Aquat Ecol (2007) 41:511–523
DOI 10.1007/s10452-007-9125-z

Production and biomass of picophytoplankton and larger autotrophs in Andean ultraoligotrophic lakes: differences in light harvesting efficiency in deep layers

Cristiana Callieri · Beatriz Modenutti · Claudia Queimaliños · Roberto Bertoni · Esteban Balseiro

Published online: 10 July 2007

Abstract We measured biomass and primary production of picophytoplankton (PicoPhy: 0.2–2 μm) and of autotrophic size fraction >2 μm in six deep ultraoligotrophic lakes in the Andean-Patagonian region (around 41°S) during summer stratification. Surface Photosynthetically Active Radiation (PAR) ranged from 1277 to 1849 μmol photons m^{-2} s^{-1}, and the euphotic zone, generally deeper than the mixed layer, varied between 28 m and 49 m. We found a strong photoinhibiting effect of high PAR and UV-A at surface levels, whereas UV-B radiation (<320 nm) had low extra contribution in the photosynthesis inhibition. As a consequence, cell numbers, Chl *a* and primary production rates of both fractions increased towards deep layers in all lakes. The photosynthetic efficiency (Chl-specific production per photon unit) of both fractions increased with depth, although this increase was higher in PicoPhy, indicating a higher fitness to low-light. The per cent contribution of PicoPhy production to total production, showed an inverse significant relation with total dissolved phosphorus (TDP). Moreover our data fitted the existing database showing a significant trend towards a decrease of PicoPhy biomass and an increase of its relative contribution to total biomass with decreasing trophic state. At very low-phosphorus concentration, typical of north Patagonian lakes, we found good evidence of the competitive advantage of PicoPhy. Low-light and low TDP may interact to create the most favourable conditions for the smaller photosynthetic organisms. In conclusion, we found that at low-light and very low nutrient regime PicoPhy achieves higher photosynthetic efficiency than the larger autotrophic organisms.

Keywords Deep chlorophyll maxima · Picocyanobacteria · Patagonian lakes · Photosynthetic efficiency · Primary production

C. Callieri (✉) · R. Bertoni
CNR – Institute of Ecosystem Study, Largo Tonolli 50, Verbania Pallanza 28922, Italy
e-mail: c.callieri@ise.cnr.it

B. Modenutti · C. Queimaliños · E. Balseiro
CONICET – UNC Laboratory of Limnology, Universidad Nacional del Comahue, Bariloche, Argentina

Introduction

Picophytoplankton (PicoPhy: 0.2–2 μm) has been recognized as an important component within the pelagic communities in both, freshwater (Stockner 1991; Weisse 1993; Stockner et al. 2000) and marine environments (Li 1995; Partensky et al. 1996; Agawin et al. 2000). The absolute and relative importance of the different sizes of phytoplankton has been explained by environmental variables such as nutrients (Stockner 1991; Bell and Kalff 2001; Drakare et al. 2003), temperature and water column stability (Weisse and Kenter 1991; Padisák et al. 1997; Callieri and Piscia 2002; Camacho et al. 2003).

Compared with larger-sized phytoplankton, PicoPhy do best in resource-poor habitats, a phenomenon that has been linked to the greater surface area to volume ratio of small organisms promoting efficient uptake of nutrients, which gives them a competitive advantage over larger organisms when nutrients are scarce (Friebele et al. 1978; Raven 1986). An increase in relative importance of PicoPhy with the decrease of phosphorus concentration has been observed in marine and freshwater environments (Stockner 1991; Bell and Kalff 2001). In addition, picophytoplankton seems to be pre-adapted to low levels of Photosynthetically Active Radiation (PAR) (Callieri et al. 1996; Gervais et al. 1997), and through experimental manipulation, Wehr (1993) suggested that PicoPhy is a superior competitor under low-phosphorus and low-light conditions.

In the Andean-Patagonian region (around 41°S), many lakes are ultraoligotrophic (Morris et al. 1995; Markert et al. 1997) and could, therefore, be important picophytoplankton environments. Furthermore, in these lakes the concentration of dissolved organic carbon (DOC) are very low and consequently the light penetrates to deeper layers (Morris et al. 1995). Previous contributions have indicated a strong photosynthesis inhibition of phytoplankton caused by both UV-R (290–400 nm) and PAR (400–700 nm) (Helbling et al. 2001; Modenutti et al. 2004; Villafañe et al. 2004) that influences the vertical profile of the organism abundance. Due to the strong irradiance inhibition, phototrophic organisms segregate to the deep layers and develop a deep chlorophyll maximum (DCM) (Pérez et al. 2002; Modenutti et al. 2004). In these ultraoligotrophic lakes DCM forms below the thermocline and is caused by population of mixotrophic ciliates (*Ophrydium naumanni*), nanoflagellates, dinoflagellates (*Gymnodinium paradoxum*) and PicoPhy (Queimaliños et al. 1999; Modenutti and Balseiro 2002). During summer, the depths of 1% and 10% surface PAR receive on average 13 and 150 µmol photons m^{-2} s^{-1}, respectively (Pérez et al. 2002). This range of photon flux can be optimal for PicoPhy, which are considered as shade tolerant (Stockner et al. 2000), as well as for low-light adapted phytoplankton (Pan et al. 1996; Regel et al. 2004). The result might be a niche overlap of autotrophic organisms of different sizes: the winner being determined by the ability to overcome the light and nutrient-limitation.

In this study, we selected six Andean ultraoligotrophic lakes with low-phosphorus concentrations, where high PAR and UVR irradiances forced planktonic organisms towards the deeper layers. We tested, as working hypothesis, that PicoPhy, under low-light and low-phosphorus conditions, might be a superior competitor of larger phytoplankton. We performed in-lake experiments comparing the best features for photosynthetic production and efficiency of two autotrophic size fractions: PicoPhy and >2 µm.

Material and methods

Study area

This study was carried out in six Andean lakes located between 41°S and 71°W, and included in the Nahuel Huapi National Park, Patagonia, Argentina (Fig. 1). The selected lakes (Lakes Moreno, Gutiérrez, Mascardi, Nahuel Huapi, Correntoso and Espejo) are large (Area, >5 km^2) and deep ($Z_{max,}$ >90 m) (Table 1). The

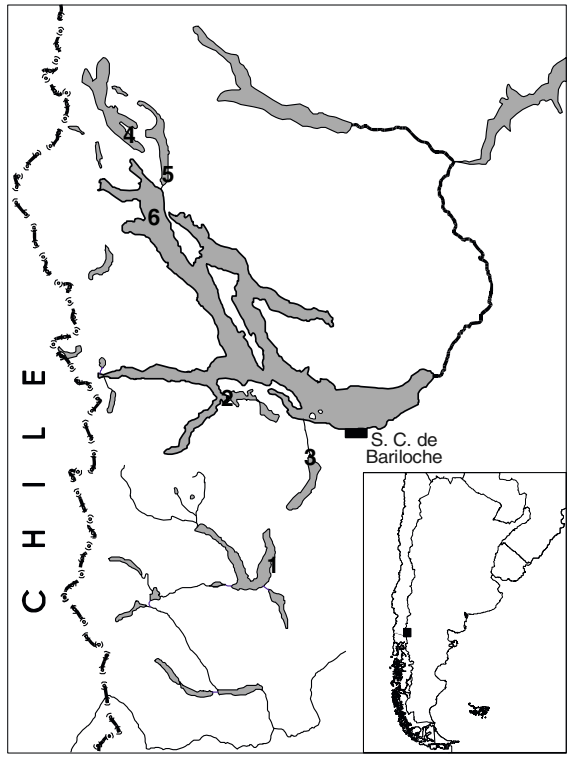

Fig. 1 Geographical location of the six studied lakes. 1: Lake Mascardi, 2: Lake Moreno, 3: Lake Gutiérrez, 4: Lake Espejo, 5: Lake Correntoso and 6: Lake Nahuel Huapi

Table 1 Location and morphometry of Patagonian lakes during January 2005

Lake	Location	Area (km^2)	Z_{max} (m)	$Z_{1\%}$ 305 nm (m)	$Z_{1\%}$ 340 nm (m)	$Z_{1\%}$ PAR (m)	Z_{therm}	K_d PAR (m^{-1})	Mean ± s.d. TDP ($\mu g\ l^{-1}$)
Mascardi	41°20′S 71°34′W	39	218	6.2	9.2	27.9	11.3	0.165	1.88 ± 0.30
Moreno	41°05′S 71°32′W	5.2	90	6	9.2	32.8	19.3	0.14	2.27 ± 0.64
Gutiérrez	41°15′S 71°33′W	16.4	111	8.3	13.4	37.4	22	0.127	1.52 ± 0.31
Espejo	40°40′S 71°41′W	30	245	9.2	14.46	41.4	15	0.111	1.65 ± 0.59
Correntoso	40°44′S 71°39′W	19.5	>100	9.7	14.9	43.4	13.3	0.106	1.46 ± 0.35
Nahuel Huapi	40°47′S 71°39′W	557	464	10.8	17.9	48.8	$>Z_{1\%}$	0.094	1.75 ± 0.60

References: Z_{max} = maximum depth; $Z_{1\%}$ = depth of 1% of surface irradiance; Z_{therm} = thermocline depth; K_d PAR = extinction coefficient of photosynthetically active radiation; TDP = total dissolved phosphorus

climate is temperate cool with an annual precipitation of 1500 mm and a mean annual temperature of 8.7 ± 4.5°C (Paruelo et al. 1998). The surrounding vegetation is constituted by a mixed forest of *Nothofagus dombeyi* (Mirb.) Blume and *Austrocedrus chilensis* (D.Don) Florin et Boutleje.

The lakes exhibit a warm monomictic thermal regime, being thermally stratified from late spring to summer (Quirós and Drago 1985). The lakes are ultraoligotrophic, with low dissolved carbon and corresponding high PAR and UVR transparency (Morris et al. 1995).

Sampling and data collection

The six lakes were sampled during the warm season (January 2005). Vertical profiles (0–60 m) of temperature, UV bands (305, 320, 340 and 380 nm), Photosynthetically Active Radiation (PAR, 400–700 nm) and in situ chlorophyll *a* distribution on the basis of the natural fluorescence, were measured with a PUV 500B submersible radiometer (Biospherical Instruments). Samples for determinations of nutrient concentration, chlorophyll *a* and abundance of PicoPhy and autotrophic fraction >2 μm were obtained at ca. 100, 50, 25, 10, and 1% of surface PAR in a sampling point located at the deepest part of each basin. All samplings were carried out in duplicates, at mid-day, 1 h before astronomic noon.

Chlorophyll *a* concentration (Chl *a*) was determined for the entire phytoplanktonic community as well as for PicoPhy. For the entire phytoplanktonic community 100 ml of the samples were filtered on Whatman GF/F filters. For PicoPhy, up to 250 ml of sampled water were filtered through a 2.0 μm pore size polycarbonate filter (Nuclepore) and subsequently, the filtrate was filtered through a 0.2-μm pore size polycarbonate filter (Nuclepore) to concentrate PicoPhy (Callieri and Piscia 2002). The filters were processed, chlorophyll *a* was extracted with hot ethanol (Nusch 1980) and measured with a 10–AU fluorometer (Turner Design).

Total dissolved phosphorus (TDP) was determined on 150 ml of filtered (GF/F) lake water. The samples were digested with potassium persulphate at 125°C at 1.5 atm for 1 h. The phosphorus concentrations were obtained through the ascorbate-reduced molybdenum method (APHA 1992). Total Inorganic Carbon (TIC) was estimated from pH and alkalinity (using Gran titration) after correction by temperature and ionic strength.

Samples for enumeration of autotrophic picoplankton were fixed with 20% formaldehyde (0.2 μm filtered) buffered with sodium cacodylate 0.1 M (final concentration 2% vol/vol), stored in darkness at 4°C and processed within 2 weeks (Callieri and Stockner 2002). They were counted on black polycarbonate filters (Poretics, 0.2 μm pore size) by autofluorescence

of phycoerythrin (Zeiss Axioplan microscope equipped with an HBO 100 W lamp, a Neofluar 100× objective, 1.25× additional magnification, and filter sets for blue and green light excitation, Zeiss filter set 09: BP450–490, FT510, LP520, Zeiss filter set 14: LP510–KP560, FT580, LP590). Cells were measured using an image analysis system (Image ProPlus; Media Cybernetics, Silver Spring, MD, USA). Cell volumes were transformed to carbon using a conversion factor of 200 fg C μm^{-3} (Weisse 1993).

A volume of 250 ml of the lake water at each depth was fixed with acid Lugol solution for enumeration of the autotrophic fraction >2 µm. Phytoplankton and mixotrophic ciliate were quantified in 50 ml chambers with an inverted microscope following the Utermöhl Technique (Utermöhl 1958). Number of endosymbiotic *Chlorella* inside ciliates was estimated according to Queimaliños et al. (1999). Biovolume of the autotrophic fraction >2 µm was based on measurements and calculations applying Sun and Liu (2003) geometric models. Cellular carbon content was estimated according to Menden-Deuer and Lessard (2000).

Primary production measurements

Primary production (PP) was measured in each lake using the ^{14}C technique (Steeman-Nielsen 1952). Dark bottle measurements were substituted by the "time 0" organic ^{14}C measurement by adding the isotope to the dark bottle and immediately filtering and analyzing (Fahnenstiel et al. 1994). Lake water was sampled at different depth with varying PAR (100, 50, 25, 10, and 1% of surface PAR) before starting the incubations. Immediately, 18 quartz tubes (volume, 14 ml each) were filled with lake water. To each tube, 2.22 kBq $NaH^{14}CO_3$ ml^{-1} (Amersham) was added and then incubated in situ for 4 h symmetrically around noon. Incubations were carried out with the tubes held horizontally in a frame at different levels of the euphotic zone, coinciding with the sampling depths (ca.100, 50, 25, 10, and 1% of surface PAR). The upper level incubation (100% of PAR) was run in two treatments, one exposed to full sunlight (quartz tubes) and the other only to PAR + UV-A (quartz tubes wrapped with Mylar D™ foil with a cutoff at 320 nm). At each treatment depth, the tubes were placed in three replicates each.

After incubation, 500 µl aliquots were taken from each tube to check total radioactivity measuring the dpm ml^{-1}. In order to estimate the size fractionated primary production (>2 µm and <2 µm) the samples were filtered using disposable plastic syringes and 25-mm plastic filter holders. Polycarbonate filters (Osmonics™) with a mesh size of 2 µm (diameter: 25 mm) were used for the autotrophic fraction >2 µm. The filtrate was concentrated on 0.22-µm pore size nitrocellulose membranes (Millipore™). Filters were acidified with 200 µl 1 N HCl for 60 min in 20 ml-scintillation vials. After adding 10 ml of scintillation liquid (Filter Count, Beckman) the vials were counted in a Beckman LS6000 Scintillation Counter, which automatically corrects for the quenching and gives the dpm values. Photosynthetic carbon assimilation was calculated based on the proportion between ^{14}C uptake and TIC availability (Steeman-Nielsen 1952).

For the calculation of the primary productivity at each depth, the following formula is used (Danish Standard Association 1982, modified, see Callieri and Stockner 2002):

$$PP = \left[LB - \left(o.r. \times \frac{TA_{LB}}{TA_{o.r.}}\right)\right] \times totCi \times 1.05 \times 1.06 \times \frac{1000}{TA_{LB} \times \Delta t}$$

where: PP = primary productivity (mg C m^{-3} h^{-1}), LB = activity in the light bottle (d.p.m. ml^{-1}), $o.r.$ = activity of the organic residual in the dark bottle (d.p.m. ml^{-1}), $totCi$ = total inorganic carbon (mg l^{-1}), 1.05 = correction factor for slower assimilation of ^{14}C with respect to ^{12}C, 1.06 = correction factor for respiration of assimilated ^{14}C, TA_{LB} = total activity of the LB (d.p.m. ml^{-1}), $TA_{o.r}$ = total activity of the organic residual in the dark bottle (d.p.m. ml^{-1}) and Δt = incubation time (in hours).

Data analysis

Chlorophyll profiles were calculated based on the PUV 500B Chl *a* profiles and the <2 and >2 µm Chl *a* laboratory measurements with extraction.

Differences between surface samples incubated using Quartz and Quartz wrapped with Mylar were tested with *t*-test. Normality and homoscedasticity were checked before analysis. Multiple correlation analysis was carried out with nutrient and light (TDP

concentration and PAR) as independent variables and primary production of <2 μm or >2 μm autotrophic fractions as dependent ones. All statistical analyses were performed with SigmaStat 3.01.

Finally, we included our data on the extensive freshwater database of Vörös et al. (1998) and recalculated the regressions between Chl *a* and PicoPhy abundance, biomass and percent contribution of PicoPhy to total biomass.

Results

During our summer study (January 2005) the euphotic zone of the lakes were large, varying between 28 m and 49 m depth (Table 1). Five lakes (Moreno, Gutiérrez, Mascardi, Correntoso and Espejo) exhibited a thermal stratification with thermocline depth varying from 11 m to 22 m (Table 1). The depth of the euphotic zone in these lakes was deeper than the mixed layer (Fig. 2). Illumination extended up to the upper part of the hypolimnion (Fig. 2). Epiliminion of Lake Espejo was not thermally homogenous with a slight decrease from surface to thermocline depth. In the largest lake, Nahuel Huapi, thermocline extended to a depth of $>Z_{1\%}$: therefore, the whole sampled water column corresponded to the epilimnion. During the study, surface PAR irradiance ranged from 1277 to 1849 μmol photons m^{-2} s^{-1} and the diffuse attenuation coefficients (K_d) were very low in all lakes, with lakes Nahuel Huapi ($K_d = 0.094$ m^{-1}) and Mascardi ($K_d = 0.165$ m^{-1}) representing the extremes of the transparency gradient (Table 1). Lake Mascardi receives glacial clays from a glaciar located in the Tronador Mountain and this caused its lower transparency (Modenutti et al. 2000).

Concentrations of phytoplankton (expressed as Chl *a* values) and TDP were always low, typical of ultraoligotrophic lakes, in a decreasing gradient from Lake Moreno to Lake Correntoso (Table 1, Fig. 2). TDP concentration did not vary much along the water column (see s.d. in Table 1) and amongst lakes (Moreno: 2.27 μg l^{-1}, Correntoso 1.46 μg l^{-1}). Chlorophyll *a* showed an increase towards the deeper layers in all lakes and a more or less pronounced deep chlorophyll maxima (DCM) were situated below the thermocline in almost all lakes (Fig. 2). Both phytoplankton fractions (<2 μm and >2 μm) exhibited the same pattern of increase in Chl *a* towards the lower boundary of the euphotic zone where there was always <2% of surface PAR (Fig. 2). The range of Chl *a* concentrations was 0.03–1.88 μg l^{-1} for the PicoPhy and 0.08–2.13 μg l^{-1} for the >2 μm fraction.

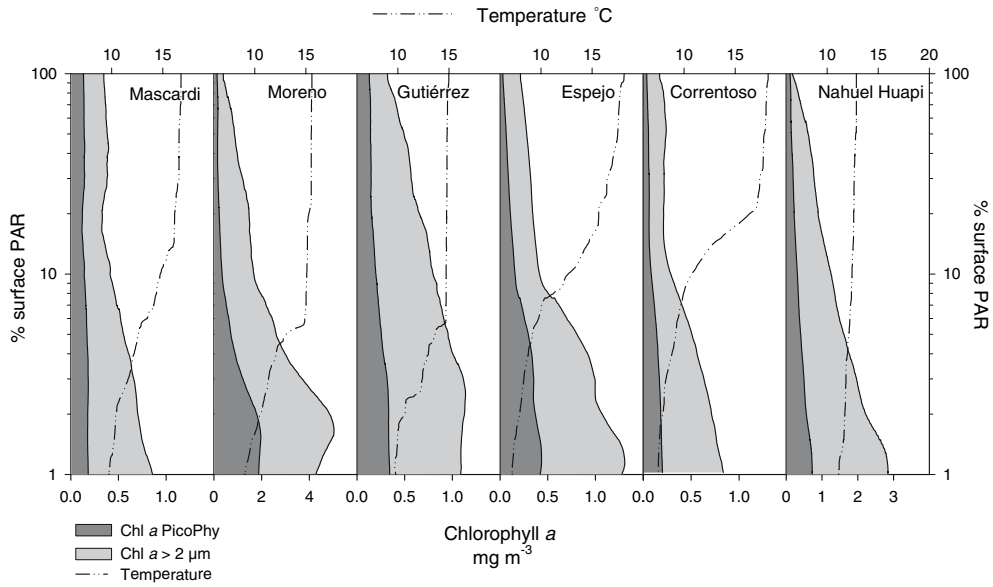

Fig. 2 Vertical profiles of temperature and chlorophyll *a* of picophytoplankton (PicoPhy) and >2 μm fractions in the six lakes within the euphotic zone (100–1% of surface PAR)

The contribution of PicoPhy to total Chl *a* concentration in phytoplankton varied from 13% to 53% (mean 28%). In Lakes Mascardi, Correntoso and Nahuel Huapi PicoPhy Chl *a* was lower and quite homogenously distributed along the depth profile (Fig. 2). In surface water, Chl *a* concentration of both fractions was very low (Fig. 2), and PicoPhy Chl *a*, although low, contributed more than 30% (in Nahuel Huapi even 53%) except in Lake Espejo and Correntoso. The Chl *a* concentration of the >2 μm fraction increased towards the base of the euphotic zone so that the percentage of PicoPhy contribution to Chl *a* was lower. In addition we observed an exponential relationship between Chl *a* and cell number in the > 2 μm fraction ($r^2 = 0.483$, $P < 0.001$).

PicoPhy in Lakes Moreno, Gutiérrez, Mascardi, Correntoso and Espejo was composed exclusively of small prokaryotic cells (*Synechococcus* spp.; mean biovolume: 0.31 ± 0.1 μm^3 per cell). In Lake Nahuel Huapi, eukaryotic picoplanktonic cells (ovoid-shaped cells, mean biovolume: 0.46 ± 0.07 μm^3) were also recorded. However these cells were low in numbers, representing only 3–6% of total PicoPhy cells. The larger autotrophic fraction (>2 μm) included nanophytoplankton (2–20 μm), net phytoplankton (>20 μm) and endosymbiotic *Chlorella* inside mixotrophic ciliates (*Ophrydium naumanni* and *Stentor araucanus*). Nanophytoplankton was dominated by nanoflagellates (*Chrysochromulina parva* and *Rhodomonas lacustris*) whilst the fraction >20 μm was dominated by dinoflagellates, mainly *Gymnodinium paradoxum*. At the DCM level, Lake Gutiérrez presented the lower C content because of the dominance of smaller cells (2–20 μm), whereas in Lake Moreno the highest C content was due to the higher contribution of the autotrophic fraction >20 μm (Table 2).

PicoPhy production ranged from 0.04 mg to 0.48 mg C m^{-3} h^{-1} with very low values at the subsurface level and with higher values at depths with 150–350 μmol photons m^{-2} s^{-1} (data not shown). A similar pattern was observed for the >2 μm fraction with production rates in the range 0.01–0.56 mg C m^{-3} h^{-1}. Except for Lake Moreno, where PicoPhy production comprised 26% of the total production, for all the other lakes the production of PicoPhy was around 50% of the total, as average in the water column (Table 2).

Chlorophyll-specific Primary Production (PP_{chl}) was observed to be lower in surface water layers, probably caused by a strong photoinhibition of the two autotrophic fractions (Fig. 3). PP_{chl} of PicoPhy was higher in all lakes, compared with that of the >2 μm fraction. At incubation depths 50, 25, and 10% of surface PAR, PP_{chl} of PicoPhy increased substantially, whereas at DCM level (around 1% of surface PAR) PP_{chl} of both fractions decreased. The >2 μm fraction varied less along the water column than the fraction comprising picoplankton.

The photosynthetic efficiency (mg C × (mg Chl *a*)$^{-1}$/mol photons m^{-2}) gradually increased in all the lakes (except Lake Moreno, Fig. 4) from the surface layers to the 1% surface PAR, for both fractions. The number of cells of PicoPhy had a similar pattern of vertical distribution, reaching the highest abundance at the deepest part of the euphotic layer.

The UV-B effect on the primary production of the two size fractions, was evaluated by using

Table 2 Cell abundance, biomass and size of PicoPhy and of the >2 μm autotroph fraction, at the DCM. Daily production (PPD) of the two fractions in the euphotic zone, and the percent of PicoPhy production on total autotrophic production (% PicoPhy)

Lake	Abundance (cell ml^{-1})		Size (μm^3)		Biomass (μg C l^{-1})		PPD (mg C m^{-2} d^{-1})		PicoPhy %
	PicoPhy	>2 μm	PicoPhy	>2 μm	PicoPhy	>2 μm	PicoPhy	>2 μm	
Mascardi	136346	480	0.29	402	7.88	23.7	71.9	61.3	54
Moreno	81429	972	0.26	645	4.28	87.3	44.7	128.3	26
Gutiérrez	94306	853	0.47	74	8.91	8.9	95.2	98.2	49
Espejo	65260	1670	0.38	104	4.92	22.7	49.3	47.9	51
Correntoso	134528	1213	0.26	245	7.08	39.1	54.1	33.0	62
Nahuel Huapi	69753	884	0.20	420	2.93	46.8	89.7	94.5	49

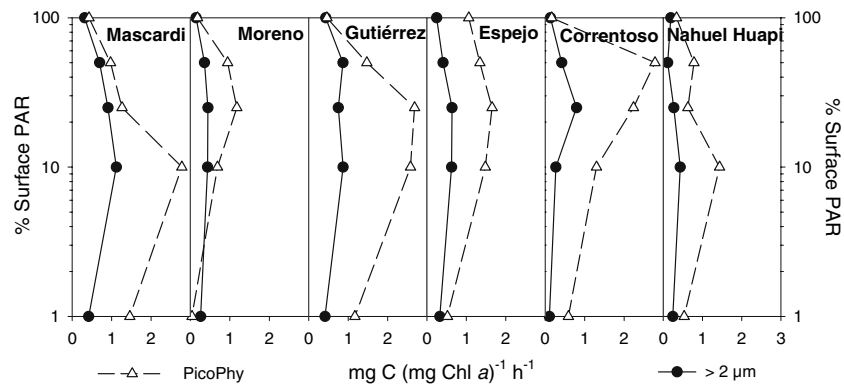

Fig. 3 Vertical profiles of the Chlorophyll *a* specific primary production (PP_{Chl}) of picophytoplankton (PicoPhy) and >2 µm fractions in the six lakes within the euphotic zone (100–1% of surface PAR)

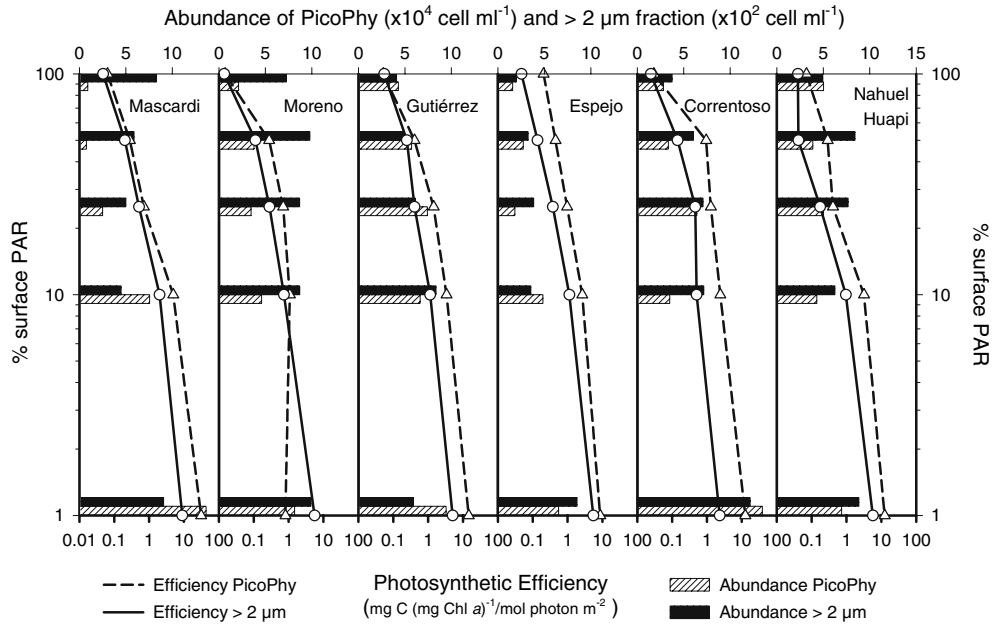

Fig. 4 Vertical profiles of photosynthetic efficiency and cell abundances of picophytoplankton (PicoPhy) and >2 µm fractions in the six lakes within the euphotic zone (100–1% of surface PAR)

Quartz (Q = PAR + UV-A + UV-B) and Mylar (M = PAR + UV-A) protected quartz tubes incubated at 100% of surface PAR. We compared the production measured at the two treatment conditions (Quartz and Mylar) with the production at 10% of surface PAR ($PAR_{10\%}$) (from 15 m to 25 m, with ~150 µmol photons m^{-2} s^{-1}) (Table 3). At this depth there is <1% of UV-B and UV-A (305, 320, 340 nm). A Pairwise Multiple Comparison (Dunn's method) showed a statistically significant difference ($P < 0.05$) amongst the production at $PAR_{10\%}$ and the other treatments, but no difference between Quartz and Mylar for both the fractions, indicating a low contribution of UV-B radiation (<320 nm) in the photosynthesis inhibition and a photoinhibiting effect of high PAR and UV-A.

Multiple linear regression analysis using pooled data of all lakes of the production of the two fractions vs. PAR and TDP showed different results depending on the fraction considered (Fig. 5): a significant inverse correlation of PicoPhy to PAR ($P < 0.001$) but not to TDP ($P = 0.425$) was observed, whereas the >2 µm fraction was directly correlated to TDP ($P = 0.004$) but inversely to PAR ($P = 0.001$).

With the same data set but excluding the surface samples (strongly photoinhibited), the per cent

Table 3 Primary production (mg C $m^{-3}h^{-1}$) of the two autotrophic size fractions (PicoPhy and >2 μm) incubated at different depths: 100% and 10% of surface PAR in quartz tubes and in quartz tubes covered with Mylar D film

Lake	PicoPhy						>2 μm					
	Quartz 100% PAR		Mylar 100% PAR		Quartz 10% PAR		Quartz 100% PAR		Mylar 100% PAR		Quartz 10% PAR	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
Mascardi	0.055	0.004	0.075	0.018	0.354	0.085	0.064	0.007	0.097	0.020	0.280	0.011
Moreno	0.010	0.009	0.029	0.011	0.182	0.072	0.028	0.020	0.061	0.022	0.464	0.119
Gutiérrez	0.039	0.007	0.032	0.011	0.293	0.047	0.064	0.020	0.048	0.034	0.307	0.065
Espejo	0.036	0.002	0.049	0.031	0.179	0.054	0.051	0.009	0.055	0.074	0.121	0.022
Correntoso	0.007	0.007	0.004	0.002	0.157	0.051	0.024	0.016	0.013	0.022	0.078	0.036
Nahuel Huapi	0.031	0.004	0.048	0.018	0.326	0.085	0.016	0.007	0.018	0.020	0.299	0.011

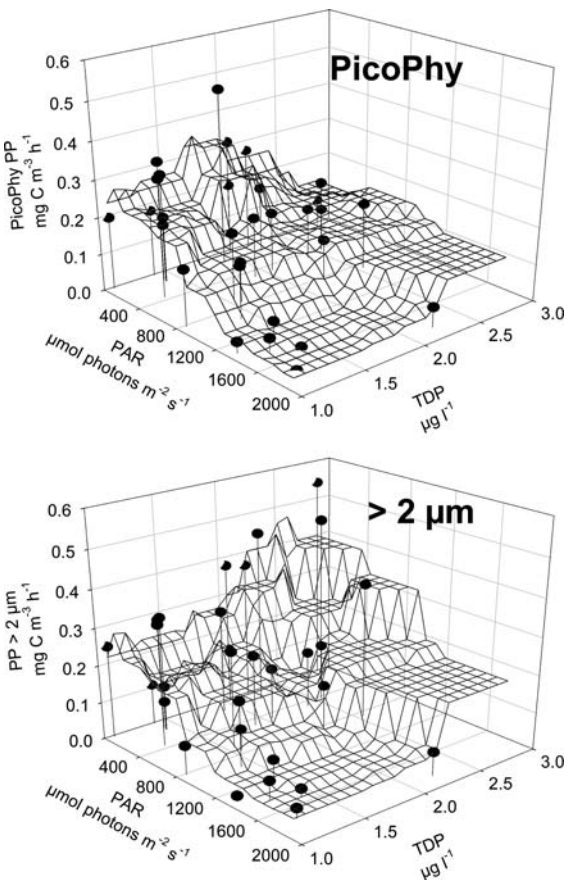

Fig. 5 Primary production of picophytoplankton (PicoPhy, upper panel) and >2 μm fraction (lower panel) in relation with total dissolved phosphorus and irradiance. The black dots are the experimental points

contribution of picophytoplankton production to total autotrophic production, showed an inverse significant relationship with TDP concentrations (r^2 = 0.437, $P < 0.001$, d.f. = 22) (Fig. 6).

The inclusion of Andean-Patagonian lakes in the extended database of Vörös et al. (1998) fitted very well in the three observed trends of North Hemisphere lakes: an increase of PicoPhy cell number and biomass (Chl *a*) and a decrease of its relative contribution to total biomass as trophic state increases. However, our data fell in the oligotrophic left extreme of the dataset (Fig. 7, empty symbols). A remarkable fit of the data was found showing a significant trend towards a decrease in abundance

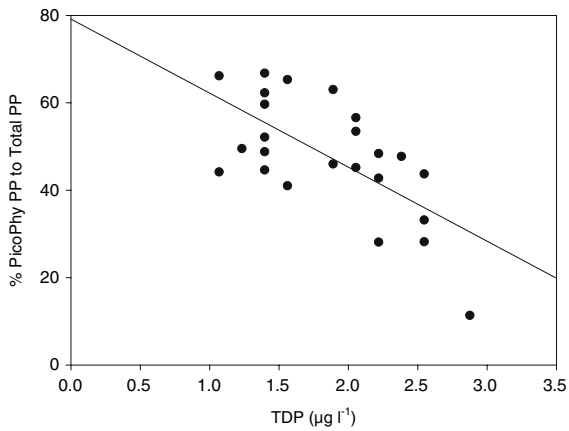

Fig. 6 Percent of contribution of PicoPhy PP to total PP versus total dissolved phosphorus (TDP). Surface data were excluded due to high photoinhibition

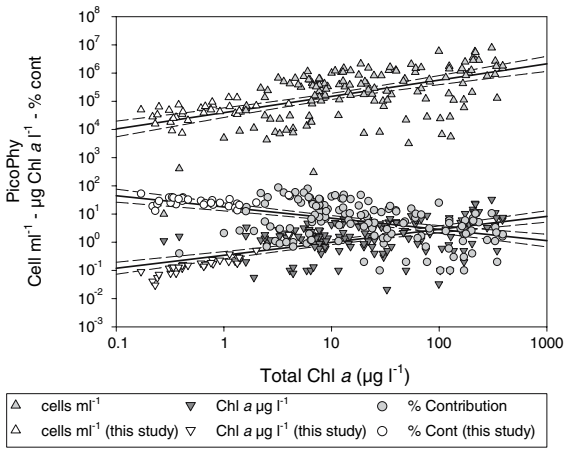

Fig. 7 Relationship between total autotrophic biomass (µg Chl *a* l^{-1}) and picophytoplankton abundance, biomass and its percent contribution to total autotrophic biomass. (From: Vörös et al. 1998, modified). The empty symbols correspond to data from this study

(r^2 = 0.327, n = 172, P < 0.0001) and biomass (r^2 = 0.350, n = 170, P < 0.0001) of PicoPhy with the decrease in trophic state, whereas the percent PicoPhy contribution to total biomass showed a significant increase (r^2 = 0.256, n = 167, P < 0.0001).

Discussion

The Patagonian ultraoligotrophic lakes are characterized by extremely high transparency of their water column (K_d = 0.1–0.2), which creates a thick layer where PAR and UV radiation photoinhibit the organisms living in this layer or transported to it by mixing. The inhibitory effect of UVR on primary production in a wide range of freshwater planktonic environments has been largely documented (e.g. Karentz et al. 1994; Neale et al. 2001). Particularly in ultraoligotrophic, clearwater lakes with very low extinction coefficients, as North Patagonian lakes, a large portion of the epilimnion is under the influence of high PAR and UVR. The effect of high PAR and UVR on two representatives of the >2-µm autotrophic fraction (the mixotroph *Ophrydium naumanni* and the autotroph *Gymnodinium paradoxum*) is to reduce primary production in the upper layers (Modenutti et al. 2004), but not in the pigmented mixotrophic ciliate *Stentor araucanus* (Modenutti et al. 2005).

Here we did not measure the photoinhibition of individual organisms of the >2 µm fraction but the primary production of the assemblages > and <2 µm. Picocyanobacteria dominated the <2 µm fraction, as was usually observed in other lakes (Callieri and Pinolini 1995; Lavallée and Pick 2002; Camacho et al. 2003) whereas the larger fraction (>2 µm) included different organisms forming a more heterogeneous assemblage.

We observed a photoinhibiting effect by PAR and UV-A on both the fractions and a little additional inhibition by UV-B. Similar results have been obtained in other lakes (Moeller 1994; Villafañe et al. 1999), and in particular, in a high-mountain lake a stronger effect of UV-A than UV-B on photosynthesis was found, both with in situ measurements and with the Biological Weighting Functions (Neale et al. 2001; Callieri et al. 2001). However one should be cautious to draw conclusions as static incubations, i.e. at fixed depths, of water samples for measuring primary production may fail to predict the performance of vertically moving individuals and may offer a distorted picture of the effects of UV in nature (Zagarese et al. 1998; Modenutti et al. 2005; Bertoni and Balseiro 2005).

For both the fractions, primary production per unit Chlorophyll *a* is higher at intermediate depths (25 and 10% of surface PAR) where cells seem to be in the best conditions of production relative to their chlorophyll *a* content. Therefore, if we do not consider the photon flux density arriving to the cells or the water mixing effect, the best conditions for photosynthesis are at intermediate depths. The organisms below the thermocline receive low irradiance, though sufficient for positive PP and with an efficient photon flux harvesting (Figs. 3 and 4).

The development of a deep chlorophyll maximum (DCM) observed in the clear North Patagonian lakes during summer stratification (Pérez et al. 2002) has been explained as an exploitation of a favourable niche (Modenutti et al. 2004). We focussed our attention on the differences in the performance of photosynthesis of the fractions > and <2 µm. We also compared the relationships amongst PAR, TDP and PP of PicoPhy and of larger heterogeneous photosynthetic assemblage. We found that the increase in the cell numbers of autotrophs in the two fractions at DCM level was coupled to the increase in their respective photosynthetic efficiencies. This has been also found in other lakes of the Northern Hemisphere with different community

structure (Fahnenstiel et al. 1991; Frenette et al. 1996; Callieri and Piscia 2002).

In >2 μm eukaryotic cells, an increment of the Chl *a* per cell has been described as an acclimation response to dim light (Berner et al. 1989). The optimization of the photosynthesis at low-light intensity is, therefore, the result of the increase of the photosynthetic unit (PSU) size or change in the PSU numbers (Falkowski and Raven 1997). We observed this photoacclimation in all lakes, indicating that cells >2 μm found in these deep metalimnetic or hypolimnetic layers are pre-adapted to the low-light intensities. In addition, in these deeper layers, these cells are isolated from the epilimnion where they would be exposed to higher irradiances in a turbulent gradient.

The increase in both abundance and photosynthetic efficiency of PicoPhy at 1% of surface irradiance has been observed in other aquatic ecosystems and has been attributed to other factors, including the presence of phycobiliproteins, thermocline stability, increase of PSU amongst others (Pick and Agbeti 1991; Padisák et al. 1997; Malinsky-Rushansky et al. 2002; Camacho et al. 2003).

Picocyanobacteria have been reported to perform well under low, green light conditions because of the presence of phycoerythrin (Hauschilds et al. 1991; Callieri et al. 1996). In this sense, the phycoerythrin-rich *Synechococcus* cells tend to saturate photosynthesis and growth rate at very low irradiances (Stockner and Antia 1986). This explains the increase in relative abundance of these organisms compared with other phytoplankton at DCM level of the deep ultraoligotrophic Patagonian lakes (Fig. 4), where the favourable climate for blue-green light is prevailing (Pérez et al. 2002). Malinsky-Rushansky et al. (2002) observed an increase in Chl *a* per cell in PicoPhy (one picoeukaryot and two *Synechococcus* strains) under low-light treatments. We did not, however, observe a significant increase of Chl *a* per cell in PicoPhy, but we did observe an increase in photosynthetic efficiency with depth. Although we did not measure accessory pigments, we suggest that this increase in efficiency may be associated with an increase in the relative content of phycoerytrin that contributes to light harvesting (Gervais et al. 1997; Camacho et al. 2003).

During the period of thermal stratification, Lakes Mascardi, Moreno, Gutiérrez and Correntoso developed a thermally homogeneous epilimnion (Fig. 2). The increase in these lakes of photosynthetic efficiency of PicoPhy below the thermocline averaged 60-fold that of surface (Fig. 4). On the contrary, Lakes Nahuel Huapi and Espejo did not show such a pattern. These differences between lakes may be related to the onset of the thermocline and to its stabilization, conditions that influence the development of DCM (Padisák et al. 1997).

The oxygen uptake of marine *Synechococcus* is near zero at low-light and increases only when increasing the intensity of the growth irradiance (Kana 1992). In our lakes, picocyanobacteria below the thermocline at low irradiance and cold water would be near their zero respiration rates, which will constitute as another positive selection factor. Thus, picocyanobacteria could maintain their metabolism at a low-level, prolong their life remaining at the same time photosynthetically active to the low photon quanta that reach them (they are in fact highly efficient). Therefore, the presence of an abundant population of highly efficient PicoPhy in the deep cold layer appears to be likely and indicates a high fitness at these low-light layers. Low respiration rates imply an increase in net production without a necessary increase in the gross production. The high efficiencies observed at these levels may be a consequence of the combined effect of increase photon harvesting and reduction of respiration rates that increase *per se* the net production.

The debate on the importance of nutrient for the PicoPhy success in the competition with larger phytoplankton in terms of cell number, growth and photosynthesis is still open. The vertical distribution of PicoPhy has been found to be influenced by nutrients (Padisák et al. 1997; Gervais et al. 1997). In a decreasing range of trophic conditions, PicoPhy relative contribution to total phytoplankton biomass and production increases (Stockner 1991; Bell and Kalff 2001; Callieri and Stockner 2002). We obtained a good evidence of this inverse relation between TDP and the relative contribution of PicoPhy to total production at the lower extreme of phosphorus concentration range (Fig. 6). This is supported by the absence of a significant relationship between PP of PicoPhy and TDP we found in the ultraoligotrophic Patagonian lakes (Fig. 5, multiple linear regression $P = 0.425$), indicating that PicoPhy activity (production and growth) is independent from P even at very

low concentrations. This result agrees with the findings of Moutin et al. (2002) who explained the abundance of *Synechococcus* in the open ocean, both in the P-depleted zone and on occasional episodic P nanopulses, with the high affinity of *Synechococcus* for ortophosphate. Lavallée and Pick (2002) also found lack of correlation between PicoPhy growth rates and any form of dissolved P. All these results can be better understood looking at the kinetic of P uptake. The maximum specific cell phosphorus-based uptake rate (U_m) was found to be higher for cyanobacteria than for green algae (Vadstein 2000), giving to the former a competitive ability for P uptake.

The result of the interaction of light and nutrient-limitation (mainly P) in the natural environment supports the idea that low-light and low P may interact to create very favourable conditions for the smaller photosynthetic organisms. PicoPhy can be successful at very low P concentration because they possess superior uptake capabilities of inorganic P (Stockner and Antia 1986; Vadstein 2000). Therefore, this high nutrient uptake per biomass unit of PicoPhy may determine their success in layers where their niche overlaps with larger phytoplankton.

The available data on the relationship between total phytoplankton biomass, as indicator of trophic gradient, abundance and relative importance of PicoPhy (Vörös et al. 1998; Bell and Kalff 2001; Callieri and Stockner 2002) does not include those from ultraoligotrophic large deep lakes. We used our data from North Andean-Patagonian lakes to expand the database of Vörös et al. (1998) in order to cover the trophic range in the ultraoligotrophic extreme of the gradient (Fig. 7). These results strengthened the model outlined by Stockner (1991) and facilitate inclusion of ultraoligotrophic deep lakes.

In conclusion ultraoligotrophic clear-water, deep lakes can be considered as highly suitable environments for growth and development of PicoPhy. Our present study reveals that PicoPhy at the DCM, can do better than other larger autotrophic organisms probably as a consequence of their high P assimilation capacity and light harvesting efficiency. In these deep cold and dim light layers PicoPhy can succeed and moreover, they are able to exploit every single piece of energy that arrives there under photon form, thus showing a high-photosynthetic efficiency.

Acknowledgements We thank LIBIQUIMA, Universidad Nacional del Comahue, for laboratory facilities. This work was possible as a result of the International Cooperation Programme between CNR (Italy) and CONICET (Argentina). This work was partially supported by FONCyT 01-13395, CONICET PIP 6507 and UNC B119.

References

Agawin NSR, Duarte CM, Agustí S (2000) Nutrient and temperature control of the contribution of picoplankton to phytoplankton biomass and production. Limnol Oceanogr 45:591–600

APHA (1992) Standard methods for the examination of water and wastewater. Am Publ Health Ass, Washington pp 1134

Bell T, Kalff L (2001) The contribution of picophytoplankton in marine and freshwater systems of different trophic status and depth. Limnol Oceanogr 46:1243–1248

Berner T, Dubinsky Z, Whyman K, Falkowsky PG (1989) Photoadaptation and the "package effect" in *Dunaliella tertiolecta* (Chlorophyceae). J Phycol 25:70–78

Bertoni R, Balseiro E (2005) Mixing Layer Running Incubator (MIRI): an instrument for incubating samples while moving vertically in the mixing layer. Limnol Oceanogr Methods 3:158–163

Callieri C, Pinolini ML (1995) Picoplankton in Lake Maggiore, Italy. Int Revue ges Hydrobiol 80:491–501

Callieri C, Piscia R (2002) Photosynthetic efficiency and seasonality of autotrophic picoplankton in Lago Maggiore after its recovery. Freshwat Biol 47:941–956

Callieri C, Stockner JG (2002) Freshwater autotrophic picoplankton: a review. J Limnol 61:1–14

Callieri C, Amicucci E, Bertoni R, Vörös L (1996) Fluorometric characterization of two picocyanobacteria strains from different underwater light quality. Int Revue ges Hydrobiol 81:13–23

Callieri C, Morabito G, Huot Y, Neal P, Lichman E (2001) Photosynthetic response of pico- and nanoplanktonic algae to UVB, UVA and PAR in a high mountain lake. Aquat Sci 63:286–293

Camacho A, Miracle MR, Vicente E (2003) Which factors determine the abundance and distribution of picocyanobacteria in inland waters? A comparison among different types of lakes and ponds. Arch Hydrobiol 157:321–338

Danish Standard Association (1982) Water Quality-Incubator method for determining the carbon assimilation by plankton algae using ^{14}C technique. Dansk Standard, DS/R 293

Drakare S, Blomqvist P, Bergström AK, Jansson M (2003) Relationships between picophytoplankton and environmental variables in lakes along a gradient of water colour and nutrient content. Freshwater Biol 48:729–740

Fahnenstiel GL, Carrick HJ, Iturriaga R (1991) Physiological characteristics and food-web dynamics of *Synechococcus* in Lakes Huron and Michigan. Limnol Oceanogr 36:219–234

Fahnenstiel GL, Redalje DG, Lohrenz SE (1994) Has the importance of photoautotrophic picoplankton been overestimated? Limnol Oceanogr 39:432–438

Falkowski PG, Raven JA (1997) Aquatic photosynthesys. Blackwell Science, Malden, Massachusetts

Frenette JJ, Demers S, Legendre L, Boulé M (1996) Size-related photosynthetic characteristics of phytoplankton during periods of seasonal mixing and stratification in an oligotrophic multibasin lake system. J Plankton Res 18:45–61

Friebele ES, Correl DL, Faust MA (1978) Relationship between phytoplankton cell size and the rate of orthophosphate uptake: in situ observations of an estuarine population. Mar Biol 45:39–52

Gervais F, Padisák J, Koschel R (1997) Do light quality and low nutrient concentration favour picocyanobacteria below the thermocline of the oligotrophic Lake Stechlin? J Plankton Res 19:771–781

Hauschild CA, McMurter HJG, Pick FR (1991) Effect of spectral quality on growth and pigmentation of picocyanobacteria. J Phycol 27:698–702

Helbling EW, Villafañe V, Barbieri ES (2001) Sensitivity of winter phytoplankton communities from andean lakes to artificial ultraviolet-B radiation. Rev Chil Hist Nat 74:273–282

Kana TM (1992) Relationship between photosynthetic oxygen cycling and carbon assimilation in *Synechococcus* WH7803 (Cyanophyta). J Phycol 28:304–308

Karentz D, Bothwell ML, Coffin RB, Hanson A, Herndl GJ, Kilham SS, Lesser MP, Lindell M, Moeller RE, Morris DP, Neale PJ, Sanders RW, Weiler CS, Wetzel RG (1994) Impact of UV-B radiation on pelagic freshwater ecosystems: Report of working group on bacteria and phytoplankton. Arch Hydrobiol Beih 43:31–69

Lavallée B, Pick FR (2002) Picocyanobacteria abundance in relation to growth and loss rates in oligotrophic to mesotrophic lakes. Aquat Microb Ecol 27:37–46

Li WKW (1995) Composition of ultraphytoplanktonin the central North Atlantic. Mar Ecol Prog Ser 122:1–8

Malinsky-Rushansky N, Berman T, Berner T, Yacobi YZ, Dubinsky Z (2002) Physiological characteristics of picophytoplankton, isolated from Lake Kinneret: responses to light and temperature. J Plankton Res 24:1173–1183

Markert B, Pedrozo F, Geller W, Friese K, Korhammer S, Baffico G, Díaz M, Wölfl S (1997) A contribution to the study of the heavy-metal and nutritional element status of some lakes in the southern Andes of Patagonia (Argentina). Sci Tot Environ 206:1–15

Menden-Deuer S, Lessard EJ (2000) Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol Oceanogr 45:569–579

Modenutti BE, Balseiro EG (2002) Mixotrophic ciliates in an Andean lake: dependence on light and prey of an *Ophrydium naumanni* population. Freshwater Biol 47:121–128

Modenutti BE, Pérez GL, Balseiro EG, Queimaliños CP (2000) Relationship between light availability, chlorophyll *a* and total suspended solid in a glacial lake of South Andes. Verh int Ver Limnol 27:2648–2651

Modenutti BE, Balseiro EG, Callieri C, Queimaliños C, Bertoni R (2004) Increase in photosynthetic efficiency as a strategy of planktonic organisms exploiting deep lake layers. Freshwater Biol 49:160–169

Modenutti BE, Balseiro EG, Callieri C, Bertoni R, Queimaliños C (2005) Effect of UV-B and different PAR intensities on the primary production of the mixotrophic planktonic ciliate *Stentor araucanus*. Limnol Oceanogr 50:864–871

Moeller RE (1994) Contribution of ultraviolet radiation (UV-A, UV-B) to photoinhibition of epilimnetic phytoplankton in lakes of differing UV transparency. Arch Hydrobiol Beih 43:157–170

Morris DP, Zagarese HE, Williamson CE, Balseiro EG, Hargreaves BR, Modenutti BE, Moeller R, Queimaliños C (1995) The attenuation of UV radiation in lakes and the role of dissolved organic carbon. Limnol Oceanogr 40:1381–1391

Moutin T, Thingstad TF, Wambeke FV, Marie D, Raimbault GS, Raimbault P, Claustre H (2002) Does competition for nanomolar phosphate supply explain the predominance of the cyanobacterium *Synechococcus*? Limnol Oceanogr 47:1562–1567

Neale P, Litchman E, Sobrino C, Callieri C, Morabito G, Montecino V, Huot Y, Bossard P, Lehmann C, Steiner D (2001) Quantifying the response of phytoplankton photosynthesis to ultraviolet radiation: biological weighting functions versus in situ measurements in two Swiss lakes. Aquat Sci 63:265–285

Nusch EA (1980) Comparison of different methods for chlorophyll and phaeopigment determination. Arch Hydrobiol Beih Ergen Limnol 14:14–36

Padisák J, Krienitz L, Koschel R, Nedoma J (1997) Deep-layer autotrophic picoplankton maximum in the oligotrophic Lake Stechlin, Germany: origin, activity, development and erosion. Eur J Phycol 32:403–416

Pan YL, Rao DVS, Mann KH (1996) Acclimation to low light intensity in photosynthesis and growth of *Pseudo-nitzschia* multiseries Hasle, a neurotoxigenic diatom. J Plankton Res 18:1427–1438

Partensky F, Blanchot J, Lantoine F, Neveux J, Marie D (1996) Vertical structure of picophytoplankton at different sites of the subtropical Northeastern Atlantic Ocean. Deep-Sea Res 43:1191–1213

Paruelo JB, Beltrán A, Jobbágy E, Sala OE, Golluscio RA (1998) The climate of Patagonia: general patterns and controls on biotic processes. Ecol Aust 8:85–101

Pérez GL, Queimaliños CP, Modenutti BE (2002) Light climate and plankton in the deep chlorophyll maxima in North Patagonian Andean lakes. J Plankton Res 24:591–599

Pick FR, Agbeti DM (1991) The seasonal dynamic and composition of photosynthetic picoplankton communities in temperate lakes in Ontario, Canada. Int Revue ges Hydrobiol 76:565–580

Queimaliños CP, Modenutti BE, Balseiro EG (1999) Symbiotic association of the ciliate *Ophrydium naumanni* with *Chlorella* causing a deep chlorophyll *a* maximum in an oligotrophic South Andes lake. J Plankton Res 21:167–178

Quirós R, Drago E (1985) Relaciones entre variables fisicas, morfométricas y climáticas en lagos patagónicos. Rev Asoc Cienc Nat Lit 16:181–199

Raven JA (1986) Physiological consequences of extremely small size for autotrophic organisms in the sea. In: Platt T, Li WKW (eds) Photosynthetic picoplankton. Can Bull Fish Aquat Sci 214:1–70

Regel RH, Brookes JD, Ganf GG (2004) Vertical migration, entrainment and photosynthesis of the freshwater dinoflagellate *Peridinium cinctum* in a shallow urban lake. J Plankton Res 26:143–157

Steeman-Nielsen E (1952) The use of radioactive carbon (^{14}C) for measuring organic production in the sea. J Con Int Expl Mer 18:117–140

Stockner JG (1991) Autotrophic picoplankton in freshwater ecosystems. Int Revue ges Hydrobiol 76:664 pp

Stockner JG, Antia NJ (1986) Algal picoplankton from marine and freshwater: a multidisciplinary perspective. Can J Fish Aquat Sci 43:2472–2503

Stockner J, Callieri C, Cronberg G (2000) Picoplankton and other non-bloom forming cyanobacteria in lakes. In: Whitton B, Potts M (eds) Ecology of Cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp 195–238

Sun J, Liu DY (2003) Geometric models for calculating cell biovolume and surface area for phytoplankton. J Plankton Res 25:1331–1346

Utermöhl H (1958) Zur Vervollkommung der quantitative Phytoplankton Methodik. Mitt Int Verein Limnol 9:1–38

Vadstein O (2000) Heterotrophic, planktonic bacteria and cycling of phosphorus. In: Schink B (ed) Advances in microbial ecology, vol 16. Kluwer Academic, Plenum, pp 115–167

Villafañe VE, Andrade M, Lairana V, Zaratti F, Helbling EW (1999) Inhibition of phytoplankton photosynthesis by solar ultraviolet radiation: studies in Lake Titicaca, Bolivia. Freshwater Biol 42:215–224

Villafañe VE, Buma AGJ, Boelen P, Helbling EW (2004) Solar UVR-induced DNA damage and inhibition of photosynthesis in phytoplankton from Andean lakes of Argentina. Arch Hydrobiol 161:245–266

Vörös L, Callieri C, Balogh KV, Bertoni R (1998) Freshwater picocyanobacteria along trophic gradient and light quality range. Hydrobiologia 369/370:117–125

Wehr JD (1993) Effects of experimental manipulation of light phosphorus supply on competition among picoplankton and nanoplankton in a oligotrophic lake. Can J Fish Aquat Sci 50:936–945

Weisse T (1993) Dynamics of autotrophic picoplankton in marine and freshwater ecosystems. Adv Microb Ecol 13:327–370

Weisse T, Kenter U (1991) Ecological characteristics of autotrophic picoplankton in a prealpine lake. Int Revue ges Hydrobiol 76:493–504

Zagarese HE, Tartarotti B, Cravero W, Gonzalez P (1998) UV damage in shallow lakes: the implications of water mixing. J Plankton Res 20:1423–1433