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Bacterial Community Composition in Lake Tanganyika: Vertical and Horizontal Heterogeneity

Aaike De Wever,[1]* Koenraad Muylaert,[1] Katleen Van der Gucht,[1] Samuel Pirlot,[2] Christine Cocquyt,[1] Jean-Pierre Descy,[2] Pierre-Denis Plisnier,[2,3] and Wim Vyverman[1]

Ghent University, Department of Biology, Section of Protistology and Aquatic Ecology, Ghent, Belgium[1]; University of Namur, Department of Biology, Laboratory of Freshwater Ecology, URBO, Namur, Belgium[2]; and Royal Museum for Central Africa, Tervuren, Belgium[3]

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Vertical and latitudinal differences in bacterial community composition (BCC) in Lake Tanganyika were studied during the dry season of 2002 by means of denaturing gradient gel electrophoresis analysis of PCR-amplified 16S RNA fragments. Dominant bands were sequenced and identified as members of the *Cyanobacteria*, *Actinobacteria*, *Nitrospirae*, green nonsulfur bacteria, and *Firmicutes* divisions and the *Gamma-* and *Deltaproteobacteria* subdivisions. The BCC in the lake displayed both vertical and latitudinal variation. Vertical changes in BCC were related to the thermal water column stratification, which influences oxygen and nutrient concentrations. Latitudinal variation was related to upwelling of deep water and increased primary production in the south of the lake. The number of bands per sample increased with bacterial production in the epilimnion of the lake, suggesting a positive diversity-productivity relationship.

Since the first application of molecular tools to the study of the ecology of aquatic bacteria, bacterial community composition (BCC) has been studied in a wide variety of aquatic ecosystems, ranging from shallow to deep lakes to coastal seas and oceans. Molecular studies of BCC in lakes and rivers have revealed a consistent set of typical freshwater bacteria. Zwart et al. (51) discerned 34 putative phylogenetic clusters which occur in a wide range of freshwater environments. The dominant divisions include the *Proteobacteria*, *Bacteroidetes*, *Verrucomicrobia*, *Cyanobacteria*, *Actinobacteria*, and green nonsulfur bacteria. Urbach et al. (46), however, detected unusual bacterial communities in the ultraoligotrophic Crater Lake, in which there was a greater dominance of *Gammaproteobacteria*. So far, few studies have focused on the large, ancient lakes of the world or on tropical lakes. To our knowledge, no information is available yet on BCC in tropical lakes. Of the large lakes, data are available only for Lake Baikal (1, 3, 6, 40). Lake Tanganyika is the third largest lake by volume and the deepest lake after Lake Baikal. Studies on bacteria in Lake Tanganyika have been limited to monitoring data on bacterial abundance and production (15, 39) or taxonomic studies dealing with specific bacterial groups (9, 10). As this lake is well known for its rich endemic fauna (4), studies on the BCC may be worthwhile.

Lake Tanganyika is a meromictic, permanently temperature-stratified lake with strong vertical oxygen concentration gradients. This lake is anoxic below a depth of 100 to 200 m, and it contains the largest volume of anoxic freshwater in the world. The permanent temperature and oxygen gradients may affect the BCC in the lake. In stratified lakes and seas, vertical zonation of BCC has been found along temperature and/or oxygen concentration gradients (21). Several studies have demonstrated a relationship between BCC and oxygen concentration (21) or temperature (42). During the dry season, Lake Tanganyika also displays pronounced latitudinal differences in the depth of the thermocline. The differences in thermocline depth affect nutrient concentrations in the epilimnion and result in differences in phytoplankton biomass between the north and south of the lake (4). So far, horizontal variation in BCC has been studied mainly in marine ecosystems rather than in lakes. Horizontal variation in BCC has been observed along transects crossing marine fronts (33) or in marine upwelling regions (18). The causes of horizontal differences in BCC were changes in nutrient concentrations and phytoplankton biomass or community composition.

For this study, samples from the entire water column were collected along a north-south transect in Lake Tanganyika during the dry season of 2002, and the BCC in these samples was analyzed using denaturing gradient gel electrophoresis (DGGE). The results of this study provide the first description of the bacterial community in Lake Tanganyika and are the first data on BCC in a tropical lake. Vertical and horizontal differences in the BCC were related to important environmental gradients in the lake.

MATERIALS AND METHODS

Study site. Lake Tanganyika is situated in East Africa and is bordered by Burundi, Tanzania, Zambia, and Congo. This lake is part of the East African rift valley. With a maximum depth of 1,470 m, it is the second deepest lake in the world. The lake measures 650 by 50 km (average width) and can be divided into three subbasins: the Kigoma basin in the north (1,310 m), the central Kalemie basin (800 m), and the East Marungu basin in the south (1,470 m) (4). The lake has an annually recurrent limnological cycle related to the monsoon season (35, 36). During the dry season (May or June to September or October), southeast monsoon winds tilt the thermocline toward the surface in the southern part of the lake. This causes an upwelling of deep water in the south of the lake and an

* Corresponding author. Mailing address: Ghent University, Department Biology, Section Protistology and Aquatic Ecology, 9000 Ghent, Belgium. Phone: 32 (0)9 264 85 05. Fax: 32 (0)9 264 85 99. E-mail: Aaike.DeWever@UGent.be.

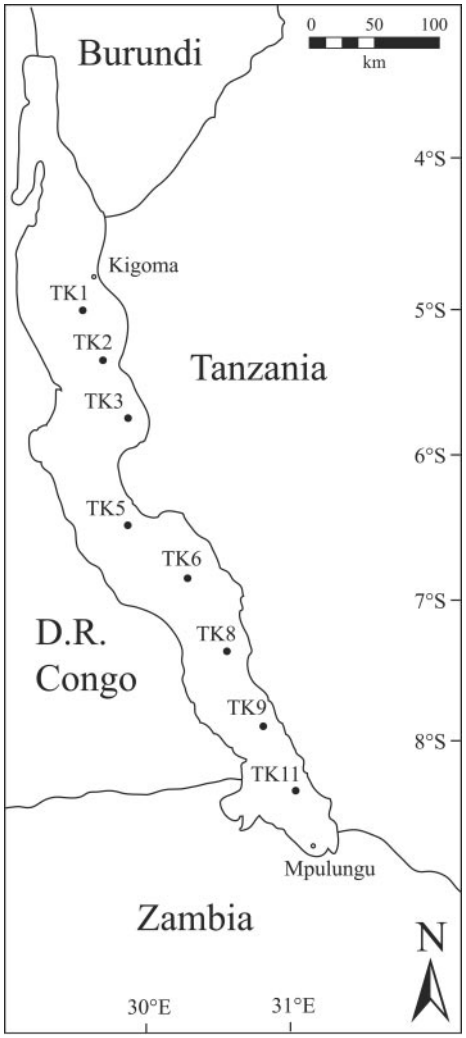

FIG. 1. Sampling sites along a longitudinal transect in Lake Tanganyika.

accumulation of warm surface water in the north of the lake. When the monsoon winds subside during the rainy season, the thermocline exhibits dampened oscillations. These oscillations cause internal waves and alternated upwelling in the north and south of the lake. The maximum stability of the lake is reached at the end of the rainy season in January to April. Long-term monitoring suggests that tilting of the thermocline and upwelling of deep water are reduced during El Niño years (34).

Field sampling. For this study, samples were collected on a 7-day cruise from the north to the south of the lake on 10 to 14 July, during the dry season of 2002. Water samples were collected at eight sites situated along a north-south transect in the lake (Fig. 1) using Hydrobios (5 liter) or Go-Flo (up to 12 liter) sampling bottles. At each site, samples were collected each 20 m down to 100 m. Below a depth of 100 m, samples were collected every 100 to 200 m down to 400 or 1,200 m, depending on the depth of the lake at the site. In some cases different hauls were required to collect large enough volumes for the different analyses. At each site temperature, conductivity, dissolved oxygen, and pH depth profiles were recorded using a SeaBird 19 CTD (conductivity-temperature-depth) instrument. For determination of bacterial production, equal volumes of water samples from 0, 10, 20, and 30 m, representing the upper mixed layer of the water column, were pooled. Subsamples for nutrient analysis were stored refrigerated for analysis within 24 h on board the ship. For pigment analysis, equal volumes of water from depths of 0, 20, 40, and 60 m were pooled to obtain a composite epilimnetic sample. The pooled sample was filtered with a GF/F filter. Subsamples for enumeration of bacteria, phytoplankton (both <5 μm and ≥ 5 μm), heterotrophic nanoflagellates (HNF), and ciliates were fixed by the lugol-formalin-thiosulfate method (41). For analysis of the BCC, water was prefiltered with a 5-μm polycarbonate filter to sample only free-living bacteria. The 5-μm filtrate was then filtered with a 0.22-μm membrane filter, which was folded, wrapped in aluminum foil, and stored frozen.

Analysis of samples. Water used for analysis of dissolved inorganic nutrients was first filtered with a GF/F filter. Nitrate concentrations were determined spectrophotometrically using Macherey-Nägel kits. Soluble reactive phosphorus (SRP) and total phosphorus (TP) contents were determined using standard methods (14). Unfortunately, NH_4 measurements proved to be unreliable. The procedure used for pigment extraction and analysis was based on the procedures of Pandolfini et al. (32) and Descy et al. (8). Phytoplankton that were ≥ 5 μm in diameter were identified when possible and enumerated using an inverted microscope. Phytoplankton biomass was estimated from cell biovolume measurements and previously published biovolume-to-carbon conversion data (28). Ciliates were also enumerated by inverted microscopy, but the quantitative protargol staining technique was used for identification of the dominant species (29). The biovolume of ciliates was converted to biomass as described by Putt and Stoecker (38). Bacteria, phytoplankton that were <5 μm in diameter, and HNF were enumerated using epifluorescence microscopy. Bacteria were stained with DAPI (4′,6′-diamidino-2-phenylindole) (37) and were filtered onto a 0.2-μm-pore-size membrane filter. At least 400 cells were counted in a minimum of 10 randomly chosen fields using UV illumination. When filamentous bacteria were encountered, the total length of filaments in the field of view was recorded. For enumeration of phytoplankton that were <5 μm in diameter, a subsample was filtered onto a 0.8-μm-pore-size membrane filter. At least 400 cells were enumerated using violet-blue illumination (395- to 440-nm excitation filter and 470-nm emission filter) and a Zeiss Axioplan microscope at a magnification of $\times 1{,}000$. A distinction was made between picophytoplankton (diameter, <2 μm) and phytoplankton that were 2 to 5 μm in diameter. For the picophytoplankton, prokaryotic cells were discriminated from eukaryotic cells by switching to green illumination (510- to 560-nm excitation filter and 590-nm emission filter) (26). Heterotrophic nanoflagellates were stained with DAPI and filtered onto 0.8-μm-pore-size filters. A minimum of 100 cells were counted using UV illumination (365-nm excitation filter and 397-nm emission filter). The biovolume of HNF was estimated from cell measurements and was converted to C as described by Putt and Stoecker (38).

Bacterial production. Bacterial production was estimated by determining the rate of incorporation of tritiated thymidine into DNA (11, 12). Subsamples (20 ml) were incubated with [^{3}H]thymidine (20 nM) for 2 h at the lake temperature in the dark. Incubations were ended by adding cold 15% trichloroacetic acid (10 ml) and storing the preparations for at least 15 min at 4°C. Subsamples were filtered through 0.2-μm cellulose nitrate filters. The filters were rinsed with 5% trichloroacetic acid and, when dry, stored in scintillation vials. Subsamples were radioassayed (Beckman LS6000IC) after addition of scintillation cocktail (FilterCount; Packard). A conversion factor of 1×10^9 cells per nanomole of thymidine and indigenous bacterial carbon contents were used to convert the thymidine incorporation into carbon units.

DGGE analysis. Part of the filter for DGGE analysis was cut out with a sterile scalpel, and DNA was extracted using the extraction protocol described by Muyzer et al. (30). DNA was purified on a Wizard column (Promega, Madison, WI) used according to the manufacturer's recommendations. For DGGE analysis, a small 16S rRNA gene fragment was amplified with primers F357-GC (5′-CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCCCCTACGGGAGGCAGCAG-3′) and R518 (5′-ATTACCGCGGCTGCTGG-3′). PCR amplification was performed with these primers specific for the domain *Bacteria* as described by Van der Gucht et al. (47) by using a Genius temperature cycler with 4 to 8 μl of template DNA. The presence of PCR products and their concentration were determined by analyzing 5 μl of product on 1% (wt/vol) agarose gels, staining with ethidium bromide, and comparison with a molecular weight marker (Smartladder; Eurogentec). Equal amounts of PCR product were applied to the DGGE gel using a denaturing gradient containing 35 to 70% denaturant and run as described by Muyzer et al. (30) with the slight modification described by Van der Gucht et al. (47). As standards, we used a mixture of DNA from nine clones obtained from a clone library of the 16S rRNA genes from a small eutrophic lake. On every gel, three or four standard lanes were analyzed parallel to the samples. To obtain a matrix with the relative intensity of each band in all samples, the gels were analyzed using the software package Bionumerics 5.1 (Applied Maths BVBA, Kortrijk, Belgium). A number of bands with more than 40% relative band intensity in at least two samples were selected for sequencing. These bands were excised and sequenced after reextraction and amplification. Sequencing was performed with an ABI-Prism sequencing kit (PE Biosystems) using primer Stef1Tex (5′-GCGTTCATCGTTGCGAG-3′) and an automated sequencer (ABI-Prism 377). A nucleotide BLAST search (2; http://www.ncbi.nlm

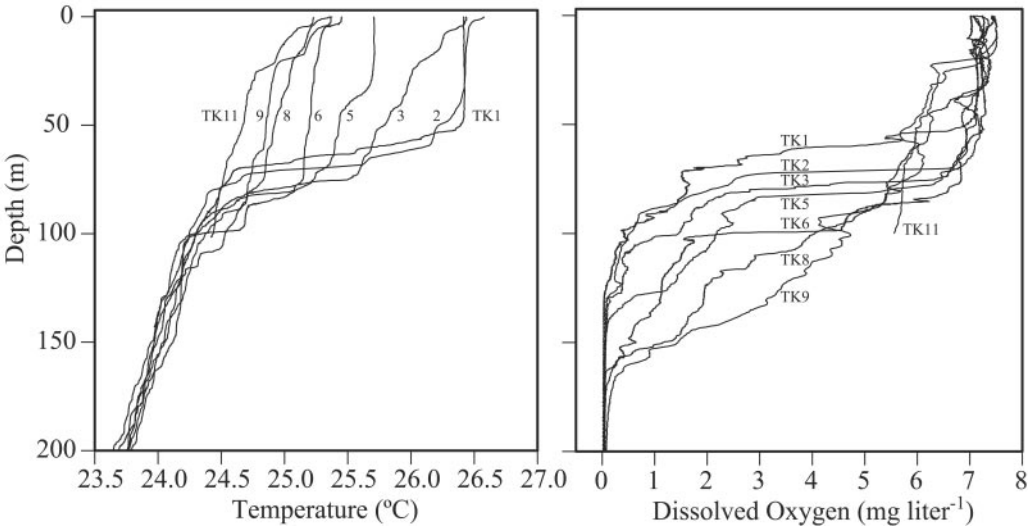

FIG. 2. Temperature (left panel) and oxygen (right panel) profiles for 200 m at the sampling sites.

.nih.gov/BLAST/) was performed in order to obtain sequences with the greatest significant alignment.

Data analysis. To obtain a quantitative measure of the water column stability at each site, the potential energy anomaly (PEA) for the upper 100 m of the water column was calculated as described by Simpson et al. (43). Pigment data were processed with the CHEMTAX software to estimate the contribution of major algal groups to total phytoplankton biomass; an initial pigment ratio matrix was derived from a previously published study of oligotrophic lakes (8). Details of the analysis of pigment data can be found in reference 7. The similarity of the bacterial community in the lake to communities from other freshwater systems was explored by aligning the sequenced DGGE bands with representatives from related freshwater clusters as defined by Zwart et al. (51) and Warnecke et al. (49) and with close relatives retrieved from GenBank. The alignment was carried out with ClustalX (45), and the aligned sequences were used with PAUP4b10 (44) to construct a 1,000× bootstrapped neighbor-joining tree rooted with the archaeon *Pyrodictium occultum*.

Nucleotide sequence accession numbers. The sequences determined in this study have been deposited in the GenBank database under accession numbers AY845326 to AY845337.

RESULTS

Environmental variables. The surface water temperature declined from 26.5°C in the north to 25.2°C in the south of the lake. The thermocline depth simultaneously increased from 60 m in the north to about 120 m in the south of the lake (Fig. 2). At depths below 120 m, the temperature differences between the north and south of the lake were very small. As a result of the latitudinal variation in the thermocline depth, the PEA decreased from the north to the south of the lake (Fig. 3). The dissolved oxygen concentrations at the lake surface did not differ much between the north and the south of the lake. Anoxic conditions were reached at a depth of about 120 m in the north of the lake and at a depth of about 180 m in the south. The conductivity increased below the thermocline at all sites. The conductivity at the water surface was higher in the north than in the south of the lake (Fig. 4). The pH decreased below the thermocline at all sites and did not differ between the north and the south of the lake (Fig. 4). The SRP and TP concentrations (Fig. 4) increased below the thermocline throughout the lake. The epilimnetic concentrations of SRP were similar in the north and in the south of the lake, while the TP concentrations were slightly higher in the south. At depths below 700 m, the SRP and TP concentrations were slightly higher in the north than in the south of the lake. The concentrations of nitrate generally peaked between the thermocline and the oxycline. In the surface waters, the nitrate concentrations were higher in the south of the lake than in the north of the lake. Nitrate was absent at depths below 500 m due to conversion to ammonia in anoxic conditions.

Biological components. The total phytoplankton biovolume at a depth of 20 m was low at all sites (<50 mm^3 m^{-3}) and increased from the north to the south of the lake. The contribution of phytoplankton smaller than 5 μm in diameter to the total phytoplankton biomass increased toward the south of the lake (Fig. 3). The chlorophyll *a* concentrations measured in pooled samples obtained from depths of 0 to 60 m ranged from 0.3 to 1.0 μg liter^{-1} and increased from the north to the south of the lake (Fig. 3). The concentrations in the euphotic zone were slightly higher (range, 0.5 to 1.3 μg liter^{-1}) (7). Using processing of pigment data with CHEMTAX, *Cyanobacteria* of the *Synechococcus* pigment type and *Chlorophytes* were identified as the main phytoplankton groups, contributing about 90% of the total chlorophyll *a* concentration. The biomass of heterotrophic nanoflagellates at a depth of 20 m ranged from 2.2 to 3.3 μg C liter^{-1}, and there was not a clear latitudinal trend. The mean size of the HNF was between 2 and 5 μm. The ciliate biomass at a depth of 20 m ranged from 0.1 to 8.8 μg C liter^{-1} and was maximal at station TK1 and minimal at stations TK9 and TK11. The ciliate community was dominated by the peritrichous organisms *Pseudohaplocaulus* sp. and *Vorticella aquadulcis*.

The bacterial abundance ranged from 1×10^6 to 4×10^6 cells ml^{-1} in surface waters and from 3×10^5 to 4×10^5 cells ml^{-1} at depths below 200 m (Fig. 4). At depths below 200 m, colonies of filamentous bacteria were present in all samples. Due to the low numbers of filaments encountered during the counting, the total filament length varied greatly between samples (1.6×10^5 to 8.8×10^5 μm ml^{-1}). The average total length of filaments for all hypolimnetic samples was 6×10^5 μm ml^{-1}. Like

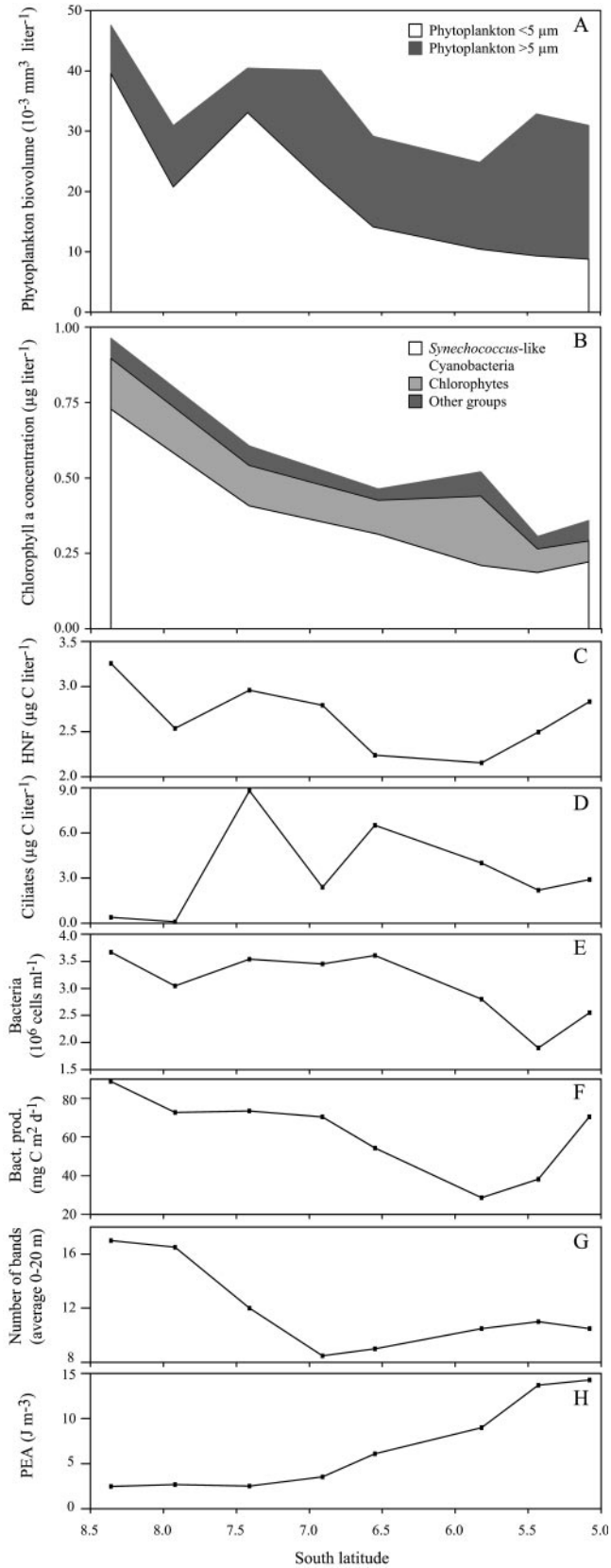

FIG. 3. Horizontal profiles (from south to north) of measured biotic parameters and the PEA. Each dot represents a sampling station, with TK11 on the left in the graph and TK1 on the right. (A) Horizontal changes in phytoplankton biomass at a depth of 20 m, obtained from inverted microscope (≥5 μm) and epifluorescence (<5 μm) counts. (B) Results of depth-integrated (0 to 60 m) chlorophyll *a* measurements and CHEMTAX analysis. Data for *Synechococcus*-like *Cyanobacteria*, *Chlorophytes*, and other phytoplankton groups are expressed as chlorophyll *a* equivalents determined by the CHEMTAX analysis. (C to H) HNF biomass (C), ciliate biomass (D), bacterial density (E), bacterial production (Bact. prod.) (F), number of DGGE bands (G), and PEA calculated for the upper 100 m (H).

bacterial abundance, the bacterial production in epilimnion samples also increased from the north to the south of the lake.

DGGE analysis. In the 56 samples that were analyzed by DGGE, 35 band classes were discerned. The number of bands encountered per sample varied between 4 and 19 and was on average 12. The number of bands generally increased from the lake surface to deep water. In the surface water the number of bands increased from the north to the south of the lake (Fig. 3).

Band sequencing. Eleven bands belonging to different band classes were sequenced. These sequenced bands accounted for on average 71% of the relative band intensity in the samples. Only five of the sequenced bands were closely related (>98% similarity) to sequences deposited in the GenBank database (genotypes 1, 2, 3, and 6). The six remaining genotypes exhibited lower sequence similarity (92 to 97%; genotypes 5, 7, 8, 10, and 11). Phylogenetic relationships between our sequences and the database sequences are shown in Fig. 5.

The distribution of the sequenced bands is shown in Fig. 6. Genotypes 1, 3, 4, 5, and 10 were almost exclusively found in the oxic epilimnion of the lake. These genotypes exhibited the highest similarity with *Gammaproteobacteria* (genotypes 1 and 5), *Actinobacteria* (genotype 3 and 10), and *Cyanobacteria* (genotype 4). Genotype 1 occurred mainly in the south of the lake, while genotypes 5 and 10 were more common in the north. The closest matches during a BLAST search for genotype 1 were a member of the *Gammaproteobacteria* isolated from ocean floor basalt (98% sequence similarity) (25) and an *Acinetobacter* sp. from effluent from a bioremediation site (99%). The closest relative of genotype 5 was a member of the *Gammaproteobacteria* belonging to the *Legionellales* isolated from a Swedish lake. Genotypes 3 and 10 exhibited high sequence similarity with *Actinobacteria* from Swedish lakes and belonged to the acI-B and acIV-A clusters, respectively, as defined by Warnecke et al. (49). Genotype 4 was identified as *Synechococcus*. This genotype also occurred in some hypolimnetic samples. The short length of the sequence did not allow discrimination between freshwater (accession no. AY224198) and marine (accession no. AY135672) *Synechococcus* sequences.

Genotypes 2, 8, and 9 were found mainly in hypolimnetic waters and exhibited high similarity to the *Gammaproteobacteria* (genotypes 2 and 8) and *Deltaproteobacteria* (genotype 9). Genotype 2 exhibited high similarity (99%) to *Actinobacter calcoaceticus*. Genotype 8 exhibited the highest similarity (97%) to a member of the *Gammaproteobacteria* found on the roots of Proteaceae (accession no. AY827046) and the gammaproteobacterium *Stenotrophomonas* isolated from effluent from a water treatment plant (accession no. AY803991). Genotype 9 exhibited the highest similarity to an uncultured bacterium from deep groundwater (95%) and to a member of the

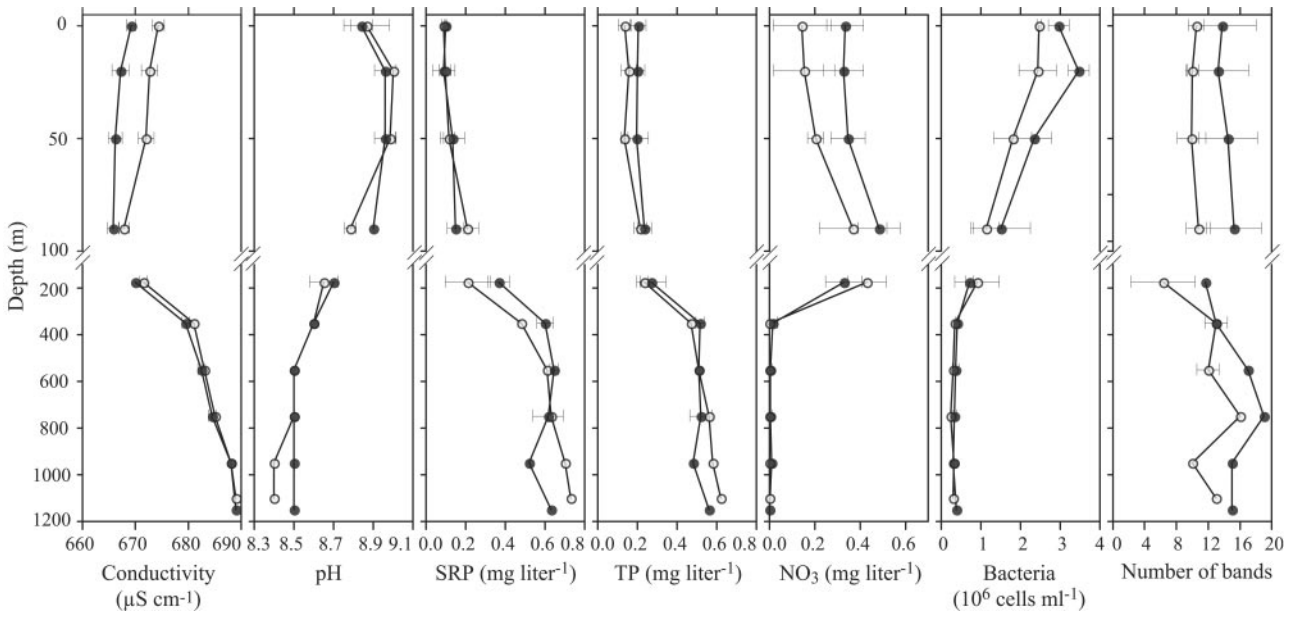

FIG. 4. Depth profiles over 1,200 m for conductivity, pH, SRP, TP, NO_3, bacterial density, and the number of bands for the northern (open circles) and southern (solid circles) stations. The values are averages of the data for sites TK1, TK2, and TK3 for the northern sites and averages of the data for sites TK5, TK6, TK8, TK9, and TK11 for the southern sites.

Deltaproteobacteria isolated from uranium mining waste piles (94%).

Genotypes 6, 7, and 11 were abundant in the hypolimnion throughout the lake but were also detected in epilimnetic samples in the south of the lake. These genotypes were identified as *Nitrospirae*, green nonsulfur bacteria, and *Firmicutes*, respectively. Genotype 6 exhibited 99% sequence similarity to an unidentified member of the *Nitrospirae* detected in groundwater from a deep-well injection site. Genotype 7 exhibited the highest similarity (95%) to a green nonsulfur bacterium isolated from subseafloor sediment from the Sea of Okhotsk (NT-B3 cluster) (16) and an uncultured genotype found in polluted groundwater. Genotype 11 exhibited 96% sequence similarity (74 of 93 bp) to an *Alicyclobacillus* sp. and an *Alicyclobacillus vulcanalis* sequence isolated from hot springs.

DISCUSSION

Lake Tanganyika is a meromictic lake characterized by permanent vertical temperature stratification. Many other environmental variables, like dissolved oxygen, pH, conductivity, and inorganic nutrient concentrations, were strongly linked to the vertical temperature gradient. Eight of the 11 sequenced band classes displayed a clear vertical zonation that was related to the water column stratification (Fig. 6). The number of bands in the DGGE analysis also differed for epi- and hypolimnetic samples, with the hypolimnetic samples having a higher number of bands than the epilimnetic samples. Filamentous bacteria were observed in all samples from the anoxic hypolimnion but in no epilimnetic samples. As filamentous bacteria were excluded from the DGGE analyses due to prefiltration of the samples, differences in the BCC between the epilimnion and the hypolimnion were probably more pronounced than the DGGE data suggest.

As in most tropical temperature-stratified lakes, the vertical and horizontal temperature differences were relatively small in Lake Tanganyika (only 1.5 to 3°C). It is unlikely that such small temperature differences were responsible for the pronounced horizontal and vertical differences in the BCC. Therefore, the influence of vertical and latitudinal temperature differences in Lake Tanganyika on the BCC was probably indirect and due to regulation of the degree of mixing of deep and surface waters. Throughout the lake, the transition from epilimnion to hypolimnion was associated with a transition from oxic to anoxic conditions. As bacteria require different metabolic pathways to survive under oxic and anoxic conditions, differences in oxygen concentration may to a large extent explain differences in the BCC between the epilimnion and the hypolimnion. Genotype 9, which was closely related to a member of the *Deltaproteobacteria*, was found mainly in deep water samples. *Deltaproteobacteria* are known to occur mainly in benthic environments and are rarely found in oxygenated water columns (31). This suggests that differences in oxygen concentration indeed contributed to vertical differences in the BCC. Pronounced vertical differences in BCC have been observed previously in stratified lakes with anoxic deep waters. Konopka et al. (21) observed differences in BCC between oxic and anoxic water samples from 10 thermally stratified lakes in northeastern Indiana. Koizumi et al. (20) found important vertical changes in the BCC in the saline meromictic Lake Kaiike. However, vertical differences in BCC were also observed in stratified lakes without an anoxic hypolimnion. Denisova et al. (6), for example, showed that there were significant differences in BCC with depth in Lake Baikal. Lindström et al. (23) observed greater dominance of *Verrucomicrobia* in the oxic hypolimnion of a Swedish lake.

Genotypes 6, 7, and 11 were found in anoxic deep waters throughout the lake but also occurred in the epilimnion in the south of the lake. This suggests that some genotypes are not restricted to either aerobic or anaerobic conditions. Instead of

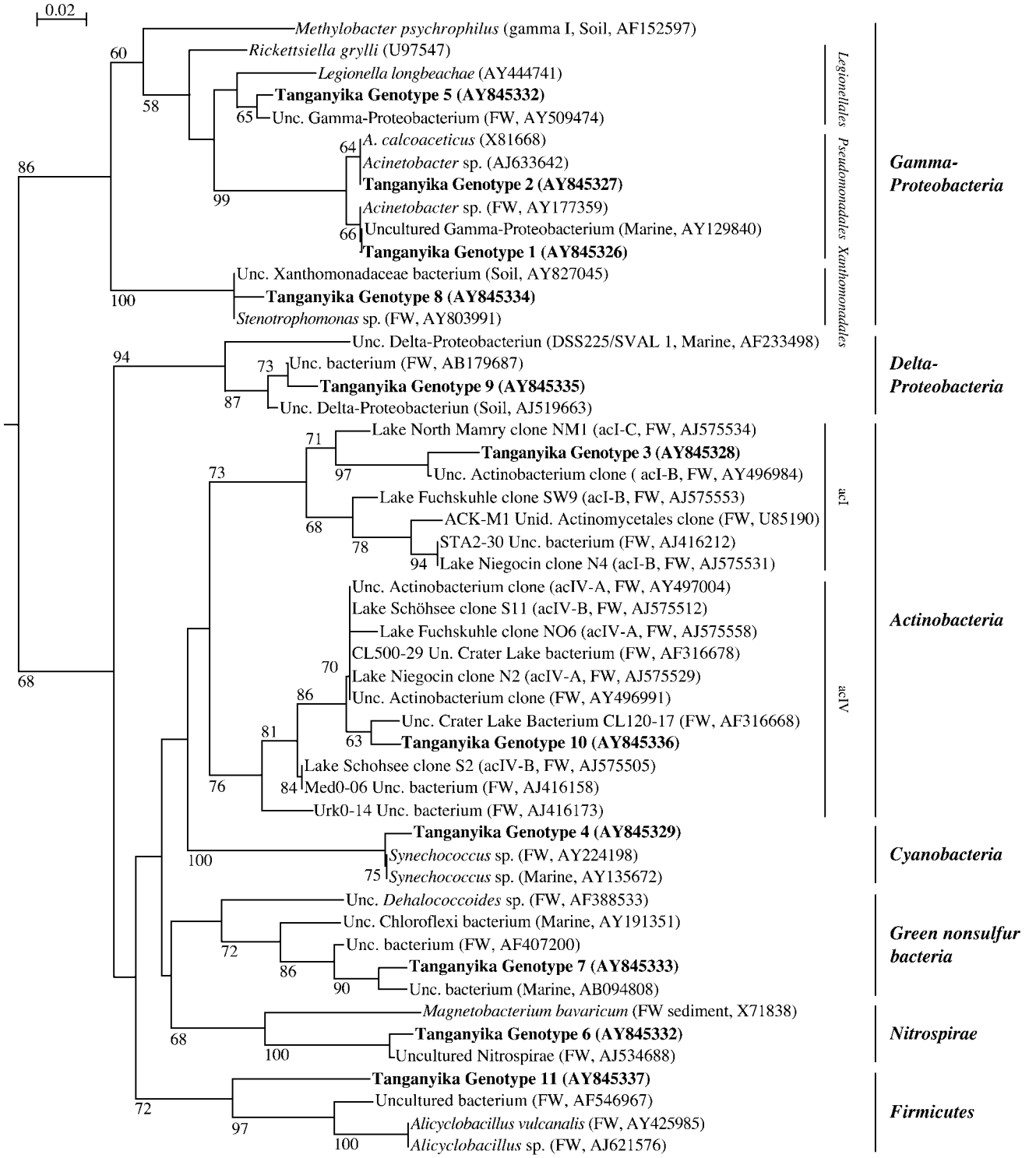

FIG. 5. Neighbor-joining tree showing phylogenetic relationship between sequenced genotypes, their closest matches during a BLAST search, and relevant cluster representatives from the studies of Zwart et al. (51) and Warnecke et al. (49). Bootstrap percentages greater than 50 are indicated at the nodes and show the support for a cluster 1,000 replicates. GenBank accession numbers are indicated in parentheses. Unc., uncultured; FW, genotypes isolated from freshwater environments.

being linked to oxygen concentration, the distribution of these genotypes may be related to nutrient concentrations. Nutrient concentrations in Lake Tanganyika were high in the hypolimnion throughout the lake. Due to upwelling of deep nutrient-rich water, nutrient concentrations were also increased in the epilimnion in the south of the lake. This was particularly clear for nitrate. The occurrence of hypolimnetic bacteria in surface waters enriched with nutrients is in agreement with observa-

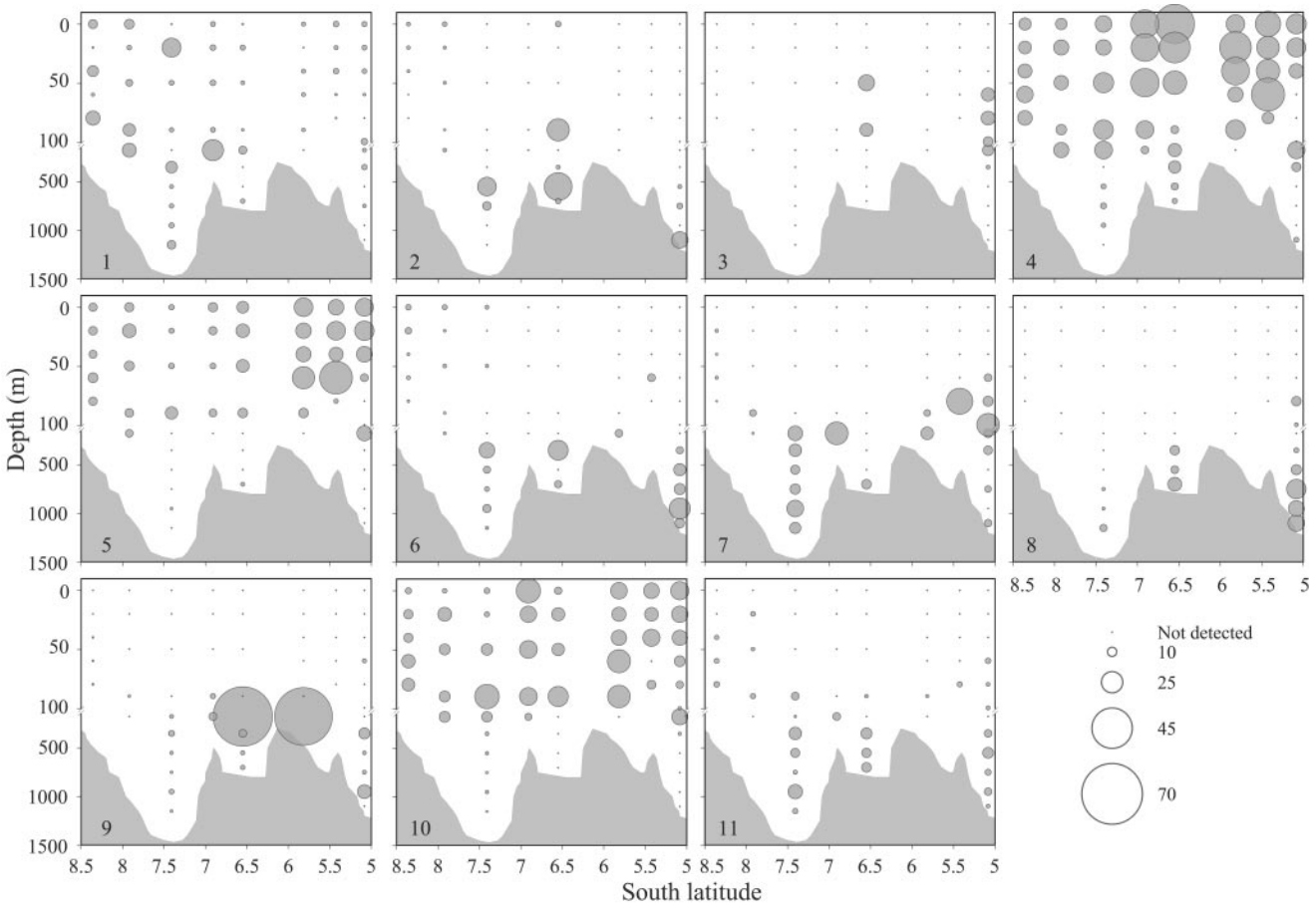

FIG. 6. Distribution of different genotypes throughout the lake. The panel numbers correspond to the band class numbers in Fig. 5. The sizes of the circles correspond to the relative band intensities of the genotypes on the DGGE gel.

tions of Lindström et al. (23). These authors found that typical hypolimnetic bacteria appeared in epilimnetic samples to which nutrients were added. In a transplant experiment carried out in a eutrophic reservoir, a significant effect of nutrients on BCC has also been observed (13).

In addition to the pronounced vertical water column stratification of Lake Tanganyika we also observed a clear latitudinal environmental gradient in the epilimnion of this lake. From the north to the south of the lake, the thermo- and oxycline depth increased, the PEA decreased, and the nitrate concentration increased. This indicated that there was erosion of the thermocline and upwelling of deep water in the south of the lake. Upwelling of deep water in the south of Lake Tanganyika is an annually recurring phenomenon during the dry season that is related to a tilting of the thermocline by southeasterly monsoon winds (35). Upwelling of nutrient-rich water resulted in a higher phytoplankton biomass and, surprisingly, in increased importance of small phytoplankton (diameter, <5 μm) in the phytoplankton community. The upwelling event was also reflected in a latitudinal gradient in the BCC. Compared to the vertical gradient in the BCC, a smaller number of genotypes showed clear latitudinal variation (6 of the 11 band classes sequenced). The influence of spatial variations in water column stability or upwelling on BCC has to our knowledge not been studied previously in lakes. In marine ecosystems, however, local hydrodynamic phenomena like upwelling regions or fronts have also been found to influence BCC. Kerkhof et al. (18) observed changes in BCC during an upwelling event in the Mid-Atlantic Bight. Pinhassi et al. (33) observed strong changes in BCC along the Kattegat-Skagerak front in the North Sea. Schauer et al. (39a) related changes in BCC in a coastal embayment of the Mediterranean to upwelling of deep water from a submarine canyon. Upwelling of deep water in the south of Lake Tanganyika may influence the BCC directly by increasing nutrient concentrations in the epilimnion. However, the effect of nutrients on BCC in the upwelling region may also be indirect. Upwelling of deep, nutrient-rich water in the south of Lake Tanganyika was associated with an increase in the total phytoplankton biomass and a higher contribution of small (<5-μm) phytoplankton. Changes in the total biomass and community composition of phytoplankton may influence the quantity and quality of organic matter supplied to bacteria and in that way influence BCC (27). In a study of BCC in arctic lakes, the bacterial community was found to change with the dominant source of organic matter (5). In phytoplankton cultures, different bacterial communities are often associated with different phytoplankton species (48). In the epilimnion, the number of bands was higher in the south than in the north of

the lake. The mean number of bands per sample in the epilimnion was positively correlated with bacterial production, suggesting that there is a positive diversity-productivity relationship. Yannarell and Triplett (50) also found a positive diversity-productivity relationship in different Wisconsin lakes. Horner-Devine et al. (15a), however, found that the responses of bacterial diversity to productivity in a mesocosm experiment were different for different major taxonomic groups.

In the hypolimnetic samples from Lake Tanganyika, the genotypes with the greatest relative band intensity were identified as members of the *Deltaproteobacteria*, *Nitrospirae*, green nonsulfur bacteria, and *Firmicutes*. The sequences exhibited similarity to genotypes isolated from other freshwater environments, albeit different from hypolimnetic lake water, ranging from deep groundwater to acidic hot springs. However, they were not included in the typical freshwater clusters defined by Zwart et al. (51). This is not surprising since anoxic environments were not included in the study of Zwart et al. (51). The genotypes with the highest relative band intensities in the epilimnion were identified as members of the *Actinobacteria*, *Gammaproteobacteria*, and *Cyanobacteria*. *Actinobacteria* are generally an important component of freshwater bacterial communities (31), and the *Actinobacteria* found in our study belong to the typical freshwater clusters defined by Zwart et al. (51). The representatives of the *Gammaproteobacteria* were not identified as members of the freshwater gamma I cluster (51), but they exhibited high levels of similarity to species observed in other freshwater systems, like Swedish lakes and effluent from a bioremediation site. The importance of *Gammaproteobacteria* in the epilimnion of Lake Tanganyika was rather unexpected, as these organisms are often of minor importance in freshwater ecosystems (31). Moreover, we did not detect *Alpha-* and *Betaproteobacteria*, which tend to be major components of bacterial communities in freshwater systems. The prevalence of *Gammaproteobacteria* and the lack of *Alpha-* and *Betaproteobacteria* in our samples, however, may be related to bias in the DGGE analysis. The relative band intensity of a genotype in a DGGE analysis does not necessarily reflect the relative abundance of the genotype in the prokaryotic community (22). For instance, Kirchman and Castle (19) detected *Betaproteobacteria* using fluorescent in situ hybridization but not using DGGE. The high relative band intensity for *Synechococcus* in our samples also illustrates this bias in the DGGE method. While *Synechococcus* often contributed more than 50% of the relative band intensity in DGGE profiles of the epilimnetic samples, epifluorescence counts indicated that the contribution of picocyanobacteria to the total prokaryotic cell numbers could not have been greater than 10%. Moreover, cell counts and pigment data indicate that there was an increase in picocyanobacteria, while the DGGE data revealed a decrease in the relative band intensity for *Synechococcus*.

Conclusions. This study demonstrated the presence of pronounced vertical and latitudinal gradients in the BCC in Lake Tanganyika during the dry season of 2002. Vertical gradients in the BCC could be related to vertical differences in oxygen and/or nutrient concentrations. As Lake Tanganyika is a permanently stratified lake, vertical gradients in the BCC are probably permanently present. Latitudinal gradients in the BCC were related to upwelling of deep water in the south of the lake. Upwelling may influence BCC by influencing nutrient concentrations and phytoplankton biomass and community composition. Latitudinal differences in BCC may be less pronounced at the end of the rainy season, when stratification is similar throughout the lake. Since 2002 was an El Niño year (although a weak event) and upwelling in Lake Tanganyika seems to be relatively weak during El Niño years (34), latitudinal differences in BCC may be more pronounced during the dry season of other years. Sequencing of the dominant bands in the DGGE gels revealed the presence of *Actinobacteria* and *Gammaproteobacteria* in the epilimnion, while *Gammaproteobacteria*, *Nitrospirae*, green nonsulfur bacteria, *Actinobacteria*, *Deltaproteobacteria*, and *Firmicutes* were observed in the hypolimnion. A thorough analysis of the BCC using clone libraries, fluorescent in situ hybridization, and/or reverse line blotting is required to obtain more detailed information on the occurrence of specific bacterial clusters in this lake.

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