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Photosynthetic picoplankton in Lake Tanganyika: biomass distribution patterns with depth, season and basin

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Photosynthetic picoplankton (PPP), particularly picocyanobacteria (PCya), are known to be a major component of phytoplankton in oligotrophic waters. We investigated the dynamics of PPP and heterotrophic bacteria (HBact) in Lake Tanganyika during the dry and rainy seasons of 2004 to 2007, in the two basins of this large lake. Flow cytometry analyses showed that PPP was mainly composed by PCya of the phycoerythrin-rich Synechococcus type, with maximal abundances (2.3×10^4–8.2×10^5 cells mL^{-1}) found in the mixolimnion (10–20 m depth). PPP biomass integrated over the water column depth ranged between 0.41 and 3.09 g C m^{-2}, with maximal values in the south basin during the dry season (2.28 ± 0.62 g C m^{-2} on average). The contribution of PPP to total phytoplankton biomass ranged from 41 to 99%, with highest values in the south basin in the dry season. Cellular measurements by image analysis of epifluorescence microscopy images showed a significant increase of the cell volume of the PCya during this period. Flow cytometry also allowed enumeration of photosynthetic picoeukaryotes. Assuming a conversion factor of 530 fg C $cell^{-1}$, they contributed on average to 6% to PPP biomass, except during the dry season in the south basin, where their contribution increased to up to 20% of PPP biomass. Integrated over a 100-m water column depth, PCya biomass was on average 1.4 times higher than HBact biomass. This study establishes reference values for the biomass contribution of this part of the microbial food web, covering for the first time, spatial (different sites), vertical (profiles at different depths) and seasonal variations on a multi-year basis. The results strengthen the view of a major role of PPP in the pelagic food web of large tropical Lake Tanganyika.

INTRODUCTION

Photosynthetic picoplankton (PPP) is typically defined as the fraction of phytoplankton whose size is comprised between 0.2 and 2 μm (Sieburth *et al.*, 1978), and its importance in marine and freshwater ecosystems was first appreciated in the 1970s (Paerl, 1977; Johnson and Sieburth, 1979; Waterbury *et al.*, 1979). PPP is now widely recognized as a major contributor to the carbon flow in both types of systems, especially in oligotrophic waters (Agawin *et al.*, 2000; Bell and Kalff, 2001). It is composed of picocyanobacteria (PCya) and eukaryotic phototrophs (Callieri, 2008). The genera *Prochlorococcus* (Vaulot *et al.*, 1990; Campbell *et al.*, 1994) and *Synechococcus* (Johnson and Sieburth, 1979; Waterbury *et al.*, 1979, Scanlan and West, 2002) dominate the PCya community in marine waters, where they can contribute the major fraction of primary production (Waterbury *et al.*, 1986; Partensky *et al.*, 1999).

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In freshwaters, the genus *Synechococcus* is the most commonly observed, although one single report of *Prochlorococcus*-like cells in a Spanish eutrophic reservoir exists (Corzo *et al.*, 1999).

Extensively studied in marine waters, PPP, particularly PCya, have also been widely investigated in freshwater systems (e.g. Bell and Kalff, 2001), including European lakes (e.g. Callieri and Piscia, 2002), North American Great Lakes (Fahnenstiel *et al.*, 1991; Ivanikova *et al.*, 2007), North Patagonian lakes (Callieri *et al.*, 2007) and oligotrophic Lake Baikal (Nagata *et al.*, 1994; Belykh and Sorokovikova, 2003). Widely distributed and different from each other, all these systems are, however, situated in the temperate or high latitude zones. While one would expect to find data on high abundances of PCya in warm, oligotrophic waters, conditions known to favour dominance by PPP (Agawin *et al.*, 2000), records from tropical lakes are quite scarce (but see Sarmento *et al.*, 2008).

Located in East Africa, Lake Tanganyika is a permanently stratified, large oligotrophic and meromictic lake, composed of two deep basins. As in other tropical lakes, seasonality, in the form of a rainy and a dry season, is one of the main sources of variability in the ecology of the lake. The depth of the mixed layer varies with season and location. Particularly, during the dry season (May–September) when south-east winds are blowing, deeper vertical mixing occurs in the oxic layer, and an upwelling takes place at the southern end (Coulter, 1991; Plisnier *et al.*, 1999). Then, vertical transport brings up nutrient-rich waters from below the thermocline (Coulter, 1991). The first record of PPP from Lake Tanganyika was from a survey by Vuorio *et al.* (Vuorio *et al.*, 2003), who detected PCya using epifluorescence microscopy and assessed their abundance in a few samples. Descy *et al.* (Descy *et al.*, 2005) and Pirlot *et al.* (Pirlot *et al.*, 2005), using epifluorescence microscopy counts and pigment analyses, confirmed that PCya were important contributors to Chl *a* biomass, and provided evidence of contrasting distribution between the two main lake basins: while cyanobacteria having the *Synechococcus* pigment type (Jeffrey *et al.*, 1997) were the second most abundant phytoplankton group in the northern basin of Lake Tanganyika, they were largely dominant in the southern basin of the lake. According to the previous work on the ecology of East African lakes (Hecky and Kling, 1987), this PCya dominance in the lake's southern basin was quite unexpected: this basin has a weaker temperature density gradient and experiences upwelling, with deeper mixing and higher nutrient availability; conditions which are known to favour diatoms over other phytoplankton (Reynolds, 1984, 1989). Worth mentioning is the identification of Lake Tanganyika PCya as *Synechococcus*, confirmed by Stenuite (Stenuite, 2009), who used 16S DNA cloning and sequencing of samples collected at various locations at different times, and found that PCya of Lake Tanganyika were closely related to two groups of *Synechococcus*, without apparent differences.

Recently, Sarmento *et al.* (Sarmento *et al.*, 2008) used flow cytometry and epifluorescence microscopy to collect information on picoplankton abundance and distribution in another large East African Rift lake, Lake Kivu. In this paper, a broad comparison of PPP abundance among various marine and freshwater bodies was made, which showed exceptionally high *Synechococcus* cell numbers per unit area in large, oligotrophic tropical lakes, even higher than in oceanic waters. The first available data from Lake Tanganyika showed *Synechococcus* maximal abundance slightly greater than 3000 10^{10} cells m^{-2}, i.e. the highest figure in this comparison across aquatic systems. Lake Kivu PPP numbers in the range of 0.05–0.2 10^6 cells mL^{-1} and lower maximal water column abundance, with 630×10^{10} cells m^{-2} (Sarmento *et al.*, 2008).

As in marine systems, heterotrophic bacteria (HBact) are an important component of picoplankton in large tropical lakes. The first data on Lake Tanganyika HBact biomass were from Hecky and Kling (Hecky and Kling, 1981), who reported figures similar to those of Pirlot *et al.* (Pirlot *et al.*, 2005) who used epifluorescence microscopy to estimate HBact numbers and biomass in Lake Tanganyika. The range of HBact abundance in this study was 2.3–5.3 10^6 cells mL^{-1}, and the biomass range in the oxic layer was 1.65–2.27 g C m^{-2}, i.e. in the same order of magnitude as that of total phytoplankton, including PPP. PCya were not estimated directly in that study, however, but indirectly from biomass of cyanobacteria having the *Synechococcus* pigment type. Sarmento *et al.* (Sarmento *et al.*, 2008) used enumeration of HBact of Lake Kivu by flow cytometry, and reported bacterial numbers in this Rift lake between 1 and 2×10^6 cells mL^{-1}, i.e. slightly lower than in Lake Tanganyika. It is also worth mentioning that Sarmento *et al.* (Sarmento *et al.*, 2008) demonstrated a significant correlation between high nucleic acid bacteria (HNA, assumed to be "active" bacteria) and phytoplankton biomass in Lake Tanganyika and Lake Kivu, suggesting a coupling between actively growing HBact and primary producers in these two tropical lakes.

While being less concentrated than PCya, photosynthetic picoeucaryotes (PicoEuk) in the ocean can be dominant within the PPP in terms of total production (Worden, 2004). Little is known of these organisms in large tropical lakes. They were detected in the

cytograms from Lake Kivu (see Sarmento *et al.*, 2008, Fig. 2), where they were present in abundances ranging from 1 to 8 10^3 cells mL^{-1}.

Here, we focus on the PPP of Lake Tanganyika, using epifluorescence microscopy and flow cytometry, and we expand the analysis of the significance of PPP in the lake by presenting accurate estimates of their numbers and biomass. The main objectives of this study are to investigate further the PPP response to seasonal changes in the two major basins and to address the question as to why PCya have such a contrasted distribution in this large tropical lake.

METHOD

Sample collection

Samples were collected at two pelagic sites in Lake Tanganyika (Fig. 1): off Kigoma (north basin, Tanzania) and off Mpulungu (south basin, Zambia), during seven cruises: three in the rainy season (26 January to 17 February 2005, 30 January to 17 February 2006, and 24 February to 9 March 2007) and four in the dry season (24 August 2004 to 9 September 2004, 23 July to 9 August 2005, 11 July to 15 August 2006, and 19 to 21 September 2007). Samples were collected using a Niskin 5 L bottle every 10 m from the surface to a depth of 100 m, which comprises most of the oxygenated layer. Over the whole study, 11 vertical profiles were sampled during the wet season (3 in the north basin and 8 in the south basin), and 20 vertical profiles were sampled during the dry season (5 in the north basin and 15 in the south basin).

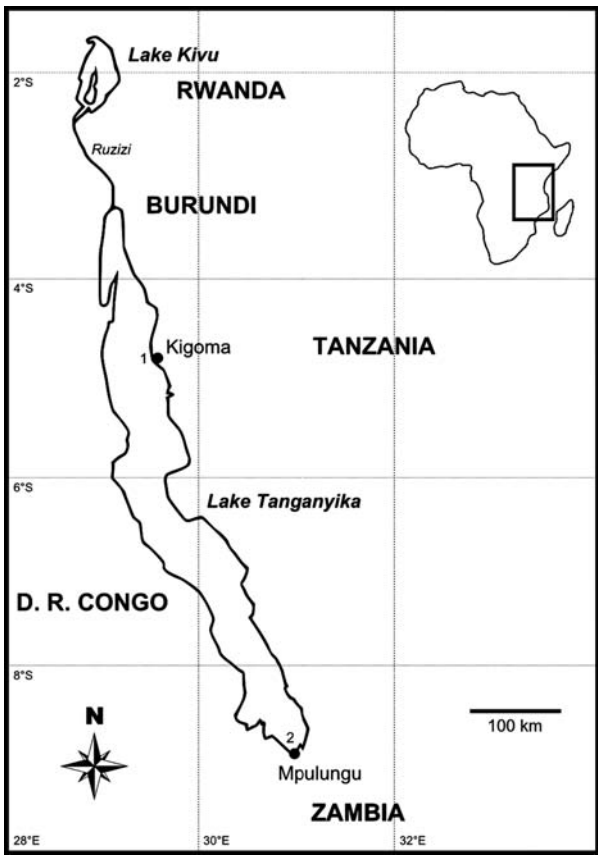

Fig. 1. Map of Lake Tanganyika, with location of the sampled stations (1: Kigoma; 2: Mpulungu).

Limnological variables

Vertical profiles were obtained using a CTD probe (Hydrolab DS4a) and euphotic depth measurements (1% of the subsurface light) were obtained by measuring the PAR downward attenuation with a LICOR LI-1400 quantum sensor. Nutrient analyses were carried out using standard spectrophotometric techniques (A.P.H.A., 1992), or Macherey-Nägel® analytical kits (Düren, Germany).

Pigment analysis

Chlorophylls and carotenoid pigments were quantified by HPLC analysis of all water column samples, using the Wright *et al.* (Wright *et al.*, 1991) gradient elution method after extraction in 90% HPLC grade acetone, with a HPLC system comprising a Waters 600E multisolvent delivery system, a Waters 996 PDA detector and a Waters 470 fluorescence detector. Calibration was made using commercial external standards (DHI, Denmark). A more detailed description of the procedure, including processing with CHEMTAX for estimating biomass of phytoplankton groups, can be found in Descy *et al.* (Descy *et al.*, 2000, 2005). CHEMTAX is a matrix factorization program developed by Mackey *et al.* (Mackey *et al.*, 1996), in order to estimate biomass (in Chl *a* units) of algal classes from concentrations of marker pigments determined by HPLC analysis of water column samples. Phytoplankton groups quantified by the pigment method were chlorophytes, diatoms and chrysophytes, cryptophytes, photosynthetic dinoflagellates and two cyanobacteria pigment types: cyanobacteria T1 (type 1, *Synechococcus* pigment type, Jeffrey *et al.*, 1997), and cyanobacteria T2, corresponding mostly to filamentous taxa with heterocytes (Descy *et al.*, 2005). We considered that cyanobacteria T1 corresponded to PCya, as Chl *a* of cyanobacteria T1 correlated significantly (Pearson's $r = 0.42$, $P < 0.001$, $n = 63$, data not shown) with *Synechococcus* abundance determined by flow

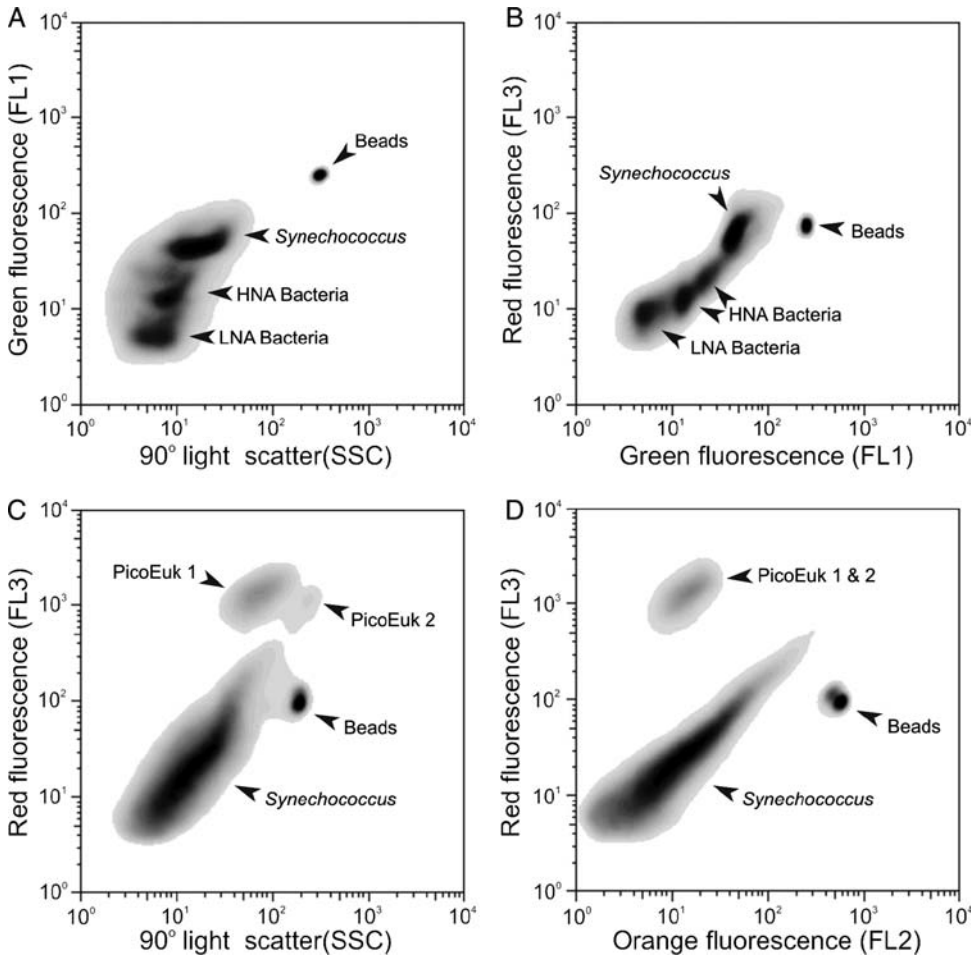

Fig. 2. Examples of cytograms (density plots obtained by flow cytometry) of Lake Tanganyika water samples. (**A** and **B**) The Syto-13 stained picoplankton samples. Identification of the three populations (HNA, high nucleic acid bacteria; LNA, low nucleic acid bacteria; *Synechococcus* spp.) and the polysciences 1 μm beads. (**C** and **D**) Unstained samples showing the red and orange autofluorescence of the three picophytoplankton groups: *Synechococcus* spp., small picoeukaryotes (PicoEuk1) and larger picoeukaryotes (PicoEuk2).

cytometry. Estimations of the contribution of PPP to total Chl *a* biomass in the euphotic zone were also obtained by filter-fractionation: the fraction passing through a 2-μm polycarbonate filter (Poretics, Osmonic) was assumed to be the picoplanktonic fraction.

Picoplankton enumeration and biomass estimation

Abundance and biomass of PCya, HBact and picoeukaryotes were estimated in all water column samples. Heterotrophic and PPP abundance was determined by flow cytometry. Biomass was estimated by combining flow cytometry cell numbers and estimates of cell carbon from cell biovolume measurements by epifluorescence microscopy. Results are expressed per unit water volume or per unit area of the water column, by integrating over a 100-m depth.

Picoplankton was enumerated with a FACSCalibur (Becton Dickinson) flow cytometer equipped with a 15 mW Argon-ion laser (488 nm emission). Four millilitres of water were collected and fixed immediately with cold glutaraldehyde 10% (final concentration 1%), left in the dark for 10 min at room temperature and stored at −20°C, usually for maximum 2 weeks, before being transferred to Belgium and stored at −80°C until analysis. Individual samples were divided in two subsamples for counts of HBact and PPP. To count HBact, 400 μL of sample was stained with a diluted SYTO-13 (Molecular Probes Inc., Eugene, OR, USA) stock (10:1) at 2.5 μmol L^{-1} final concentration, left for ∼10 min in the dark to complete the staining, and run in a flow cytometer. At least 30 000 events were acquired for each subsample. Fluorescent beads (1 μm, Fluoresbrite carboxylate yellow-green microspheres, Polysciences Inc., Warrington, PA, USA) were added at a known

density as internal standards. The bead standard concentration was determined by epifluorescence microscopy. HBact were detected by their signature in a plot of side scatter (SSC) versus FL1 (green fluorescence) and were separated into two populations (Fig. 2): HNA (high nucleic acid content) and LNA (low nucleic-acid content) bacteria in the SCC versus FL1 plot (Gasol and del Giorgio, 2000). For PPP, we ran the sample without stain. We identified three different populations of picophotosynthetic organisms (Fig. 2): one PCya (*Synechococcus*-like) and two picoeukaryotes (PicoEuk 1 and PicoEuk 2) in plots of SSC versus FL3 (red fluorescence), and FL2 (orange fluorescence) versus FL3. Data acquisition and analysis were performed with the software CellQuest and Paint-A-Gate (Becton Dickinson).

Biovolumes of photosynthetic and heterotrophic picoplankton were measured in most samples using an epifluorescence microscope Zeiss Axioplan (Carl Zeiss MicroImaging GmbH, Göttingen, Germany), at $\times 1000$ magnification (samples which did not show a homogenous distribution on the filter were discarded). Ten millilitre of water sample was filtered onto a 0.2-μm pore-size black membrane filter, and cells from 10 randomly chosen fields were enumerated. HBact stained with DAPI (4,6 diamidino-2-phenylindole; 10 μg mL^{-1}, final concentration) were measured under UV-wavelength excitation (G365, FT395, LP420) and their biovolume (V) was calculated assuming a sphere. The relationship $Cbact = 92 \times V^{0.598}$, determined from the data of Simon and Azam (Simon and Azam, 1989), gave an average cell carbon content of 15.0 ± 3.8 fg C $cell^{-1}$ (Stenuite *et al.*, 2009) which was used to calculate bacterial biomass from HBact abundance data.

Combining green (BP546/12, FT580, LP590) and blue-wavelength excitation (BP450 to BP490, FT510, LP520), PCya were measured and their biovolume estimated by image analysis (using Zeiss KS 300 3.0 software) considering them as prolate spheroids (Sun and Liu, 2003). Assuming a conversion factor of 230 fg C μm^{-3} (Worden, 2004), average PCya cell carbon contents were calculated. These values were then used as conversion factors, to allow biomass estimation when epifluorescence microscopy measurements were not performed.

No systematic epifluorescence microscopy observations of picoeukaryotes were performed, so we used a conversion factor of 530 fg C $cell^{-1}$ (Worden, 2004) to determine their biomass. This value is in the low side of the range of values used in the literature to calculate picoeukaryote biomasses (Grob *et al.*, 2007).

RESULTS

During the period studied, the depth of the mixed layer (Zm) was in the range of values usually found in the Lake (Descy *et al.*, 2005), with lower values during the wet seasons, and increasing depth during the dry seasons, especially in the south basin (Fig. 3). Seasonal patterns are also clear in the euphotic depths which are greater during the wet seasons, with values of 39 and

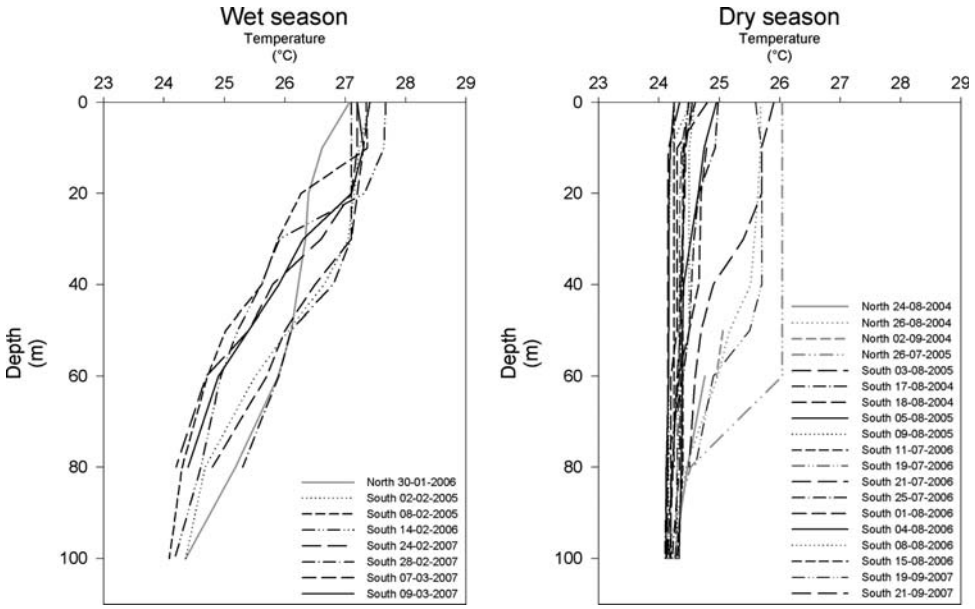

Fig. 3. Vertical profiles of water temperature (°C), during the wet and dry seasons, in the north and south basins of Lake Tanganyika.

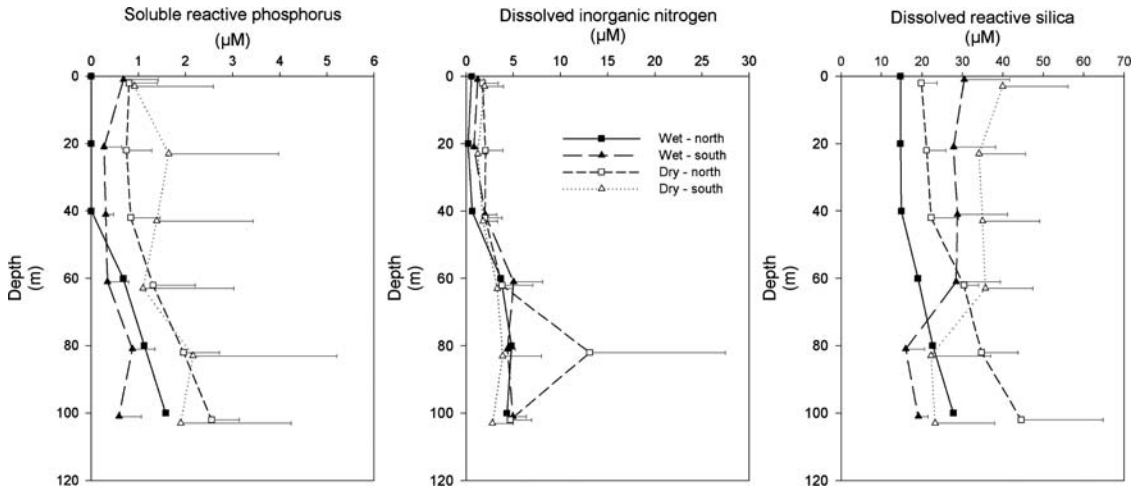

Fig. 4. Average concentration (μM) of soluble reactive phosphorus, dissolved inorganic nitrogen and dissolved reactive silica at 0, 20, 40, 60, 80 and 100 m depth during the wet and dry seasons, in the north and south basins of Lake Tanganyika, with standard deviations (for readability, only the positive standard deviations are shown, and depth values are slightly shifted forward).

43 m in the north and south basins, respectively. Values found during the dry season were lower, with 37 and 26 m, respectively, in the north and south basins. Values of the mixed layer to euphotic depth ratio (Zm:Zeu) were also seasonally different, with 0.5 and 0.6 for the north and south basins during the wet season, but with values of 2.3 and 3.7 during the dry season, respectively, in the north and the south basins. Average water temperatures in the euphotic zone ranged from 24.6 to 26.8°C, with lower values during the dry season, and average pH was in the range 8.1–8.7. Nutrient concentrations were always low, sometimes below the detection limits of the methods used (Fig. 4). When considering all data collected in the period 2002–2006 (Descy *et al.*, 2006), it is apparent that macronutrient availability varies with site and season in Lake Tanganyika. SRP in the euphotic zone was significantly different ($P < 0.01$, Student's *t*-test) at both sites between seasons, with an average concentration of 0.48–0.55 μM in the dry season versus 0.19 μM in the rainy season, with often concentrations below the detection limit (~0.03 μM in our analytical conditions). DIN was low off Kigoma at all times (average ~2.14 μM whatever the season), but was higher in the southern basin than in the northern basin ($P < 0.01$); DIN varied seasonally off Mpulungu, with an average ~3.07 μM in the wet season versus 4.07 μM in the dry season ($P < 0.005$).

Average Chl *a* concentrations in the euphotic zone in the northern basin were 0.86 ± 0.57 μg L^{-1} during the wet season and 0.82 ± 0.16 μg L^{-1} during the dry season. In the southern basin, Chl *a* concentrations were 0.95 ± 1.34 and 1.25 ± 0.46 μg L^{-1}, during the wet and dry seasons, respectively. When integrated over a 100-m water column, differences were more contrasting between seasons and stations. Average levels of Chl *a* were 48.8 ± 16.6 (wet season) and 56.4 ± 13.4 mg m^{-2} (dry season), in the north basin. In the south basin, Chl *a* biomass was on average 51.6 ± 36.3 and 85.9 ± 28.7 mg m^{-2} for the wet and dry seasons, respectively. Results from HPLC pigment analysis showed that chlorophytes and cyanobacteria T1 (i.e. *Synechococcus*-like) were the main contributors to Chl *a* concentration in the north basin, whatever the season. In the south basin, chlorophyte biomass was always much lower than in the north and cyanobacteria T1 were always dominant, especially during the dry season (Fig. 5). The average contribution of PPP to total Chl *a* concentration, estimated by size-fractionation during the

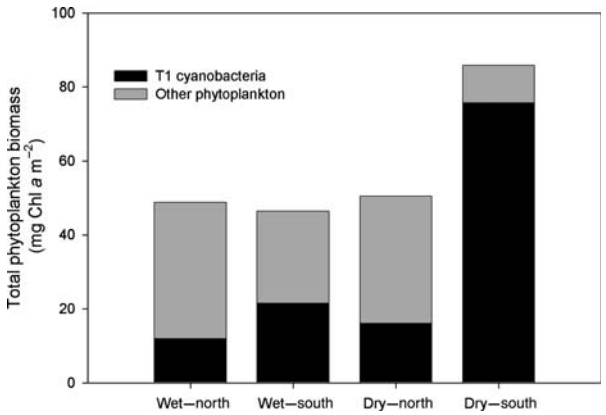

Fig. 5. Average total phytoplankton biomass (mg Chl *a* m^{-2}), integrated over a 100-m water column, during the wet and dry seasons, in the north and south basins of Lake Tanganyika.

wet season was 41% in the north basin and 65% in the south basin. These values were, on average, 57 and 84% during the dry season, in the north and south basins, respectively.

The images obtained by flow cytometry allowed the identification of three different populations of PPP: one population of phycoerythrin-rich PCya and two populations of cells larger than PCya, without phycoerythrin, identified as picoeukaryotes. SYTO-13 stained samples showed two populations of HBact, the HNA (high nucleic acid) and LNA (low nucleic acid) bacteria, but also the PCya, which appeared clearly as a peak different from that of HBact (Fig. 2).

All identified PPP populations showed clear vertical trends (Figs 6 and 7). Highest PCya cell numbers were always observed in the euphotic zone (between 2.3×10^4 to 8.2×10^5 cells mL^{-1}) (Fig. 6). Maximum values were usually encountered at around 10–20 m depth. Higher abundances were recorded during the dry season in the southern basin. All vertical profiles showed considerable decreases of PCya below the thermocline (Fig. 6). Average picoeukaryote abundance in the euphotic zone was usually around 2×10^3 cells mL^{-1}, i.e. two orders of magnitude lower than PCya numbers. During the dry season in the south basin, picoeukaryote abundances in the euphotic zone ranged

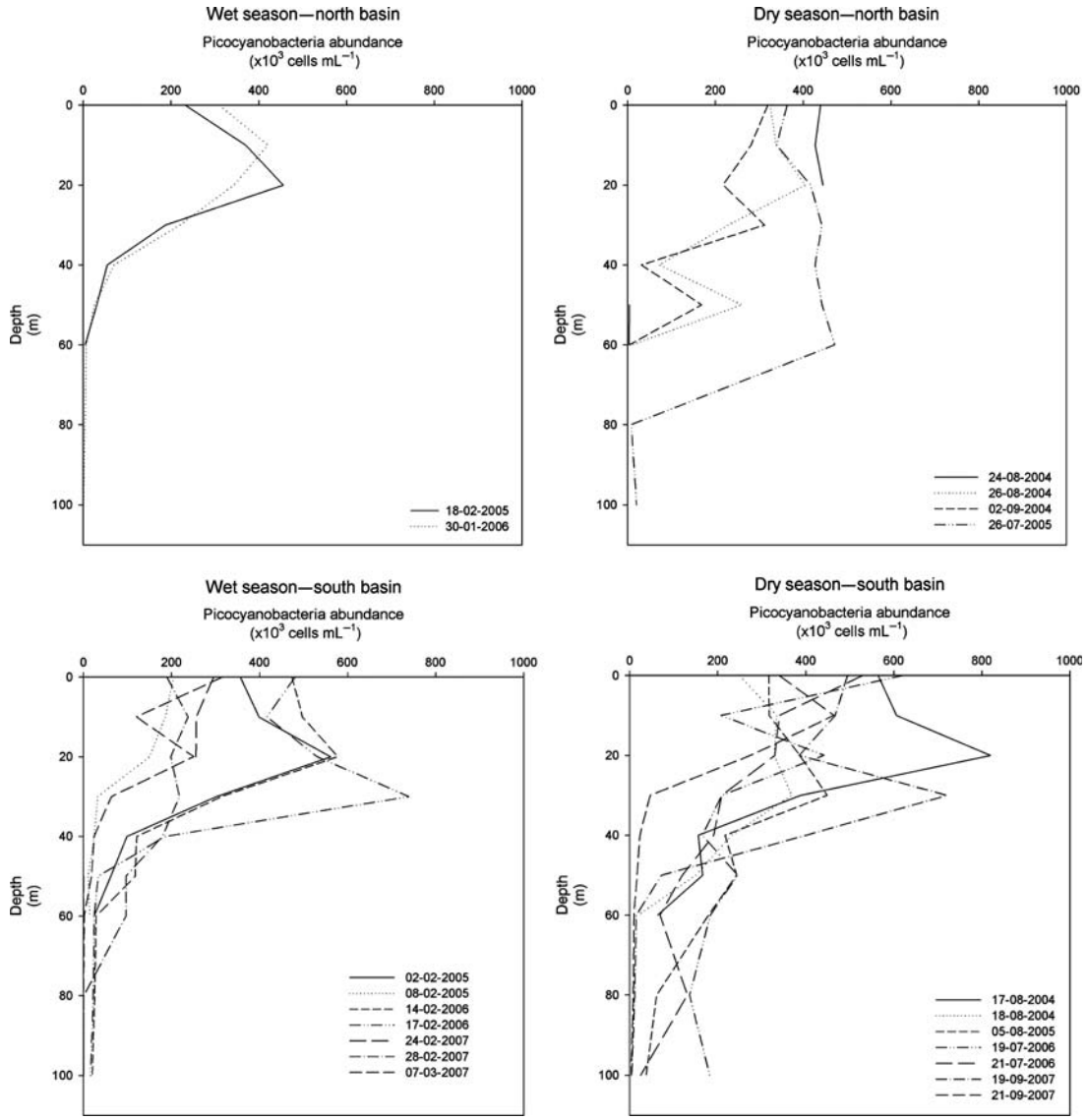

Fig. 6. Vertical profiles of picocyanobacteria abundances (cells mL^1), during the wet and dry seasons, in the north and south basins of Lake Tanganyika.

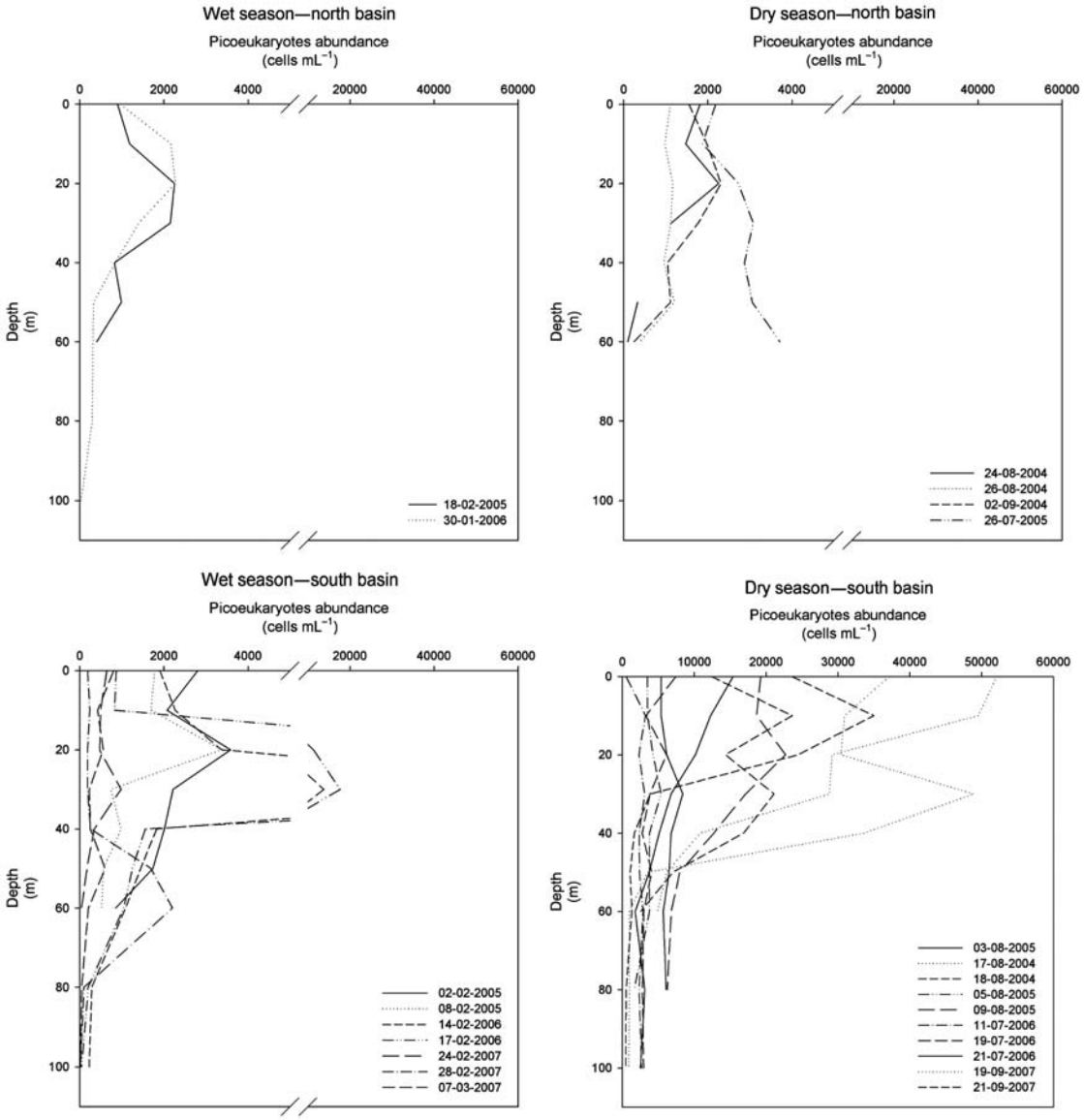

Fig. 7. Vertical profiles of picoeukaryotes (PicoEuk 1+2) abundances (cells mL^{-1}), during the wet and dry seasons, in the north and south basins of Lake Tanganyika.

from 500 to 5.2×10^4 cells mL^{-1} (Fig. 7). HBact abundances estimated by flow cytometry varied between 1.2×10^5 and 3.7×10^6 cells mL^{-1}, in the 0–100 m water column. The highest abundances were observed in the upper layers, while they slightly decreased towards the hypolimnion (Fig. 8).

In the 0–40 m layer, PCya had an average cell volume of 0.41 ± 0.05 μm^3 in the south basin, value significantly different (Mann–Whitney rank–sum test, $P < 0.001$) when compared with the values obtained in the wet season and in the north basin during the wet and dry seasons (0.31 ± 0.06 μm^3 on average). Average PCya cell carbon contents were, then, set to 93 fg C cell^{-1} for the south basin during the dry season, and to 71 fg C cell^{-1} for the other series.

Integrated over a 100-m water column depth, PCya biomass was 0.88 and 0.91 g C m^{-2} during the rainy season, for the north and south basins, respectively. Depth-integrated PCya biomass was higher during the dry season, with 1.24 and 1.82 g C m^{-2}, respectively (Fig. 9). Depth-integrated HBact biomass was similar during the wet season, with average values of 0.76 and 0.86 g C m^{-2}, for the north and south basins. In the dry season, average HBact biomass was 1.20 g C m^{-2}

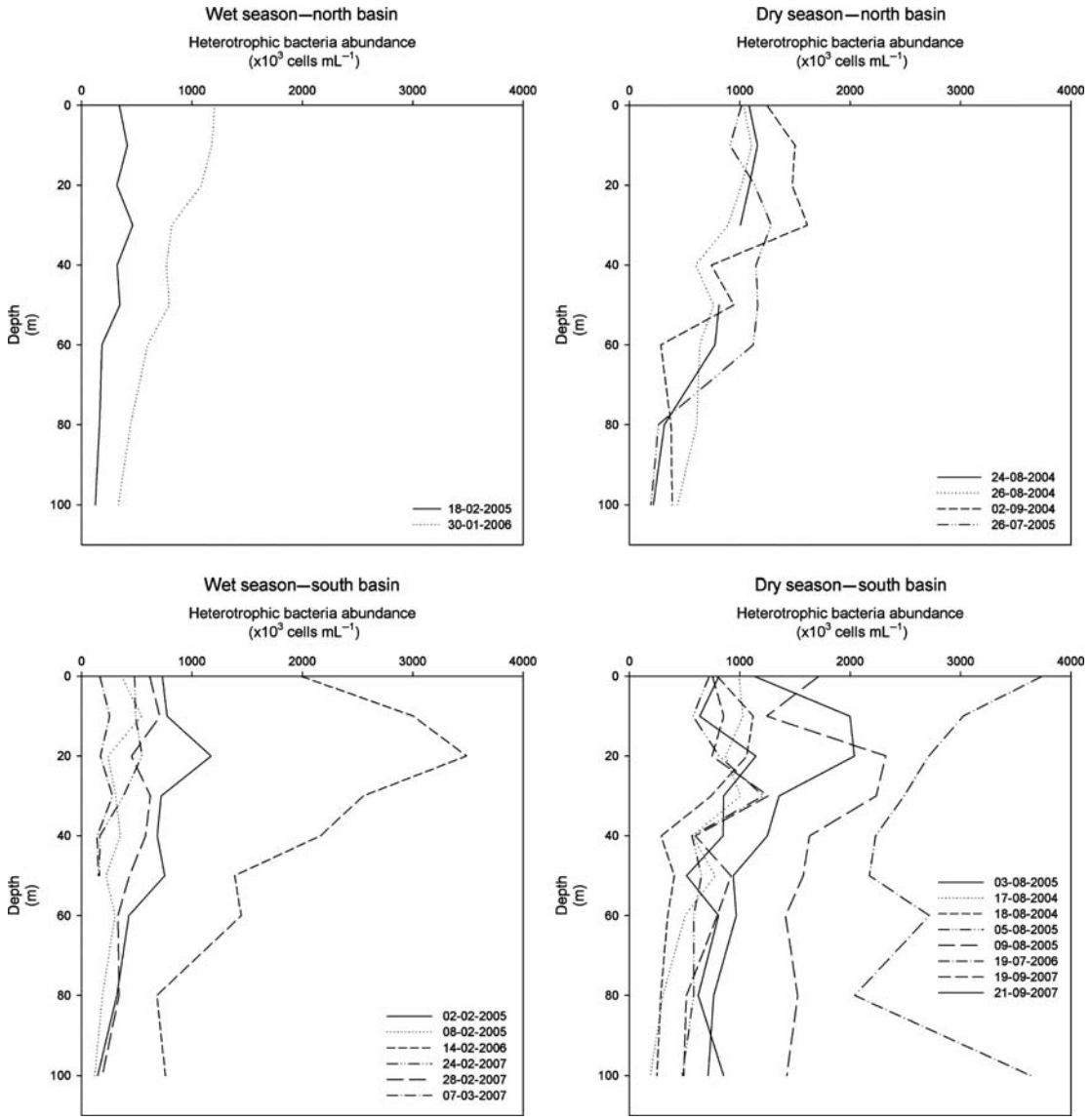

Fig. 8. Vertical profiles of heterotrophic bacteria abundance (cells mL^{-1}), during the wet and dry seasons, in the north and south basins of Lake Tanganyika.

in the north basin, and reached 1.65 g C m^{-2} in the south basin (Fig. 9). Compared with PCya and HBact biomass, picoeukaryote biomass was usually much lower, with an average value of 0.04 and 0.05 g C m^{-2} for the north basin during the wet and dry seasons, and 0.07 g C m^{-2} for the south basin during the wet season. However, a substantial increase of picoeukaryote biomass was noted during the dry season in the south basin, where values reached 0.41 g C m^{-2} on average (Fig. 9).

Considering all seasons and stations, and when integrated over a 100-m water column, PCya biomass was, on average, 1.4 times higher than HBact biomass.

DISCUSSION

Several reasons have been proposed to explain the high abundance, ubiquitous distribution of PPP and their dominance in oligotrophic systems. Among them, their small size, which provides high surface-to-volume ratios (Raven, 1986), enhances the molecular diffusion of nutrients (Chisholm, 1992) and gives PPP an advantage in nutrient uptake, helping them outcompete larger phytoplankton (Søndergaard, 1990; Agawin *et al.*, 2000; Bell and Kalff, 2001; Cotner and Biddanda, 2002), particularly under oligotrophic conditions (Weisse, 1993). Light conditions in the water column select the dominant pigment-type within the PCya assemblage:

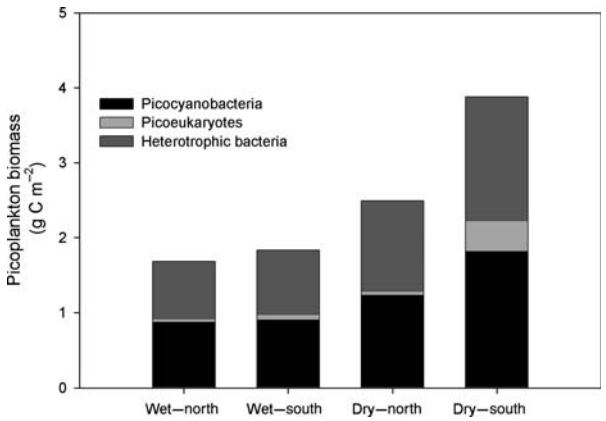

Fig. 9. Average picoplankton biomass (g C m^{-2}), integrated over a 100-m water column, during the wet and dry seasons, in the north and south basins of Lake Tanganyika.

phycocyanin-rich cells are usually found under low-light regimes, while phycoerythrin-rich cells are more typical of more transparent waters (Voros *et al.*, 1998; Stomp *et al.*, 2007). PCya also exhibit remarkable capacity for acclimation to varying light regimes, allowing growth either under high light (Loënneborg *et al.*, 1985; Kana and Glibert, 1987; Postius *et al.*, 1998), or under low light conditions, by rapidly increasing their specific chlorophyll *a* (Chl *a*) and phycobilin content (Moore *et al.*, 1995).

According to our fractionated filtrations, PPP contributed on average to more than 70% of total Chl *a* biomass, with contributions of up to 99% during the dry season in the south basin. Compared with previous estimations by Descy *et al.* (Descy *et al.*, 2005), our figures show that PPP is a dominant fraction of phytoplankton at all times in the lake. Indeed, we took into account values found during the dry season, while the filter-fractionations pigment analyses reported by Descy *et al.* (Descy *et al.*, 2005) were performed only in the south basin and during the wet season. An important contribution of PPP to total phytoplankton is typical of oligotrophic freshwater ecosystems, where PPP usually account for more than half of total phytoplankton biomass (Voros *et al.*, 1998; Bell and Kalff, 2001). Nevertheless, in contrast to temperate oligotrophic lakes, in tropical oligotrophic lakes such as Lake Tanganyika, the abundance of PPP, especially the PCya, remained high throughout the year. There were, however, significant seasonal variations in PCya biomass, which was significantly higher during the dry season (Mann–Whitney rank-sum test, $P < 0.001$) in both basins.

PCya concentrations were especially high in the euphotic zone (between 2.3×10^4 and 8.2×10^5 cells mL^{-1}), with maximum values generally observed at a depth of 20 m. PCya may be well adapted to the high light conditions which prevail at all times in the surface waters of tropical, clear lakes. In a typical rainy season situation in Lake Tanganyika, with a thermocline located at 20 m, phytoplankton in the mixed layer would be exposed to a mean irradiance of 700–800 μE m^{-2} s^{-1} around mid-day, and would experience irradiance >2000 μE m^{-2} s^{-1} in full sunlight near the surface. Thus, in the rainy season, acclimation of phytoplankton to high light is necessary. According to Descy *et al.* (Descy *et al.*, 2009), cyanobacteria T1 in Lake Tanganyika showed the highest zeaxanthin:Chl *a* ratio among a series of freshwater bodies. Similar high photoprotective carotenoid content in small planktonic cyanobacteria was found in Lake Baikal surface water by Fietz and Nicklish (Fietz and Nicklish, 2004). In marine oligotrophic waters, the zeaxanthin:Chl *a* ratio in picoplankton may be even higher in surface waters, and decrease with depth, reflecting acclimation of PCya to lower light exposure, with corresponding decrease in the amount of photoprotective pigment and increase of photosynthetic pigments (Higgins *et al.*, 2006). As a support to this fact, in Lake Tanganyika, the average red (FL3) standardized cytometric fluorescence signal, which indicates specific chlorophyll content, increased with depth (Fig. 10). In the south basin, the FL3 signals were higher during the dry season than the wet season (Fig. 10). A similar cellular Chl *a* and phycobilin increase with decreasing light intensities is well known for oceanic PCya (e.g. Moore *et al.*, 1995).

The conditions prevailing in the dry season are deeper vertical mixing driven by the SE trade winds. In the southern part of the lake, deeper mixing than in the north occurs as a result of seasonal upwelling, exposing planktonic microorganisms to lower average light exposure (~150 μE m^{-2} s^{-1} in a 100 m mixed layer) but to a greater range of irradiance as phytoplankton cells circulate in the mixed layer, i.e. from darkness below the euphotic zone to high irradiance (~2000 μE m^{-2} s^{-1}) near the surface. These conditions, which may present some variability related to a variable intensity of vertical mixing, should favour photosynthetic organisms capable of photoacclimation to low light, such as PE-rich PPP, and which have at the same time a high level of photoprotective pigment for avoiding damage to the photosystems near the lake surface.

Relatively low macronutrient concentration may explain why PPP reach very high abundance in Lake Tanganyika. However, a paradoxical fact is that PPP reach higher abundance and biomass in the southern basin than in the northern basin. Similarly, PCya were more abundant in the dry season off Mpulungu, during upwelling (higher availability in DIN and SRP).

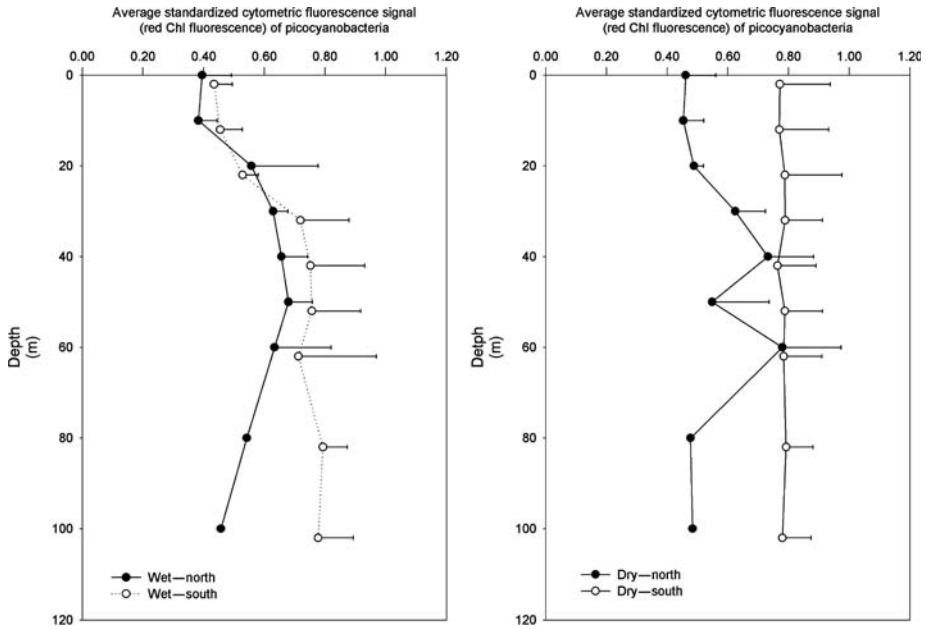

Fig. 10. Average standardized cytometric fluorescence signal (red Chl fluorescence) of picocyanobacteria, during the wet and dry seasons, in the north and south basins of Lake Tanganyika, with standard deviations (for readability, only the positive standard deviations are shown, and depth values are slightly shifted forward).

This clearly contrasts with the expectation that PCya would be more competitive at lower nutrient concentration. A possible explanation of this apparent paradox would be limitation of PPP growth by Fe availability. Indeed, De Wever *et al.* (De Wever *et al.*, 2008) conducted nutrient addition experiments on Lake Tanganyika phytoplankton and showed a high positive response of PCya to Fe addition. Therefore, one may assume that the high pH in Lake Tanganyika surface waters in the rainy season could reduce Fe bioavailability and PCya growth. An increase of PCya abundance during the dry season, especially in the south basin, would be related to the upwelling events that transport low-pH deep water to the surface, favouring Fe bioavailability. Supporting this hypothesis, an increase in concentration of Redox sensitive elements, including Mn and Fe, was observed off Mpulungu during the seasonal upwelling (Descy *et al.*, 2006).

In addition to these factors promoting PCya growth at the expense of other phytoplankton, one should bear in mind that grazers are able to exert a strong control on microorganism abundance and community composition (i.e. Calbet and Landry, 1999). In Lake Tanganyika, heterotrophic nanoflagellates (HNF), which are dominant grazers of bacteria in many aquatic ecosystems, exhibit their greater abundances during the dry season in the south basin (Pirlot *et al.*, 2005), and feed not only upon HBact, but also upon PCya (Unrein, unpublished data). More data are needed, however, to see to what extent PPP dynamics are influenced by microzooplankton in Lake Tanganyika.

Another remarkable and unusual fact is the importance of PCya biomass relative to HBact biomass in Lake Tanganyika. Whatever the station or season, the ratio PCya biomass to HBact biomass was never below 0.5 (min: 0.5; max: 2.8) in our data set. This pattern was already clearly visible in Fig. 2, when looking at the very high number of PCya cells stained with Syto-13. More studies are needed to test whether this pattern is specific to Lake Tanganyika or is widespread among other large tropical lakes, but the results found by Sarmento *et al.* (Sarmento *et al.*, 2008) seem to show that the relative importance of PCya compared with HBact is also high in Lake Kivu.

Picoeukaryotes were found at both stations and seasons, but with clear spatial and temporal patterns. Using a conversion factor of 530 fg C cell^{-1} (Worden, 2004), their biomass did not exceed, on average, 7% of the PPP biomass, except during the dry season in the south basin, where they accounted on average for 20% of the PPP biomass. However, these estimates rely entirely on the choice of C-to-volume conversion factor. A significant correlation (Spearman's $r = -0.9$, $P < 0.001$, $n = 12$) between average PCya:picoeukaryotes abundance ratio and average Chl *a* biomass was found, indicating that picoeukaryotes are more likely to be found during higher Chl *a* events. Increased nutrient concentrations are known to favour picoeukaryotes in

their competition with PCya (Callieri and Stockner, 2002). Modification of the light regime during the dry season, with an important increase of the Zm:Zeu ratio and, consequently, a decrease in the daily average dose of UV radiation in the water column, is also a factor which supports picoeukaryote development, since these organisms are known to be less resistant to high-energy radiation than PCya (Sommaruga *et al.*, 2005).

CONCLUSION

In summary, our study confirms the very high share of the picoplanktonic fraction in the phytoplankton of Lake Tanganyika. Despite they were less abundant, they represented a larger biomass than that of HBact. PCya were much more abundant than photosynthetic picoeucaryotes. Among the factors explaining high PPP abundance, high light in the transparent water column, combined with the depth of the mixed layer, may be a major factor selecting for those microorganisms capable of quick acclimation to a varying light climate. Here, macronutrients do not appear to play a major role, as PPP were more abundant in the richer water of the southern basin during upwelling events. However, experimental evidence from bioassays with Fe additions suggests that these events could bring up bioavailable Fe from the deep waters, which could favour PPP growth.

Evidence has accumulated for a recent decline of primary production in Lake Tanganyika (Stenuite *et al.*, 2007), probably linked to climate change (Verburg *et al.*, 2003). This decline has been related to surface waters warming and enhanced lake stratification, associated with a decrease in nutrient supply and increased transparency. Such changes did result in a significant shift in the phytoplankton assemblage of the lake (Descy and Sarmento, 2008), and could also have favoured PPP at the expense of larger phytoplankton. As in the oligotrophic zones of the oceans, high PPP and HBact abundance in tropical lakes could be an ecosystem response to environmental changes such as global warming. As PPP are located at the base of the food web, modifications in their dynamics would also have major consequences for the ecology of these lakes (Descy and Sarmento, 2008), by favouring the microbial food web at the expense of the classic, more efficient, food chain based on the direct phytoplankton–metazooplankton link.

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