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12
13 **The role and composition of winter picoeukaryotic assemblages in shallow Central**
14 **European great lakes**

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34
35 **Abstract**

36
37 Studies on autotrophic picoplankton (APP; <3 µm) in shallow lakes are mainly confined to
38 the spring-fall seasons, when sampling efforts are not complicated by adverse and unsafe
39 conditions that occur during winter. The aim of the present work was to study the role and
40 diversity of winter APP communities in temperate shallow lakes by means of analysis of
41 measures of size-fractionated photosynthesis and culture-based molecular taxonomic
42 identification. Our results show that APP comprised a substantial part of planktonic primary
43 production in shallow Central European great lakes (13-46% in Lake Balaton and 11-42% in
44 Lake Fertő). Better acclimation of APP than that of the larger phytoplankton (>3 µm) to low-
45 temperature and low-light winter environment was confirmed by their higher maximum
46 photosynthetic rate and light utilization parameter. Maximum photosynthetic rate and light
47 saturation parameter increased significantly with both temperature and available light, but
48 with different impact on the two size groups. Twenty-two picoeukaryotic strains were isolated
49 and identified based on 18S rRNA gene sequence analysis. Taxonomic composition of the

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

56 Introduction

57
58 Autotrophic picoplankton (APP) includes the smallest photosynthetic organisms, consisting of
59 prokaryotic and eukaryotic taxa within the size range of 0.2-2 or 3 μm . The term was
60 originally defined by Sieburth et al. (1978) to the 0.2-2 μm size range, but was later expanded
61 to 3 μm because of the larger cell size of picoeukaryotes (Vaulot et al., 2008). APP is a major
62 component of the photosynthetic biomass in many aquatic ecosystems, particularly in
63 oligotrophic lakes and oceans (Agawin et al., 2000; Callieri et al., 2007; Stockner, 1991;
64 Weisse, 1993). Thus, it constitutes an important source of energy in aquatic food webs as an
65 integral part of the microbial loop (Azam et al., 1983; Callieri 2008 and references therein).
66 APP cells are more effective in nutrient and light acquisition than larger phytoplankton owing
67 to their high surface area (Irwin et al., 2006) and their reduced chromophore self-shading
68 (Raven, 1998). Accordingly, these tiny cells are assumed to be good competitors in resource-
69 poor habitats (Callieri, 2008) and under low light conditions (Callieri, 2016). Besides nutrient
70 supply and light availability, other factors such as water temperature, salinity, grazing or viral
71 infection also influence the occurrence and dynamics of APP in aquatic ecosystems (Callieri,
72 2008; Stockner, 1991).

73 The contribution of APP to total phytoplankton biomass and primary production can
74 be significant. In the tropical Pacific Ocean, for example, Li et al. (1983) attributed 25-80% of
75 the inorganic carbon fixation to APP, while in the South East Pacific Ocean, APP constituted
76 36–57% of the total primary production (Rii et al., 2016). APP was also found to constitute
77 60-80% of the total primary production in the Sargasso Sea (Glover et al., 1985), an average
78 of 44% in the subtropical and tropical regions of the North Atlantic Ocean (Jardillier et al.,
79 2010) and 12-83% in the south Atlantic and Atlantic sectors of the Southern Ocean
80 (Froneman et al., 2001). In a temperate coastal ecosystem APP accounted for a mean annual
81 value of 51% (4 to 76%) of total carbon fixation (Morán, 2007). Global estimates indicate that
82 these small cells provide 39% of the planktonic primary production in the world's ocean
83 (Agawin et al., 2000). These results show that APP dominate phytoplankton biomass and
84 production in nutrient-poor, warm ($> 26^{\circ}\text{C}$) waters, but represent only a minor part of the
85 autotrophic biomass and production in nutrient-rich and cold ($< 3^{\circ}\text{C}$) waters (Agawin et al.,
86 2000).

87 Less information is available for freshwater lakes, despite the fact that the contribution
88 of APP could be as high as in marine waters (Bell and Kalff, 2001). In North American deep
89 lakes, for example, APP production comprised 25-60% (Lake Huron) or 10-70% (Lake
90 Michigan) of carbon fixation (Fahnenstiel and Carrick, 1992). Higher contribution values
91 were described in a small meromictic lake (Little Round Lake, USA), where APP composed
92 22-97% of the planktonic primary production. In a mesotrophic deep lake in Austria (Lake
93 Mondsee) APP constituted 16-58% of the total, area-integrated primary production
94 (Greisberger et al., 2008). In eutrophic, humic shallow lakes from the floodplain of the Lower
95 Parana River (Argentina) picoplankton production represented 36-93% of the total carbon
96 fixation (Izaguirre et al., 2010; Rodriguez et al., 2012). In a meso-eutrophic humic shallow
97 lake in Finland, APP constituted 5-57% of the primary production (Peltomaa and Ojala,
98 2010), while in a eutrophic, river-dominated estuary, picoplankton accounted for 42-55% of
99 total primary productivity (Gaulke et al., 2010).

100 Size-fractionated primary production measurements were also conducted in Hungarian
101 shallow lakes. In Lake Balaton, the contribution of APP varied between 40 and 50% in the
102 less productive Eastern basin and between 1 and 57% in the Western basin during the
103 productive period of the year (Vörös et al., 1991). More than a decade later in summer 2005,
104 it ranged between 23 and 54% along the longitudinal axis of the lake (Somogyi and Vörös,
105 2006). In a hypertrophic reservoir in Hungary, APP accounted for 10-55% of the total primary

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

108 Most of the fractionated photosynthesis measurements in freshwater lakes were
109 confined to a relatively short period, usually between spring and autumn at the peak of APP
110 development (e.g. Callieri et al., 2007; Malinsky-Rushansky et al., 1997; Steitz and
111 Velimirov, 1999; Vörös et al., 1991). Little is known about the role of winter APP
112 assemblages in ecosystem processes, despite the fact that studies on size-fractionated
113 phytoplankton are essential to understand food web dynamics in aquatic ecosystems (Callieri,
114 2008). Undersampling is particularly obvious in the freshwater lakes of the temperate zone,
115 where sampling in winter is difficult and sometimes virtually impossible because of variable
116 and unsafe ice conditions (Dokulil and Herzig, 2009; Felföldi et al., 2016). There have been
117 some size-fractionated primary production measurements performed in the winter season, but
118 these studies were done mainly in deep lakes. In the case of Lake Kinneret (Israel), for
119 example, the photosynthetic activity of APP and its contribution to total photosynthesis was
120 lower in winter than during the productive period (Malinsky-Rushansky et al., 1997). In an
121 alkaline, deep lake (Mono Lake, California), APP accounts for nearly 25% of the primary
122 production during the winter bloom and more than 50% at other times of the year (Roesler et
123 al., 2002). Similar results were found in the case of Lake Simcoe (Canada), where winter APP
124 contribution was 40% on average at nearshore sites and 30% at offshore sites (Kim et al.,
125 2015), and showed a higher share (up to 90%) from spring to autumn. In contrast, in Lake
126 Mondsee, APP contribution to total primary production was highest (as much as 58%) in the
127 autumn- winter period (Greisberger et al., 2008). However, it should be noted that in the case
128 of Lake Kinneret, the minimum temperature in winter does not drop below 10 °C (Malinsky-
129 Rushansky et al., 1997), as opposed to the abovementioned lakes, which usually possess ice
130 cover.

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

140

141 **Methods**

142

143 *Study site and sampling*

144 Lake Balaton (Hungary) is the largest lake in Central Europe with a surface area of 596 km²
145 and an average depth of 3.2 m (Fig. 1). Specific conductivity (SC, 700–800 µS/cm) and pH
146 (8.3–8.6) are relatively high and constant. In winter, the lake is usually frozen for 42 ± 27
147 days, but years without ice cover can also occur (Vörös et al., 2009). There is a depth and
148 trophic gradient along the longitudinal axis of the lake, which results in higher inorganic
149 turbidity and higher algal biomass in the western parts of the lake (Felföldi et al., 2011a;
150 Vörös et al., 2009). Two stations were chosen for photosynthesis measurements, to represent
151 different areas of the lake: Station 1 in the deeper Eastern basin (46°55.327' N, 17°55.649' E,
152 4 m at the sampling station) with annual maximum chlorophyll *a* concentration between 10-
153 20 µg/L and Station 2 (46°44.095' N, 17°16.583' E) in the shallower (3.1 m) western part of
154 the lake with annual maximum chlorophyll *a* concentration between 30–40 µg/L (Vörös et al,

155 2009). For strain isolation, an additional sampling station was chosen with an average depth
156 of 3.7 m (Station 3; 46°44.552' N, 17°26.308' E) close to Station 2.

157 Lake Fertő/Neusiedlersee is a wind-exposed, extremely shallow (~ 1.3 m), eutrophic
158 (annual maximum chlorophyll *a*: 30-40 µg/L) steppe lake (SC between 1300 and 3200 µS/cm,
159 pH between 7.8 and 9.3) straddling the Austrian/Hungarian border (Fig. 1; Löffler, 1979;
160 Somogyi et al., 2010). The total surface area of the lake is 309 km², of which ~55% is covered
161 by reed (Löffler, 1979). There are numerous reedless brown-water ponds (inner ponds) of
162 variable size within the reed belt, which is intersected with artificial canals connecting the
163 inner ponds with the open water areas. The open water area and the inner ponds have similar
164 ionic composition, pH and SC, but there are big differences in their transparency (Dinka et al.,
165 2004). As a result of wind-induced sediment resuspension, the open water of the lake is
166 characterized by high inorganic turbidity and usually low Secchi-disk transparency (Löffler,
167 1979). During winter, however, when the lake is ice covered, the amount of suspended solids
168 declines due to the absence of turbulence (Dokulil and Herzig, 2009). In winter, the lake is
169 usually frozen for 56 days, but there is a declining tendency of ice cover duration at a rate of
170 ca. 1 day per year, and as in Lake Balaton, ice development was not observed in some years
171 (Dokulil and Herzig, 2009). Two sampling stations were chosen, representing different water
172 bodies within the lake: an open water sampling station (Station 1; 47°45.424' N, 16°43.389' E)
173 and an inner lake, Ruster Poschen (Station 2; 47°46.597' N, 16°45.260' E).

174 For photosynthesis measurements, water samples were taken with a 4.5 m long tube
175 sampler from Lake Balaton at Station 1 and Station 2 on five winter sampling dates between
176 2009 and 2014 and from Lake Fertő/Neusiedlersee (Station 1 and Station 2) in winter 2010,
177 2012 and 2014 (Table 1). For strain isolation, water samples were taken from Lake Balaton at
178 Station 2 in winter 2007 and at Station 1 and 3 in winter 2009. Additional water samples were
179 taken from Lake Fertő at Station 2 in October 2008. When the lakes were covered by ice,
180 sampling and measurements were done through a sawed, 30 cm wide hole.

181 182 *Physicochemical measurements*

183 Photosynthetically active radiation (PAR, 400-700 nm) in the water column was measured at
184 0.25 m increments with a Li-COR underwater radiometer, using a flat (2π) quantum sensor.
185 The sensor was placed directly under the ice on a metal frame 50 cm away from the 30 cm
186 sawed hole and slowly lowered into the water column. The hole was covered again with the
187 ice block and the snow cover (if present) was undisturbed to acquire realistic values for PAR
188 transmission. Diminution of light within the ice cover (and snow if present) – as a result of
189 light reflection and attenuation – was calculated as the difference between PAR measured
190 above and just below the ice cover. Mean irradiance (I_{mean}) in the water column was
191 calculated according to Ferrero et al. (2006) and Allende et al. (2009) adapted to shallow
192 waters. Assuming a fully mixed water column, I_{mean} was calculated as follows:

$$193 \quad I_{\text{mean}} = I_0 \times (1 - \exp(-K_d \times z)) / K_d \times z$$

194 where I_0 is the average irradiance measured between 12 and 14 h, K_d is the downwelling
195 diffuse attenuation coefficient for PAR, and z is the depth at the sampling site (Allende et al.,
196 2009). I_0 irradiance values were calculated from the hourly averages of global radiation
197 between 12 and 14 h for the day of sampling provided by the Hungarian Meteorological
198 Service, assuming that $1 \text{ W/m}^2 = 4.6 \text{ µmol/m}^2/\text{sec}$ (Wetzel and Likens, 2000). PAR was
199 considered to be 47% of global radiation according to Wetzel and Likens (2000). Light
200 attenuation of the ice and snow cover, which was measured in the field, was also taken into
201 account. Water temperature, pH and conductivity of the water samples were measured with a
202 Wissenschaftlich-Technische-Werkstätten (WTW) pH 315i and a Hanna HI9033 portable
203 field meter.

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

210

211 *Microscopic analysis*

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

234

235 *Photosynthesis measurement*

236 Photosynthesis of the phytoplankton community was measured using the ^{14}C -technique
237 (Steemann-Nielsen, 1952). 20 mL subsamples were put into glass vials, preincubated for 1
238 hour, and after adding $\text{NaH}^{14}\text{CO}_3$ (0.1 - 0.13 MBq) incubated for 3 hours in a self-designed
239 photosynthetron (Üveges et al. (2011)). Vials were incubated in triplicate at eight different
240 irradiances ranging from 5 to 1300 $\mu\text{mol}/\text{m}^2/\text{sec}$ at ambient lake temperature (measured
241 during sampling). Three vials were incubated in darkness for dark carbon uptake.

242 For photosynthesis measurement of the total phytoplankton, 5 mL of each incubated
243 sample was filtered onto a 0.4 μm pore sized cellulose-acetate membrane (Millipore) under
244 low vacuum pressure. In order to estimate size fractionated primary productivity ($>3 \mu\text{m}$ and
245 $<3 \mu\text{m}$) the remainder (15 mL) was filtered through polycarbonate filters of 3 μm pore size
246 (Millipore, diameter 47 mm) using plastic disposable syringes and plastic 47 mm filter
247 holders without vacuum. The filtrate obtained in this step was then filtered through a 0.4 μm
248 pore size cellulose-acetate membrane filter (Millipore) to concentrate APP cells.
249 Photosynthesis of the entire phytoplankton community, as well as that of the size-fractions
250 were determined separately by this method modified by Callieri et al. (2007).

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

254 carbon assimilation was calculated based on the proportion between ^{14}C uptake and DIC
255 availability using an isotope discrimination factor of 1.05 (Stemann-Nielsen, 1952). The
256 obtained results were normalized to wet weight (determined as described above).
257 Photosynthesis-irradiance (P-I) curves were fitted using the model of Eilers and Peeters
258 (1988) with the data analysis software OriginPro 2015. Definitions of photosynthetic
259 parameters are given in Table S1. I_{mean} provides a reference for comparing I_k values, i.e.,
260 phytoplankton primary production in lakes for which $I_{\text{mean}} < I_k$ can be safely assumed to be
261 light limited according to Allende et al. (2009). Areal primary production was estimated using
262 the P-I curves and the light intensity profile of the water column at 0.1 m increments.

263

264 *Isolation and molecular identification of picoeukaryotic algal strains*

265 Picoeukaryotic strains ACT1001-ACT1006 (Algal Culture Tihany) were isolated from the
266 water of Lake Fertő in October 2008, whereas strains ACT1007-ACT 1022 were isolated
267 from Lake Balaton in January 2007 and 2009. Isolation was carried out using a modified
268 BG11 medium, in which only one tenth of the recommended micronutrient solution was used
269 (Rippka et al., 1979) as previously described (Somogyi et al., 2009). Unialgal cultures were
270 established by serial streaking on 1.5% agar plates and single colony isolations at 8 °C under
271 25 $\mu\text{mol}/\text{m}^2/\text{sec}$ cool white fluorescent light (Tungsram F33) on a 12:12 hour light:dark cycle.
272 The strains were later transferred to liquid media (modified BG11 medium) and were
273 maintained at 21 °C under 40 $\mu\text{mol}/\text{m}^2/\text{sec}$ on a 14:10 hour light:dark cycle.

274 Genomic DNA was extracted according to the procedure described previously by
275 Somogyi et al. (2009). PCR amplification of the 18S rRNA gene was performed with a final
276 volume of 50 μL using approximately 2 μL of genomic DNA, 0.2 mM of each
277 deoxynucleotide, 2 mM MgCl_2 , 1 U LC *Taq* DNA polymerase (Fermentas), 1X PCR buffer
278 (Fermentas), 0.325 μM of Euk528f and CHLO02 primers (Elwood et al., 1985; Zhu et al.,
279 2005) and 400 ng of BSA (Fermentas). PCR amplicons were purified with the PCR-MTM
280 Clean Up System (Viogene). Sequencing was carried out with the BigDye[®] Terminator v3.1.
281 Cycle Sequencing Kit (Applied Biosystems). Chromatograms were corrected manually with
282 Chromas 1.45 software (Technelysium Pty Ltd.). The generated sequences were compared to
283 the GenBank nucleotide database using the BLAST program (Altschul et al., 1997). The
284 obtained 18S rRNA gene sequences were submitted to GenBank under the accession numbers
285 HQ594495- HQ594515.

286

287 *Statistical analysis*

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

293

294 **Results**

295

296 *Physical and chemical characteristics (light environment)*

297 Lake Balaton was frozen at Station 1 with an ice thickness of 4 cm in winter 2010 and 13 cm
298 in winter 2011 (Table 1). As a result of light attenuation within the ice, 83% of surface PAR
299 was detected entering the water column in winter 2011. At Station 2, ice cover was formed
300 only in winter 2010 and the 16 cm thick ice absorbed more than half of the surface irradiance
301 (42% of PAR reached the ice/water interface). Snow cover was found only at Station 2 in
302 winter 2010, with a thickness of about 0.5 cm. Vertical attenuation coefficients ranged

303 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
304 result, mean irradiance in the water column was between 30 and 300 $\mu\text{mol}/\text{m}^2/\text{sec}$ at Station 1
305 and between 45 and 190 $\mu\text{mol}/\text{m}^2/\text{sec}$ at Station 2 (Table 1). Water temperature (0.6-5.3 °C),
306 pH (8.1-8.7), and specific conductance (690-820 $\mu\text{S}/\text{cm}$) varied in ranges typical for the lake
307 during winter.

308 Ice coverage on Lake Fertő was observed in winter 2010 and 2012 with an average
309 thickness of 13-15 cm and 15-19 cm, respectively (Table 1). Ice cover absorbed on average
310 37% of surface irradiance (63% of PAR reached the ice/water interface at both stations in
311 winter 2010, and 57% at Station 1 and 70% at Station 2 in winter 2012). The vertical
312 attenuation coefficients ranged between 1.6/m and 3.6/m at Station 1, and between 2/m and
313 2.2/m at Station 2 (Table 1). Mean irradiance of the water column varied between 110 and 190
314 $\mu\text{mol}/\text{m}^2/\text{sec}$ at Station 1 and between 130 and 280 $\mu\text{mol}/\text{m}^2/\text{sec}$ at Station 2 (Table 1). Water
315 temperature ranged between 0.5 and 7.6 °C (Table 1). pH values (between 8 and 8.6) in Lake
316 Fertő were similar to those measured in Lake Balaton, but specific conductance was higher
317 (between 1900 and 2400 $\mu\text{S}/\text{cm}$).

318

319 *Phytoplankton biomass and APP contribution*

320 In Lake Balaton, chlorophyll *a* concentration was between 1.5 and 13 $\mu\text{g}/\text{L}$ at Station 1, with
321 the highest values measured in February 2009. At Station 2, chlorophyll *a* concentration
322 varied between 7 and 21 $\mu\text{g}/\text{L}$ and the highest concentration was measured in February 2010
323 (Table 1). APP constituted 12-26% of the total phytoplankton biomass at Station 1 with
324 values between 30 and 220 $\mu\text{g}/\text{L}$ (Table 1, Fig. 2). APP was composed of both
325 picocyanobacteria and picoeukaryotic algae with abundances between 11 and 104 x 10³
326 cells/mL for CyAPP (5-98 $\mu\text{g}/\text{L}$ biomass) and between 11 and 60 x 10³ cells/mL for EuAPP
327 (20- 120 $\mu\text{g}/\text{L}$ biomass). With the exception of winter 2010, picoplankton biomass was
328 dominated by EuAPP (Table 1). At Station 2, APP biomass (100-520 $\mu\text{g}/\text{L}$) and contribution
329 (6-43%) to total phytoplankton biomass was higher than at Station 1 (Table 1, Fig. 2). As in
330 the case of Station 1, picocyanobacteria and picoeukaryotic algae were also observed. CyAPP
331 abundance varied between 26 and 110 x 10³ cells/mL (14- 60 $\mu\text{g}/\text{L}$ biomass), whereas EuAPP
332 abundance reached 40-250 x 10³ cells/mL (86-500 $\mu\text{g}/\text{L}$ biomass) (Table 1). EuAPP was
333 clearly dominant within the pico fraction at each sampling date. Larger-sized phytoplankton
334 biomass varied between 141 and 1419 $\mu\text{g}/\text{L}$ and between 579 and 1578 $\mu\text{g}/\text{L}$ at Station 1 and
335 Station 2, respectively, with *Cryptomonas sp.*, *Rhodomonas lacustris var. nannoplanctica* and
336 *Chrysochromulina parva* as the dominant taxa.

337 Chlorophyll *a* concentration in Lake Fertő was between 4.5 and 12 $\mu\text{g}/\text{L}$ at Station 1
338 and between 6 and 7 $\mu\text{g}/\text{L}$ at Station 2 (Table 1). APP contribution was higher than in Lake
339 Balaton: at Station 1 APP constituted 29-35% of the total phytoplankton biomass with values
340 between 230 and 370 $\mu\text{g}/\text{L}$, while at Station 2 their biomass ranged from 160 to 220 $\mu\text{g}/\text{L}$,
341 constituting 21-24% of the total phytoplankton biomass (Table 1). Both picocyanobacteria
342 and picoeukaryotes were present, with EuAPP dominance at Station 2. At Station 1, however,
343 picoplankton was dominated by CyAPP at every sampling date: approximately half of the
344 APP biomass was composed of characteristic, *Aphanothece*-like colonial picocyanobacteria
345 (Table 1). CyAPP abundance varied between 410 and 680 x 10³ cells/mL (biomass between
346 210 and 350 $\mu\text{g}/\text{L}$) at Station 1, while it was much lower (abundance: 4-80 x 10³ cells/mL,
347 biomass: 2 and 40 $\mu\text{g}/\text{L}$) at Station 2. An opposite tendency was observed in the case of
348 EuAPP, whose abundance ranged between 3 and 20 x 10³ cells/mL (biomass between 10 and
349 70 $\mu\text{g}/\text{L}$) at Station 1 and between 30 and 50 x 10³ cells/mL (biomass between 120 and 220
350 $\mu\text{g}/\text{L}$) at Station 2 (Table 1). Larger-sized phytoplankton biomass reached 423-916 $\mu\text{g}/\text{L}$ at
351 Station 1 and 570-822 at Station 2. *Cryptomonas sp.* and *Rhodomonas lacustris var.*

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

354

355 *Role of APP in winter primary production*

356 The maximum photosynthetic rate (P_{\max}) of the total phytoplankton varied between 3 and 10
357 $\mu\text{g C/L/h}$ at Station 1 of Lake Balaton (Table S2). Based on the P-I curves and the average
358 ambient light intensities, depth-integrated primary production (PP) varied between 40 and 120
359 $\text{mg C/m}^2/\text{day}$ (Table 2). At Station 2, P_{\max} and PP values were higher (5-23 $\mu\text{g C/L/h}$ and 80-
360 280 $\text{mg C/m}^2/\text{day}$, respectively) (Table S2, Table 2). The contribution of APP to total primary
361 production was between 13 and 46% at both stations (Table 2, Fig. 2). These contribution
362 values were higher than those in terms of total phytoplankton biomass (Fig. 2). At Station 2,
363 the contribution of APP to total phytoplankton biomass and to total PP was similar (Fig. 2). In
364 Lake Fertő, P_{\max} of the total phytoplankton varied between 3 and 26 $\mu\text{g C/L/h}$ at Station 1 and
365 between 4 and 12 $\mu\text{g C/L/h}$ at Station 2 (Table S2). At Station 1, depth-integrated primary
366 production varied between 24 and 125 $\text{mg C/m}^2/\text{day}$. At Station 2, PP values (40 and 80 mg
367 $\text{C/m}^2/\text{day}$) were lower (Table 2). The contribution of APP to total primary production was
368 much lower at Station 1 (10-20%) than at Station 2 (30-40%, Fig. 2). At Station 1, these
369 contribution values were much lower than could be expected from the distribution of biomass
370 between the two size fractions. At Station 2, however, APP contribution to PP was higher
371 than the contribution to total phytoplankton biomass (Fig. 2).

372 The biomass-specific maximum photosynthetic rate (P_{\max}^B) of APP was significantly
373 higher than that of larger-sized phytoplankton (Fig. 3). In Lake Balaton, P_{\max}^B of APP varied
374 between 10 and 67 $\text{ng C}/\mu\text{g Ww/h}$ at Station 1 and between 10 and 56 $\text{ng C}/\mu\text{g Ww/h}$ at
375 Station 2 (Table S2). In the case of nano+microplankton, P_{\max}^B was between 4 and 15 $\text{ng C}/\mu\text{g}$
376 Ww/h at Station 1, and between 5 and 23 $\text{ng C}/\mu\text{g Ww/h}$ at Station 2 (Table S2). A similar
377 tendency was found in Lake Fertő with higher P_{\max}^B of APP (Station 1: 8-39 $\text{ng C}/\mu\text{g Ww/h}$,
378 Station 2: 9-78 $\text{ng C}/\mu\text{g Ww/h}$) than that of larger-sized phytoplankton (Station 1: 3-18 ng
379 $\text{C}/\mu\text{g Ww/h}$, Station 2: 3-12 $\text{ng C}/\mu\text{g Ww/h}$). There were no clear differences between the
380 light saturation parameter (I_k) and the optimal light intensity (I_{opt}) of the different size
381 fractions, but these parameters were somewhat lower for APP than for larger-sized
382 phytoplankton in many cases (Fig. 3, Table S2). The light saturation parameter ranged
383 between 40 and 120 $\mu\text{mol/m}^2/\text{sec}$ in Lake Balaton and between 20 and 120 $\mu\text{mol/m}^2/\text{sec}$ in
384 Lake Fertő (Table S2). We observed large differences between the size fractions in terms of
385 the biomass-specific light utilization parameter (α^B) (Fig 2, Table S2). In Lake Balaton, α^B of
386 APP ranged between 0.2 and 0.7 ($\text{ng C}/\mu\text{g Ww/h}/(\mu\text{mol/m}^2/\text{sec})$), while in the case of
387 nano+microplankton, α^B was between 0.07 and 0.18 ($\text{ng C}/\mu\text{g Ww/h}/(\mu\text{mol/m}^2/\text{sec})$). A
388 similar tendency was found in Lake Fertő with higher α^B of APP [0.2-1.1 ($\text{ng C}/\mu\text{g}$
389 $\text{Ww/h}/(\mu\text{mol/m}^2/\text{sec})$)] than that of larger-sized phytoplankton [0.06-0.1 ($\text{ng C}/\mu\text{g}$
390 $\text{Ww/h}/(\mu\text{mol/m}^2/\text{sec})$].

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

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

402 I_{mean} and $P_{\text{max}}^{\text{B}}$: the slopes were much higher for APP than for larger-sized phytoplankton in
403 both lakes (Table 3).

404
405

406 *Taxonomic composition of the picoeukaryotic community*

407 The winter picoeukaryotic community of Lake Fertő (Station 2) was dominated by 1.6–3 μm
408 sized spherical cells. On the basis of their 18S rRNA gene sequences, the isolated strains
409 ACT1001–ACT1005 belonged to the genus *Choricystis* (Table 4). Strain ACT1006 was
410 affiliated with the genus *Mychonastes*. The winter picoeukaryotic community of Lake
411 Balaton consisted of 1.8–2.7 μm sized single spherical cells and rod-shaped, small green algae
412 (0.8–1.2 μm wide, 4–6 (12) μm long). The majority of the isolated strains had spherical to
413 ovoid cells. Among them, ACT1007–ACT 1013 (isolated from Station 1), ACT1018–ACT
414 1019 (isolated from Station 3) and ACT1021–ACT 1022 (isolated from Station 3) belonged to
415 the genus *Choricystis* (Table 4). Isolates ACT1016–ACT 1017 (isolated from Station 3)
416 belonged the genus *Mychonastes*. Only two isolates had rod-shaped cell morphology:
417 ACT1014 (isolated from Station 3) was identified as *Stichococcus bacillaris* (Table 4),
418 whereas ACT1015 (isolated from Station 3) as *Nannochloris bacillaris* (Table 4).

419

420 **Discussion**

421

422 *Eukaryotic dominance in winter picoplankton*

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

438 Regarding their temperature preference, picoeukaryotes are well adapted to harsh polar
439 environmental conditions and dominate Arctic pelagic phytoplankton communities for most
440 of the year (Metfies et al., 2016). In shallow lakes within the temperate zone, picoplankton is
441 dominated by CyAPP in summer, whereas EuAPP are dominant from autumn until spring
442 (Callieri, 2008; Somogyi et al., 2009; Vörös et al., 2009). Picocyanobacteria isolated from
443 freshwater lakes usually have higher temperature optima and higher light requirements than
444 picoeukaryotes resulting in clear seasonal niche partitioning. Thus, the observed winter
445 predominance of EuAPP cells was in good agreement with previous findings (Callieri, 2008;
446 Malinsky-Rushansky et al., 2002; Somogyi et al., 2009; Vörös et al., 2009). On the other
447 hand, deep temperate oligotrophic lakes, such as Lake Superior (Ivanikova et al., 2007), can
448 harbour dominant winter CyAPP communities. We observed a similar phenomenon at Station
449 1 in Lake Fertő, which suggests the presence of a winter-acclimated CyAPP ecotype.

450

451 *Taxonomic composition of winter picoeukaryotic communities*

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

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

495

496 *Photosynthetic characteristics and primary production of winter phytoplankton*

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

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

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

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

534 The higher biomass-specific maximum photosynthetic rate and higher light utilization
535 parameter of APP than that of larger-sized phytoplankton in the studied shallow lakes implied
536 a better acclimation of APP to low-temperature and low-light winter environment. No
537 difference was observed in this regard between CyAPP- and EuAPP-dominated picoplankton.
538 In agreement with this, more effective light acquisition and faster metabolic rates were
539 described for smaller cells than larger ones owing to their high surface area and reduced
540 chromophore self-shading (Callieri, 2008; Irwin et al., 2006; Raven, 1998). Similarly higher
541 $P_{\text{max}}^{\text{B}}$ values were obtained for APP than for the larger-sized phytoplankton fraction in a
542 temperate coastal ecosystem (Morán, 2007) and in Lake Mondsee (Greisberger et al., 2008).
543 In a relict oxbow lake, however, there were no significant differences between the
544 phytoplankton size fractions in terms of their $P_{\text{max}}^{\text{B}}$, α and I_{k} (Rodriguez et al., 2012).

545 546 *Light acclimation in winter*

547 Light limitation did not occur in the studied shallow lakes as I_{k} values for both APP and
548 larger-sized phytoplankton were lower than I_{mean} except at Station 1 of Lake Balaton in
549 February 2009 (Table 1, Table S2). However, the observed I_{k} values were much lower than
550 usual during the autumn to spring period in temperate shallow lakes (Dokulil et al., 2014;
551 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 $\mu\text{g/l}$ (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 photosynthetic
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 homogeneously 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

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

604 The effect of nutrient availability cannot be neglected in winter, however, different
605 factors can result in different scenarios: convection mixing in winter promotes nutrient
606 resuspension in deep lakes (Hampton et al., 2015), on the other hand, ice cover reduces the
607 input of dissolved and particulate nutrients from the atmosphere and nearby terrestrial
608 surroundings (Bertilsson et al., 2013). However, despite the important role of nutrients, winter
609 in the temperate zone is accompanied by the dominance of physical constraints. In Lake
610 Balaton, the concentration of dissolved inorganic nitrogen and phosphorus increases during
611 the winter months (Présing et al., 2001), because phytoplankton (including
612 picophytoplankton) have a limited ability to take up nutrients due to reduced temperature.
613 Other studies have come to similar conclusion, winter phytoplankton primary production has
614 been found to primarily depend on light rather than on nutrient availability in various water
615 bodies, such as an alkaline pan (Pálffy et al., 2014) and a humic, boreal lake (Tulonen et al.,
616 1994). Grazing might also be a determining factor, but in winter zooplankton activity is
617 greatly limited. Winter zooplankton abundance and biomass in Lake Fertő/Neusiedlersee
618 varied among years but correlated positively with chlorophyll *a* concentration. Zooplankton
619 dynamics fundamentally depend on phytoplankton biomass in autumn and on conditions
620 during the winter that follow, and ice cover is particularly important in this respect (Dokulil et
621 al., 2014). Crustacean zooplankton ingestion rates were also found to be considerably
622 restrained at lower temperatures (Loiterton et al., 2004).

623

624 *Climate change implications*

625 Ice cover duration has been found to show a decreasing trend on many lakes as mean air
626 temperatures have increased as a result of global climate change (Weyhenmeyer et al., 2011).
627 For this reason, the study of winter phototrophic communities is particularly important for
628 understanding the dynamics and functioning of seasonally frozen lakes (Bertilsson et al.,
629 2013). Changes in the duration of ice and snow cover patterns influence phytoplankton
630 succession (Bertilsson et al., 2013), however, estimating the effects of such climate-driven
631 changes is difficult. Worldwide reduction in the duration of snow cover is expected to
632 enhance light transmission, since clear ice can transmit as much as 95% of ambient PAR
633 (Hampton et al., 2015). As a result, light transmission can promote complex convection
634 patterns, which can keep small celled organisms in suspension (Hampton et al., 2015). On the
635 other hand, a reduction in ice cover duration also involves habitat loss for ice-associated algae
636 and enhances resuspension, which restricts light transmission and helps large celled
637 organisms to remain in suspension (Beall et al., 2016). In the case of shallow lakes, all these
638 processes affect not only phytoplankton but also benthic productivity, which could be
639 important in whole-lake primary production. In Lake Balaton, benthic algae have a substantial
640 role in the shallow littoral regions with a contribution of 60-95% to total primary production
641 (phytobenthos+phytoplankton) in summer, while in the deeper pelagic region, only 15% is
642 attributable to phytobenthos (Üveges et al., 2011). In Lake Fertő, benthic algae are virtually
643 absent from spring to autumn due to high inorganic turbidity (Dokulil and Herzig, 2009) but
644 the winter situation is unexplored. So far, we have limited information on how climate change
645 will affect different algal groups. Our results suggest that a higher average winter temperature
646 and light availability in shallow lakes could be more advantageous for APP than
647 nano+microplankton, which can reorganize energy flows, providing more energy to
648 picoplankton grazers (heterotrophic and mixotrophic nanoflagellates, ciliates, etc.). However,
649 as some of the dominant winter nanoplankton taxa can feed mixotrophically, they can also
650 benefit from APP production.

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

666

667 **Concluding remarks**

668

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

679

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681

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688

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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 μ m).

Sampling station	Sampling date	WT (°C)	Ice cover (cm)	PAR%	K_{dPAR} (1/m)	I_{mean} (μ mol/m ² /sec)	Chl (μ g/L)	EuAPP abundance (10 ³ cells/mL)	CyAPP abundance (μ g/L)	EuAPP biomass (μ g/L)	CyAPP biomass (μ g/L)	APP _{col} biomass (μ g/L)	NP biomass
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 ($^{\circ}\text{C}$), I_{mean} – mean irradiance of the water column ($\mu\text{mol}/\text{m}^2/\text{sec}$), $P_{\text{max}}^{\text{B}}$ – biomass-specific maximum photosynthetic rate ($\text{ng C}/\mu\text{g Ww/h}$), I_{k} – light saturation parameter ($\mu\text{mol}/\text{m}^2/\text{sec}$), α^{B} – biomass-specific light utilization parameter ($P_{\text{max}}^{\text{B}}/I_{\text{k}}$), APP – autotrophic picoplankton, NP – nano+microplankton.

Sampling site	Variable 1	Variable 2	Intercept	Slope	n	R^2
Lake Balaton	WT	$P_{\text{max}}^{\text{B}}$ of APP	-0.54	12.28	30	0.84***
Lake Balaton	WT	$P_{\text{max}}^{\text{B}}$ 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}	$P_{\text{max}}^{\text{B}}$ of APP	2.42	0.17	30	0.43***
Lake Balaton	I_{mean}	$P_{\text{max}}^{\text{B}}$ 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	$P_{\text{max}}^{\text{B}}$ of APP	-1.94	7.89	18	0.79***
Lake Fertő	WT	$P_{\text{max}}^{\text{B}}$ 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}	$P_{\text{max}}^{\text{B}}$ of APP	-33.98	0.36	18	0.69***
Lake Fertő	I_{mean}	$P_{\text{max}}^{\text{B}}$ 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	<i>Choricystis</i> sp.	HQ594495
ACT1002	Lake Fertő, Stn. 2	26/10/2008	<i>Choricystis</i> sp.	HQ594496
ACT1003	Lake Fertő, Stn. 2	26/10/2008	<i>Choricystis</i> sp.	HQ594497
ACT1004	Lake Fertő, Stn. 2	26/10/2008	<i>Choricystis</i> sp.	HQ594498
ACT1005	Lake Fertő, Stn. 2	26/10/2008	<i>Choricystis</i> sp.	HQ594499
ACT1006	Lake Fertő, Stn. 2	26/10/2008	<i>Mychonastes</i> sp.	HQ594500
ACT1007	Lake Balaton, Stn. 1	25/01/2009	<i>Choricystis</i> sp.	HQ594501
ACT1008	Lake Balaton, Stn. 1	25/01/2009	<i>Choricystis</i> sp.	HQ594502
ACT1009	Lake Balaton, Stn. 1	25/01/2009	<i>Choricystis</i> sp.	HQ594503
ACT1010	Lake Balaton, Stn. 1	25/01/2009	<i>Choricystis</i> sp.	HQ594504
ACT1011	Lake Balaton, Stn. 1	25/01/2009	<i>Choricystis</i> sp.	HQ594505
ACT1012	Lake Balaton, Stn. 1	25/01/2009	<i>Choricystis</i> sp.	HQ594506
ACT1013	Lake Balaton, Stn. 1	25/01/2009	<i>Choricystis</i> sp.	HQ594507
ACT1014	Lake Balaton, Stn. 3	25/01/2009	<i>Stichococcus bacillaris</i>	HQ594508
ACT1015	Lake Balaton, Stn. 3	25/01/2009	<i>Nannochloris bacillaris</i>	HQ594509
ACT1016	Lake Balaton, Stn. 3	25/01/2009	<i>Mychonastes</i> sp.	HQ594510
ACT1017	Lake Balaton, Stn. 3	25/01/2009	<i>Mychonastes</i> sp.	HQ594511
ACT1018	Lake Balaton, Stn. 3	25/01/2009	<i>Choricystis</i> sp.	HQ594512
ACT1019	Lake Balaton, Stn. 3	25/01/2009	<i>Choricystis</i> sp.	HQ594513
ACT1021	Lake Balaton, Stn. 2	16/01/2007	<i>Choricystis</i> sp.	HQ594514
ACT1022	Lake Balaton, Stn. 2	16/01/2007	<i>Choricystis</i> 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).

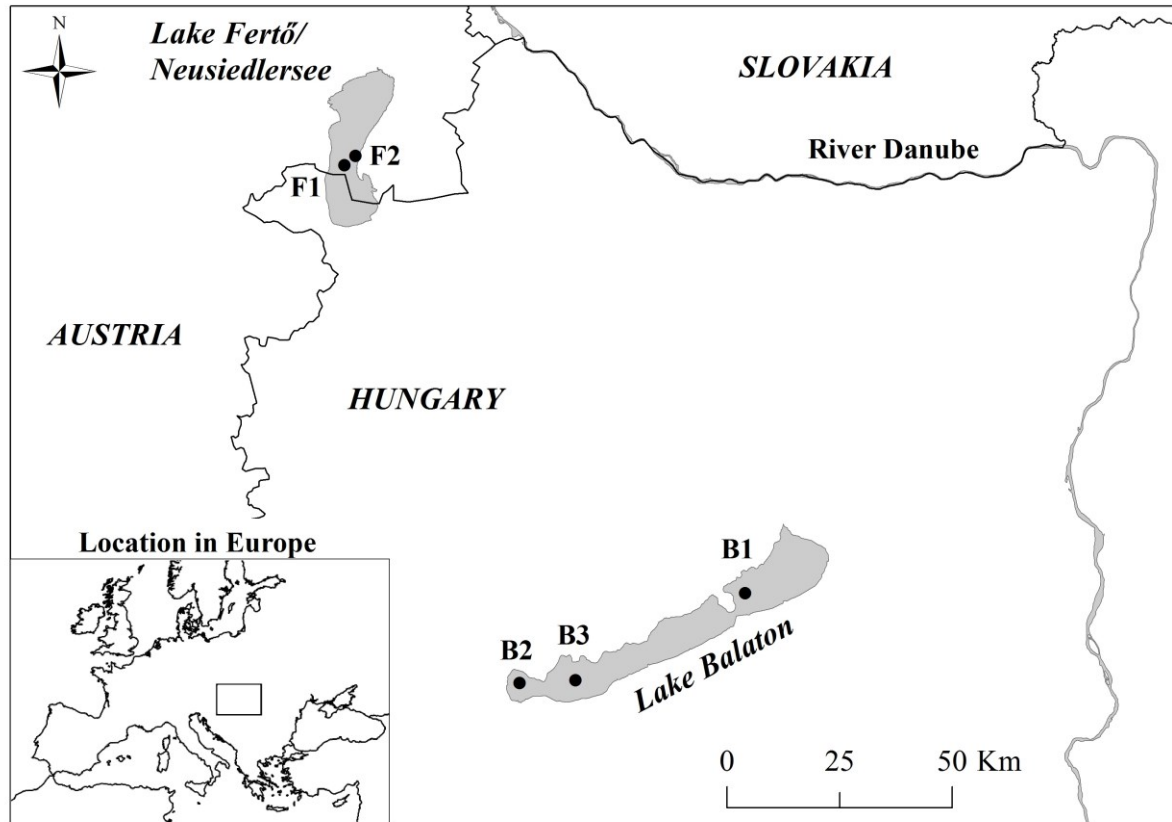


Fig. 2 Contribution of autotrophic picoplankton (APP) to total phytoplankton biomass (A) and total planktonic primary production (B) in shallow Central European great lakes (LB – Lake Balaton, LF – Lake Fertő/Neusiedlersee; Stn. 1 – Station 1, Stn. 2 – Station 2) in winter 2009-2014. The plotted rectangles 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.

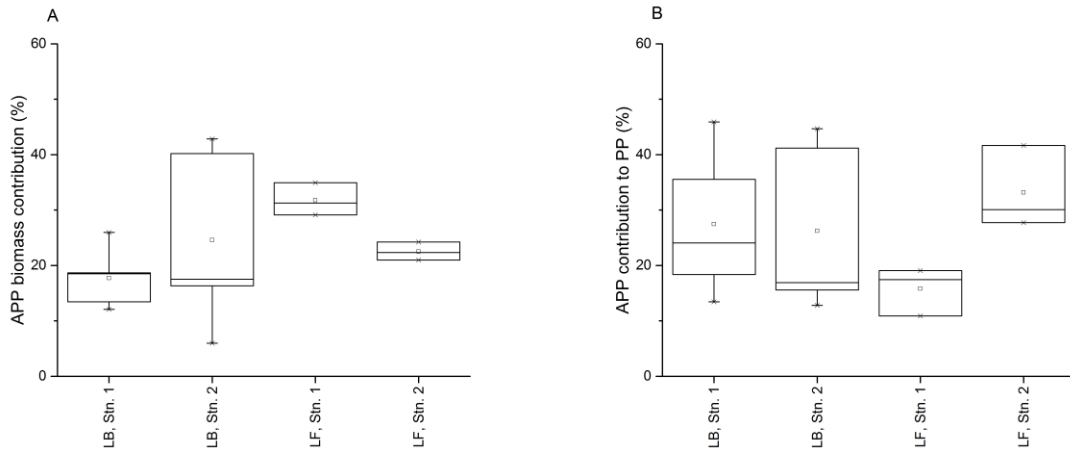


Fig. 3 P-I parameters [A: biomass-specific maximum photosynthetic rate (P_{max}^B), B: light saturation parameter (I_k), C: biomass-specific light utilization parameter (α^B) and D: optimal light intensity (I_{opt})] of the different size fractions (APP – autotrophic picoplankton, NP – nano+microplankton) 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).

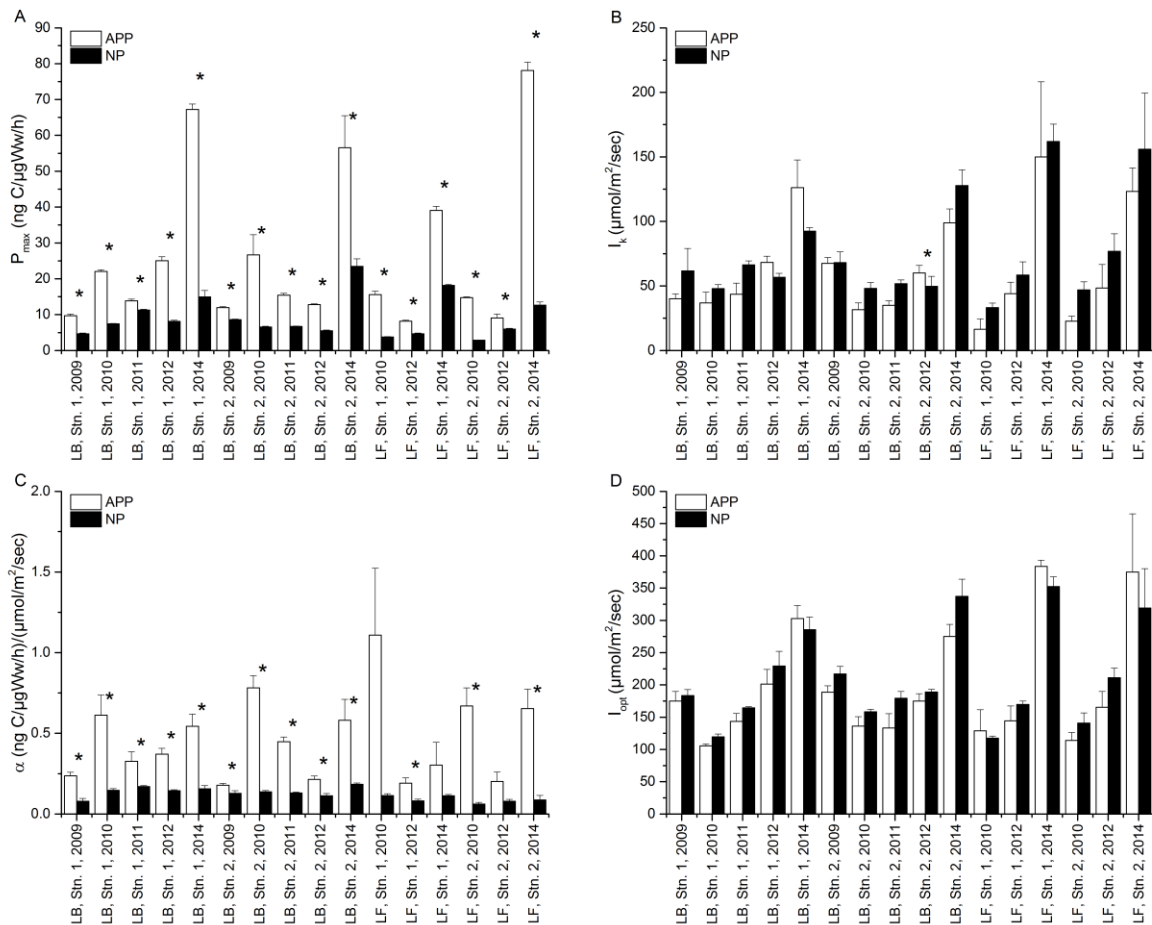


Fig. 4 Biomass-specific maximum photosynthetic rate (P_{max}^B) as a function of water temperature (A and B), and the light saturation parameter (I_k) as a function of mean water column irradiance (C and D) of the different size fractions (APP – autotrophic picoplankton, NP – nano+microplankton) in the 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).

