Bacterial Diversity and Physicochemical Stratification of the Water Column during the Rainy Season in Las Cumbres Lake, Panama

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Citation

Abstract
Bacterial diversity was studied in Las Cumbres Lake, Panama City, in relation to the physicochemical variables of the lake during the rainy season. Water samples were taken at different depths, and physicochemical variables (temperature, dissolved oxygen, total solids, phosphate and nitrate concentrations) and a biological indicator of wastewater pollution, the total coliform count, were determined.

To examine bacterial diversity, 16S rRNA clone libraries from each depth were analyzed. In total, 255 bacterial sequences were obtained and assigned to OTUs (operational taxonomic units) corresponding to a similarity threshold of 97% (0.03 cutoff). This resulted in 24 total OTU assignments from different phyla, such as Proteobacteria, Bacteriodetes, Verrucomicrobia, Actinobacteria and Chlorobi. Alpha (a) diversity was assessed by rarefaction curves. At three depths (0, 5 and 10 meters), this analysis demonstrated that the sampling effort was sufficient to represent the diversity of the communities. Beta (b) diversity was analyzed by means of a Venn diagram and revealed a large number of unique species at each depth.

This study pioneers and establishes methods that can be used for similar studies in freshwater and marine environments in Panamá, and suggests that surveys of water quality should sample from deeper strata, in addition to surface waters.

INTRODUCTION
Physicochemical variations throughout a body of water such as a lake are dependent in part on microbial activities (Hohnk, 1962). Microbes are responsible for providing essential elements of life through biogeochemical cycling (Kirchman, 2008). Some essential processes that maintain life on earth and are performed by microbes are, among others, organic matter degradation, recycling of CO2, and nitrogen fixation from the atmosphere.

Microorganisms also have a large impact on the distribution of organic and inorganic nutrients in the ocean because they efficiently and thoroughly penetrate both the water and sediment layers by virtue of by their high numbers (Surajit, et al., 2006). To this end, microbes possess extracellular catalytic enzymes that decompose various dissolved and particulate substrates, and the release of these nutrients makes life possible in an aquatic environment (Meyer-Reil 1994).

Of equal importance, the biodiversity of the bacteria that participate in these processes is dependent upon environmental conditions such as the seasons of the year, wind fluctuations, geomorphology of the lake, and anthropogenic influences. Human activities in the form of household and industrial wastes and nutrient status alterations impact heavily upon aquatic ecosystems. Microbes respond readily to anthropogenic pollution, which is responsible for changes in microbial diversity and shifts in dominant phylotype representation (Kuznetsov, 1970, Matcher, et al., 2011). Determining physical and chemical factors, such as temperature, pH, and geography, that correlate with differences between diverse microbial communities will reveal how easily microbes tolerate different environmental changes and will increase our understanding of microbial ecology (Lozupone and Knight, 2007).

Metagenomics, a culture-independent method to study bacterial biodiversity, gives more insights into true bacterial
diversity than culture-dependent methods, since it is estimated that only 1% of all bacteria have been cultured (Zengler et al., 2002; Schloss and Handelsman, 2005). Phylogenetic analysis of the 16S rRNA gene is a cornerstone for such studies, and permits classification of bacteria into approximately 23 known phyla, most of which can be found in aquatic environments (Whitman et al., 1998; Streit WR et al., 2004). In both freshwater and marine environments, unclassified environmental bacteria have been detected as well (Spring et al., 2000; Gentile et al., 2006). Cyanobacteria, along with the Alpha Proteobacteria, constitute the major fraction of bacteria that have previously been documented in lake surface waters (Pandey and Pandey, 2002; Zwart et al., 2002; EileBertilsson, 2004).

Located in Panamá City, Las Cumbres Lake (09°05'54.9" N, 79°32'21.3" W) is a vertically stratified ecosystem due to the physical and chemical gradients (oxygen, nitrates, phosphate, temperature) established along its water column. The lake is affected by sewage water, and possibly by runoff from industrial activities originating in factories surrounding the lake (B.Chial, personal communication).

The aim of this study was to determine the bacterial diversity of Las Cumbres Lake using 16S rRNA gene analysis, and to relate this diversity to the physicochemical variables found in the water column. This study pioneers and establishes methods that may then be used for other similar studies in freshwater and marine environments in Panamá.

**EXPERIMENTAL PROCEDURES**

**Study Site:** Our study area consisted of Las Cumbres Lake, which has a surface area of 1.5 km² and is located in Panama province in the Republic of Panama (09°05'54.9" N, 79°32'21.3" W) (Figure 1). The lake is affected by sewage water and possibly by industrial activities from factories around the lake (B. Chial, personal communication). Since the lake has a specific geomorphology that shows some stratification, the sample site chosen was at the deepest part of the lake, where the depth reached 21 meters (0660579 E / 1006143 N) (B.Chial, personal communication).

**Sample collection:** Water samples were collected during the month of November of 2009, during the rainy season in Panama. Samples were collected from 0 m, 5 m, 10 m and 16 m using a Van Dor horizontal sampler. These depths correspond to a stratification profile previously defined using the physicochemical and biological parameters of this lake (B.Chial, personal communication). Samples were kept on ice in a cooler until processed in the laboratory.

**Physicochemical and Biological Parameters:** The in situ determination of oxygen, temperature, and total suspended solids was performed with a multiparameter probe instrument according to manufacturer’s instructions (Horiba).

Chemical and microbiological analyses for total coliforms, nitrates, and phosphates were carried out by the laboratory of the National Authority of the Environment of Panama, according to methodology standardized for water and wastewater methods (Standard Methods for the Examination of Water and Wastewater, 20th Edition, 2005, APHA, AWWA, WPCF.1992).

**DNA Extraction:** Water samples were filtered through a Millipore sterivex filter cartridge with a pore size of 0.22 mm. Lysis buffer was added to each cartridge and the samples were stored at -80°C until processed, as previously described (Ferrari and Hollibaugh, 1999).

**PCR Amplification:** Small subunit rRNA genes were amplified using the following primers: 9F, a primer specific for the domain Bacteria (forward, 5’-GAGTTTGATCCTGGCTCAG-3’, positions 9 to 27 in E.
coli), and the universal primer 1490R (reverse, 5’GGTTACCTTGTACGACCTT-3’, positions 1490 to 1508 in E. coli), as previously described (Weisenburg et al., 1991).

A PCR Master mix was prepared according to manufacturer’s instructions (Promega, Master Mix), and PCR conditions were performed as previously described (Weisenburg et al., 1991).

Metagenomic libraries: PCR products were cloned into a pCR2.1 vector and transformed into Top10F’ competent cells according to manufacturer’s instructions (Invitrogen). Clones were selected from Luria Broth (LB) agar plates containing 40 mg/ml 5-bromo-4-chloro-3-indolylβ-D-galactopyranoside (X-Gal), 100 mM isopropylβ-D-thiogalactopyranoside (IPTG) and 100-µg/ml ampicillin. PCR screening was performed using primers 9F and 1490R. For each depth the clone libraries were assigned as follows: PA0 (0m), PA1 (5m), PA2 (10m) and PA3 (16m).

Sequence determination and taxa identification: Fifty insert clones of each depth were sequenced at the Molecular Biology Facilities of the Smithsonian Tropical Research Institute in Panama. Sanger sequencing was performed on 303 sequences using 9F and 1490R primers, and ABI PRISM Big Dye Terminator v3.1, as described by the manufacturer (Applied Biosystem), run on the Genetic Analyzer ABI 3130XL (Applied Biosystem), and verified using Sequencher 4.7 (Gene Codes). The sequence data set was screened for potential chimeric structures by using Bellerophon Chimera program V3.0 on the green genes webpage (http://greengenes.lbl.gov/cgi-bin/nph-bel3_interface.cgi).

Sequences were compared using the BLASTX algorithm against the National Center for Biotechnology information (NCBI, (http://www.ncbi.nlm.nih.gov) database and with the Ribosomal DataBase Project (RDP) (Cole et al., 2009; Wang et al., 2007). Only sequences with similarities to the bacterial domain (255 sequences) were selected for phylogenetic analysis.

Nucleotide sequence accession numbers: The partial 16S rRNA gene sequences obtained in this study have been deposited in Genbank under the accession numbers JX013233- JX013484.

Sequence quality filtering and alignment: Sequences used in the present study were converted to fasta format using the READSEQ conversion tool (http://www-bimas.cit.nih.gov/molbio/readseq/). The 16S rRNA sequence curation pipeline of MOTHUR software, version 1.24.1 (Schloss et al., 2009) was used for sequence processing. Sequence processing including making groups and a name file using the make.groups and unique.seq commands, respectively. Sequence alignment was performed using the align.seqs command, which aligned the sequences to the SILVA-compatible alignment database reference alignment. The screen.seqs command was then executed to remove any sequence in the alignment that did not belong to the same alignment space. Aligned sequences were then filtered to remove columns that corresponded to ‘.’ or ‘-’ in all sequences. Unique.seqs command then removed redundant sequences. Next, the pre.cluster command merged and removed sequences that contained mismatches. Chimeras were removed using the chimera.uchime command and the reference database from Silva that is compatible with the Goldsequence database. Sequences affiliated with mitochondria and chloroplasts were removed using the classify.command and the RDP reference files.

OTU based-Sequence analysis:

Filtered alignments were then used to generate a pair wise distance matrix using the dist.seqs command in MOTHUR. To assign sequences into OTUs, a further neighbor-clustering algorithm was employed using the cluster.split command and the classify.otu command to assign each OTU on the basis of a similarity distance cutoff of 0.03 (OTU0.03). The OTUs were then assigned to class taxa. MOTHUR was used to calculate bacterial richness and diversity with both the Chao1 and inverse Simpson indices, respectively. Using MOTHUR output (rarefaction.single command), the maximum observed rarefaction OTU values and rarefaction curves were generated. Community overlap was examined using a Venn diagram generated by MOTHUR.

RESULTS

Physicochemical and microbiological variables:

Water Column Profile:

The vertical distributions of physicochemical and microbial variables were measured at the surface and at each 5 meters to a depth of 16 meters for a column of water in Las Cumbres Lake (Figure 2). The exact coordinates for the monitored station were 0660579 E / 1006143 N, and are shown pictorially in Figure 1.
A temperature gradient was observed running from the surface (29.2°C) to five meters deep (26.8°C) and spanning approximately three degrees Celsius. Below five meters, the temperature remained relatively constant at about 26.7 °C (Figure 2A). A thermocline is not observed as such, however two strata were present: a surface layer up to five meters deep followed immediately by a monimolimnion of 26.7 °C down to 16 meters (the depth of the final measurement).

A similar pattern was observed for the oxygen profile, showing a 50% reduction from its concentration at the surface (4.8 mgO2/L) to 5 meters deep, whereas below this depth the oxygen concentration was maintained relatively constant (2.4 mgO2/L to 2.2 mgO2/L) (Figure 2B).

The concentration of total suspended solids (Figure 2C), showed relatively constant values in the first 10 meters (137.5 mgTSS/L to 136.9 mgTSS/L) and then increased in concentration by 16 meters (159.0 mgTSS/L).

The nitrate concentration (Figure 2D) increased slightly from the surface (0.89 mgNO3/L) at 5 and 10 meters deep (0.98 mg NO3/L to 1.0 mg NO3/L), decreasing again at 16 meters (0.88 mg NO3/L). Likewise, for the orthophosphates profile (Figure 2E), the surface value started at 0.11 mgPO4/L, at 5 meters deep reached 0.14 mg PO4/L, continued to increase until at 10 meters at 0.16 mg PO4/L, and finally decreased its value to 0.12 mg PO4/L at 16 meters.

Total coliforms, meanwhile, increased from the surface (20 CFU/100ml) to 5 meters deep (120 CFU/100ml), and then decreased sharply from 10 to 16 meters deep (30 CFU/100ml). The average values of 3 replicate measurements for the physicochemical and microbiological parameters are presented in Table 1.

**Table 1**

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>T(°C)</th>
<th>DO (mg/L)</th>
<th>Total Suspended Solids (mg/L)</th>
<th>Nitrate-NO3 (mg/L)</th>
<th>Phosphate-PO4 (mg/L)</th>
<th>Total Coliforms (CFU/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>29.2</td>
<td>6.8</td>
<td>137.5</td>
<td>0.89</td>
<td>0.11</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>29.8</td>
<td>2.4</td>
<td>130.9</td>
<td>0.66</td>
<td>0.14</td>
<td>110</td>
</tr>
<tr>
<td>10</td>
<td>26.7</td>
<td>2.3</td>
<td>136.95</td>
<td>1.0</td>
<td>0.16</td>
<td>26</td>
</tr>
<tr>
<td>15</td>
<td>26.7</td>
<td>2.2</td>
<td>159.03</td>
<td>0.88</td>
<td>0.12</td>
<td>40</td>
</tr>
</tbody>
</table>

Pearson correlation analysis (product moment correlation) was performed between all physicochemical and microbiological parameters (Table 2), and the Inverse Simpson Index for the various groups of bacteria were determined for each depth.
Table 2
Correlation matrix between physicochemical and microbial parameters, and the Coverage and Inverse Simpson Index. Values marked in red are significant at p < 0.05.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Depth (m)</th>
<th>T (°C)</th>
<th>pH</th>
<th>DO (mg/L)</th>
<th>NO₂⁻</th>
<th>NO₃⁻</th>
<th>TP (mg/L)</th>
<th>TD-S (mg/L)</th>
<th>NPD</th>
<th>Coverage</th>
<th>NI Simpson</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth (m)</td>
<td>0.0000</td>
<td>-0.968</td>
<td>0.000</td>
<td>-0.886</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>T (°C)</td>
<td>-0.968</td>
<td>1.000</td>
<td>-0.514</td>
<td>-0.477</td>
<td>-0.514</td>
<td>1.000</td>
<td>-0.982</td>
<td>-0.982</td>
<td>-0.982</td>
<td>0.171</td>
<td>0.171</td>
</tr>
<tr>
<td>pH</td>
<td>0.000</td>
<td>-0.514</td>
<td>1.000</td>
<td>-0.982</td>
<td>-0.982</td>
<td>-0.982</td>
<td>1.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>-0.886</td>
<td>-0.477</td>
<td>-0.982</td>
<td>1.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>0.000</td>
<td>-0.514</td>
<td>-0.982</td>
<td>0.000</td>
<td>1.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>1.000</td>
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</tr>
<tr>
<td>NO₃⁻</td>
<td>0.000</td>
<td>-0.514</td>
<td>-0.982</td>
<td>0.000</td>
<td>0.000</td>
<td>1.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>1.000</td>
<td>0.000</td>
</tr>
<tr>
<td>TP (mg/L)</td>
<td>-0.982</td>
<td>-0.982</td>
<td>1.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>1.000</td>
<td>1.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>TD-S (mg/L)</td>
<td>-0.982</td>
<td>-0.982</td>
<td>0.000</td>
<td>1.000</td>
<td>0.000</td>
<td>0.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>NPD</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
</tbody>
</table>

All relationships between the physicochemical factors and depth, and the inverse Simpson Index with depth, showed a high correlation (0.78 to 0.92), albeit at a significance not less than p < 0.05. Levels of significance determined for these relationships ranged between p = 0.077 to p = 0.218. However, the regression curves for each case showed a clear trend with respect to depth (Figure 3).

The relationship between depth and dissolved oxygen, temperature, total suspended solids, total coliforms, and the Inverse Simpson Index, showed an inverse relationship. In contrast, the relationship between depth and nitrates and phosphates showed a positive relationship.

Composition of clone libraries
1. PA0 clone library (depth = 0 meters): A total of 50 clones were sequenced from this library. All sequences were analyzed using the BLAST algorithm of the GenBank and RDP databases and 39 sequences were selected for analysis due to their similarities to the Bacterial domain. After the filtering process, one sequence was removed and the unique.seqs command identified 20 unique sequences. No chimeras were found. Seven different OTUs were identified from a total of 37 clones after the sequence filtering process. Of these 62% corresponded to Proteobacteria, 27% corresponded to Verrucomicrobia and 11% to Bacteriodetes (Figure 4). The dominant class was the Betaproteobacteria.
and among them the order Burkholderiales.

**Figure 4**

Bacterial phyla of the PA0 library (depth = 0 meters) as determined by MOTHUR using 16S rRNA sequences.

To characterize bacterial species richness and diversity we used MOTHUR with the Chao1 and inverse Simpson indices, both of which corresponded well to the rarefaction analysis (Figure 5). The rarefaction curves for the PA0 library reached a plateau, revealing that enough sampling effort had been done to represent the diversity of the community.

**Figure 5**

Rarefaction curve (red) showing the observed bacteria diversity of 16S rRNA genes retrieved from water trough samples at 0 meters (PA0 library). Light blue lines indicate upper and lower 95% confidence intervals. The dark blue line represents a theoretical 1:1 sampling.

2. **PA1 clone library (depth = 5 meters):** A total of 67 clones were sequenced from this library. All sequences were analyzed using the BLAST algorithm of GenBank and the RDP database, and 54 sequences were further processed due to their similarities with the Bacterial domain. After the filtering process, two sequences were removed and the unique.seqs command identified 23 unique sequences. One chimera was found and was removed. Nine different OTUs were identified from a total of 40 clones after the sequence filtering process. Of these, 87.5% corresponded to Proteobacteria, 7.5% to Bacteriodetes, 2.5% corresponded to Verrucomicrobia and 2.5% to unclassified bacteria (Figure 6). The dominant class was the Gammaproteobacteria, and among them the order Enterobacteriales.

**Figure 6**

Bacterial phyla of PA1 library (depth = 5 meters) as determined by MOTHUR using 16S rRNA sequences.

Bacterial richness and diversity, as well as a rarefaction curve, were calculated as described above (Figure 7). Once again the rarefaction curve reflected that adequate sampling had been performed to reflect community diversity.

**Figure 7**

Rarefaction curve (red) showing the observed bacteria diversity of 16S rRNA genes retrieved from water trough samples at 5 meters (PA1 library). Light blue lines indicate upper and lower 95% confidence intervals. The dark blue line represents a theoretical 1:1 sampling.

3. **PA2 clone library (depth = 10 meters):** A total of 51 clones were sequenced from this library. All sequences were analyzed using the BLAST algorithm of GenBank and the RDP database, and 46 sequences were further processed due to their similarities with the Bacterial domain. After the
filtering process, three sequences were removed and the unique.seqs command identified 21 unique sequences. No chimeras were found. Seven different OTUs were identified from a total of 40 clones after the sequence filtering process. Of these, 98% corresponded to Proteobacteria and 2% to Actinobacteria (Figure 8). The dominant class was the Gammaproteobacteria and the order Enterobacteriales.

**Figure 8**
Bacterial phyla of PA2 library (depth = 10 meters) as determined by MOTHUR using 16S rRNA sequences.

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteobacteria</td>
<td>98%</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>2%</td>
</tr>
</tbody>
</table>

The rarefaction curve for the PA2 library indicated that the sampling effort did represent the diversity of the community, since the curve reached a plateau (Figure 9). Richness and diversity analyses with MOTHUR were also performed.

**Figure 9**
Rarefaction curve (red) showing the observed bacteria diversity of 16S rRNA genes retrieved from water trough samples at 10 meters (PA2 library). Light blue lines indicate upper and lower 95% confidence intervals. The dark blue line represents a theoretical 1:1 sampling.

4. PA3 clone library (depth = 16 meters): A total of 37 clones were sequenced from this library. All sequences were analyzed using the BLAST algorithm of GenBank and the RDP database, and 22 sequences were selected for further processing due to their similarities with the Bacterial domain. After the filtering process, two sequences were removed and the unique.seqs command identified 16 unique sequences. No chimeras were found. Nine unique OTUs were identified from a total of 10 clones after the sequence filtering process. Of these, 3 sequences corresponded to Proteobacteria, 4 to Bacteriodetes and 2 to unclassified bacteria. The rarefaction curve revealed that a larger number of sequences needed to be sampled, because although the number of OTUs increased with an increasing number of sequences, total coverage of bacterial richness was not achieved (Figure 10).

**Figure 10**
Rarefaction curve (red) showing the observed bacteria diversity of 16S rRNA genes retrieved from water trough samples at 16 meters (PA3 library). Light blue lines indicate upper and lower 95% confidence intervals. The dark blue line represents a theoretical 1:1 sampling.

Beta diversity (b) between the four different sample depths in Las Cumbres Lake during the rainy season: The MOTHUR venn command elaborated a Venn diagram at a distance of 0.03 (97% similarity) that showed the overall distribution of OTUs observed among the four samples. Altogether, the four clone libraries produced 24 OTUs at a 3% distance. All bacterial communities contained more unique OTUs than shared OTUs, but the PA3 library only contained 2 unique OTUs. Samples PA0 and PA1, PA0 and PA3, PA1 and PA2, PA1 and PA3, all shared 3 OTUs (Figure 11).
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Figure 11
Venn Diagram at distance 0.03 The number of species in group PA0 is 9 The number of species in group PA1 is 9 The number of species in group PA2 is 8 The number of species in group PA3 is 8 The number of species shared between groups PA0 and PA1 is 3 The number of species shared between groups PA0 and PA2 is 1 The number of species shared between groups PA0 and PA3 is 3 The number of species shared between groups PA1 and PA2 is 3 The number of species shared between groups PA1 and PA3 is 3 The number of species shared between groups PA2 and PA3 is 1 The number of species shared between groups PA0, PA1 and PA2 is 1 The number of species shared between groups PA0, PA1 and PA3 is 2 The number of species shared between groups PA2 and PA3 is 0 The number of species shared between groups PA1, PA2 and PA3 is 1 The total richness of all the groups is 2

The shared OTUs among the samples corresponded to the taxa alpha, beta and gammaproteobacteria, unclassified, members of the Proteobacteria and Bacteriodetes.

DISCUSSION
To our knowledge, this is the first molecular study on the freshwater bacterial biodiversity of Las Cumbres Lake, Panama. Analysis of physicochemical variables indicated that there was a vertical stratification of multiple parameters, such as temperature, conductivity, pH, total nitrogen, total phosphorus, and oxygen concentration, as well as the microbiological measurement of total fecal coliforms. Overall, during this sampling date in November, the gradients were relatively weak, except for the total coliforms and dissolved oxygen.

The profiles, obtained up to 16 meters deep for each of the physicochemical variables, showed some agreement, as we can see in their profile patterns. For example, the concentration of dissolved oxygen decreased from the surface (4.8 mg/L) to half of that value at 5 meters deep (2.4 mg/L), and reached its lowest value at a depth of 16 meters (2.2 mg/L). Notably, the dissolved oxygen concentration fell below the minimum acceptable value to support aerobic life (4 mg/L) at just 5 meters below the surface. Thus, from the point of view of dissolved oxygen, there were two distinct layers, from 0 m to 5 m, and from 5 m to 16 m. This low oxygen level could be one of the causes of the increase in fish death reported by lake users and residents to the national environmental authority of Panama (Natalia Young, personal communication). The same pattern was observed for the temperature profile.

This observation is noteworthy, because the dissolved oxygen concentration would more typically increase as the water temperature decreases. The fact that the opposite phenomenon is observed suggests that something else, possibly the microbial community, is acting to decrease dissolved oxygen at the lower depths of the lake.

From a depth of 5 meters to 10 meters, there was a similar increase in concentration for nitrates and phosphates. This may be further evidence of microbial activity, as this layer contains potential products of biodegradation (nitrates, phosphates and oxygen consumption), with a corresponding oxygen consumption being decreased by 16 meters deep. Also, an increase in total suspended solids was observed from 10 meters to 16 meters deep. The Inverse Simpson Index also indicates that microbial diversity diminishes with depth (Figure 3), suggesting a specific bacterial community that could be generating the chemical constituents as a bioproduct.

Together, these results reveal that different environmental conditions are present on the surface than at various depths, therefore imposing conditions that would establish a vertical distribution of the microbial community.

Accordingly, sequencing of 16S rRNA clone libraries from multiple depths of the lake indicated changes in bacterial diversity, as characterized by OTUs with a 0.03 separation, with the PA2 library being the most diverse of all four libraries. The most representative phyla in Las Cumbres Lake were Proteobacteria (ab and g) and Verrucomicrobia, followed by Bacteriodetes and Actinobacteria. The taxa Betaproteobacteria and Gammaproteobacteria of the Proteobacteria were the most abundant in all clone libraries. Analysis of bacterial diversity, as reflected by rarefaction curves, confirmed that in three of the four libraries there was
sufficient sampling effort applied to characterize bacterial diversity at that depth.

The Betaproteobacteria taxon has previously been reported as characteristic and widely distributed in different freshwater environments, and usually dominate total bacterial communities at freshwater sites (Eiler and Bertilsson, 2004; Zwart et al., 2002, Allgaier, Grossart, 2006; El Saied, 2007). Even though Las Cumbres is a freshwater lake, Gammaproteobacteria dominated in our clone libraries, a finding which nevertheless had also been found to be true in lakes, rivers and seawater near islands in previous studies (Silveira et al., 2011, Wu et al., 2007). Members of this sub-phylum, such as the Enterobacteriales, are considered transient members of freshwater lakes dispensed from anthropogenic or zoonotic sources (Zwart et al., 2002, Newton et al., 2011). In fact, members of the Enterobacteriaceae have been used for source tracking of pollutants in surface waters (Newton et al., 2011). One study on bacterial diversity associated with sewage waters indicated that Proteobacteria, specifically those that normally occur in the environment, dominated the sewage samples, and these were mainly found to be Gammaproteobacteria, followed by Beta- and Epsilonproteobacteria (McLellan et al., 2010) and the results obtained in this study are consistent with our suggestion of a human impact on Las Cumbres Lake.

The Verrucomicrobia phylum was present in the PA0 library, which according to previous studies is characteristic of surface waters (Newton et al., 2011).

The taxon Actinobacteria was only present in the PA2 clone library (Figure 8). Actinobacteria have been recovered from the epilimnia of lakes, and this phylum is associated with nucleic and amino acid metabolism and harbors rhodopsins (actinorhodopsins), a potential mechanism for supplemental energy generation by light harvesting (Newton et al., 2011; Phylosof et al., 2009).

Bacteriodetes play an important role in the detritus food chain and carbon cycling in aquatic ecosystems of both freshwater and marine ecosystems (Matcher et al., 2011), and this phylum was present among three of the clone libraries, as shown by the venn diagram (Figure 11).

Las Cumbres Lake is a manmade lake in an urban environment and, as stated previously, it has been affected by human activities such as household wastewaters. Given this fact, an indicator of fecal pollution, total coliforms, was determined. Additionally, recent studies have demonstrated that Bacteriodetes may also be a potential indicator of fecal contamination (Ferguson and Signoretto, 2011, Ju-Jeong et al., 2011, Ibekwe et al., 2012). All of our clone libraries contained OTUs assigned to Bacteriodetes, which it may be a further indicator that this lake is polluted with sewage water.

Variations of physical and chemical parameters in the water column of Las Cumbres Lake did not correlate significantly (at a level of p=0.95), but did show a clear relationship with the specific bacterial distribution, since OTU variation was observed for each library (Figure 3). Other studies with similar results have demonstrated that the bacterial composition of lakes varies temporally and spatially within habitats (ElSaid, 2007, Hahn, 2006). Important factors that can influence a bacterial community include water chemistry and temperature in freshwater environments. This study has confirmed this association by showing how microbial diversity corresponds to changes in physicochemical parameters, and at the same time shows the tolerance of the microbial communities to environmental changes. In order to better understand the ecology of microbial communities, studies such as this one are essential in order to define the interplay between these biotic and abiotic factors.

**CONCLUSION**

In this study of physicochemical parameters and microbial diversity distributions at Las Cumbres Lake, Panama, all measurements were shown to vary with depth, dividing the lake into two differential environments between 0 to 5 meters and from 5 to 16 meters during November (the rainy season).

The oxygen profile indicated that from 5 meters depth down to the bottom, environmental conditions could not have supported macroscopic aquatic life during this sampling period in November. This low level of oxygen also indicated a possible discharge of sewage water into the lake.

Due to the stratification of physicochemical and biological parameters observed at the Las Cumbres Lake, the current sampling program for lakes to be implemented by the national environmental authority of Panama should be modified to take samples from at least two depths, for example at the surface and at 10 meters, in order to properly evaluate water quality conditions.
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