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Photosynthetic picoplankton dynamics in Lake Tahoe: temporal and spatial niche partitioning among prokaryotic and eukaryotic cells

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Dynamics of prokaryotic and eukaryotic picophytoplankton were investigated over a 2-year time period using flow cytometry and combined with an in situ *experiment in Lake Tahoe, USA to better characterize to which extent environmental factors control these communities. Pronounced seasonal patterns and clear temporal and spatial partitioning were observed between picocyanobacteria and picoeukaryotes. Picocyanobacteria dominated in the nutrient deficient upper water column during the stratified season, while picoeukaryotes reached maximum abundance during isothermal conditions and maintained high numbers in deep-water layers during the stratified season. Picocyanobacteria were more sensitive to high solar and UV radiation compared with picoeukaryotes, which were not affected by high solar radiation and nutrient enrichment stimulated their growth. The opposing response of these two populations is consistent with their vertical distribution: picocyanobacteria dominate below the 30% isolume and above the nitrocline depth, whereas picoeukaryotes increase in the vicinity of the nitrocline and thus increased nutrient concentration. This spatial separation of picophytoplankton groups along environmental gradients in Lake Tahoe is consistent with other deep-oligotrophic lakes and the marine environment, suggesting that these marine and freshwater organisms have similar ecophysiological requirements. These results highlight that the smallest photosynthetic communities show taxon-specific responses to mixing and resource availability, which affect the structure and dynamics of picophytoplankton.*

Featured Article

INTRODUCTION

Autotrophic picoplankton include the smallest photosynthetic organisms, consisting of pro- and eukaryotic cells within the size range of 0.2–2 and 3 μm, respectively. They are a major component of the photosynthetic biomass in many aquatic ecosystems, particularly in oligotrophic lakes and oceans (Stockner, 1991; Weisse, 1993; Boyd *et al.*, 2000; Callieri *et al.*, 2007b). In these systems, up to 70% of the carbon fixed annually is attributed to this size range (Stockner and Antia, 1986; Bell and Kalff, 2001; Callieri and Stockner, 2002) and consequently photosynthetic picoplankton (PPIC) are an important source of energy to aquatic food webs, which is recycled by other microorganisms in the water column (Azam *et al.*, 1983) or exported to the sediment (Richardson and Jackson, 2007). The PPIC community is composed predominantly of picocyanobacteria. While picoeukaryotes are numerically less abundant, they can reach similar biomass due to their higher carbon content (Worden *et al.*, 2004). The freshwater picocyanobacteria community is dominated by phycoerythrin- or phycocyanin-rich chroococcoid of the genus *Synechococcus* and picoeukaryotes by *Chlorella*-like chlorophytes (Weisse, 1993; Stomp *et al.*, 2007). Owing to the advent of analytical improvements such as flow cytometry (Olsen *et al.*, 2000) and molecular biological tools

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(Burton, 1996), their role in ecosystem processes is becoming much better understood (Schallenberg and Burns, 2001; Crosbie *et al.*, 2003; Tittel *et al.*, 2003). The ecological significance of PPIC has been demonstrated particularly for the oceans and observations have lead to novel insights regarding these photosynthetic cells including niche partitioning of ecotypes along environmental gradients (Bouman *et al.*, 2006; Johnson *et al.*, 2006) and unique nutrient-acquisition capacities (Zehr *et al.*, 2008). The temporal succession and spatial distribution along environmental gradients of different taxonomic picophytoplankton populations and the extent to which environmental factors regulate their dynamics is, however, less well characterized for freshwater systems (Crosbie *et al.*, 2003; Callieri *et al.*, 2007a).

Compared to larger eukaryote algal cells, PPIC have higher growth rates and possess superior nutrient uptake capabilities (Raven, 1998; Bec *et al.*, 2008). Consequently, the relative importance of PPIC to total phytoplankton biomass and production increases with declining nutrient concentration (Burns and Stockner, 1991; Sondergaard, 1991; Bell and Kalff, 2001), particularly for picocyanobacteria (Burns and Schallenberg, 1998; Tzaras *et al.*, 1999; Schallenberg and Burns, 2001). A complex response to nutrient enrichment is, however, expected among different picophytoplankton groups (Schallenberg and Burns, 2001; Bec *et al.*, 2005; Davey *et al.*, 2008) and positive growth responses have been reported for some picoeukaryotes.

Similarly, PPIC are more efficient in photon absorption owing to reduced chromophore self-shading (Raven, 1998) in low-resource environments compared with larger-sized cells. The smaller package effect, however, increases the possibility of cell damage due to photosynthetically active radiation (PAR) and ultraviolet radiation (UVR, 290–400 nm) owing to smaller capacity for screening out damaging UVR and reduced photorepair mechanisms (Kirk, 1994; Raven, 1998). In oligotrophic systems with high solar radiation, PPIC are episodically exposed to high PAR and UVR both of which can have detrimental effects (Neale *et al.*, 2003). Photoautotrophic organisms have developed several mechanisms that protect the photosynthetic apparatus from excess exposure to UVR and PAR (Demming-Adams and Adams, 1992) and the sensitivity varies among species. Some studies have shown that the smallest freshwater phytoplankton cells are relatively resistant to UVR and no difference in photoinhibition has been observed between pico-and microphytoplankton (Laurion and Vincent, 1998). In contrast, other studies showed that PPIC growth is strongly affected by high levels of UVR (Callieri *et al.*, 2001; Callieri *et al.*, 2007b). One reason for these contrasting results is that PPIC taxa have differential sensitivity to solar radiation and this indicates the importance of separation between functional groups.

Consequently, environmental conditions of resource availability of light and nutrients exert a strong effect on the seasonal dynamics of PPIC. In oligo-mesotrophic temperate lakes, picocyanobacteria usually show a bimodal seasonal cycle and peak in spring and late summer (Weisse, 1988; Callieri and Stockner, 2002). Density decline during summer is largely due to grazing impacts. The vertical distribution of picocyanobacteria is variable among lakes, ranging from density maxima in surface waters (Maeda *et al.*, 1992), metalimnion (Weisse and Kenter, 1991; Padisak *et al.*, 1997; Crosbie *et al.*, 2003) to upper hypolimnetic waters (Fahnenstiel and Carrick, 1992; Callieri *et al.*, 2007b). *Synechococcus*-like cyanobacteria are highly plastic and can acclimate to different environmental conditions and irradiance levels by adjusting their pigment composition. Picoeukaryotes, on the other hand, tend to have a pronounced peak in spring or early summer during stratification onset (Weisse, 1993) and are distributed over the mixed depth layer (Pick and Agbeti, 1991; Crosbie *et al.*, 2003).

Differential responses of picocyanobacteria and picoeukaryotes to environmental variation suggest that the role of resource availability in structuring the community is complex. The present study describes detailed temporal and spatial PPIC dynamics in the oligotrophic Lake Tahoe over a 2-year study period. The high transparency and great depth of Lake Tahoe allow for extensive vertical habitat gradients and spatial partitioning of planktonic organisms, making it a suitable ecosystem to study ecological differences and resource requirements of PPIC. Using flow cytometry, picocyanobacteria and picoeukaryotes were differentiated and their response to nutrient enrichment and UVR variation were experimentally investigated. This study shows that the mixing pattern and pronounced vertical gradients of Lake Tahoe together with high solar radiation affect the spatio-temporal structure of the PPIC community. Dynamics of picocyanobacteria differed greatly from those of picoeukaryotic cells and this suggests that these taxonomic groups show differential responses to changes in mixing and resource availability.

METHOD

Site description

Lake Tahoe is a subalpine lake located at an elevation of 1898 m a.s.l. in the Sierra Nevada mountain range of California and Nevada, USA (39°N, 120°W). The lake has a surface area of 500 km^2, maximum depth of 501 m, mean depth of 333 m and is free of ice the entire

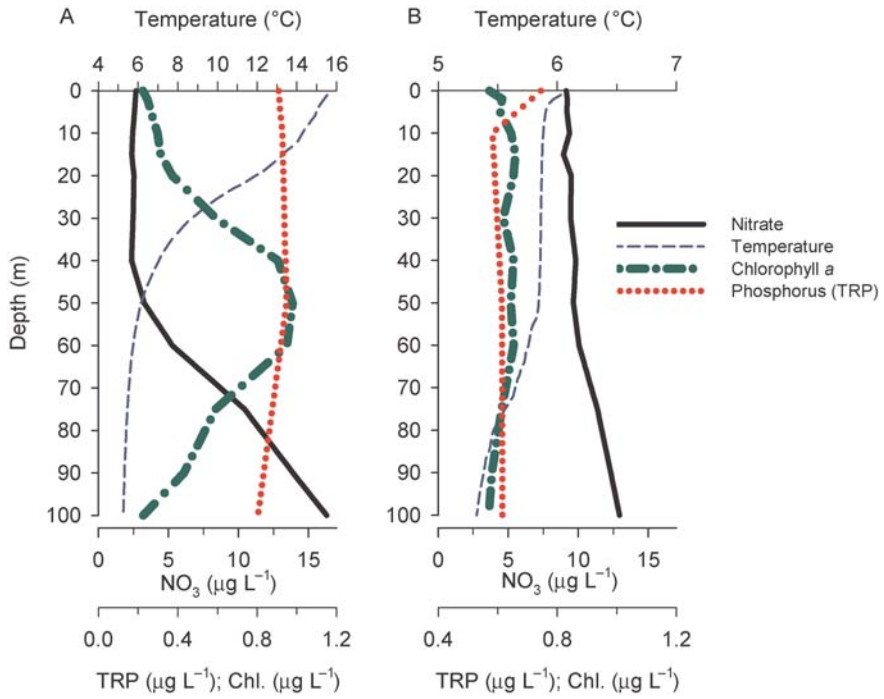

Fig. 1. Vertical profiles (0–100 m) of nitrate, phosphorus (total reactive phosphorus, TRP), temperature and chlorophyll *a* (Chl *a*) averaged over (**A**) the summer stratification (May–Oct) and (**B**) the winter mixing (Dec–Mar) season in Lake Tahoe between 2006 and 2008.

year. The lake starts to stratify in April, and by October thermal stratification begins to break down with a deepening of the thermocline; the average thermocline depth is 21 m in August and increases to 32 m in October (Coats *et al.*, 2006). Lake Tahoe is oligotrophic and average annual total phosphorus (P) concentrations in the upper 100 m were 10.3 μg L^{-1} ($\pm$1.8 SD) and total reactive phosphorus (TRP) concentrations were around 0.9 μg L^{-1} ($\pm$0.5 SD) between 2006 and 2008 (Fig. 1). During the stratified period, average nitrate (N) concentrations were around 2.5 μg L^{-1} ($\pm$1.7 SD) in the upper water column (above 40 m) and increased to 16 μg L^{-1} at 100-m depth (Fig. 1). This vertical gradient leveled off during the mixing period and nitrate concentration increased to $\sim$10 μg L^{-1} in the upper water layer. Current annual average Secchi depth is around 21 m and is located above the euphotic zone depth, which ranges between 52 and 66 m (Winder and Hunter, 2008), and the deep-water chlorophyll maximum that typically develops during the summer between 40 and 60 m depth (Fig. 1).

Sampling and measurement of environmental parameters

Physico-chemical data used in this study were collected from a near-shore station (Index station, maximum depth of 125 m) and a mid-lake station (maximum depth of 460 m) (for a detailed map see Jassby *et al.*, 2003) with an average sampling interval of 12 and 26 days, respectively, between 2006 and 2008. Vertical profiles of temperature and PAR were taken with a Seabird (SBE 25 Sealogger CTD). Daily interpolated temperature profiles at 1-m intervals were used to calculate vertical density profiles. Density (ρ) was calculated as a function of temperature, salinity (assumed constant at 0.042 psu) and pressure (depth). The density difference between the mean density of 30–50 m and 0–10 m water was used as an indicator of strength of stratification (see Winder *et al.*, 2009). Chlorophyll *a* (Chl *a*), N and TRP were measured at the Index station at 0, 2, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90 and 105 m depths and mid-lake station at 0, 10, 50 and 100 m depths. Further details of nutrient measurements are given in Winder and Hunter (Winder and Hunter, 2008). Chl *a* concentration was determined fluorometrically (Turner Designs fluorometer calibrated with pure Chl *a*) after methanol extraction for particles collected from 100 mL water onto GF/C filters. The nitrocline depth was defined by the depth in the water column where nitrate concentration differences are greater than 0.5 μg L^{-1} $depth^{-1}$. Euphotic zone depth was defined as the depth where PAR reaches 1% of surface radiation. The vertical attenuation coefficient (K_d) for scalar irradiance was

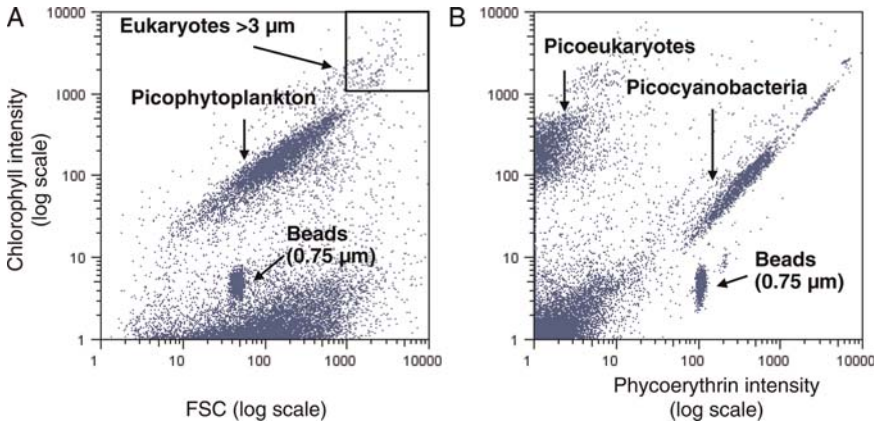

Fig. 2. Cytogram of the (**A**) forward scatter (FSC) and chlorophyll, and (**B**) phycoerythrin and chlorophyll fluorescence profile for a sample collected from 100-m depth at Lake Tahoe end of May 2006. Fluorescent latex beads of 0.75 µm diameter are added to the sample. Picocyanobacteria and picoeukaryotes were differentiated based on their size and fluorescence signal.

calculated according to Kirk (Kirk, 1994). K_d is the linear regression coefficient of $\ln Ed(z)$ with respect to depth. Average monthly PAR within the mixed layer (I_m) was estimated after Bouman *et al.* (Bouman *et al.*, 2006) using the following equation:

$$I_m = \frac{I_0 * [1 - \exp(-z_m K_d)]}{z_m K_d}$$

where I_0 is the daily PAR irradiance just below the sea surface, z_m is the depth of the mixed layer and K_d is the vertical attenuation coefficient of down-welling irradiance. Surface PAR radiation was derived from solar radiation measured continuously at Lake Tahoe by assuming a 0.47 PAR fraction of total radiation (Papaioannou *et al.*, 1993) and binned by month. To estimate irradiance below the surface, 4% surface reflection was assumed.

Samples for identifying the seasonal dynamics of PPIC and nano- and microphytoplankton cells were collected at 0, 10, 20, 30, 40, 50, 60, 75, 90 and 105 m depths at the Index station and 0, 10, 50 and 100 m at the mid-lake station, respectively, once a month between May 2006 and July 2008. Samples for PPIC enumeration were fixed with glutaraldehyde (0.25% final concentration) and stored at −80°C prior to analysis. Samples for larger phytoplankton cell enumeration were preserved with Lugol's solution.

Phytoplankton cell enumeration

PPIC cells were analyzed using flow cytometry (Influx Mariner, Cytopeia Corp., Seattle, WA, USA) and counted in the logarithmic mode using forward scatter (FSC) as well as Chl *a* and phycoerythrin (PE) pigment autofluorescence excited by a 488 nm laser (Sapphire Coherent) with 692/40 and 572/27 fluorescence detectors, respectively. This PE/Chl *a* emission ratio discrimination procedure was robust and produced a clear clustering throughout the sampling period (Fig. 2). Fluorescent latex beads of 0.75 µm diameter were used to calibrate the sidescatter signal into units of size. After flow rate stabilization of the flow cytometer, sample volume was measured and samples were run for at least 4 min at high flow rate so that at least 200 000 cells per sample were counted. Sample volume was measured again after analysis and volume difference (measured in weight) was used for flow-cytometric estimates of PPIC abundances. Flow cytometry data were analyzed using the FlowJo software (TreeStar) and cells enumerated on the basis of light scatter and autofluorescence properties. The size threshold of <3 µm in all dimensions was used to define PPIC, which produces similar results compared with <2 µm threshold (Stockner and Antia, 1986). PPIC groups in the size range between 0.5 and 3 µm were differentiated according to their size and photopigment fluorescence: photoautotrophic bacteria (picocyanobacteria) were selected based on a combination of low Chl *a* and high PE content, picoeukaryotes based on high Chl *a* and low PE content. Picocyanobacteria were counted by gated window based on PE and Chl *a* fluorescence and subsequently removed for enumerating picoeukaryotes because picocyanobacteria frequently overlap with eukaryote cells (Fig. 2). Abundances of PPIC were linearly integrated between dates, and monthly depth integrated abundances were calculated for both sampling stations for the upper 100-m water layer.

Enumeration of nano- and microphytoplankton followed the Utermöhl method and is described in detail in Winder and Hunter (Winder and Hunter, 2008).

Phytoplankton carbon (C) biomass estimation

PPIC biomass was estimated from cell counts by assigning an average C concentration to each taxonomic group. For picocyanobacteria, an average C content of 82 fg C $cell^{-1}$ was assumed and for picoeukaryotes 530 fg C $cell^{-1}$ according to Worden *et al.* (Worden *et al.*, 2004), which represents conservative C estimates (for comparison see Verity *et al.*, 1992; DuRand *et al.*, 2001). Seasonality of C content in picocyanobacteria is largely determined by changes in cell abundances because cell sizes are relatively robust on a seasonal scale (Worden *et al.*, 2004). Seasonal C variation in picoeukaryotes is often a combination of change in cell size and abundance; consequently, the actual C content for this group may deviate from the estimates. Carbon conversion factors for larger eukaryote phytoplankton were derived from Menden-Deuer and Lessard (Menden-Deuer and Lessard, 2000) by applying the conversion factors to cell biovolume for each major taxonomic group.

Contribution of PPIC to total primary productivity (PPr) and Chl *a* concentration

The contribution of picophytoplankton primary production (PPr) to total phytoplankton production was quantified three times during the summer stratification period in 2007. Size-fractionated PPr rates were measured by determining the *in situ* C assimilation rate at 2, 10, 20, 30, 40, 50 and 60 m depths using the standard ^{14}C technique at the Index station (described in Goldman *et al.*, 1989). After incubation, water samples were sequentially filtered through 3 and 0.45 μm Isopore Membrane filters and hourly rates of integrated PPr (mg C m^{-2} h^{-1}) over the upper 60-m depth calculated for each sampling date. In addition, size-fractionated Chl *a* concentration was determined fluorometrically (see above) for particles collected on 3 and 0.45 μm pore size filters. Photosynthetic rates were normalized to the concentration of Chl *a*.

In situ UV and nutrient enrichment experiment

The sensitivity of PPIC taxonomic groups to the exposure of solar radiation and nutrient addition was investigated *in situ* using an experimental setup in August 2006. This allowed for testing of their response under well-defined (natural) UVR and PAR conditions. A representative phytoplankton assemblage over the euphotic depth zone was collected from 5, 20, 40 and 60 m depths at the mid-lake station. The water from each depth was combined and filtered through an 80 m size mesh. For the "nutrient-addition" treatments, lake water was enriched with N added in the form of $NaNO_3$ (20 g NO_3N L^{-1}) and P added in the form of NaH_2PO_4 (10 g PO_4^{3-} L^{-1}) (nutrient ratios followed Goldman *et al.*, 1993). Previous experiments demonstrated that these concentrations result in a positive phytoplankton growth response (Goldman *et al.*, 1993). Samples were filled in 2-L liquid-tight specimen bags (Bitran S Series) made of UVR-transmitting polyethylene (transmits 94% PAR and 86% of solar UVR 295–399 nm, 50% transmittance at 234 nm). For the +UVR treatment, bags were placed in UVR-transparent Aclar sleeves [long-wave-pass plastic that transmits 100% PAR in water and most UVR (98% of UV-B 295–319 nm, 99% UV-A 320–399 nm, with a sharp wavelength cutoff and a 50% transmittance point at 212 nm)] and for the −UVR treatment in Courtgard sleeves [long-wave-pass plastic that transmits 95% PAR in water but blocks most UVR (transmits no UV-B 295–319 nm, and only 9% of UV-A 320–400 nm with a sharp wavelength cutoff and a 50% transmittance point at 400 nm)]. Four replicates of each treatment (+UVR +NP; −UVR +NP; +UVR −NP; −UVR −NP) were incubated at 2, 6, 8, 10 and 12 m depths at the mid-lake station and exposed for 7 days. These depths were chosen because vertical irradiance profiles showed a strong UV gradient in surface waters and declining UV-B below 10 m depth. Cell concentration for initial and final glutaraldehyde-preserved samples was analyzed using flow cytometry (see above). The percentage of change is obtained as: $100 \times (cell_2 - cell_1)/cell_1$, where $cell_1$ is the cell concentration for the subpopulation at the start of the experiment and $cell_2$ at the end of the experiment.

UVR profiles were collected at the beginning of the experimental set-up using a Biospherical Instruments Cosine (BIC) radiometer (Biospherical Instruments Inc., San Diego, CA, USA) that measured 305, 320 and 380 nm UV and PAR irradiance (for further details see Rose *et al.* in press).

Data analysis

Measurements for both sampling stations were similar and data from both stations were combined for further analysis and binned by month. To examine the environmental effects on PPIC abundances, generalized linear models with a Gaussian error distribution were used. Irradiance, stratification strength and nutrient (TRP, N) concentrations were considered predictive variables. Model selection was based on a step-wise procedure and

the improvement of the fit gained was assessed using the χ^2 change in deviance at the 5% level. To identify the most parsimonious model, the Akaike Information Criterion (AIC) corrected for small sample size (AIC_c) (Burnham and Anderson, 2002) was used. Finally, the retained predictor variables were included in a generalized least square model including a periodic function to describe seasonality (Shumway and Stoffer, 2006):

$$\log \mathrm{PPIC}_t = \beta_0 + \beta_1 \log X_n + \beta_3 \sin(2\pi T) + \beta_4 \cos(2\pi T) + \eta_t$$

where PPIC_t is the picocyanobacteria or picoeukaryote abundances at time t, X_n the selected environmental variables, T the trend in decimal years (y), β_i the constants, η_t the residuals and the sine and cosine terms describe the periodic function. Statistical analyses were performed using the statistical software R (R Development Core Team 2005).

For the *in situ* experiment, a linear regression analysis was performed with the arcsine-transformed percentage of PPIC change versus depth. The null hypothesis was that the slope of the regression was 1, i.e. there was no difference in phytoplankton change across depths. Linear regressions were performed for picocyanobacteria and picoeukaryotes and elevations across treatments were compared to test whether UVR exclusion and/or nutrient enrichment showed different responses in cell density changes.

RESULTS

Seasonal succession of picocyanobacteria and picoeukaryotes

Depth-integrated cell numbers and carbon estimates for picocyanobacteria and picoeukaryotes followed relatively similar patterns over the 2-year study period in Lake Tahoe; however, the two photosynthetic picoplankton groups revealed distinctly different seasonal and vertical patterns (Figs 3 and 4). Picocyanobacteria were dominated by phycoerythrin-containing *Synechococcus* (Winder, unpublished data) and this group represented 26–98% of total PPIC cell density and achieved numerical dominance during the summer stratification period. These prokaryotes typically start to increase exponentially by the end of June after stratification onset and reached depth-integrated abundances of up to 5.8×10^{12} cells m^{-2} over the upper 100-m water column during the stratified season (Fig. 3A). The depth distribution of picocyanobacteria is characterized by peak densities in the depth layers between 20 and 50 m of maximum densities up to 120×10^3 cells mL^{-1} (Fig. 4A). During the summer season, picocyanobacteria occupied the nitrate-depleted water layer above the nitrocline (Fig. 4A), while this group was equally distributed in the water column during the winter mixing season. Picocyanobacteria cell concentrations in the upper 100-m water column are significantly correlated with the nitrocline depth ($r = 0.78$, $n = 29$, $P < 0.001$), indicating increased abundances with deepening of the nitrocline depth and thus with nutrient-deficient conditions in surface waters.

Picoeukaryotes showed opposing seasonal abundances compared with picocyanobacteria and reached their maximum during the mixing period with depth-integrated densities of up to 0.87×10^{12} cells m^{-2} in the upper 100 m water column (Fig. 3A). This PPIC group maintained high abundances until mid-summer. Picoeukaryotes are equally distributed over the entire water column during the mixing period with maximum densities of 13×10^3 cells mL^{-1}. After the onset of stratification, this group dominated between 40 and 70 m depth where they reached localized abundances in the order of 8×10^3 to 12×10^3 cells mL^{-1} (Fig. 4B). Similarly, total Chl *a* followed the same seasonality and vertical distribution patterns as the picoeukaryotes with a maximum between 40 and 80 m depth during the stratified season (Fig. 4C). This characterizes the vertical distribution of pico- and larger (>3 m) eukaryotic cells and the deep chlorophyll maxima in the vicinity of or below both the nitrocline (shown in Fig. 4A) and euphotic depth zone (shown in Fig. 4C). Overall depth-integrated picoeukaryotes were significantly correlated with total Chl *a* concentration ($r = 0.43$, $P = 0.02$, $n = 27$), whereas total PPIC and picocyanobacteria densities were not correlated with Chl *a* concentration ($r = 0.29$, $P = 0.13$ and $r = 0.32$, $P = 0.08$, respectively; $n = 27$).

The most parsimonious model that explained the pronounced seasonality for picocyanobacteria included stratification intensity, nitrogen concentration and PAR, which was, however, not a significant predictor after accounting for seasonality (Table I). In contrast, the seasonal pattern of picoeukaryote densities was not related to any environmental variable (P for all retained variables >0.05). This PPIC taxon showed pronounced seasonal vertical distribution patterns and localized high abundances throughout the season (Figs 2A and 3B). During the stratified season, picoeukaryote densities were positively correlated with localized nitrate concentration between 40 and 60 m depth ($r = 0.65$, $P = 0.008$; $n = 23$). This suggests that nutrient inputs are an important factor controlling picophytoplankton and that nutrients stimulate picoeukaryote growth whereas picocyanobacteria can tolerate nutrient-depleted conditions.

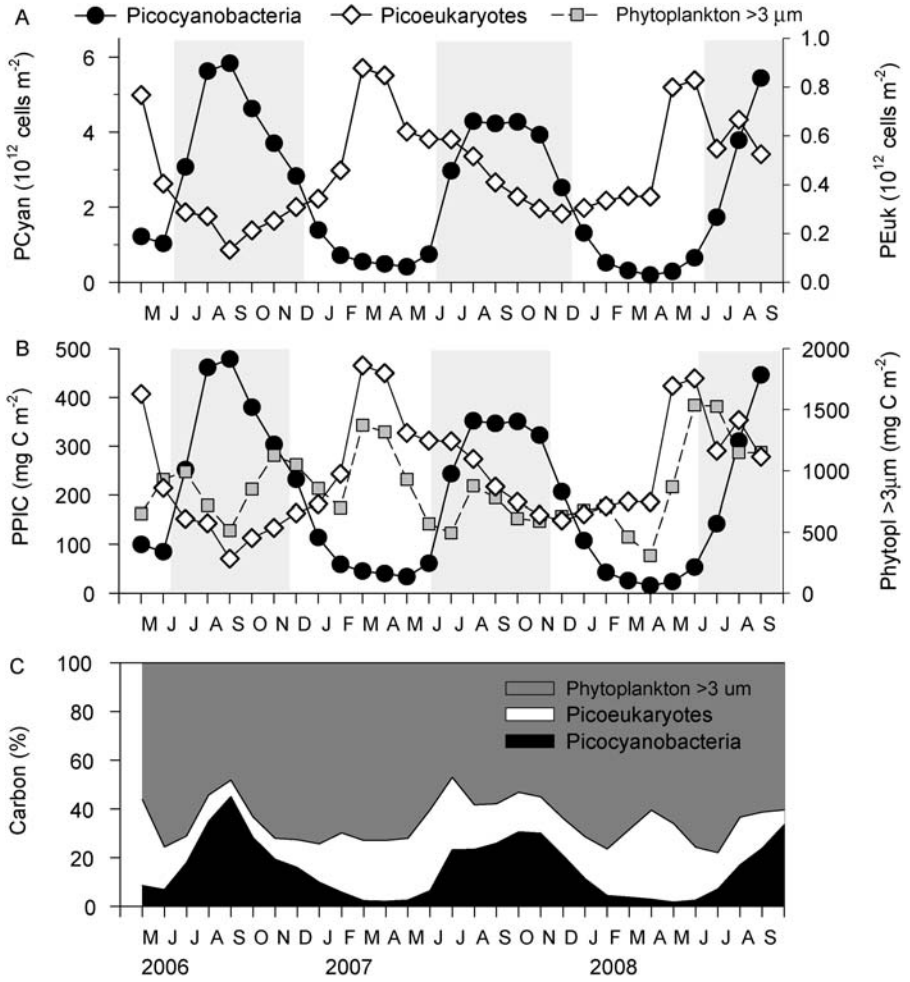

Fig. 3. Seasonal succession of phytoplankton in Lake Tahoe between May 2006 and September 2008. (**A**) Picocyanobacteria (PCyan) and picoeukaryote (PEuk) cell abundances; (**B**) picocyanobacteria and picoeukaryotes (PPIC) and larger eukaryotes (>3 m) carbon content; and (**C**) relative carbon contribution of picocyanobacteria, picoeukaryotes and larger eukaryotes (>3 m). Values are depth-integrated over the upper 100-m water column. Shaded area in (A) and (B) represents time periods of vertical stratification.

The estimated C content of picocyanobacteria and picoeukaryotes reached about equal levels (Fig. 3B). Depth integrated maximum C concentration for picocyanobacteria was up to 478 mg C m^{-2} during the summer period and for picoeukaryotes 465 mg C m^{-2} during the spring season. In comparison, maximum depth integrated carbon concentration for larger eukaryotes was about three times higher (1535 mg C m^{-2}) (Fig. 3B). Picocyanobacteria represented on average 15%, picoeukaryotes 20% and larger eukaryotes 65% of the total integrated biomass (Fig. 3C). During the stratified season, the contribution of PPIC was highest and reached up to 53% of total C biomass.

Contribution of PPIC to total PPr

Depth-integrated PPr for the entire phytoplankton community ranged from 32 to 40 mg C m^{-2} h^{-1} between July and November and size-fractionated PPr rates showed that PPIC contributed between 36 and 56% (Table II). The overall contribution of PPIC to total PPr was relatively consistent throughout the different water depths (ranged between 29 and 77% of total PPr). Net PPr to biomass ratios (P/B) ranged between 1.6 and 2.7 mg C mg Chl a^{-1} h^{-1} and were slightly higher for larger eukaryotes compared with PPIC (Table II).

Response of PPIC to UVR and nutrient enrichment

Picocyanobacteria densities decreased under ambient conditions and UVR exclusion and nutrient-enriched treatments in the upper 8-m water column, except in the UVR exclusion at 8-m depth (Fig. 5A). The 8-m depth corresponds to about 10% UV-B of subsurface

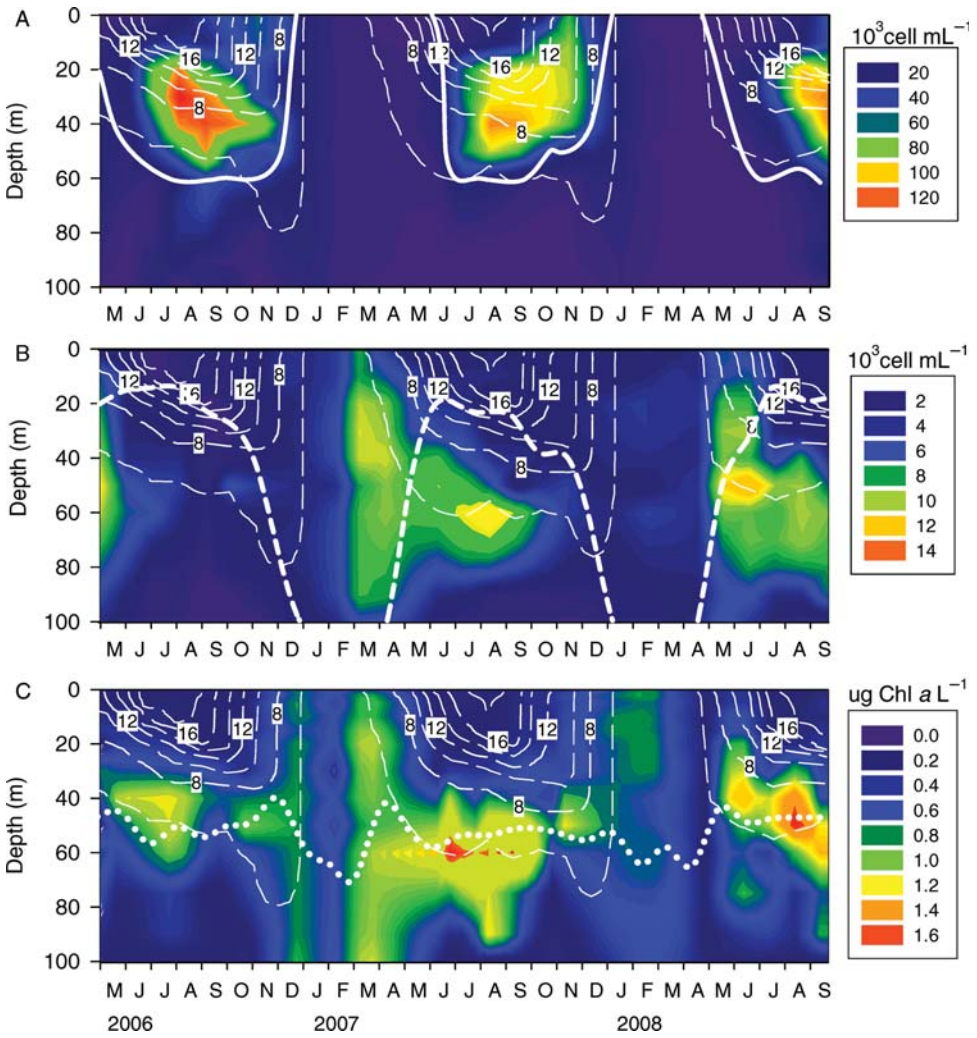

Fig. 4. Temporal vertical distribution of photosynthetic picoplankton abundances and chlorophyll *a* concentration in Lake Tahoe between May 2006 and September 2008. (**A**) Picocyanobacteria overlaid with temperature contour lines (dashed lines) and nitrocline depth (solid line); (**B**) picoeukaryotes overlaid with temperature contour lines (dashed lines) and thermocline depth (heavy dashed line); (**C**) chlorophyll *a* concentration overlaid with temperature contour lines (dashed lines) and euphotic depth (dotted line) overlaid.

irradiance (Fig. 5C); below this critical depth, picocyanobacteria increased significantly under ambient nutrient conditions and UVR did not affect their growth. Picocyanobacteria maintained constant cell densities at depths below 8 m with nutrient enrichment under both light treatments, suggesting that nutrient addition did not stimulate their growth. Overall picocyanobacteria showed a significant nutrient effect, whereas the effect of UVR was not significant (Table III). In contrast, nutrient addition stimulated growth of picoeukaryotes and cell abundances increased in both light treatments with nutrient addition (Fig. 5B and Table III). Similarly, at ambient nutrient levels, picoeukaryote densities increased under both light treatments, although the increase was less pronounced than with nutrient enrichment, indicating that picoeukaryotes are not sensitive to UVR and longer wavelength radiation.

DISCUSSION

The present study shows that separation of taxonomic picophytoplankton groups using flow cytometry provides a refined picture of the seasonal variation and the controlling factors for pro- and eukaryotic picophytoplankton cells. Recurrent seasonal patterns of picocyanobacteria and picoeukaryotes were observed in Lake Tahoe and these functional taxa showed opposing responses to environmental gradients, resulting in clear temporal and seasonal niche partitioning. Some

patterns of these smallest photosynthetic organisms in Lake Tahoe differ from other freshwater systems and showed striking similarities with the oceanic environment, suggesting that Lake Tahoe functions similar to open ocean ecosystems.

Picocyanobacteria largely outnumbered picoeukaryotes, which is consistent with other clear-water lakes (Pick and Agbeti, 1991); however, the latter population reached similar levels of carbon standing stock owing to their higher carbon content (Worden *et al.*, 2004). On average, PPIC contributed to more than a third and reached up to 56% of total carbon fixation during the stratification period. These measurements are in the range of previous observations showing that PPIC contributed between 30 and 60% to total PPr on a seasonal basis in Lake Tahoe (Lane, 1983). The highest contribution of PPIC to carbon uptake was during the stratified season (Lane, 1983), which is likely due to some combination of greater sedimentation and grazing of larger eukaryotic cells by the copepod-dominated zooplankton community, and higher growth efficiency of PPIC in nutrient-depleted environments (Raven, 1998). In general, the range of PPIC cell densities and contribution to carbon fixation in Lake Tahoe is similar to values observed in other oligotrophic lakes (e.g. Lake Huron, Michigan, Ontario, Baikal, Maggiore, Mondsee, Fahnenstiel and Carrick, 1992; Crosbie *et al.*, 2003; Katano *et al.*, 2005; Callieri *et al.*, 2007a) and the oceans (Blanchot *et al.*, 2001; DuRand *et al.*, 2001; Worden *et al.*, 2004).

Table I: Analysis of variance of the most parsimonious model for predicting seasonal abundance dynamics of picocyanobacteria in Lake Tahoe between May 2006 and September 2008

Predictor	Value	*t*	*P*-value
Intercept	−0.59 ± 3.01	−0.19	0.85
Irradiance	−0.22 ± 0.28	0.79	0.43
Stratification	0.69 ± 0.28	2.41	0.02
Nitrogen (50 m for pico)	−0.38 ± 1.13	−2.84	0.01
Phosphorus	–	–	–
NP	–	–	–
Sine	0.49 ± 0.39	1.27	0.75
Cosine	1.25 ± 0.22	5.83	<0.001
AICc	30.27		

AICc, Akaike Information Criterion (AIC) corrected for small sample size (AIC_c) (Burnham and Anderson, 2002).

In contrast to picocyanobacteria, picoeukaryotes significantly increased with Chl *a*, and this size group showed similar seasonal and spatial patterns compared with total Chl *a* and thus larger eukaryotes. Further, nutrient addition stimulated picoeukaryote growth and their response is similar to that of larger eukaryotic algal cells (Reynolds, 2006). Picocyanobacteria, on the other hand, appeared competitively advantaged under nutrient-depleted conditions as was observed in the experimental treatment with nutrient addition and as has been reported in other studies (Schallenberg and Burns, 2001). Suppressed growth of these unicellular organisms under elevated nutrient concentration may be attributable to reduced light availability due to the increase in larger phytoplankton or possibly even to the potential increased viral activity and cell lysis (Callieri and Pinolini, 1995; Wilson *et al.*, 1996; Scanlan and Wilson, 1999). Phosphorus concentrations in Lake Tahoe are often below 1 g L^{-1}, which may arrest the lytic cycle of viruses in host cells (Wilson *et al.*, 1996) and may facilitate the growth of picocyanobacteria in the nutrient-depleted upper water layers. In addition, *Synechococcus* has a high affinity for orthophosphate and higher uptake rates than eukaryotic algae, which can explain their abundance in nutrient-depleted environments (Lavallee and Pick, 2002; Moutin *et al.*, 2002). This indicates that species-specific responses of the PPIC community are expected with changes in the nutrient environment.

The photosynthetic community showed strong seasonal niche differentiation among taxonomic groups in Lake Tahoe. Picocyanobacteria and picoeukaryotes

Table II: Size-fractionated primary production rate (PPr), chlorophyll a *(Chl* a*) concentration and Chl* a*-normalized primary production during 2007 in Lake Tahoe integrated over the upper 60 m water column*

		PPr (%)		Chlorophyll *a* (μg m^{-2})		Assimilation rate (mg C Chl a^{-1} h^{-1})	
Date	Total PPr (mm C m^{-2} h^{-1})	<3 μm	>3 μm	<3 μm	>3 μm	<3 μm	>3 μm
16 July 2007	35.64	36.29	63.71	7.86	8.45	1.64	2.69
30 August 2007	40.41	35.93	64.07	7.74	10.08	1.88	2.57
16 November 2007	32.51	55.88	44.12	na	7.76	na	1.85

Values are shown for picophytoplankton (<3 m) and microphytoplankton (>3 m), respectively (na, not available).

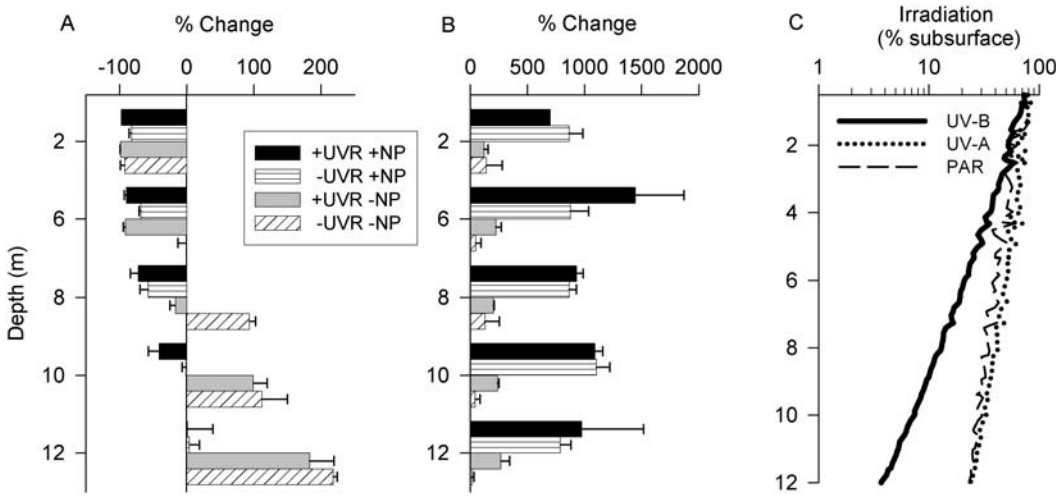

Fig. 5. Changes in cell densities along a depth gradient of (**A**) picocyanobacteria and (**B**) picoeukaryotes in response to light and nutrient variation (see methods) and (**C**) ambient irradiance of UV-B (320 nm), UV-A (380 nm) and PAR during the incubation experiment. +UVR indicates full sunlight spectrum, −UVR excluding UVR, +NP nutrient enrichment with nitrate and phosphorus, −NP ambient nutrient concentration. Error bars indicate standard deviations.

Table III: Linear regression analysis of the effect of UV radiation (UVR) and nutrient addition (NP) on picocyanobacteria and picoeukaryote growth (the model is %Change=b Depth+a)

	Picocyanobacteria				Picoeukaryotes			
Treatment	Slope *b*	Intercept *a*	R^2	*P*-value	Slope *b*	Intercept *a*	R^2	*P*-value
+UVR +NP	9.6 ± 2.5[a]	−132.5	0.83	0.031	28.9 ± 23.3[a]	866.1	0.34	0.30
+UVR −NP	29.2 ± 7.4[b]	−207.2	0.84	0.029	15.0 ± 3.5[a]	130.0	0.86	0.02
−UVR +NP	9.4 ± 2.4[a]	−112.7	0.84	0.028	26.7 ± 19.6[a]	867.7	0.48	0.31
−UVR −NP	29.2 ± 4.3[b]	−160.5	0.94	0.006	28.5 ± 14.0[a]	66.2	0.58	0.14

Different lowercase letters indicate that slopes are statistically different.

were temporarily inversely correlated ($r = -0.49$, $P = 0.006$, $n = 29$) and the former group peaked during the stratified season, which is typical for deep oligotrophic temperate lakes and oceanic environments (Pick and Agbeti, 1991; Stockner and Shortreed, 1991; Crosbie *et al.*, 2003; Callieri *et al.*, 2007a). In some lakes, a spring peak has been reported for picocyanobacteria (Postius and Ernst, 1999) but does not seem to be consistent across systems (Weisse, 1993). The seasonal dynamics of picocyanobacteria in Lake Tahoe was best explained by the strength of stratification and nitrogen concentration, and confirms the importance of physical and chemical conditions driving the seasonality of picocyanobacteria. Picoeukaryotes showed opposing seasonal dynamics and peaked in late winter to early spring during periods of mixing and isothermal conditions. Concentrations of this population peaked at or slightly prior to the timing of the spring peak of larger eukaryotes. The dominance of eukaryotic picoplankton during isothermal conditions is consistent with some deep oligotrophic lakes and marine upwelling zones (e.g. lakes in Ontario, Sargasso Sea, Glover *et al.*, 1988; Pick and Agbeti, 1991). In other lakes, eukaryotic picoplankton typically peak in spring at the start of stratification onset (Fahnenstiel and Carrick, 1992; Padisak *et al.*, 1997; Crosbie *et al.*, 2003) and their cell abundances usually decrease to low values during the stratified season. In Lake Tahoe, both PPIC taxa maintained relatively high concentrations during the summer season and this pattern differed from that reported in Lake Constance and Mondsee where PPIC values dropped considerably during the clear-water phase as the result of grazing and/or reduced light availability (Weisse, 1993; Crosbie *et al.*, 2003). High PPIC densities,

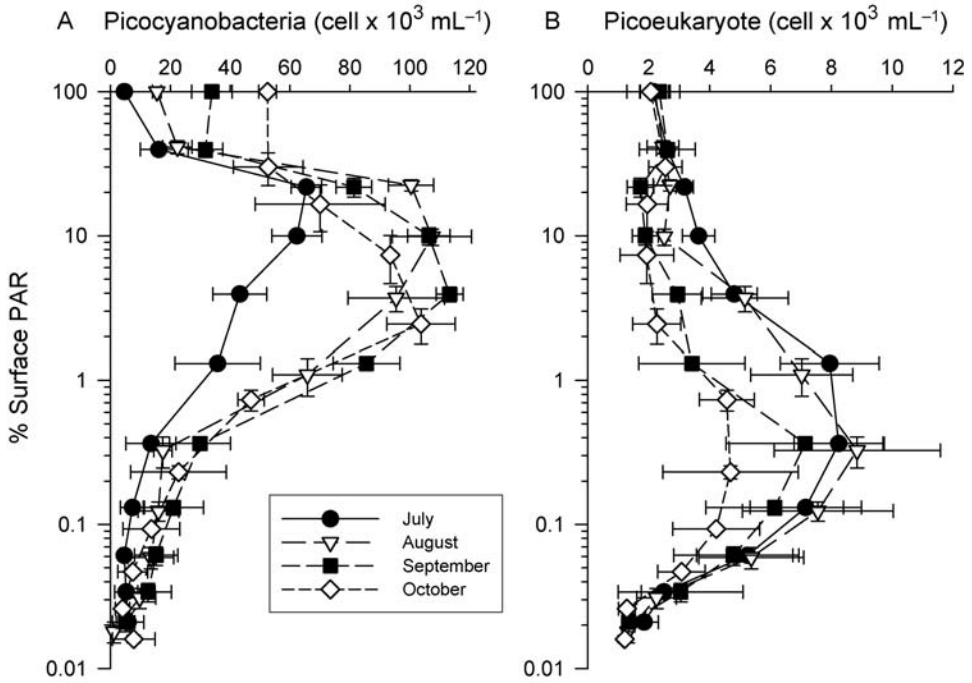

Fig. 6. Vertical monthly average profiles of (**A**) picocyanobacteria and (**B**) picoeukaryotes during the stratified period against the light gradient as % of surface PAR in Lake Tahoe between 2006 and 2008. Error bars represent standard errors.

particularly of cyanobacteria throughout the summer season, in Lake Tahoe suggest low grazing impact, although this hypothesis remains to be tested.

In addition to nutrients, picocyanobacteria and picoeukaryotes showed differential sensitivity to solar radiation. Picocyanobacteria cell concentrations increased below 30% PAR (Fig. 6A) and the 6% UV-B isolume (preferred light level) relative to surface light levels. The *in situ* experiment supports the idea that these cells are exposed to lethal solar radiation in surface waters. Sensitivity of picocyanobacteria cells to UVR and high PAR radiation can be attributed to their small size and low concentration of photoprotective compounds (Llabres and Agusti, 2006), because cells smaller than 2 m cannot effectively use UVR-absorbing compounds as sunscreens owing to an unfavorable surface-to-volume ratio (Garcia-Pichel, 1994). The high light sensitivity of *Synechococcus* in Lake Tahoe contrasts with findings for *Synechococcus* in oceanic Atlantic communities, where they showed higher resistance to solar radiation compared with eukaryotes (Boelen *et al.*, 2002). These different responses reflect the variability in photoprotection capacity within this taxonomic group. Further, picocyanobacteria abundances decreased considerably below 1% PAR isolume (Fig. 6A) and thus below the nitrocline and deep-water chlorophyll maximum. In addition to the tolerance of *Synechococcus* for more nutrient-depleted conditions, their restriction to the upper water layer may also be the result of light limitation in deeper layers imposed by less efficient accessory pigments (Moore *et al.*, 1995).

In contrast, picoeukaryotes dominated in the vicinity of the nitrocline and euphotic depth during summer stratification and formed a deep-water maximum that overlapped with the chlorophyll maximum. Although densities of this functional group increased considerably below 1% surface PAR (Fig. 6B), the *in situ* experiment demonstrated that these cells are not sensitive to solar radiation in surface waters. This implies that eukaryotic cells take advantage of increased nutrient concentration in the vicinity of the nitrocline at greater depth. Additionally, grazing in surface waters may also be an important factor for their deep-water appearance during the summer season. The deep-water chlorophyll maximum in Lake Tahoe is formed largely by accumulation of phytoplankton cells caused by cell sinking from overlaying waters, *in situ* cell growth (Coon *et al.*, 1987; Carney *et al.*, 1988), while zooplankton grazing has a negligible effect (Cullen, 1982). The fact that PPIC contributed to about 50% to total carbon fixation at 60-m depth suggests that the deep-water picoeukaryote maximum is controlled by the same mechanisms and that these cells are efficient in harvesting light and adapted to low light conditions.

The vertical niche partitioning of the phototrophic picoplankton community in Lake Tahoe (prokaryote dominance between the 30 and 1% isolume, eukaryotes at the bottom of the euphotic depth zone) is consistent with patterns observed in other deep oligotrophic lakes, but also showed some striking differences that are more representative of marine environments. Clear vertical partitioning along a habitat gradient is typical for some deep transparent lakes (e.g. the Laurentian Great Lakes). For instance, a metalimnion peak of picocyanobacteria above the deep-water chlorophyll maximum was observed in Lake Huron and Lake Michigan during thermal stratification (Fahnenstiel and Carrick, 1992), although picocyanobacteria contributed to the deep-chlorophyll maximum. In contrast, picocyanobacteria increase with depth and dominate in the deep-chlorophyll maximum in deep transparent Andean-Patagonian lakes (Callieri *et al.*, 2007b). Similar to Lake Tahoe, in the oligotrophic tropical Pacific and Atlantic Ocean, picocyanobacteria (*Synechococcus* and *Prochlorococcus*) dominate in surface layers (Blanchot *et al.*, 2001; DuRand *et al.*, 2001; Moran *et al.*, 2004). In these ecosystems, *Prochlorococcus* often occupies the same vertical niche as *Synechococcus* does in Lake Tahoe, i.e. above the deep-chlorophyll maximum and the nitracline depth (e.g. Sargasso Sea, Bermuda and subtropical North Pacific at Station ALOHA, Campbell *et al.*, 1997; DuRand *et al.*, 2001), while *Synechococcus* typically dominates above the thermocline depth (Moore *et al.*, 1995).

Like picocyanobacteria, the picoeukaryote vertical distribution in Lake Tahoe is comparable with oceanic environments and likely reflects the similarity in nutrient gradients and spectral light penetration. For example, picoeukaryotes increase in deep-water layers in the oligotrophic oceans and contribute to the deep-water chlorophyll maximum (DuRand *et al.*, 2001; Moran *et al.*, 2004). Lake Tahoe and the oligotrophic ocean share similar physical characteristics, including high transparency, nutrient-depleted surface water layers, low grazing rates and periodic nutrient pulses from the hypolimnion. The fact that the freshwater and marine PPIC community occupies similar niches suggests that these organisms have similar ecophysiological requirements.

These findings demonstrate that changes in resource availability and the mixing regime also affect the smallest photosynthetic planktonic organisms. The relation between nutrient availability, light penetration and mixing depth are important mechanisms in determining the dominance of prokaryotic and eukaryotic picophytoplankton cells and the structure of this community. Taxon-specific sensitivity to light and nutrients acts as a dominant driver of the temporal and spatial PPIC dynamics particularly in clear-water oligotrophic systems. Given the high phylogenetic diversity of PPIC (Becker *et al.*, 2004; Fuller *et al.*, 2006) more refined patterns among picoeukaryotes and picocyanobacteria can be expected. Understanding the controlling mechanisms of this carbon source is a necessary step towards understanding carbon fluxes in freshwater systems.

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