the field, was also taken into account. Water temperature, pH and conductivity of the water samples were measured with a Wissenschaftlich-Technische-Werkstätten (WTW) pH 315i and a Hanna HI9033 portable field meter.

Freshly collected samples were maintained under cool, dark conditions until transport to the lab within several hours of collection. Chlorophyll *a* concentration was determined spectrophotometrically after hot methanol extraction using the absorption coefficients determined by Wellburn (1994). Dissolved inorganic carbon (DIC) was measured using an Elementar High TOC analyser in water samples filtered through a precombusted GF-5 glass fibre filter (nominal pore size is 0.4 µm).

Microscopic analysis

Nano- and microplankton samples were fixed with Lugol's solution and their abundance and composition was determined with an inverted microscope (Utermöhl, 1958). Cell volume of the observed taxa was calculated using the formulas of Hillebrand et al. (1999). Total biovolume of the nano- and microplankton was calculated on the basis of cell volume and abundance values. Biomass (wet weight) was estimated from the total biovolume of the fractions assuming a specific gravity of 1.0 g/cm^3. The abundance and composition of the picoplankton was determined in fresh, unpreserved samples according to MacIsaac and Stockner (1993). Briefly, samples were concentrated on 0.4 µm pore size black cellulose-acetate filters (Macherey-Nagel), which were subsequently embedded into 50% glycerol on a microscope slide. The slides were examined with an Olympus BX51 epifluorescence microscope at 1,000x magnification using blue–violet (U-MWBV2) and green (U-MWG2) excitation light. Twenty fields (~400 cells) were photographed with an Olympus DP71 colour camera and APP was counted on the images to avoid fluorescence fading. Picoeukaryotes (EuAPP) fluoresce vivid red under blue–violet excitation due to chlorophyll *a* and show none or only weak fluorescence when excited with green light. Picocyanobacteria (CyAPP) can be distinguished from eukaryotes owing to the presence of phycobiliproteins, which exhibit greatly enhanced red fluorescence when using the green wave band. Phycoerythrin-rich picocyanobacteria fluoresce bright yellow, while phycocyanin-rich picocyanobacteria show weak red autofluorescence under blue–violet excitation light (MacIsaac and Stockner 1993). Autotrophic picoplankton abundance was converted to biomass (wet weight) by measuring the dimensions of 50 cells under dia-illumination, calculating their biovolume and considering an average density of 1 g/cm^3.

Photosynthesis measurement

Photosynthesis of the phytoplankton community was measured using the ^{14}C–technique (Steemann-Nielsen, 1952). 20 mL subsamples were put into glass vials, preincubated for 1 hour, and after adding $NaH^{14}CO_3$ (0.1 - 0.13 MBq) incubated for 3 hours in a self-designed photosynthetron (Üveges et al. (2011). Vials were incubated in triplicate at eight different irradiances ranging from 5 to 1300 µmol/m^2/sec at ambient lake temperature (measured during sampling). Three vials were incubated in darkness for dark carbon uptake.

For photosynthesis measurement of the total phytoplankton, 5 mL of each incubated sample was filtered onto a 0.4 µm pore sized cellulose-acetate membrane (Millipore) under low vacuum pressure. In order to estimate size fractionated primary productivity (>3 µm and <3 µm) the remainder (15 mL) was filtered through polycarbonate filters of 3 µm pore size (Millipore, diameter 47 mm) using plastic disposable syringes and plastic 47 mm filter holders without vacuum. The filtrate obtained in this step was then filtered through a 0.4 µm pore size cellulose-acetate membrane filter (Millipore) to concentrate APP cells. Photosynthesis of the entire phytoplankton community, as well as that of the size-fractions were determined separately by this method modified by Callieri et al. (2007).

After that, filters were placed into HCl vapour to volatilize remaining inorganic ^{14}C. Next the filters were dissolved in 10 mL Bray scintillation mixture, after which radioactivity was measured with an LKB 1211-RACKBETA liquid scintillation counter. Photosynthetic

carbon assimilation was calculated based on the proportion between ^{14}C uptake and DIC availability using an isotope discrimination factor of 1.05 (Steemann-Nielsen, 1952). The obtained results were normalized to wet weight (determined as described above). Photosynthesis-irradiance (P–I) curves were fitted using the model of Eilers and Peeters (1988) with the data analysis software OriginPro 2015. Definitions of photosynthetic parameters are given in Table S1. I_{mean} provides a reference for comparing I_k values, i.e., phytoplankton primary production in lakes for which $I_{mean} < I_k$ can be safely assumed to be light limited according to Allende et al. (2009). Areal primary production was estimated using the P–I curves and the light intensity profile of the water column at 0.1 m increments.

Isolation and molecular identification of picoeukaryotic algal strains

Picoeukaryotic strains ACT1001-ACT1006 (Algal Culture Tihany) were isolated from the water of Lake Fertő in October 2008, whereas strains ACT1007-ACT 1022 were isolated from Lake Balaton in January 2007 and 2009. Isolation was carried out using a modified BG11 medium, in which only one tenth of the recommended micronutrient solution was used (Rippka et al., 1979) as previously described (Somogyi et al., 2009). Unialgal cultures were established by serial streaking on 1.5% agar plates and single colony isolations at 8 °C under 25 μmol/m^2/sec cool white fluorescent light (Tungsram F33) on a 12:12 hour light:dark cycle. The strains were later transferred to liquid media (modified BG11 medium) and were maintained at 21 ^oC under 40 μmol/m^2/sec on a 14:10 hour light:dark cycle.

Genomic DNA was extracted according to the procedure described previously by Somogyi et al. (2009). PCR amplification of the 18S rRNA gene was performed with a final volume of 50 μL using approximately 2 μL of genomic DNA, 0.2 mM of each deoxynucleotide, 2 mM $MgCl_2$, 1 U LC *Taq* DNA polymerase (Fermentas), 1X PCR buffer (Fermentas), 0.325 μM of Euk528f and CHLO02 primers (Elwood et al., 1985; Zhu et al., 2005) and 400 ng of BSA (Fermentas). PCR amplicons were purified with the PCR-M™ Clean Up System (Viogene). Sequencing was carried out with the BigDye® Terminator v3.1. Cycle Sequencing Kit (Applied Biosystems). Chromatograms were corrected manually with Chromas 1.45 software (Technelysium Pty Ltd.). The generated sequences were compared to the GenBank nucleotide database using the BLAST program (Altschul et al., 1997). The obtained 18S rRNA gene sequences were submitted to GenBank under the accession numbers HQ594495- HQ594515.

Statistical analysis

Simple linear regression was used to test relationships between P-I parameters and environmental factors (water temperature, light conditions) using OriginPro 2015 software. Relationships were considered to be significant at $p < 0.05$. Normality of the data was tested, using a graphical approach (Q-Q plot). Differences between P-I parameters of the different fractions were tested with *t*-test.

Results

Physical and chemical characteristics (light environment)

Lake Balaton was frozen at Station 1 with an ice thickness of 4 cm in winter 2010 and 13 cm in winter 2011 (Table 1). As a result of light attenuation within the ice, 83% of surface PAR was detected entering the water column in winter 2011. At Station 2, ice cover was formed only in winter 2010 and the 16 cm thick ice absorbed more than half of the surface irradiance (42% of PAR reached the ice/water interface). Snow cover was found only at Station 2 in winter 2010, with a thickness of about 0.5 cm. Vertical attenuation coefficients ranged

between 0.6/m and 5.6/m at Station 1 and between 0.9/m and 2/m at Station 2 (Table 1). As a result, mean irradiance in the water column was between 30 and 300 µmol/m^2/sec at Station 1 and between 45 and 190 µmol/m^2/sec at Station 2 (Table 1). Water temperature (0.6-5.3 °C), pH (8.1-8.7), and specific conductance (690-820 µS/cm) varied in ranges typical for the lake during winter.

Ice coverage on Lake Fertő was observed in winter 2010 and 2012 with an average thickness of 13-15 cm and 15-19 cm, respectively (Table 1). Ice cover absorbed on average 37% of surface irradiance (63% of PAR reached the ice/water interface at both stations in winter 2010, and 57% at Station 1 and 70% at Station 2 in winter 2012). The vertical attenuation coefficients ranged between 1.6/m and 3.6/m at Station 1, and between 2/m and 2.2/m at Station 2 (Table 1). Mean irradiance of the water column varied between 110 and 190 µmol/m^2/sec at Station 1 and between 130 and 280 µmol/m^2/sec at Station 2 (Table 1). Water temperature ranged between 0.5 and 7.6 °C (Table 1). pH values (between 8 and 8.6) in Lake Fertő were similar to those measured in Lake Balaton, but specific conductance was higher (between 1900 and 2400 µS/cm).

Phytoplankton biomass and APP contribution

In Lake Balaton, chlorophyll *a* concentration was between 1.5 and 13 µg/L at Station 1, with the highest values measured in February 2009. At Station 2, chlorophyll *a* concentration varied between 7 and 21 µg/L and the highest concentration was measured in February 2010 (Table 1). APP constituted 12-26% of the total phytoplankton biomass at Station 1 with values between 30 and 220 µg/L (Table 1, Fig. 2). APP was composed of both picocyanobacteria and picoeukaryotic algae with abundances between 11 and 104 x 10^3 cells/mL for CyAPP (5-98 µg/L biomass) and between 11 and 60 x 10^3 cells/mL for EuAPP (20- 120 µg/L biomass). With the exception of winter 2010, picoplankton biomass was dominated by EuAPP (Table 1). At Station 2, APP biomass (100-520 µg/L) and contribution (6-43%) to total phytoplankton biomass was higher than at Station 1 (Table 1, Fig. 2). As in the case of Station 1, picocyanobacteria and picoeukaryotic algae were also observed. CyAPP abundance varied between 26 and 110 x 10^3 cells/mL (14- 60 µg/L biomass), whereas EuAPP abundance reached 40-250 x 10^3 cells/mL (86-500 µg/L biomass) (Table 1). EuAPP was clearly dominant within the pico fraction at each sampling date. Larger-sized phytoplankton biomass varied between 141 and 1419 µg/L and between 579 and 1578 µg/L at Station 1 and Station 2, respectively, with *Cryptomonas sp.*, *Rhodomonas lacustris var. nannoplanctica* and *Chrysochromulina parva* as the dominant taxa.

Chlorophyll *a* concentration in Lake Fertő was between 4.5 and 12 µg/L at Station 1 and between 6 and 7 µg/L at Station 2 (Table 1). APP contribution was higher than in Lake Balaton: at Station 1 APP constituted 29-35% of the total phytoplankton biomass with values between 230 and 370 µg/L, while at Station 2 their biomass ranged from 160 to 220 µg/L, constituting 21-24% of the total phytoplankton biomass (Table 1). Both picocyanobacteria and picoeukaryotes were present, with EuAPP dominance at Station 2. At Station 1, however, picoplankton was dominated by CyAPP at every sampling date: approximately half of the APP biomass was composed of characteristic, *Aphanothece*-like colonial picocyanobacteria (Table 1). CyAPP abundance varied between 410 and 680 x 10^3 cells/mL (biomass between 210 and 350 µg/L) at Station 1, while it was much lower (abundance: 4-80 x 10^3 cells/mL, biomass: 2 and 40 µg/L) at Station 2. An opposite tendency was observed in the case of EuAPP, whose abundance ranged between 3 and 20 x 10^3 cells/mL (biomass between 10 and 70 µg/L) at Station 1 and between 30 and 50 x 10^3 cells/mL (biomass between 120 and 220 µg/L) at Station 2 (Table 1). Larger-sized phytoplankton biomass reached 423-916 µg/L at Station 1 and 570-822 at Station 2. *Cryptomonas sp*. and *Rhodomonas lacustris var.*

nannoplanctica were abundant at both stations, while *Monoraphidium irregulare* and *Ulnaria delicatissima var. angustissima* were characteristic only at Station 1.

Role of APP in winter primary production

The maximum photosynthetic rate (P_{max}) of the total phytoplankton varied between 3 and 10 µg C/L/h at Station 1 of Lake Balaton (Table S2). Based on the P-I curves and the average ambient light intensities, depth-integrated primary production (PP) varied between 40 and 120 mg C/m^2/day (Table 2). At Station 2, P_{max} and PP values were higher (5-23 µg C/L/h and 80-280 mg C/m^2/day, respectively) (Table S2, Table 2). The contribution of APP to total primary production was between 13 and 46% at both stations (Table 2, Fig. 2). These contribution values were higher than those in terms of total phytoplankton biomass (Fig. 2). At Station 2, the contribution of APP to total phytoplankton biomass and to total PP was similar (Fig. 2). In Lake Fertő, P_{max} of the total phytoplankton varied between 3 and 26 µg C/L/h at Station 1 and between 4 and 12 µg C/L/h at Station 2 (Table S2). At Station 1, depth-integrated primary production varied between 24 and 125 mg C/m^2/day. At Station 2, PP values (40 and 80 mg C/m^2/day) were lower (Table 2). The contribution of APP to total primary production was much lower at Station 1 (10-20%) than at Station 2 (30-40%, Fig. 2). At Station 1, these contribution values were much lower than could be expected from the distribution of biomass between the two size fractions. At Station 2, however, APP contribution to PP was higher than the contribution to total phytoplankton biomass (Fig. 2).

The biomass-specific maximum photosynthetic rate (P^B_{max}) of APP was significantly higher than that of larger-sized phytoplankton (Fig. 3). In Lake Balaton, P^B_{max} of APP varied between 10 and 67 ng C/µg Ww/h at Station 1 and between 10 and 56 ng C/µg Ww/h at Station 2 (Table S2). In the case of nano+microplankton, P^B_{max} was between 4 and 15 ng C/µg Ww/h at Station 1, and between 5 and 23 ng C/µg Ww/h at Station 2 (Table S2). A similar tendency was found in Lake Fertő with higher P^B_{max} of APP (Station 1:8-39 ng C/µg Ww/h, Station 2: 9-78 ng C/µg Ww/h) than that of larger-sized phytoplankton (Station 1:3-18 ng C/µg Ww/h, Station 2: 3-12 ng C/µg Ww/h). There were no clear differences between the light saturation parameter (I_k) and the optimal light intensity (I_{opt}) of the different size fractions, but these parameters were somewhat lower for APP than for larger-sized phytoplankton in many cases (Fig. 3, Table S2). The light saturation parameter ranged between 40 and 120 µmol/m^2/sec in Lake Balaton and between 20 and 120 µmol/m^2/sec in Lake Fertő (Table S2). We observed large differences between the size fractions in terms of the biomass-specific light utilization parameter (α^B) (Fig 2, Table S2). In Lake Balaton, α^B of APP ranged between 0.2 and 0.7 (ng C/µg Ww/h)/(µmol/m^2/sec), while in the case of nano+microplankton, α^B was between 0.07 and 0.18 (ng C/µg Ww/h)/(µmol/m^2/sec). A similar tendency was found in Lake Fertő with higher α^B of APP [0.2-1.1 (ng C/µg Ww/h)/(µmol/m^2/sec)] than that of larger-sized phytoplankton [0.06-0.1 (ng C/µg Ww/h)/(µmol/m^2/sec)].

There were strong positive relationships between temperature and all parameters derived from the P-I curves for both size fractions in Lake Balaton (Table 3, Fig. 4). In the case of Lake Fertő, a positive correlation was found between temperature and P^B_{max} and between temperature and I_k of both size fractions but no relationship was found between temperature and α^B (Table 3, Fig. 4). The temperature-P^B_{max} relationship differed between the size fractions: the slopes were much higher for APP than for larger-sized phytoplankton in both lakes. The same difference was observed in the case of the relationship between temperature and α^B in Lake Balaton (Table 3).

A clear, positive relationship was observed between the mean irradiance of the water column and both P^B_{max} and I_k of the different size fractions, but it was more pronounced in the case of APP (Table 3, Fig. 4). The two size fractions showed different relationship between

I_{mean} and P^B_{max}: the slopes were much higher for APP than for larger-sized phytoplankton in both lakes (Table 3).

Taxonomic composition of the picoeukaryotic community

The winter picoeukaryotic community of Lake Fertő (Station 2) was dominated by 1.6-3 μm sized spherical cells. On the basis of their 18S rRNA gene sequences, the isolated strains ACT1001-ACT1005 belonged to the genus *Choricystis* (Table 4). Strain ACT1006 was affiliated with the genus *Mychonastes*. The winter picoeukaryotic community of Lake Balaton consisted of 1.8-2.7 μm sized single spherical cells and rod-shaped, small green algae (0.8–1.2 μm wide, 4–6 (12) μm long). The majority of the isolated strains had spherical to ovoid cells. Among them, ACT1007-ACT 1013 (isolated from Station 1), ACT1018-ACT 1019 (isolated from Station 3) and ACT1021-ACT 1022 (isolated from Station 3) belonged to the genus *Choricystis* (Table 4). Isolates ACT1016-ACT 1017 (isolated from Station 3) belonged the genus *Mychonastes*. Only two isolates had rod-shaped cell morphology: ACT1014 (isolated from Station 3) was identified as *Stichococcus bacillaris* (Table 4), whereas ACT1015 (isolated from Station 3) as *Nannochloris bacillaris* (Table 4).

Discussion

Eukaryotic dominance in winter picoplankton

The < 3 μm size class was dominated by picoeukaryotic cells, except Station 1 in Lake Fertő, where mainly picocyanobacteria were found. EuAPP and CyAPP cells are considerably different in terms of both structure and physiology. The first and most obvious difference is the larger cell size of picoeukaryotes owing to their more complex internal structure, which results in higher metabolic requirements for EuAPP cells compared with picocyanobacteria (Callieri, 2008; Crosbie et al., 2003). As a result, the growth rates of CyAPP can be up to three times as high as those of EuAPP (Jasser and Arvola, 2003). EuAPP cells have a lower light requirement than CyAPP cells, although the latter group also includes low-light adapted microorganisms (Callieri, 2008). In the Pacific Ocean, for example, picoeukaryotes yield a larger share of picoplankton productivity compared with picocyanobacteria in the well-lit region (>15% surface irradiance) than in the lower regions (1–7% surface irradiance) of the euphotic zone (Rii et al., 2016). Moreover, picocyanobacteria were found to be more sensitive to high PAR and UV radiation than picoeukaryotes in high mountain lakes (Winder, 2009). Besides that, EuAPP have higher salinity tolerance (Budinoff and Hollibaugh, 2007), which explains their predominance in hypersaline environments (Somogyi et al., 2014).

Regarding their temperature preference, picoeukaryotes are well adapted to harsh polar environmental conditions and dominate Arctic pelagic phytoplankton communities for most of the year (Metfies et al., 2016). In shallow lakes within the temperate zone, picoplankton is dominated by CyAPP in summer, whereas EuAPP are dominant from autumn until spring (Callieri, 2008; Somogyi et al., 2009; Vörös et al., 2009). Picocyanobacteria isolated from freshwater lakes usually have higher temperature optima and higher light requirements than picoeukaryotes resulting in clear seasonal niche partitioning. Thus, the observed winter predominance of EuAPP cells was in good agreement with previous findings (Callieri, 2008; Malinsky-Rushansky et al., 2002; Somogyi et al., 2009; Vörös et al., 2009). On the other hand, deep temperate oligotrophic lakes, such as Lake Superior (Ivanikova et al., 2007), can harbour dominant winter CyAPP communities. We observed a similar phenomenon at Station 1 in Lake Fertő, which suggests the presence of a winter-acclimated CyAPP ecotype.

Taxonomic composition of winter picoeukaryotic communities

Picoeukaryotic green algal taxa have evidently evolved by convergent evolution and are usually unidentifiable by microscopy (Krienitz and Bock, 2012 and references therein). Thus, the study of picoeukaryotic algal composition requires molecular tools mainly based on 18S rRNA gene sequence analysis, either by analysing the water samples directly or using culture-based techniques (strain isolation and identification). Both methods have their own limitations. Culture-independent techniques are subject to bias as a result of problematic DNA extraction from freshwater picoeukaryotic cells because of their multi-layered, sporopollenin-containing cell wall, which provides defence against enzymatic disintegration (e.g. Somogyi et al., 2011). The weak point of culture-based approaches is the isolation itself because of the strong selection of culturing conditions and the limited number of species able to survive under such conditions (Andersen, 2005). However, the obtained isolates may subsequently be the targets of various types of taxonomic, physiological or other studies (e.g. deciphering ecological properties, description of new ecotypes/species, searching for bioactive compounds, mass-cultivation for biofuel production). Being aware of these limitations and advantages, a cultivation-based approach was chosen to study the taxonomic composition of picoeukaryotic assemblages in Lake Balaton and Lake Fertő for the first time.

In freshwater lakes, a number of green algal lineages contain picoplankton species, including the genera *Choricystis*, *Meyerella, Marvania* and *Nannochloris* within the Trebouxiophyceae (Chlorophyta) as well as the *Mychonastes* within the Chlorophyceae (Chlorophyta). The only freshwater non-chlorophyte species is *Nannochloropsis limnetica* (Eustigmatophyceae, Heterokontophyta), which was described from small ponds in Germany (Krienitz et al., 2000). All of our isolates belonged to Chlorophyta, which corresponded well with previous findings of chlorophyte dominance in freshwater picoplankton (Krienitz and Bock, 2012; Somogyi et al., 2013). The majority of the isolates from Lake Balaton and Lake Fertő were identified as members of the genus *Choricystis* (Table 4). This genus is widely distributed in freshwater lakes, with strains isolated from Europe, Asia and North-America (Belykh et al.. 2000; Fawley et al., 2004; Hepperle and Krienitz, 2001; Hepperle and Schlegel, 2002). The considerable genetic variation (mainly based on *rbcL*) suggests the presence of several cryptic species within the *Choricystis* clade, but additional sequences will be necessary to clarify the phylogenetic relationships (Fawley et al., 2005; Hepperle and Krienitz, 2001; Hepperle and Schlegel, 2002). *S. bacillaris* and *N. bacillaris*, which were isolated from Lake Balaton, are also common freshwater taxa. Members of the *Mychonastes* clade, which were found in both lakes, are also typical freshwater algal taxa described from European (Hepperle and Krienitz, 2001; Hepperle and Schlegel, 2002; Krienitz et al., 1999) and North-American lakes (Fawley et al., 2004) as well as from Lake Kinneret, Israel (Hanagata et al., 1999). In summary, the taxonomic composition of the picoeukaryotic community in the studied shallow lakes was similar to other freshwater lakes in the temperate zone, however this is the first report from the composition of winter picoeukaryotic algal assemblages in Central European great lakes. It should be noted that, our previous studies (Felföldi et al. 2011a, 2011b) demonstrated that picocyanobacteria present in the studied lakes were affiliated with different clades of the non-marine *Synechococcus/Cyanobium* group: to group A (=*Cyanobium gracile* cluster) in the case of Lake Fertő and mainly to cluster with PD1 in the case of Lake Balaton.

Photosynthetic characteristics and primary production of winter phytoplankton

In the studied shallow lakes, autotrophic picoplankton comprised a substantial part of phytoplankton biomass (21% in Lake Balaton and 27% in Lake Fertő on the average) and primary production (27% in Lake Balaton and 24% in Lake Fertő on the average), suggesting that APP is an important component of winter aquatic communities. In the case of Lake Fertő, the higher contribution of APP in biomass than in terms of primary production was a

consequence of methodology. According to Stockner et al. (2000), microcolonies in freshwater lakes comprising a few to <50 individual cells were traditionally included in the picophytoplankton despite of the larger size of the colonies (Callieri, 2008). Colonial forms of picocyanobacteria constituted approximately half of the total APP biomass at Station 1 of Lake Fertő. During the fractionated photosynthesis measurement, however, the 3 μm pore-sized filter retained these microcolonies within the larger-sized phytoplankton fraction. Thus, the role of APP in primary production was likely underestimated for Lake Fertő (Station 1).

In previous studies on Lake Balaton, APP production was measured between May and October (Vörös, 1991), reaching values between 23 and 66 mg C/m^2/h at Station 1 and between 9 and 104 at Station 2 (Table S3). Summer rates were generally found to be higher than those in spring and autumn, except a filamentous cyanobacterial bloom at Station 2. If we compare the estimated winter rates of the present study with the previous findings (Table S3), we can conclude that APP production is considerably lower (1-8 mg C/m^2/h) in winter than in the spring-fall seasons. Larger phytoplankton show a similar seasonal trend with a smaller peak in spring and a larger maximum in summer (Herodek et al., 1982; Vörös, 1991). We have a limited knowledge on Lake Fertő in this regard, with only one fractionated measurement in summer 2010, showing APP production values one order of magnitude higher than in winter.

In general, the winter contribution of APP to phytoplankton biomass and primary production in the studied lakes was similar to values observed in other meso-eutrophic freshwater lakes (e.g. Greisberger et al., 2008; Kim et al., 2015; Ochs and Rhew, 1997; Peltomaa and Ojala, 2010). According to the earlier study on Lake Balaton, their contribution showed no seasonal trend at Station 1, ranging between 43 and 56% from May to October (Vörös, 1991). On the other hand, there was a strong seasonal trend at Station 2, where APP contribution was as low as 1% during the summer filamentous nitrogen-fixing cyanobacterial bloom (Table S3). All these findings suggest that trophic state is a better determinant of APP contribution than seasonality *per se*, which is a widely observed phenomenon in aquatic ecosystems (Stockner, 1991; Bell and Kalff, 2001; Callieri, 2008). In Lake Fertő, the winter and summer values showed no significant difference (Table S3). Information on the winter contribution of APP to primary production, however, is scarce particularly in temperate freshwater shallow lakes. Thus, the results obtained help to evaluate the winter role of APP within the aquatic food web in temperate shallow lakes.

The higher biomass-specific maximum photosynthetic rate and higher light utilization parameter of APP than that of larger-sized phytoplankton in the studied shallow lakes implied a better acclimation of APP to low-temperature and low-light winter environment. No difference was observed in this regard between CyAPP- and EuAPP-dominated picoplankton. In agreement with this, more effective light acquisition and faster metabolic rates were described for smaller cells than larger ones owing to their high surface area and reduced chromophore self-shading (Callieri, 2008; Irwin et al., 2006; Raven, 1998). Similarly higher P^B_{max} values were obtained for APP than for the larger-sized phytoplankton fraction in a temperate coastal ecosystem (Morán, 2007) and in Lake Mondsee (Greisberger et al., 2008). In a relict oxbow lake, however, there were no significant differences between the phytoplankton size fractions in terms of their P^B_{max}, α and I_k (Rodriguez et al., 2012).

Light acclimation in winter

Light limitation did not occur in the studied shallow lakes as I_k values for both APP and larger-sized phytoplankton were lower than I_{mean} except at Station 1 of Lake Balaton in February 2009 (Table 1, Table S2). However, the observed I_k values were much lower than usual during the autumn to spring period in temperate shallow lakes (Dokulil et al., 2014; Somogyi and Vörös, 2006; Vörös et al., 1991). Low I_k is characteristic of phytoplankton

552 adapted to low light, which is capable of developing maximal photosynthetic rate at low mean
553 irradiance and has accessory photosynthetic pigments (Darchambeau et al., 2014). High
554 cellular chlorophyll *a* content of the phytoplankton biomass is also a result of low-light
555 acclimation of the winter assemblage. In Lake Balaton, the chlorophyll *a* content of the
556 phytoplankton biomass varies between 0.3 and 0.5% from spring to autumn, but increased to
557 1.5-2% in winter, when active movement or a high surface area is assumed to be
558 advantageous (Dokulil et al., 2014). This was also reflected in the phytoplankton composition
559 of the studied lakes with cryptophytes and *Monoraphidium* sp. as dominant taxa.
560 Snow and ice cover varies widely in thickness and optical properties, affecting the
561 under-ice light field and, consequently, phytoplankton photosynthesis (Arst and Sipelgas,
562 2004). Winter photoautotrophic activity is often limited by the availability of light,
563 particularly under deep snow cover (Salmi et al., 2014). Phytoplankton may nevertheless
564 persist and even form transient blooms under the ice. Such a winter bloom of picoeukaryotes
565 was described from a soda pan with a chlorophyll *a* concentration reaching 1040 µg/l (Pálffy
566 et al., 2014). Under-ice diatom blooms are a characteristic winter phenomena in Lake Erie and
567 Lake Baikal (Beall et al., 2016, Jewson et al., 2009). Snow-covered ice hinders the
568 development of under-ice blooms due to insufficient light penetration, while snow-free ice
569 exhibits high PAR transmittance, which can support phytoplankton growth (Beall et al.,
570 2016). As a result, phytoplankton in Lake Erie does not suffer from light limitation under a
571 snow-free ice cover (Beall et al., 2016). During our measurements the situation was similar,
572 there was neither snow cover, nor light limitation, as indicated by the photosynthesic
573 parameters and the under-ice light field.
574 Nevertheless, we cannot exclude the possibility of light limitation near the sediment
575 surface. During our study, the water column was assumed to be stable, however, that is not
576 always the case in shallow lakes. During the ice-covered season, heat flux from the sediments
577 and the penetration of solar radiation through the ice are two of the most important drivers of
578 circulation and mixing (Kirillin et al., 2012). In seasonally frozen shallow lakes, the role of
579 these two drivers in lake dynamics changes over time, which causes fundamental changes in
580 water movements (Bertilsson et al., 2013). In the case of Lake Balaton, phytoplankton
581 biomass and primary production can distribute homogenously within the water column or
582 could be even higher near the bottom, as already described by Herodek et al. (1982). The
583 latter can be the result of higher (+1.5-2 °C) water temperature in that region due to heat
584 accumulation in the lake sediment (Bertilsson et al., 2013). This phenomenon differs from the
585 winter diatom blooms observed in deep lakes, where the cells accumulate just beneath the ice
586 cover (e.g. in Lake Erie; Beall et al., 2016).
587
588 *Factors influencing phytoplankton in winter*
589 The photosynthetic parameters showed significant changes with water temperature and mean
590 water column irradiance. Biomass-specific maximum photosynthetic rate increased
591 significantly with both temperature and available light, however, with higher increase in APP
592 than in nano+microplankton. In Lake Balaton, α^B of both fractions also showed a significant
593 positive correlation with temperature and incident irradiance. Similar results were found in a
594 eutrophic, river-dominated estuary, where APP productivity and biomass were positively
595 correlated with temperature and dissolved inorganic phosphorus concentrations (Gaulke et al.,
596 2010). The light saturation parameter showed significant changes with temperature and I_{mean}
597 in the case of both lakes. Different responses of the size fractions to light and temperature
598 suggest that winter conditions have diverse effects on carbon metabolism within the
599 phytoplankton. Besides water temperature, the presence of ice and snow cover, which
600 significantly influences the mean irradiance of the water column, has a high impact on
601 photosynthesis of the different size fractions. Thus, climate change might have different

impacts on different phytoplankton groups, especially in shallow lakes with variable ice cover.

The effect of nutrient availability cannot be neglected in winter, however, different factors can result in different scenarios: convection mixing in winter promotes nutrient resuspension in deep lakes (Hampton et al., 2015), on the other hand, ice cover reduces the input of dissolved and particulate nutrients from the atmosphere and nearby terrestrial surroundings (Bertilsson et al., 2013). However, despite the important role of nutrients, winter in the temperate zone is accompanied by the dominance of physical constraints. In Lake Balaton, the concentration of dissolved inorganic nitrogen and phosphorus increases during the winter months (Présing et al., 2001), because phytoplankton (including picophytoplankton) have a limited ability to take up nutrients due to reduced temperature. Other studies have come to similar conclusion, winter phytoplankton primary production has been found to primarily depend on light rather than on nutrient availability in various water bodies, such as an alkaline pan (Pálffy et al., 2014) and a humic, boreal lake (Tulonen et al., 1994). Grazing might also be a determining factor, but in winter zooplankton activity is greatly limited. Winter zooplankton abundance and biomass in Lake Fertő/Neusiedlersee varied among years but correlated positively with chlorophyll *a* concentration. Zooplankton dynamics fundamentally depend on phytoplankton biomass in autumn and on conditions during the winter that follow, and ice cover is particularly important in this respect (Dokulil et al., 2014). Crustacean zooplankton ingestion rates were also found to be considerably restrained at lower temperatures (Loiterton et al., 2004).

Climate change implications

Ice cover duration has been found to show a decreasing trend on many lakes as mean air temperatures have increased as a result of global climate change (Weyhenmeyer et al., 2011). For this reason, the study of winter phototrophic communities is particularly important for understanding the dynamics and functioning of seasonally frozen lakes (Bertilsson et al., 2013). Changes in the duration of ice and snow cover patterns influence phytoplankton succession (Bertilsson et al., 2013), however, estimating the effects of such climate-driven changes is difficult. Worldwide reduction in the duration of snow cover is expected to enhance light transmission, since clear ice can transmit as much as 95% of ambient PAR (Hampton et al., 2015). As a result, light transmission can promote complex convection patterns, which can keep small celled organisms in suspension (Hampton et al., 2015). On the other hand, a reduction in ice cover duration also involves habitat loss for ice-associated algae and enhances resuspension, which restricts light transmission and helps large celled organisms to remain in suspension (Beall et al., 2016). In the case of shallow lakes, all these processes affect not only phytoplankton but also benthic productivity, which could be important in whole-lake primary production. In Lake Balaton, benthic algae have a substantial role in the shallow littoral regions with a contribution of 60-95% to total primary production (phytobenthos+phytoplankton) in summer, while in the deeper pelagic region, only 15% is attributable to phytobenthos (Üveges et al., 2011). In Lake Fertő, benthic algae are virtually absent from spring to autumn due to high inorganic turbidity (Dokulil and Herzig, 2009) but the winter situation is unexplored. So far, we have limited information on how climate change will affect different algal groups. Our results suggest that a higher average winter temperature and light availability in shallow lakes could be more advantageous for APP than nano+microplankton, which can reorganize energy flows, providing more energy to picoplankton grazers (heterotrophic and mixotrophic nanoflagellates, ciliates, etc.). However, as some of the dominant winter nanoplankton taxa can feed mixotrophically, they can also benefit from APP production.

In the studied shallow lakes, production rates of both size groups were significantly lower in winter than in the spring-fall seasons (Table S3). Still, the significance of the underlying processes in winter is hard to estimate. Primary production under the ice may provide a more important contribution to higher trophic levels than would be apparent from the production rates alone (Hampton et al., 2015). Cold-adapted algae are often more abundant in key polyunsaturated fatty acids, which are essential dietary components for zooplankton and fish (Dalsgaard et al., 2003). The small, rod-shaped green alga *Stichococcus minutissimus*, a characteristic picoeukaryotic taxon in Lake Balaton, usually contains oil droplets, which may increase its nutritional value (Vörös et al., 2009). Other picoeukaryotic taxa, such as the eustigmatophycean *Nannochloropsis limnetica*, have been also found to be a high quality food for zooplankton due to its characteristic fatty acid composition (Krienitz et al., 2000). On the basis of these findings, winter phytoplankton might represent a unique nutritional cocktail – crucial for the survival of the zooplankton in winter. Exploring the key processes and their consequences in winter is therefore essential for a better understanding of whole-lake metabolism.

Concluding remarks

1. The taxonomic composition of the picoeukaryotic community in shallow Central European great lakes was similar to other freshwater lakes in the temperate zone.
2. APP production in Central European great lakes was considerably lower in winter than in the spring-fall seasons.
3. APP is an important component of winter phytoplankton with high contribution to biomass and primary production.
4. APP was better acclimated to low temperature and low light than nano+microplankton.
5. Phytoplankton photosynthesis was not light-limited in winter.
6. Higher average winter temperature and light availability in shallow lakes could be more advantageous for APP than nano+microplankton.

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TABLES

Table 1 List of investigated lakes, sampling strategy, selected physical, chemical and biological variables. Abbreviations: WT – water temperature, K_{dPAR} – vertical attenuation coefficient of photosynthetically active radiation (PAR, 400-700 nm); PAR% – % of ambient PAR below ice, I_{mean} – mean irradiance of the water column, Chl – Chlorophyll *a* concentration, EuAPP – picoeukaryotes, CyAPP –picocyanobacteria, APP_{col} – colonial forms of picoplankton, NP –nano- and microplankton (> 3 μm).

Sampling station	Sampling date	WT	Ice cover	PAR%	K_{dPAR}	I_{mean}	Chl	EuAPP abundance	CyAPP abundance	EuAPP biomass	CyAPP biomass	APP_{col} biomass	NP biomass
		(°C)	(cm)		(1/m)	(μmol/m^2/sec)	(μg/L)	(10^3 cells/mL)		(μg/L)		(μg/L)	
Lake Balaton, Stn. 1	18/02/2009	0.7	no ice	-	5.65	30	13.34	60	21	122	98	0	1419
Lake Balaton, Stn. 1	14/01/2010	1.5	4	-	1.08	106	6.04	11	104	22	55	0	560
Lake Balaton, Stn. 1	11/01/2011	1.7	13	83	0.78	98	5.99	43	46	88	24	2	488
Lake Balaton, Stn. 1	23/01/2012	2.6	no ice	-	1.15	119	2.56	28	52	56	28	12	239
Lake Balaton, Stn. 1	18/02/2014	4.2	no ice	-	0.60	304	1.54	13	11	27	5	0	141
Lake Balaton, Stn. 2	19/02/2009	0.6	no ice	-	1.60	144	13.00	246	45	500	23	0	698
Lake Balaton, Stn. 2	02/02/2010	2.1	16	42	1.97	45	21.35	42	26	86	14	0	1578
Lake Balaton, Stn. 2	25/01/2011	1.7	no ice		1.32	184	8.70	58	30	118	16	0	686
Lake Balaton, Stn. 2	16/01/2012	1.2	no ice	-	0.93	189	7.24	175	66	355	35	0	579
Lake Balaton, Stn. 2	18/02/2014	5.3	no ice	-	1.52	188	10.33	43	113	88	59	2	692
Lake Fertő, Stn. 1	08/02/2010	2.0	15	63	2.50	107	4.49	3	411	11	216	110	423
Lake Fertő, Stn. 1	06/02/2012	0.5	19	57	1.60	167	6.71	17	409	73	215	86	633
Lake Fertő, Stn. 1	24/02/2014	7.3	no ice	-	3.61	188	12.14	5	676	22	354	152	916
Lake Fertő, Stn. 2	08/02/2010	2.8	13	63	2.00	128	7.13	52	4	216	2	0	822
Lake Fertő, Stn. 2	06/02/2012	1.5	15	70	2.07	171	6.39	39	64	163	33	6	613
Lake Fertő, Stn. 2	24/02/2014	7.6	no ice	-	2.29	280	6.39	29	78	123	41	0	570

Table 2 Areal primary production of the different fractions (Total – total phytoplankton, APP – autotrophic picoplankton, NP – nano+microplankton). Abbreviation: APP% – contribution of autotrophic picoplankton to total phytoplankton primary production.

Sampling station	Date	Primary production (mg C/m^2/day)			APP %
		Total	APP	NP	
Lake Balaton, Stn. 1	18/02/2009	42	10	28	24
Lake Balaton, Stn. 1	14/01/2010	81	15	69	18
Lake Balaton, Stn. 1	11/01/2011	116	16	100	13
Lake Balaton, Stn. 1	23/01/2012	48	17	33	36
Lake Balaton, Stn. 1	18/02/2014	75	34	58	46
Lake Balaton, Stn. 2	19/02/2009	160	66	88	41
Lake Balaton, Stn. 2	02/02/2010	116	15	91	13
Lake Balaton, Stn. 2	25/01/2011	80	12	72	16
Lake Balaton, Stn. 2	16/01/2012	121	54	60	45
Lake Balaton, Stn. 2	18/02/2014	278	47	210	17
Lake Fertő, Stn. 1	08/02/2010	24	4	20	17
Lake Fertő, Stn. 1	06/02/2012	45	8	36	19
Lake Fertő, Stn. 1	24/02/2014	125	14	113	11
Lake Fertő, Stn. 2	08/02/2010	39	12	24	30
Lake Fertő, Stn. 2	06/02/2012	46	13	32	28
Lake Fertő, Stn. 2	24/02/2014	83	34	64	42

Table 3 Results of simple linear regressions between environmental factors and P-I parameters (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$) measured in winter 2009-2014 in Central European great lakes. Abbreviations (and units): WT – water temperature (°C), I_{mean} – mean irradiance of the water column (µmol/m^2/sec), P^B_{max} – biomass-specific maximum photosynthetic rate (ng C/µg Ww/h), I_k – light saturation parameter (µmol/m^2/sec), α^B – biomass-specific light utilization parameter $(P^B_{max})/(I_k)$, APP – autotrophic picoplankton, NP –nano+microplankton.

Sampling site	Variable 1	Variable 2	Intercept	Slope	n	R^2
Lake Balaton	WT	P^B_{max} of APP	-0.54	12.28	30	0.84***
Lake Balaton	WT	P^B_{max} of NP	2.52	3.30	30	0.76***
Lake Balaton	WT	I_k of APP	27.85	15.26	30	0.50***
Lake Balaton	WT	I_k of NP	37.41	13.72	30	0.61***
Lake Balaton	WT	α^B of APP	0.28	0.07	30	0.24**
Lake Balaton	WT	α^B of NP	0.11	0.01	30	0.45***
Lake Balaton	I_{mean}	P^B_{max} of APP	2.42	0.17	30	0.43***
Lake Balaton	I_{mean}	P^B_{max} of NP	4.81	0.03	30	0.21**
Lake Balaton	I_{mean}	I_k of APP	17.65	0.31	30	0.58***
Lake Balaton	I_{mean}	I_k of NP	45.61	0.15	30	0.19**
Lake Fertő	WT	P^B_{max} of APP	-1.94	7.89	18	0.79***
Lake Fertő	WT	P^B_{max} of NP	0.94	1.74	18	0.68***
Lake Fertő	WT	I_k of APP	3.33	15.54	18	0.58***
Lake Fertő	WT	I_k of NP	15.66	19.03	18	0.71***
Lake Fertő	I_{mean}	P^B_{max} of APP	-33.98	0.36	18	0.69***
Lake Fertő	I_{mean}	P^B_{max} of NP	-4.56	0.07	18	0.44**
Lake Fertő	I_{mean}	I_k of APP	-73.31	0.79	18	0.64***
Lake Fertő	I_{mean}	I_k of NP	-71.17	0.92	18	0.71***

Table 4 Origin, taxonomic affiliation and GenBank accession number of isolated picoeukaryotic algal strains.

Strain no.	Sampling station	Sampling date	Taxon	Accession no.
ACT1001	Lake Fertő, Stn. 2	26/10/2008	*Choricystis* sp.	HQ594495
ACT1002	Lake Fertő, Stn. 2	26/10/2008	*Choricystis* sp.	HQ594496
ACT1003	Lake Fertő, Stn. 2	26/10/2008	*Choricystis* sp.	HQ594497
ACT1004	Lake Fertő, Stn. 2	26/10/2008	*Choricystis* sp.	HQ594498
ACT1005	Lake Fertő, Stn. 2	26/10/2008	*Choricystis* sp.	HQ594499
ACT1006	Lake Fertő, Stn. 2	26/10/2008	*Mychonastes* sp.	HQ594500
ACT1007	Lake Balaton, Stn. 1	25/01/2009	*Choricystis* sp.	HQ594501
ACT1008	Lake Balaton, Stn. 1	25/01/2009	*Choricystis* sp.	HQ594502
ACT1009	Lake Balaton, Stn. 1	25/01/2009	*Choricystis* sp.	HQ594503
ACT1010	Lake Balaton, Stn. 1	25/01/2009	*Choricystis* sp.	HQ594504
ACT1011	Lake Balaton, Stn. 1	25/01/2009	*Choricystis* sp.	HQ594505
ACT1012	Lake Balaton, Stn. 1	25/01/2009	*Choricystis* sp.	HQ594506
ACT1013	Lake Balaton, Stn. 1	25/01/2009	*Choricystis* sp.	HQ594507
ACT1014	Lake Balaton, Stn. 3	25/01/2009	*Stichococcus bacillaris*	HQ594508
ACT1015	Lake Balaton, Stn. 3	25/01/2009	*Nannochloris bacillaris*	HQ594509
ACT1016	Lake Balaton, Stn. 3	25/01/2009	*Mychonastes* sp.	HQ594510
ACT1017	Lake Balaton, Stn. 3	25/01/2009	*Mychonastes* sp.	HQ594511
ACT1018	Lake Balaton, Stn. 3	25/01/2009	*Choricystis* sp.	HQ594512
ACT1019	Lake Balaton, Stn. 3	25/01/2009	*Choricystis* sp.	HQ594513
ACT1021	Lake Balaton, Stn. 2	16/01/2007	*Choricystis* sp.	HQ594514
ACT1022	Lake Balaton, Stn. 2	16/01/2007	*Choricystis* sp.	HQ594515

FIGURE LEGENDS

Fig. 1 Map of the studied Central European great lakes and the surrounding area. Sampling sites are marked with closed circles (Lake Fertő/Neusiedlersee: F1 – Station 1, F2 – Station 2; Lake Balaton: B1 – Station 1, B2 – Station 2, B3 – Station 3).

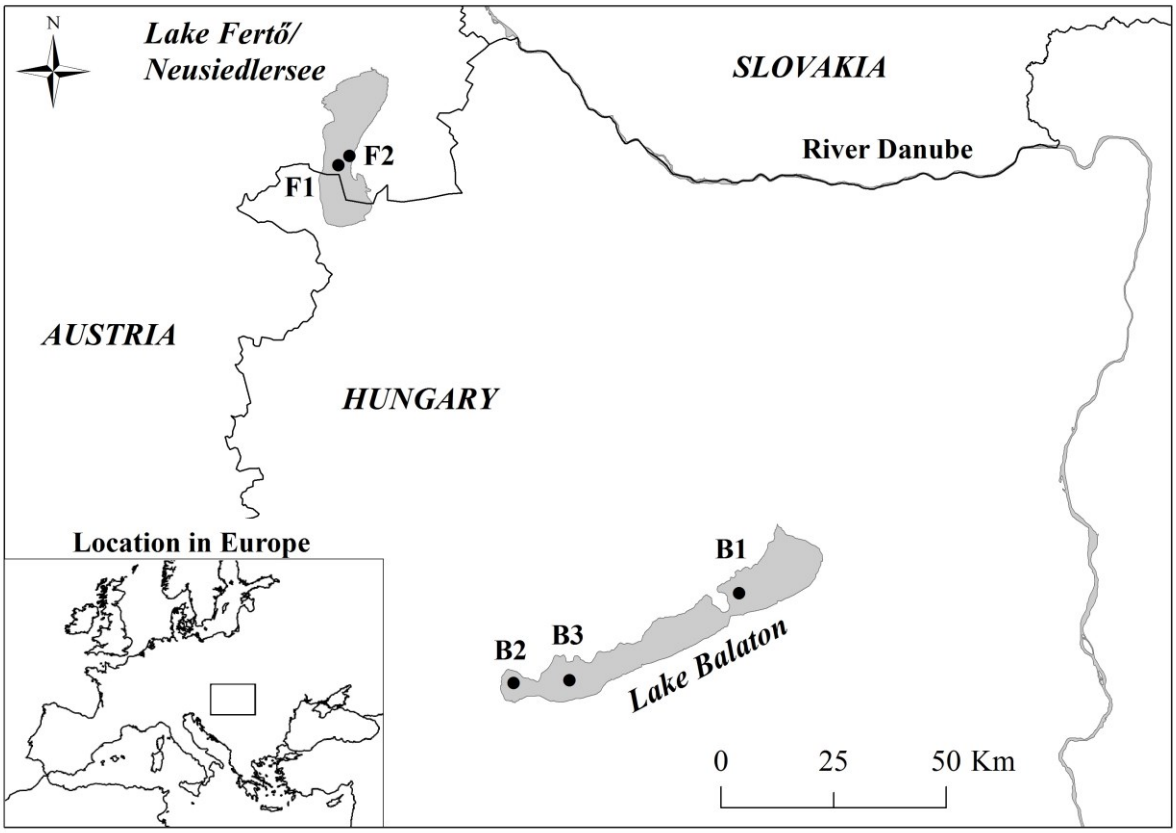

Fig. 2 Contribution of autotrophic picoplankton (APP) to total phytoplankton biomass (A) and total planktonic primary production (B) in shallow Central European great lakes (LB – Lake Balaton, LF – Lake Fertő/Neusiedlersee; Stn. 1 – Station 1, Stn. 2 – Station 2) in winter 2009-2014. The plotted rectangles represent the 25th to 75th percentiles while the lines across the rectangles represents the median. Whiskers extend to the minimum and maximum values. 1st and 99th percentiles are marked with x, mean values with squares.

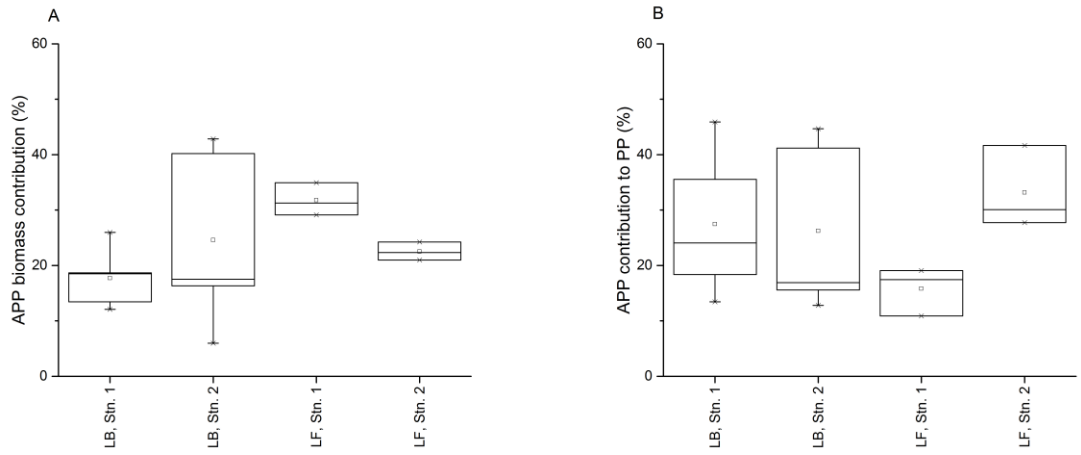

Fig. 3 P-I parameters [A: biomass-specific maximum photosynthetic rate (P^B_{max}), B: light saturation parameter (I_k), C: biomass-specific light utilization parameter (α^B) and D: optimal light intensity (I_{opt})] of the different size fractions (APP – autotrophic picoplankton, NP – nano+microplankton) in the studied shallow lakes (LB – Lake Balaton, LF – Lake Fertő/Neusiedlersee; Stn. 1 – Station 1, Stn. 2 – Station 2) in winter 2009-2014. Significant differences were marked with asterisk (*t*-test).

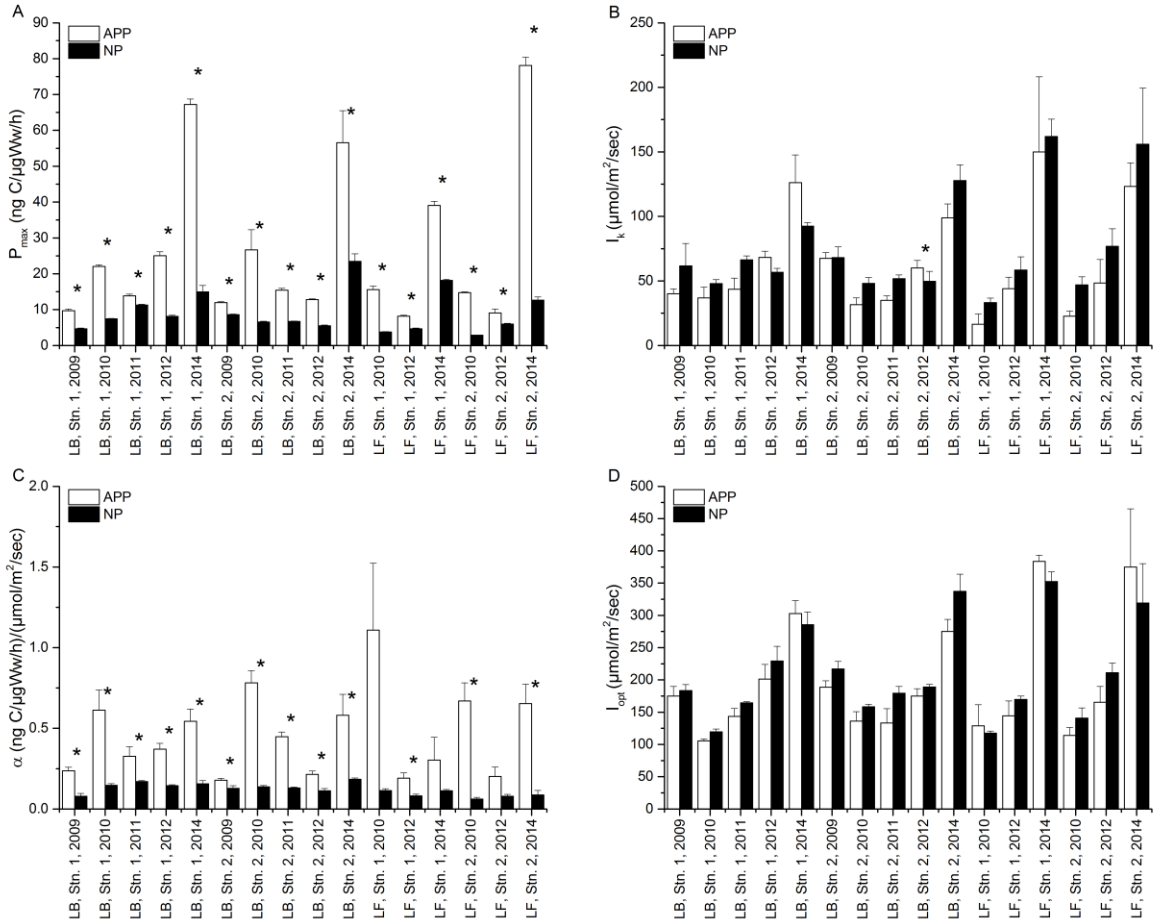

Fig. 4 Biomass-specific maximum photosynthetic rate (P^B_{max}) as a function of water temperature (A and B), and the light saturation parameter (I_k) as a function of mean water column irradiance (C and D) of the different size fractions (APP – autotrophic picoplankton, NP – nano+microplankton) in the studied shallow lakes in winter 2009-2014. Continuous (nano+microplankton) and dashed lines (autotrophic picoplankton) represent simple linear regressions (parameters can be found in Table 3).