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# Abundance and distribution of picoplankton in tropical, oligotrophic Lake Kivu, eastern Africa

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## SUMMARY

1. We used flow cytometry to characterize freshwater photosynthetic picoplankton (PPP) and heterotrophic bacteria (HB) in Lake Kivu, one of the East-African great lakes. Throughout three cruises run in different seasons, covering the four major basins, phycoerythrin-rich cells dominated the PPP. Heterotrophic bacteria and PPP cell numbers were always high and spatial variations were modest. This represents an important difference from temperate and high latitude lakes that show high fluctuations in cell abundance over an annual cycle.

2. Three populations of picocyanobacteria were identified: one corresponded to single-cells (identified as *Synechococcus* by epifluorescence microscopy, molecular methods and pigment content), and the two other that most probably correspond to two and four celled colonies of the same taxon. The proportion of these two subpopulations was greater under stratified conditions, with stronger nutrient limitation.

3. High PPP concentrations (*c.* $10^5$ cell $mL^{-1}$) relative to HB (*c.* $10^6$ cell $mL^{-1}$) were always found. Lake Kivu supports relatively less bacteria than phytoplankton biomass than temperate systems, probably as a consequence of factors such as temperature, oligotrophy, nutrient limitation and trophic structure.

4. A review of PPP concentration across aquatic systems suggests that the abundance of *Synechococcus*-like cyanobacteria in large, oligotrophic, tropical lakes is very high.

5. Photosynthetic picoplankton cell abundances in the oligotrophic tropical lakes Kivu and Tanganyika are comparable to those of eutrophic temperate lakes. This apparently contradicts the view that PPP abundance increases with increasing eutrophy. More data on PPP in tropical lakes are needed to explore further this particular pattern.

*Keywords*: East African Great Lakes, flow cytometry, heterotrophic bacteria, large tropical lake, picoplankton, *Synechococcus*

## Introduction

The importance of photosynthetic and heterotrophic picoplankton in aquatic food webs has been emphasized in the last decades (e.g. Pomeroy, 1974; Azam *et al.*, 1983; Stockner & Antia, 1986; Weisse, 1993; Fogg, 1995; Bell & Kalff, 2001; Callieri & Stockner, 2002). Freshwater scientists had been counting small plankton as *Synechococcus* or *Synechocystis* for a long time (e.g. Lewis, 1978; Lewis & Riehl, 1982) but it was in the oceans that this small sized fraction was shown to be important, a finding that led to the search for more efficient and accurate quantification methodologies.

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The great majority of the available data, especially for freshwaters, concern high latitude or temperate dimictic lakes (e.g. Bell & Kalff, 2001). Records for low latitude (pantropical) regions are rare, even if high picoplankton abundance in these warm waters should be expected according to general (temperature-based) models (Agawin, Duarte & Agustí, 2000). In addition, it is generally established that picoplankton dominates biomass and production in the tropical ocean (e.g. Li *et al.*, 1983). Lake Tanganyika is one of the tropical lakes where the picoplankton has attracted the attention of some scientists by its astonishingly high abundance (Vuorio *et al.*, 2003; Descy *et al.*, 2005; Pirlot *et al.*, 2005). Hecky *et al.* (1981) were the first to hypothesize a major role for (heterotrophic) picoplankton in the food web of Lake Tanganyika, even before the landmark paper by Azam *et al.* (1983) that firstly conceptualized the role of the microbial food web in the oceans.

Picoplankton abundance seems particularly affected by water temperature and nutrient availability. In a literature review, Agawin *et al.* (2000) demonstrated the particular relative importance of photosynthetic picoplankton (PPP) in warm and oligotrophic waters. A reduced contribution of PPP in productive waters might be related to increased loss rates (of PPP cells by grazing), whereas their dominance in warm, oligotrophic waters is commonly attributed to their size-related capacity to use nutrients and to differences in intrinsic growth rates between PPP and larger phytoplankton cells. Bell & Kalff (2001) reported a significant positive relationship between picoplankton abundance and total chlorophyll *a* (Chl *a*) in a large number of lakes and over a large biomass range. In contrast, Burns & Galbraith (2007) observed that prokaryotic picophytoplankton biomass was negatively related to indices of nutrient enrichment in a large number of New Zealand waterbodies. Actually, the relation between trophic state and PPP abundance is not straightforward. Nevertheless, it is still commonly accepted that, while PPP abundance and production increases with increasing eutrophy, their contribution to total phytoplankton biomass and production decreases with increasing trophic status, both in marine and freshwater ecosystems (Stockner, 1988, 1991; Søndergaard, 1991; Agawin *et al.*, 2000; Bell & Kalff, 2001).

Transparency (light conditions on the water column) has also a major impact in the composition of the PPP communities, by selecting the dominant pigment-type. Vörös *et al.* (1998) showed that phycocyanin-rich cells are typical under low-light regimes, while more transparent waters usually favour phycoerythrin-rich cells. These results match with laboratory experiments on marine (Glover, Keller & Guillard, 1986; Waterbury *et al.*, 1986) and freshwater (Wyman & Fay, 1987; Hauschild, McMurter & Pick, 1991; Callieri *et al.*, 1996) *Synechococcus* strains that showed that phycoerythrin-rich cells grew better in green light, the type that penetrates clear water, as opposed to red light that penetrates deeper in turbid water.

Seasonal fluctuations in the abundance of PPP usually described for temperate lakes are characterized by an annual maximum in spring (temperate) or summer (high latitudes), contrasting with relatively low numbers during the rest of the year (e.g. Gaedke & Weisse, 1998; Crosbie, Teubner & Weiss, 2003; Izaguirre, Allende & Marinote, 2003). At present, there are no reports in the literature about seasonal patterns of PPP in tropical freshwaters, although Descy *et al.* (2005) deduced from marker pigments that picocyanobacteria made a large contribution to total Chl *a* throughout the year in Lake Tanganyika.

Although several marine studies have demonstrated the efficiency and sensitivity of flow cytometry (e.g. Li & Wood, 1988; Olson, Zettler & du Rand, 1993; Gasol & del Giorgio, 2000), the technique has rarely been applied by limnologists in field studies of picophytoplankton (but see Crosbie *et al.*, 2003). Moreover, freshwater PPP and HB are usually studied separately, even though from a functional point of view it is likely that they occupy a similar position in the microbial trophic web, because both are taken by protozooplankton (heterotrophic nanoflagellates, ciliates and rotifers) as a major source of carbon (e.g. Nagata *et al.*, 1996; Simek *et al.*, 1996), and they compete for inorganic nutrients (e.g. Tanaka *et al.*, 2004).

We characterized different subpopulations of photosynthetic and heterotrophic picoplankton in a deep tropical lake (Lake Kivu, eastern Africa) and explored their seasonal variations, using flow cytometry. Through a comparison of our results with those of other studies worldwide, we attempted to test whether deep tropical lakes fit with the general pattern of picoplankton development across aquatic systems.

## Methods

Lake Kivu, located between Rwanda and Kivu Province (Democratic Republic of the Congo), is one of the Great Lakes of the East African Rift Valley. It is a deep (max. 489 m), meromictic lake, with an oxygenated epilimnion of about 70 m, and a deep hypoliminion rich in dissolved gases ($CO_2$, methane). Lake Kivu is a volcanic lake and presents some unique limnological features, such as step increases in temperature and salinity in deep water (Degens *et al.*, 1973; Spigel & Coulter, 1996). Recent studies have confirmed the oligotrophic status of Lake Kivu (Isumbisho *et al.*, 2006; Sarmento, Isumbisho & Descy, 2006). Details of nutrient concentrations, general features and phytoplankton biomass in lakes Kivu and Tanganyika can be found in Sarmento *et al.* (2006).

Samples were collected in the upper 50 m of the water column, at 10 m intervals, with a 6-L Van Dorn bottle, in the four main basins of Lake Kivu (Fig. 1): southern (02°33.94′S, 28°97.65′E), western (02°22.79′S, 28°97.35′E), northern (01°68.08′S, 29°15.69′E) and eastern (01°96.17′S, 29°12.26′E). Additional profiles were also taken in Kabuno Bay (02°61.62′S, 28°08.22′E), a very peculiar bay (Sarmento *et al.*, 2006) isolated from the rest of the lake (the only connection is a 150 m wide, 16 m deep channel). Three cruises took place: one during the dry season (26–30 August 2003), one during the rainy season (23 February–27 March 2004), and a third in the transitional period between the two (9–12 September 2004).

We measured limnological profiles using a multiparameter sonde (Hydrolab DS4a, Loveland, CO, U.S.A.), transparency measurements (Secchi depth) and photosynthetically active radiation (PAR) downward attenuation with surface and underwater Li-Cor (Lincoln, NE, U.S.A.) quantum sensors at all sites during the cruises. Samples for Chl *a* and secondary pigment analysis followed the procedure described in Descy *et al.* (2000): 3 L were filtered on Whatman GF/F (Maidstone, U.K.) or Macherey-Nägel (Düren, Germany) GF/5 filters. Pigment extraction was carried out in 8–10 mL 90 % HPLC grade acetone. After two 15 min sonications separated by an overnight period at 4 °C in the dark, HPLC analysis was carried out using the Wright *et al.* (1991) gradient elution method, with a Waters system comprising a PDA detector and a fluorescence detector. Calibration was undertaken using commercial external standards (DHI, Denmark).

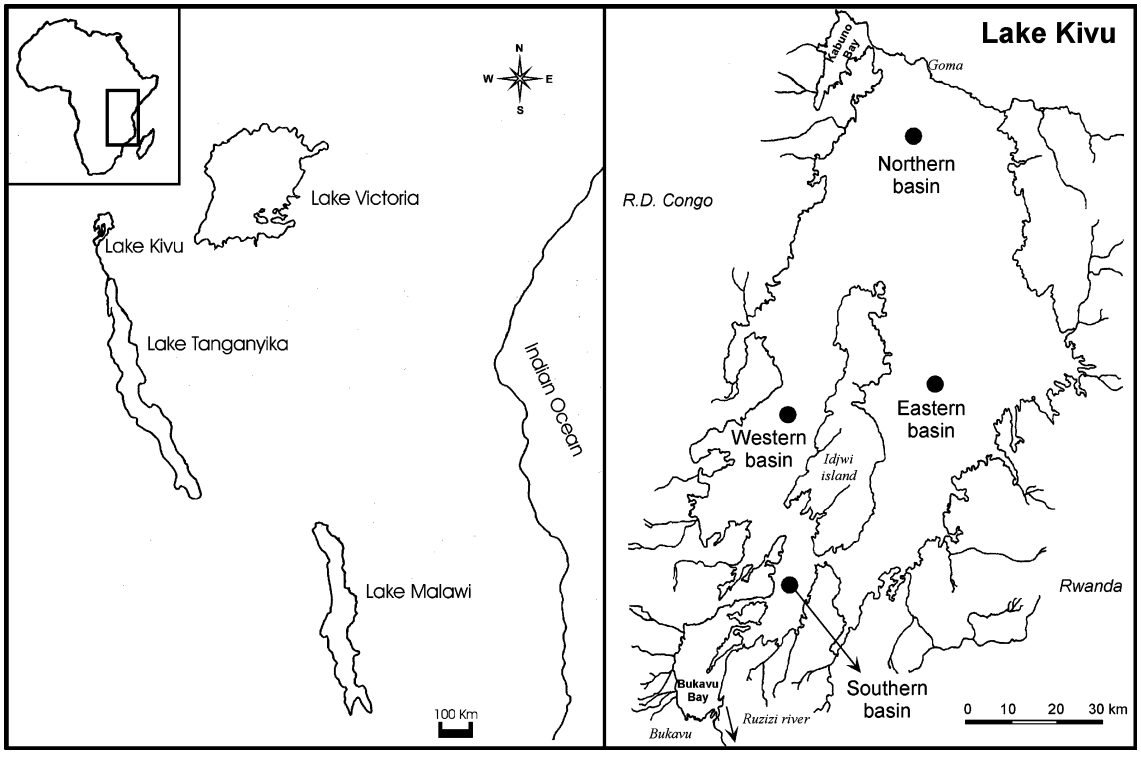


**Fig. 1** Geographic situation of Lake Kivu. Black dots are the sampling sites (see text for GPS coordinates).

Fractionated Chl *a* measurements were obtained from an average euphotic layer sample made by mixing equal volumes of lake water sampled every 5 m, from 0 to 20 m; 3 L were filtered on a Millipore membrane of 2 μm pore size (>2 μm fraction), and the filtrate was filtered on a GF5 filter (<2 μm fraction). Both filters were extracted and analysed by HPLC with the procedure described above.

Picoplankton cell number was obtained by flow cytometry, and some PPP samples were counted with an epifluorescence microscope for method intercalibration. For flow cytometry counts, 4 mL of lake water were collected from each depth and fixed immediately with cold glutaraldehyde 10% (final concentration 1%), left in the dark for 10 min at room temperature and then stored at −20 °C. Two subsamples were taken for separate counts of HB and PPP. For HB determination, 400 μL of sample was added to a diluted SYTO-13 (Molecular Probes Inc., Eugene, OR, U.S.A.) stock (10 : 1) at 2.5 μmol $L^{-1}$ final concentration, left for about 10 min in the dark to complete the staining and run in the flow cytometer. We used a FacsCalibur (Becton & Dickinson Franklin Lakes, NJ, U.S.A.) flow cytometer equipped with a 15 mW Argon-ion laser (488 nm emission). At least 30 000 events were acquired for each subsample (usually 90 000 events). Fluorescent beads (1 μm, Fluoresbrite carboxylate microspheres, Polysciences Inc., Warrington, PA, U.S.A.) were added at a known density as internal standards. The bead standard concentration was determined by epifluorescence microscopy. HB were detected by their signature in a plot of side scatter (SSC) versus FL1 (green fluorescence). Flow cytometry allows separation of two HB subpopulations: HNA (high nucleic-acid content bacteria) and LNA (low nucleic-acid content bacteria) in the SCC versus FL1 plot (Gasol *et al.*, 1999; Bouvier, del Giorgio & Gasol, 2007). In a FL3–FL1 plot (Fig. 2), beads fall in one line, HB in another, and noise in a third (respectively with more FL3 than FL1). Picocyanobacteria fall in between noise and HB. This method is based on del Giorgio *et al.* (1996) as discussed in Gasol & del Giorgio (2000). For PPP, we used the same procedure as for HB, but without addition of SYTO-13. Small algae were easily identified in plots of SSC versus FL3, and FL2 versus FL3 (Olson *et al.*, 1993). Data analysis was performed with the Paint-A-Gate software (Becton & Dickinson).

Samples for epifluorescence microscopy were preserved by the addition of filtered [0.22 μm Millex (Millipore, Billerica, MA, U.S.A.)] glutaraldehyde (1% final concentration) and were stored at −20 °C until analysis in the laboratory. For counts of PPP using epifluorescence microscopy, 10 mL of water sample were filtered onto a 0.2 μm polycarbonate black filter (Nuclepore, Whatman International Ltd, Maidstone, U.K.) using a 5 μm backing filter. We used a Zeiss Axioplan microscope equipped with plan Neofluar objectives and an AxioCam digital camera (Carl Zeiss, Gottingen, Germany). The autofluorescence of chl *a* was visible under blue light excitation (filter set 09, 450–490 nm) producing red emission (>590 nm). With green light excitation (set 15, 546 nm), phycoerythrin produces yellow/orange emission. On average, 1000 cells from 10 randomly chosen fields were counted using image analysis (KS 300 3.0 software, Carl Zeiss, Gottingen, Germany). This procedure was particularly complex in this lake, due to the constant presence of numerous *Planktolyngbya limnetica* (Lemm.) Komárková-Legnerová trichomes. Within a thin sheath, *Planktolyngbya limnetica* individual cells are very similar to *Synechococcus* (Sarmento *et al.*, 2007).

To convert biovolume to carbon we used a factor of 470 fg C $\mu m^{-3}$ for PPP (Verity *et al.*, 1992) and 20.0 fg per cell for HB (Lee & Fuhrman, 1987). For phytoplankton biomass estimation, a Chl *a* : carbon ratio of 92.8 was used, following parallel measurements of the particulate organic carbon in a Carlo-Erba 1500 elemental analyser (see Isumbisho *et al.*, 2006 for more details).

## Results

We present some of the results by geographic sectors, which reflect the exposure to dominant winds: the northern/eastern basins are exposed to winds, while the southern/western basins are smaller and more protected by the surrounding mountains.

The environmental conditions during the three cruises were slightly different as a result of the dominant south-eastern dry season winds that induce deep mixing events. The mixed layer (Zm) attained 60 m in the dry season cruise, while in the rainy season it was only 25 m (Table 1). At the transition between seasons, the lake began to re-stratify, and the epilimnion was 50 m deep, with a very weak thermal gradient. The light conditions were rather similar

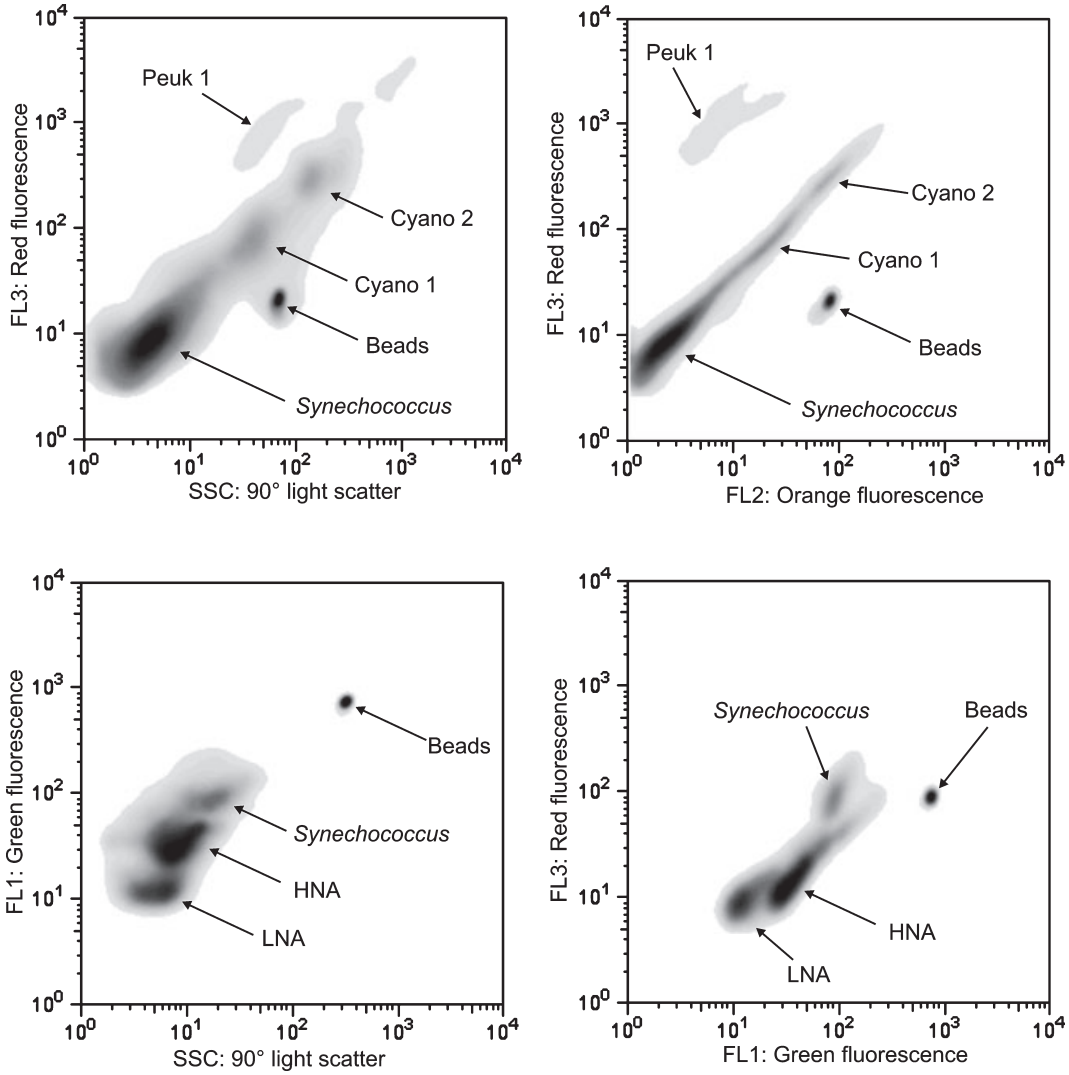


**Fig. 2** Typical cytograms of a Lake Kivu sample showing the autotrophic picoplankton populations (unstained sample, upper panels) and heterotrophic picoplankton populations (stained with SYTO-13, bottom panels). HNA, high nucleic acid content heterotrophic bacteria; LNA, low nucleic-acid content heterotrophic bacteria; Peuk1, picoeukaryotes; Cyano 1 and Cyano 2, undetermined cyanobacteria populations (see text).

between basins and seasons: the depth of the euphotic zone (Zeu) ranged between 15.3 and 18.2 m, and was slightly deeper in the rainy season (Table 1).

Chlorophyll *a* in the euphotic zone was higher in the dry season cruise, reaching 3.76 μg $L^{-1}$ in the northern/eastern basins and 2.70 μg $L^{-1}$ in the southern/western basins. The results for the rainy season and for the transition cruises corresponded to the mean values previously reported for Lake Kivu (in the range 1.25–1.62 μg Chl *a* $L^{-1}$; Sarmento *et al.*, 2006). Fractionated Chl *a* results show a relatively constant pattern: the <2 μm fraction contributed an average of 21.2% total Chl *a* (Table 1).

On the basis of size and pigment content, we identified four different populations of PPP using flow cytometry (Fig. 2): three phycoerythrin-rich picocyanobacteria (*Synechococcus*, Cyano 1 and Cyano 2) and one population of cells without phycoerythrin and larger than *Synechococcus*, which we identified as picoeukaryotes. The flow cytometry results for the *Synechococcus* population were confirmed by epifluorescence microscopy counts performed on some of the 0–20 m samples (the correlation between the results from the two methods was highly significant: $r = 0.99$, $P < 0.0001$, $n = 20$, with a 1.01 slope).

**Table 1** Summary of some general features and picoplankton results (mean values within the euphotic zone) in two sectors of Lake Kivu at different seasons

| | Temp. (°C) | Zm (m) | Zeu (m) | Mean Chl *a* (μg L$^{-1}$) | Chl *a* % <2 μm | *Synechococcus* ($10^5$ cell mL$^{-1}$) | Cyano 1 ($10^4$ cell mL$^{-1}$) | Cyano 2 ($10^4$ cell mL$^{-1}$) | Peuk ($10^3$ cell mL$^{-1}$) | HB ($10^6$ cell mL$^{-1}$) | LNA ($10^5$ cell mL$^{-1}$) | HNA ($10^5$ cell mL$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Dry season* | | | | | | | | | | | | |
| Southern/Western basins | 23.2 | 60 | 15.3 | 4.20 | 28.3 | 1.29 | 1.02 | 0.67 | 1.56 | 1.25 | 3.16 | 9.36 |
| Northern/Eastern basins | 23.1 | 60 | 16.5 | 5.33 | n. a. | 1.15 | 0.75 | 0.44 | 2.40 | 1.53 | 3.70 | 11.58 |
| *Transition* | | | | | | | | | | | | |
| Southern/Western basins | 23.2 | 50 | 17.4 | 2.27 | 17.5 | 1.39 | 1.65 | 1.36 | 8.47 | 1.77 | 8.97 | 8.77 |
| Northern/Eastern basins | 23.4 | 50 | 17.6 | 2.15 | 17.2 | 1.37 | 1.50 | 0.95 | 5.89 | 1.78 | 7.79 | 10.04 |
| *Rainy season* | | | | | | | | | | | | |
| Southern/Western basins | 24.2 | 25 | 18.2 | 2.19 | 19.0 | 1.42 | 1.85 | 0.89 | n.a. | 1.73 | 5.13 | 10.85 |
| Northern/Eastern basins | 24.1 | 25 | 17.6 | 2.29 | 23.8 | 0.74 | 0.81 | 0.57 | n.a. | 1.34 | 3.58 | 8.87 |

Zm, depth of the mixed layer; Zeu, depth of the euphotic layer; HNA, high nucleic-acid content bacteria; LNA, low nucleic-acid content bacteria; HB, heterotrophic bacteria; Peuk, picoeukaryotes; Cyano 1 and Cyano 2, undetermined cyanobacterial populations (see text); n. a.: not available.

*Synechococcus* was also clearly distinguishable in SYTO-13-stained cytograms (Fig. 2, bottom). Usually, *Synechococcus* is undistinguishable from HB in that type of plot. The *Synechococcus* abundances in the euphotic zone ranged between 0.5 and $2 \times 10^5$ cells mL$^{-1}$ (Fig. 3). As expected, the vertical profiles revealed higher abundances within the euphotic zone than at depth, with a sharp decrease around 30 m depth. In the northern/eastern basins, seasonal variation in *Synechococcus* abundance was greater than in the southern/western basins (Fig. 3).

Cells of *Synechococcus* were on average 1.21 μm long and 0.86 μm wide, with a mean cell volume of 0.43 μm$^3$. The epifluorescence microscopy measurements resulted in a conversion factor of 202 fg C cell$^{-1}$. With this factor, the mean *Synechococcus* biomass in the euphotic zone was 24.7 mg C m$^{-3}$, corresponding to 0.42 g C m$^{-2}$, integrated for this layer.

Picoeukaryote density was practically two orders of magnitude lower than that of PPP. This difference was particularly marked in the dry season cruise (Table 1).

As did Casamayor *et al.* (2007), we sporadically observed anoxygenic photosynthetic bacteria populations in the northern basin and in the isolated Kabuno bay, under the mixolimnion thermocline. These results were confirmed by HPLC detection of bacteriochlorophylls (data not shown).

Heterotrophic bacteria concentrations were relatively constant, in the 1–2 $\times$ 10$^6$ cell mL$^{-1}$ range. High nucleic-acid content (HNA) bacteria were dominant in the euphotic layer, contributing an average of 64% of the total number of HB. This proportion decreased with depth, reaching *c.* 30% in the anoxic hypolimnion (60–70 m). The vertical distribution of HB total abundance did not show any clear seasonal pattern (Fig. 4).

The mean HB biomass in the euphotic zone of Lake Kivu was 31.5 mg C m$^{-3}$ corresponding to 1.42 g C m$^{-2}$, integrated for the mixolimnion (top 60 m layer).

The correlations between the different HB subpopulations and phytoplankton (total and PPP) are summarized in Table 2. Total phytoplankton (Chl *a*) and PPP biomass were significantly correlated with HB biomass. The highest correlation coefficients were found between HNA : phytoplankton and HNA : PPP (Pearson correlation coefficients, $r = 0.90$, $n = 83$, $P < 0.001$ and $r = 0.84$, $n = 64$,

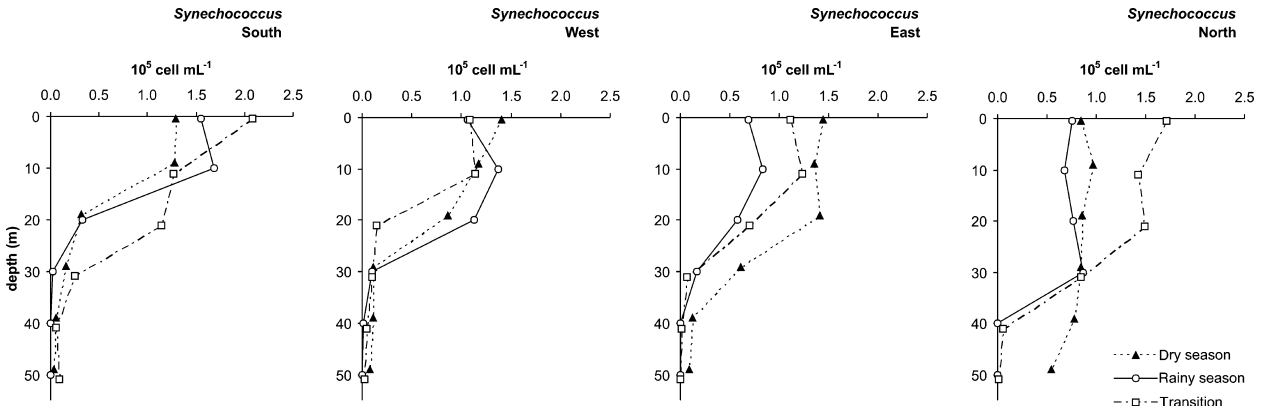


**Fig. 3** Profiles of *Synechococcus* abundance in two sectors (northern/eastern and southern/western basins) of Lake Kivu at different seasons (2003/2004).

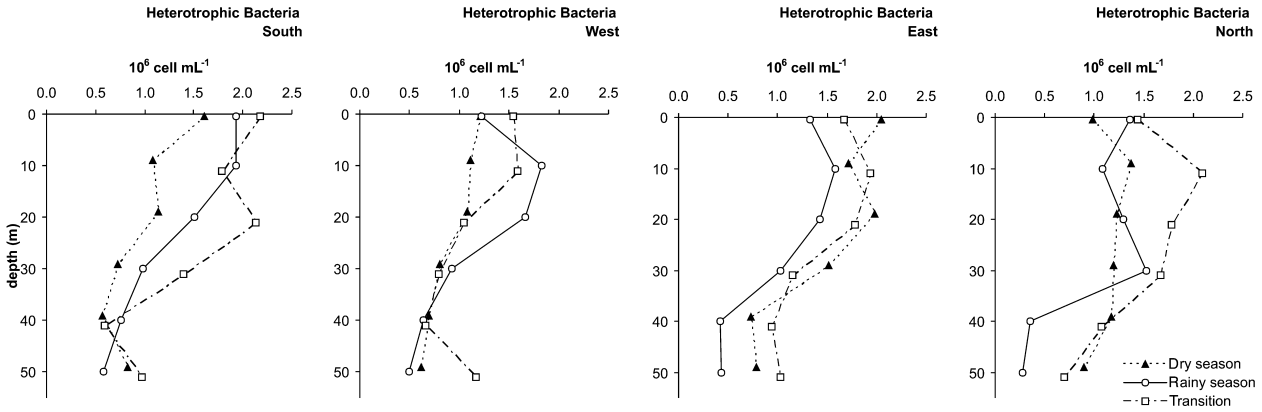


**Fig. 4** Profiles of total heterotrophic bacteria abundance in two sectors (northern/eastern and southern/western basins) of Lake Kivu at different seasons (2003/2004).

$P < 0.001$, respectively). In this analysis we used carbon biomass estimations and log–log scales in order to compare slopes with similar relationships in the literature.

On linear scales, phytoplankton biomass was, again, significantly correlated with HNA biomass ($r = 0.90$, $P < 0.001$, $n = 83$). When plotted against phytoplankton biomass, low nucleic-acid content bacteria (LNA) and total HB showed lower, although still significant, correlation coefficients. When we included HNA biomass integrated in the euphotic zone from both lakes Kivu and Tanganyika we again found a significant correlation ($r = 0.86$, $P < 0.001$, $n = 22$) with a similar slope (Fig. 5).

## Discussion

Our study of the picoplankton of a large tropical lake revealed a constantly high abundance of PPP (mainly represented by *Synechococcus*) at all seasons. Three out of the four autotrophic picoplanktonic populations identified using flow cytometry, corresponded to phycoerithrin-containing cyanobacteria (*Synechococcus*, Cyano 1 and Cyano 2). The taxonomic identity of *Synechococcus* was confirmed by microscopic observations (counts performed by epifluorescence were highly correlated with the cytometric estimates), Preliminary results of molecular analysis, performed by DGGE (Denaturing Gradient Gel Electrophoresis) with primers specific for cyanobacteria, also confirmed this identity (data not shown). Cyano 1 and Cyano 2 may comprise subpopulations (of two- and four-celled colonies) of the same single-cell taxon during the cell division phase, or possibly other taxa which are abundant in the lake (Sarmento *et al.*, 2007). Indeed, we failed to correlate these two cyanobacterial populations with the two- and four-celled *Synechococcus* sometimes observed by epifluorescence

**Table 2** Log–log slopes of the relationships between paired (*Y* : *X*) factors indicated in the left column

| | Slope | $r^2$ | *Y*-intercept | *n* |
|---|---|---|---|---|
| Total HB (mg C $m^{-3}$) : Phytoplankton (mg C $m^{-3}$) | 0.330 ± 0.028 | 0.63 | 0.673 ± 0.058 | 83 |
| HNA (mg C $m^{-3}$) : Phytoplankton (mg C $m^{-3}$) | 0.532 ± 0.028 | 0.81 | 0.002 ± 0.058 | 83 |
| LNA (mg C $m^{-3}$) : Phytoplankton (mg C $m^{-3}$) | 0.515 ± 0.063 | 0.45 | −0.232 ± 0.129 | 83 |
| Total HB (mg C $m^{-3}$) : PPP (mg C $m^{-3}$) | 0.191 ± 0.021 | 0.63 | 0.893 ± 0.018 | 64 |
| HNA (mg C $m^{-3}$) : PPP (mg C $m^{-3}$) | 0.249 ± 0.020 | 0.71 | 0.914 ± 0.023 | 64 |
| LNA (mg C $m^{-3}$) : PPP (mg C $m^{-3}$) | 0.216 ± 0.044 | 0.27 | 0.663 ± 0.052 | 64 |

All relationships were significant ($P < 0.001$).
*n*, number of individual points in the regressions; HNA, high nucleic-acid content bacteria; LNA, low nucleic-acid content bacteria; HB, heterotrophic bacteria; Peuk, picoeukaryotes; PPP, phototrophic picoplankton.

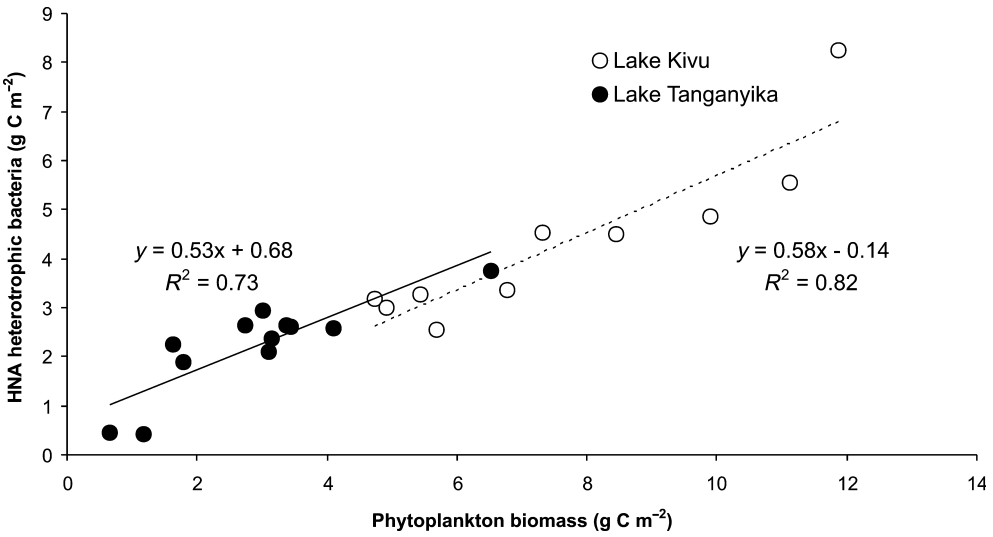


**Fig. 5** Relation between phytoplankton and HNA (high nucleic-acid content heterotrophic bacteria) biomass integrated over the euphotic layers of lakes Kivu and Tanganyika.

microscopy. At least twelve different cyanobacteria inhabit Lake Kivu, among which are several unicellular, colonial and filamentous species (Sarmento *et al*., 2007). *Chroococcus* sp. (two- or four-celled colonies), *Merismopedia trolleri* Bach. (four-, eight- or more-celled colonies), *Pseudanabaena mucicola* Lauterb. (one- to six-celled trichomes) and *Planktolyngbya limnetica* (Lemm.) Komárková-Legnerová and Cronberg are potential candidates for the two unidentified cyanobacterial populations.

In any case, independently of the taxonomic affiliation of Cyano 1 and 2, *Synechococcus* colonial forms have been observed in many freshwater environments (Pick & Agbeti, 1991; Stockner & Shortreed, 1991; Vörös, Gulyas & Nemeth, 1991) and there are some reports that indicate that, in temperate regions, these aggregates tend to be more abundant in the epilimnion in August and September, corresponding to periods of nutrient limitation (Pedrós-Alió & Brock, 1983; Klut & Stockner, 1991; Passoni & Callieri, 2000; Crosbie *et al*., 2003). Klut & Stockner (1991) suggested that the presence of colonies might have an adaptive function, both by increasing the efficiency of nutrient recycling and by providing protection against predators. Crosbie *et al*. (2003) added that microcolonies reach high concentrations in surface and near-surface waters due to the production of a photosynthate-rich mucilage resulting from active photosynthesis during periods of severe nutrient deficiency. Moreover, Schallenberg & Burns (2001) proposed that

aggregation could facilitate nitrogen fixation by allowing the development of anoxic microsites among the aggregated cells, a presumed prerequisite for the functioning of nitrogenase. It is then conceivable that, in a highly nutrient limited environment such as Lake Kivu, colonial forms might be common, with lower numbers in the dry season when deep mixing brings up some nutrients from the deep layers, thus reducing nutrient limitation (Sarmento *et al*., 2006).

The *Synechococcus* : picoeucaryotes ratio in Lake Kivu seems relatively high when compared to other systems (Table 3). Burns & Stockner (1991) reported picoeucaryote densities about one order of magnitude lower than those of chroococcoid cyanobacteria in six New Zealand lakes, in a variety of trophic states. In our study, picoeucaryote densities ($5.1 \times 10^3$ cells $mL^{-1}$ in average) were always more than one order of magnitude lower than those of picocyanobacteria ($1.2 \times 10^5$ cells $mL^{-1}$ in average), and most commonly two orders of magnitude lower. The general oligotrophic nature of the lake concurs with the trend of dominance of *Synechococcus* over picoeucaryotes (e.g. Søndergaard, Jensen & Ærtebjerg, 1991), but the light regime, constantly rich in UV, might also promote higher concentrations of *Synechococcus*, which seem to be more resistant than picoeucaryotes to high-energy radiation (Sommaruga *et al*., 2005).

As expected from literature on picoplankton (e.g. Agawin *et al*., 2000; Bell & Kalff, 2001), in a warm, oligotrophic system such as Lake Kivu, PPP cell abundance was relatively high and phycoerythrin-rich picocyanobacteria were dominant over the phycocyanin-rich cells in the transparent water column (Vörös *et al*., 1998). In addition, *Synechococcus* was very abundant all year round, despite significant seasonal changes of light exposure and nutrient supply (Isumbisho *et al*., 2006; Sarmento *et al*., 2006). This contrasts with the seasonal pattern of PPP in temperate lakes, where large fluctuations in cell abundances occur over the annual cycle.

Furthermore, studies in temperate lakes point to a general pattern, with PPP abundance increasing along the trophic gradient, while the contribution of PPP to total phytoplankton biomass decreases (Stockner, 1988, 1991; Søndergaard, 1991). Lake Kivu can still be considered as oligotrophic, based on water transparency and Chl *a*, even though it may seem more productive than Lake Tanganyika (Hecky & Kling, 1987; Sarmento *et al*., 2006). We could, then, expect higher PPP abundances and lower contribution to total phytoplankton biomass in Lake Kivu than in Lake Tanganyika. From our results, the contribution of PPP (<2 μm fraction), mainly represented by *Synechococcus*, to total phytoplankton in Lake Kivu was in the 10-32% range, while the proportion of picophytoplankton in Lake Tanganyika ranged between 30–70% (Vuorio *et al*., 2003; Descy *et al*., 2005). In terms of the absolute cell concentration of *Synechococcus*, however, average cell abundance in the euphotic zone is consistently higher in Lake Tanganyika ($3.4 \times 10^5$ cells $mL^{-1}$) than in Lake Kivu ($1.2 \times 10^5$ cells $mL^{-1}$). Although more studies would be needed to confirm such a deviation from the PPP pattern observed in temperate lakes, our results suggest a greater relative importance of PPP in tropical lakes.

The constantly high PPP abundance may be related to the higher mean primary production in large tropical lakes than in their temperate counterparts (Kilham & Kilham, 1990, 1991), because of higher and quite constant irradiance (see also Beadle, 1981), high water transparency, and more efficient nutrient recycling in the water column favoured by high biological activity in the warm mixed layer. Factors such as nutrient limitation (greater P limitation in Lake Kivu, Sarmento *et al*., 2006), variability of the light climate, competition with other phytoplankton and food web structure (Isumbisho *et al*., 2006) may be more significant in this kind of environment than trophic status in determining PPP abundance, and PPP contribution to total autotrophic biomass.

A recent study in Lake Tanganyika (Pirlot *et al*., 2005) has shown a major contribution of photosynthetic and heterotrophic picoplankton to total plankton biomass in this large tropical lake, with an heterotrophic : autotrophic ratio of about 1. Following Simon, Cho & Azam (1992) and del Giorgio & Gasol (1995), we established log-log plots of HB and phytoplankton carbon biomass. For Lake Kivu, the slope of the regression line was 0.330, i.e. steeper than the one obtained by Simon *et al*. (0.219) and slightly lower than that found by del Giorgio & Gasol (0.360). It is worth mentioning that the trophic range explored in Simon *et al*. (1992) was larger than that studied by del Giorgio & Gasol (1995), where the majority of the lakes were in the oligotrophic range. The *Y*-intercept

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**Table 3** Review of some general features and *Synechococcus* (maximum and mean) concentrations integrated in the euphotic layer (when available, otherwise in the mixed layer) in different environments, latitudes and trophic states

| Environment | Trophic state | Surface area ($km^2$) | Max depth (m) | Temp. (°C) | Zeu (m) | Mean Chl *a* ($\mu g\ L^{-1}$) | Max *Syn* ($10^{10}$ cell $m^{-2}$) | Mean *Syn* ($10^{10}$ cell $m^{-2}$) | *Syn* : Peuk (abundance) | HB : *Syn* (abundance) | Method | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Marine* | | | | | | | | | | | | |
| South Atlantic – gyre* | Ultra-oligotrophic | – | – | 20.0 | 82 | 0.2 | 297 | 69 | 1.2–6.0 | 16–164 | FC | Gasol, P. unpubl. data |
| North Atlantic (near the African coast)* | Oligo-mesotrophic | – | – | 23.1 | 62 | 0.5 | 1044 | 134 | 2.8–10 | 26–255 | FC | Gasol, P. unpubl. data |
| *Brackish water* | | | | | | | | | | | | |
| San Francisco bay (freshwater – seawater transect)‡ | Eutrophic (variable) | – | – | 17.7 | – | 9.7 | 52 | 9 | | | EM | Ning, Cloern & Cole, 2000 |
| *Freshwater* | | | | | | | | | | | | |
| 11 Antarctic lakes‡ | Different trophic status | <0.05 | 2–3 | −1.0–8.9 | – | 1.9 | 0.3 | 0.1 | 1–10 | – | FC | Andreoli *et al.*, 1992 |
| Four boreal lakes in Finland‡ | Different trophic status | 0.04 – 134 | 3.5–87 | *c.* 15 | 3.3 | 8.3 | 202 | 26 | 6–115 | – | EM | Jasser & Arvola, 2003 |
| Seven temperate lakes in Denmark | Different trophic status | 0.1–41 | 5–37 | *c.* 15 | 1–15 | 7–200 | 495 | <30 | 0–0.05 | – | EM | Søndergaard, 1991 |
| Two lakes in British Columbia‡ | Oligotrophic | 41–270 | 9–18 | 15.4 | – | 0.8 | 78 | 36 | – | *c.* 10 | EM | Stockner & Shortreed, 1985 |
| Furuike (pond) in Japan§ | Hypereutrophic | <0.01 | 1.5 | *c.* 15 | – | *c.* 100 | 152 | *c.* 60 | – | *c.* 75 | EM | Hirose *et al.*, 2003 |
| 48 lakes in North America* | Oligotrophic to mesotrophic | 0.01–210 | 3.7–108 | *c.* 22 | 3–15 | 8.7 | 140 | *c.* 100 | – | – | EM | Lavallee & Pick, 2002 |
| L. Constance (pre-alpine lake) in central Europe‡ | Meso-eutrophic | 540 | 252 | *c.* 12 | – | – | 314 | *c.* 100 | – | – | EM | Weisse, 1988 |
| L. Baikal§ | Oligotrophic | 31500 | 1620 | *c.* 6 | *c.* 15 | – | 1485 | 150 | – | – | EM | Belykh & Sorokovikova, 2003 |
| L. Maggiore (subalpine lake) in Italy§ | Oligotrophic | 212 | 370 | 11.8 | 15 | 2.9 | 825 | 185 | 10–55 | – | EM | Callieri & Piscia, 2002 |
| L. Biwa in Japan§ | Mesotrophic | 675 | 104 | *c.* 15 | 15 | 6.5 | 2170 | 186 | – | – | EM | Wakabayashi & Ichise, 2004 |

**Table 3** (*Continued*)

| Environment | Trophic state | Surface area ($km^2$) | Max depth (m) | Temp. (°C) | Zeu (m) | Mean Chl *a* ($\mu g\ L^{-1}$) | Max *Syn* ($10^{10}$ cell $m^{-2}$) | Mean *Syn* ($10^{10}$ cell $m^{-2}$) | *Syn* : Peuk (abundance) | HB : *Syn* (abundance) | Method | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| L. Skrzynka (shallow lake) in Poland‡ | Oligo-mesotrophic | 0.02 | 2.9 | *c.* 15 | – | – | 763 | 199 | 0–17 | – | EM | Szelag-Wasielewska, 1999 |
| L. Kivu (large tropical lake)§ | Oligotrophic | 2370 | 489 | 24.9 | 18 | 2.2 | 630 | 216 | 13–83 | 10–18 | FC | This study |
| Four semi-tropical shallow lakes in Florida* | Hypereutrophic | 124 | *c.* 3 | *c.* 25 | 0.8 | 111.4 | 677 | 341 | – | – | EM | Carrick & Schelske, 1997 |
| Southern Lake Baikal* | Oligotrophic | 31500 | 1620 | *c.* 12 | *c.* 15 | – | 3000 | *c.* 750 | *c.* 1.2 | *c.* 2 | EM | Nagata *et al.*, 1994 |
| L. Tanganyika (large tropical lake)§ | Oligotrophic | 32600 | 1470 | 26.8 | 44 | – | 2670 | 890 | – | – | EM | Vuorio *et al.*, 2003 |
| L. Apopka (semi-tropical, shallow lake) in Florida§ | Hypereutrophic | – | *c.* 3 | 25.0 | 0.8 | 105.1 | 2384 | 1088 | – | – | EM | Carrick & Schelske, 1997 |
| L. Tanganyika (large tropical lake)§ | Oligotrophic | 32600 | 1470 | 26.8 | 39 | 0.6 | 3190 | 1323 | 15–109 | 1.6–2.2 | FC | S. Stenuite, H. Sarmento & F. Unrein, unpubl.data |

*Prochlorococcus* was not taken into account for marine data. They are ordered by average *Synechococcus* concentration.
Syn, *Synechococcus*; Peuk, picoeukaryotes; HB, heterotrophic bacteria; EM, Epifluorescence microscopy; FC, Flow cytometry;
*single sample/cruise; ‡spring/summer; §annual mean.

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of this relationship (Table 2) was used by Simon *et al.* (1992) to show that limnetic systems supported more bacterial biomass relative to phytoplankton carbon than marine systems, since the value of this intercept was significantly higher in limnetic (1.39) than in marine (1.00) systems.

Simon *et al.* (1992) proposed several explanations for the difference, such as the fact that in limnetic systems allochthonous carbon, in addition to pelagic primary production, fuels bacterial production and leads to an enhanced biomass and production of bacteria. A second argument points to the dominance in limnetic systems of cladocerans (that graze preferentially algae and protozoans, the main consumers of bacteria), as compared to copepods in the ocean (Lehman, 1988). Therefore, a cladoceran-dominated zooplankton assemblage might, by top-down control of the microbial food web, allow higher bacteria biomass (Simon *et al.*, 1992). In the East African Great Lakes (and probably in all large tropical lakes), the contribution of cladocerans to total zooplankton biomass is very low, and copepods usually dominate (e.g. Lehman, 1988; Isumbisho *et al.*, 2006). Besides, in such large systems, allochthonous sources of carbon may not contribute significantly to bacterial productivity in the pelagic zone. In our data set, the *Y*-intercept of the HB : phytoplankton relationship was 0.673, lower than those reported by Simon *et al.* (1992) for freshwater, or even for marine systems. In other words, the tropical Lake Kivu supports much less bacterial biomass, relative to phytoplankton biomass, than temperate lakes. Whether this is a widespread pattern in large tropical lakes, possibly related to the structure of the microbial food web, remains to be explored.

Few data can be found in the literature about the HB : *Synechococcus* ratio (Table 3) but, even with this small data set, this ratio seems relatively low in the two tropical lakes comparing to the temperate ones (considering that some data from temperate lakes usually cover short spring/summer periods, when *Synechococcus* abundance is typically higher). The unusually high relative abundance of *Synechococcus* over other picoplankton is clearly visible in the SYTO-13-stained cytograms (Fig. 2).

Within the euphotic layer, the abundances of PPP and HB were in the same order of magnitude at all sampling sites, and HB were abundant throughout the year. Pirlot *et al.* (2005) also found that HB numbers were similar throughout the year in Lake Tanganyika, suggesting a balance between production and mortality losses of HB, whereas Chl *a* showed strong seasonality.

Heterotrophic bacteria and PPP abundances have been shown to correlate with water temperature in lakes and oceans (e.g. Caron, Pick & Lean, 1985; Murphy & Haugen, 1985; Jochem, 1988; Maeda, Kawai & Tilzer, 1992; Coveney & Wetzel, 1995; Li, 1998). Most studies in tropical limnology conducted so far have neglected the microbial food web, following models from temperate lakes, where PPP is only sporadically abundant in specific periods. However, in tropical large lakes, the persistently warm, transparent waters are favourable to high abundance of these microorganisms, enhancing the role of the microbial food web in whole-lake carbon cycling (Pirlot *et al.*, 2007).

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