Microb Ecol (2009) 57:94–103
DOI 10.1007/s00248-008-9398-3

MICROBIOLOGY OF AQUATIC SYSTEMS

Description of Freshwater Bacterial Assemblages from the Upper Paraná River Floodpulse System, Brazil

Michael J. Lemke · E. Kurt Lienau · Jean Rothe · Thomaz A. Pagioro · Jeff Rosenfeld · Rob DeSalle

Received: 8 July 2007 / Accepted: 9 May 2008 / Published online: 28 June 2008

Abstract Bacteria were identified from a large, seasonally flooded river (Paraná River, Brazil) and two floodplain habitats that were part of the same river system yet very different in nature: clearwater Garças Lagoon and the highly humic waters of Patos Lagoon. Bacterioplankton were collected during mid-summer (Jan. 2002) from water samples (2 l) filtered first through a 1.2-μm filter then a 0.2-μm membrane filter representing the particle-attached and free-living sub-communities, respectively. DNA was extracted from filters and purified and a 16S rRNA clone library established for each habitat. Over 300 clones were sequenced and checked for similarity to existing 16S sequences in GenBank using the BLAST algorithm with default parameters. Further classification of clones was done using a species "backbone" attachment followed by parsimony analysis. The majority (85%) of sequences, referred to here as operational taxonomic units (OTUs), were most similar to uncultured bacterium 16S sequences. OTUs from each *Proteobacteria* sub-phylum (α, β, γ, δ, ε) were present in the Upper Paraná River system, as well as members of the *Bacteroidetes*. The microbial assemblage from Patos Lagoon was least like other samples in that it had no *Firmicutes* present and was dominated by *Actinobacteria*. *Verrucomicrobia* OTUs were only found in the free-living assemblage. This study documents the presence of globally distributed phyla in Upper Paraná River and taxa unique to habitat and particle attachment.

Introduction

Most molecular studies of freshwaters have concentrated on lake (lentic) environments [e.g., 1, 4, 18, 24, 25, 52]. This study joins a small number of 16S ribosomal RNA (rRNA)-based phylogenetic analyses of Bacteria from large rivers (i.e., Danube River [6, 50] and Columbia River [15]) and contributes to limited yet growing knowledge of the bacterial assemblage from the large, floodpulse Paraná River in South America [10].

Most medium to large rivers have areas adjacent to the channel that are subject to seasonal surges of overland water runoff that result in flooding. This condition creates a large river floodplain ecosystem that is a complex mosaic of lotic (main river channel), lentic (permanent and temporal lagoons, shallow lakes, and wetlands) and semi-lotic (sloughs and side river channels) aquatic habitats. Periodic flooding, or a floodpulse, induces strong temporal variation in physical, chemical, and biotic factors that maintains a high degree of biodiversity [26, 44].

Electronic supplementary material The online version of this article (doi:10.1007/s00248-008-9398-3) contains supplementary material, which is available to authorised users.

M. J. Lemke (✉)
Biology Department, University of Illinois at Springfield,
One University Plaza,
Springfield, IL 62703, USA
e-mail: lemke.michael@uis.edu

E. K. Lienau
Department of Biology, New York University,
New York, NY 10011, USA

J. Rothe · J. Rosenfeld · R. DeSalle
Division of Invertebrate Zoology,
American Museum of Natural History,
New York, NY 10024, USA

T. A. Pagioro
Departamento Acadêmico de Química e Biologia,
Universidade Tecnológica Federal do Paraná,
Curitiba, Paraná, Brazil

The Upper Paraná River basin, with a drainage area of 2.8×10^6 km^2, supports a high species diversity among neotropical aquatic systems. Even though there are 300 phytoplankton, 55 amoeba, 218 rotifer, 40 cladoceran, 16 copepod, 80 invertebrate, 48 aquatic macrophyte, 170 fish, and 59 amphibian and reptile taxa identified [2], little is known about the prokaryote biodiversity. Given the high number of taxa in other phyla, an underlying question addressed by this study was whether prokaryotic species would also show unusually high diversity relative to other described freshwater systems.

The goal of this study was to describe select bacterial assemblages in a large river floodpulse system in Brazil in order to better understand floodpulse river ecology. Our primary objective was to compare the mid-summer, post-flood microbial assemblages from three different habitats within the Paraná River system: backwater lagoon, tributary lagoon, and mainstem river. Crump et al. [14] and Crump and Baross [13] presented convincing evidence of the high microbial activity and diversity on particles transported in a large river. For this reason, our secondary objective was to document and compare particle-attached to free-living communities in samples collected from these three habitats.

Materials and Methods

Sample Sites

Three sites on the Upper Paraná River system were sampled on 9 January 2002 (Fig. 1). With a 2.8-million-km^2 drainage area, the 4,495-km-long Paraná River and adjoining floodplains occupies the second largest hydrographic basin in South America [2]. Of the over 8-million-km^2 basin that is Brazilian territory, only the Upper Paraná River represents the last undammed reach in Brazil and thus preserves a 5–20-km-wide floodplain with a diversity of habitats (e.g., lagoons, islands, channels) [10]. The three sites were chosen to represent distinct habitat types within the floodplain that

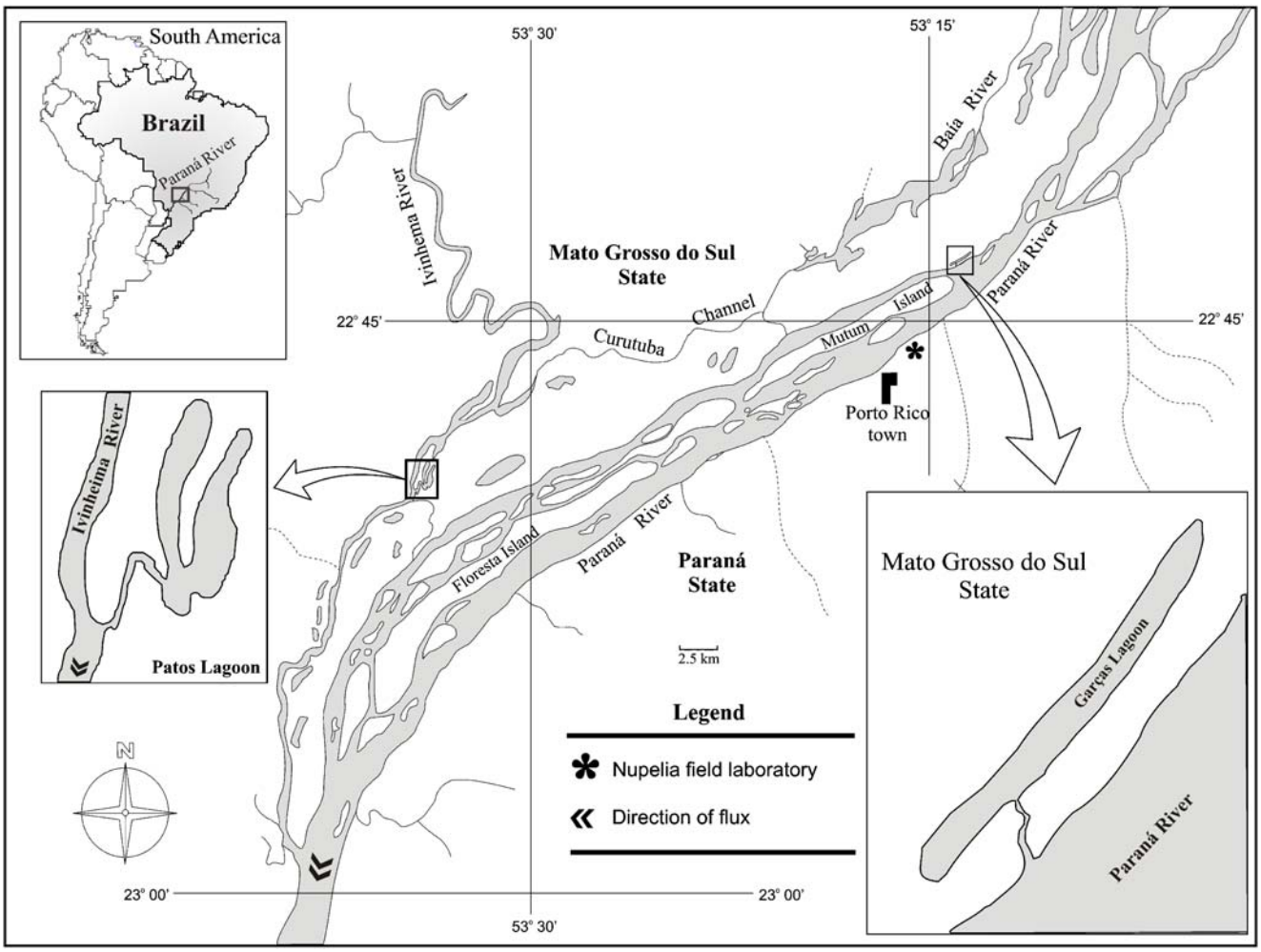

Figure 1 The Upper Paraná River floodpulse system, with *insets* showing Garças Lagoon (*lower right*) and Patos Lagoon (*left*). The sampling locations are shown by the * in each inset

Table 1 Characteristics of the 3 sample sites, Paraná River, Garças Lagoon, and Patos Lagoon, Brazil

	Paraná River	Garças Lagoon	Patos Lagoon	Ivinheima River
Surface area (km^2)	–	~1.4	1.1	–
Depth (m)		~1.2	2.8–4.8	
Habitat type	Lotic	Lentic	Lentic	Lotic
Temperature (avg.)	24.8 (18.9–29.9)[a]	26.2 (18.7–31.0)[b]	23.7 (17.7–26.4)[b]	23.9 (16.8–30.4)[a]
Oxygen (% saturation)	83.5–124.7[a]	63.5–196.4[b]	59.6[b]	43.7–116.7[a]
pH	7.3	6.3–8.1	6.4–7.1	7.0
Conductivity (μS)	54.4	21.99 (46.0–61.2)[b]	43.1 (34.0–52.0)[b]	43.0
Total phosphate (μg/l)	18.5	46.1 (25.6–87.9)[b]	43.9 (25.8–66.6)[b]	50.0
Total nitrogen (μg/l)	210 (111.0–350.0)[b]	209.2 (316.1–556.7)[b]	401.8	340
Total organic carbon (mg/l)	1–4	1.4	15.5	4.2
Avg. chlorophyll (μg/l)	2.5	~3.0–28.2[b]	9.3 (~3.0–17.3)[b]	1.6

Ivinheima River characteristics are given because it is the tributary leading into the Paraná River where Patos Lagoon is located. Ranges are given in parentheses below averages when available

[a] Thomaz et al. [44]

[b] Data not published; collected in 2000 at the sampling sites

differed considerably in their physical, chemical, and morphological characteristics (Table 1). Water measurements included pH (Digimed DM-2), conductivity (Digimed DM-3), oxygen (YSI 55), and temperature (YSI 55). Water was also analyzed for total organic carbon (Shimadzu TOC model 5000), total phosphate [23], nitrogen forms [20, 31], and chlorophyll [23].

The Paraná River was sampled near Porto Rico, Brazil where the river had a low gradient (0.09 m/km), allowing a great deal of sediment to accumulate on the river bed [2]. The water was typically low in total phosphate largely because it was sequestered by biotic components of the upstream reservoirs. Most of the nitrogen appeared as nitrate because oxygen levels were close to 100% at all times. The river had neutral pH and low chlorophyll throughout the year [44].

Garças Lagoon was located in Mato Grosso do Sul State, Brazil and was connected to the Paraná River by a narrow (~6 m) channel. The phosphate (P) and nitrogen concentration were much like the Paraná, except that some (~0.1%) of the nitrogen existed as ammonium due to oxygen levels in the lagoon. Decomposition of organic matter and ammonification can cause near-sediment waters of the lagoon to become anoxic and produce ammonia, as well as methane and hydrogen sulfide. Much more variability was seen in Garças Lagoon pH and chlorophyll levels than was seen in the river (Table 1) due to the relatively low alkalinity, high temperature, and diel primary production patterns.

Patos Lagoon was also in Mato Grosso do Sul State on the southeast bank of the Ivinheima River permanently connected by a wide channel [39]. The Ivinheima River was one of the main tributaries of the Paraná River. Its lower reach remains intact and largely unimpacted and flows (0.85 m/s) through part of the Paraná River floodplain [3, 42]. However, the Ivinheima River was surrounded by agriculture fields in its upper reaches and it carries more than twice the amount of P, making Patos Lagoon richer in nutrients than Garças Lagoon. Whereas Garças Lagoon and the Paraná and Ivinheima Rivers all have low total organic carbon (TOC) levels that range from 1 to 4 mg/l, Patos Lagoon can have exceptionally dark waters during some months, such as when the samples were collected for this study (TOC=15.5 ppm). The TOC was primarily humic compounds that were leached by rain events from organic-rich sand soils in the river floodplain. Vegetation associated with the lagoon margins were predominately *Polygonum* spp. and *Eichornia azurea*, as well as other shrubs and grasses.

Collection of Water Samples

Water samples were collected in sterile, UV-treated 1-l glass bottles and stored on ice until filtered. Water (2 l from each site) was first filtered through a 47-mm, 1.2-μm-pore Nucleopore polycarbonate filter to capture larger particles where the particle-attached bacteria fraction could be analyzed. The filtrate was then passed through 47-mm, 0.2 μm-pore Millipore Durapore filters to capture the free-living bacteria. Particle-attached and free-living are therefore operational definitions of the bacterioplankton assemblage. DNA from each filter type was extracted separately.

DNA Manipulation

Filters cut into small pieces were placed in extraction buffer (100 mM Tris-HCl [pH 8.0], 100 mM EDTA, 100 mM sodium phosphate, 1.5 M sodium chloride, and 7.4 μl/ml 10 mg/ml proteinase K). Acid-washed PVPP (1:6.75 *w*/*v*) was added to bind humic acids. The mixture was incubated (37°C) with shaking (225 rpm) for 30 min followed by addition of 20% SDS (65°C; 2 h), centrifugation (6,000×*g*;

10 min), and removal of the supernatant. Extraction of the filter was repeated two more times, the combined supernatant mixed with equal volume of chloroform/isoamyl alcohol (24:1), centrifuged (6,000×*g*, 10 min), and the aqueous phase collected. DNA was precipitated by addition of 0.6 volume of isopropanol (1 h), dried, then resuspended in nano-pure water. The extract was further purified by passing it through a 1-ml syringe spin column packed with Sepharose G-200. PCR amplification used the primer pair PRBA338F (5′ AC TCC TAC GGG AGG CAG CAG 3′) and PRUN518R (5′ ATT ACC GCG GCT GCT GG 3′) [28, 36, 37] that generated ~180-bp fragments from the ribosomal RNA SSU. These generalized primers targeted a wide variety of organisms in the domain Bacteria.

Cloning and Sequencing

After confirming the presence of amplification products on agarose gels, DNA products were cloned into JM 109 competent cells using the pGEM®-T Easy Vector System (Promega, Madison, WI, USA) followed by plating of cells on LB, ampicillin-enriched plates with IPTG/X-Gal (methods similar to [7]). Colonies of transformed cells were screened using colony PCR, and products were visualized by agarose gel electrophoresis. Appropriate-sized PCR products were purified (Sephadex spin racks), dried, and resuspended in water and sequenced using the Big Dye-based system. Two-hundred clones from each of the three study sites were sequenced for this study.

Out of 338 successfully sequenced clones, 142 were from the Paraná River, 111 from Garças Lagoon, and 85 from Patos Lagoon. The numbers of clones from particle-attached and free-living subsamples are listed in Table 2. The clones were classified into 56 operational taxonomic units (OTUs). Nucleotide sequences were submitted to GenBank and are available under accession number EU513884–EU514221.

Phylogenetic Analysis

Sequences were examined and archived using Sequencher Software (Gene Codes Corp., Ann Arbor, MI, USA). Before proceeding to the determination of bacterial diversity, we checked our sequences for chimerism using the Chimera Check program available at the MSU Center for Microbial Ecology. All clones displaying chimeric profiles were discarded from the analysis. We first analyzed our sequences using BLAST searches. Many clones showed high similarity (>97%) to known, cultured organisms, while others showed similarity only to uncultured samples in the database.

Because of these results and because BLAST results are not statements of evolutionary relationships, we used a parsimony analysis strategy named “backbone attachment” to identify our clones. Briefly, we aligned each experimental or unknown SSU sequences with a large group of SSU sequences to a matrix composed of a complete sampling or “backbone” of bacterial ssRNA sequences from the ribosomal database Project II [33]. We then did a parsimony analysis on the combined datasets and classified our unknowns based upon whether they attached to the “known” or “backbone” tree [32, 43]. To ameliorate methodological problems associated with phylogenetic analysis using sequences of different length and or different base composition called long branch attraction [19] and to control as much as possible for lack of evolutionary signal present in such short sequences, each clone was added and aligned separately from all other clones and parsimony analysis performed on each of the data sets (one per clone)[41].

Diversity Measures

Valid clone sequences were classified into OTUs and assigned to phylum and, in the case of the *Proteobacteria*, sub-phylum. The results were analyzed for (1) richness—defined as the number of species; (2) Simpson’s Index as $D=N\,(N-1))/\Sigma$

Table 2 Comparison of species number, operational taxonomic units (OTU), and diversity of OTUs by Simpson’s Index and Chao1 estimator from clones made from partial 16S rRNA sequences for total, particle-attached, and free-living communities in the surface water from the river and lagoon habitats on the Upper Paraná River floodpulse system, Brazil

	Species richness	Total clones analyzed	Detected OTUs	Chao1 estimate	Simpson’s Index	Species evenness
Paraná River						
Particle	16	74	16	18.0	0.889	0.822
Free-living	23	68	23	29.8	0.924	0.870
Total	30	142	30	34.0	0.917	0.880
Garças Lagoon						
Particle	23	66	23	47.0	0.918	0.865
Free-living	23	45	23	51.1	0.932	0.872
Total	38	111	38	118.7	0.946	0.913
Patos Lagoon						
Particle	21	58	21	35.4	0.806	0.755
Free-living	13	27	13	17.9	0.858	0.762
Total	29	85	29	37.5	0.825	0.787

($n_i(n_i-1)$—a measure of diversity that considers the number of species, the total number individuals, and the proportion of the total that occurs in each species; (3) evenness—sometimes called relative diversity—is an expression of how close a set of observed species abundances are to those from an aggregation of species having max. possible diversity; and (4) Chao-1 richness indicator [11] as $S=S_{obs}+(a^2/2b)$, where S was Chao-1 richness estimator, S_{obs} was the observed number of OTUs, a was the number of OTUs seen once, and b was the number of OTUs observed twice.

Results

Diversity

Simpson's Index provides a quantitative assessment of the probability that two individuals in the assemblage are alike and represents the probability that any taxon selected at random would be different. Thus, the higher the Simpson's Index value, the greater the likelihood that any two randomly drawn sequences would be different types (i.e., greater diversity). Species evenness is the relative distribution of individuals among the species present in an assemblage.

A relatively high Simpson's Index of diversity (>0.82) existed for all habitats (Table 2). Garças Lagoon had the highest diversity index (0.946) and showed the most even distribution of species (0.913), while Patos Lagoon had the lowest diversity (0.825) of the three habitats. Of note is that Chao 1 estimate of species richness was about three times higher at Garças Lagoon than for the other two aquatic habitats. For comparison between particle-attached and free-living bacteria, Simpson's Diversity Index and species evenness were always greater, albeit marginally, for the free-living than particle-attached assemblages (Table 2).

Phylogenetic Diagnostic Results

BLAST Searches using MEGABLAST Batch files resulted in significant BLAST scores (<e-20) for 58.3% of the clones with designation down to species for 22.1% of the clones and designation to uncultured for 77.9% of the clones. Clones from seven different bacteria phyla, including all the sub-phyla of *Proteobacteria*, were represented in the samples from the Paraná River system (Supplementary Table 1).

Water from all three sites carried a similar percentage of *Proteobacteria* (27–30%), with the *β-Proteobacteria* comprising the greatest portion of this phylum (17–21% of the total; Fig. 2). In the Paraná River system (i.e., Paraná River and Garças Lagoon), most of the remaining taxa fell into three phyla: the *Firmicutes*, *Actinobacteria*, and *Bacteroidetes*. A small percentage (≤6%) of the remaining clones was identified as *Cyanobacteria* or *Verrucomicrobia*.

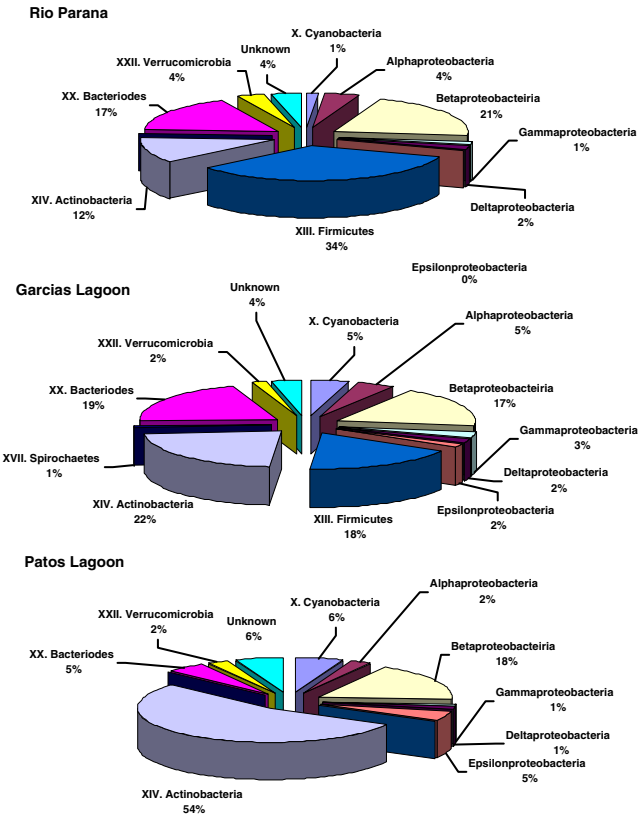

Figure 2 Comparison of the quantitative contribution of clones affiliated with different phyla and sub-phyla to the total number of clones from Paraná River, Garças Lagoon, and Patos Lagoon. Clones not associated with any known sequence are included as *Unknown*

Some differences between genera could be seen between the lotic habitat (Paraná River) and the two lentic habitats (Garças Lagoon and Patos Lagoon; Fig. 3). Of the four identified *α-Proteobacteria* genera named, only *Caulobacter* was found in the river. Though *Comamonas* was found at all sites, it was the most abundant of the *β-Proteobacteria* in the river. Taxa unique to the lentic habitat fell into *γ-* and *ε-Proteobacteria* subdivisions (e.g., *Moraxella*; *γ-Proteobacteria)*. Although *Cyanobacteria* were found in all locations, only *Pleurocapsa* was found in the lagoons.

One of the most unusual findings was the absence of *Firmicutes* from Patos Lagoon, even though a relatively high diversity of genera from this group was described in the other two habitats (i.e., >13–19% *Eubacterium* in both). However, the greatest diversity of OTUs at Patos was in the *Actinobacteria*, with *Dermatophilus* showing the greatest abundance of any genera (42%). Other genera that appeared to be habitat specific were *Actinomyces*, being unique to the river, and the *Bacteroidetes* genera *Porphyromonas* and *Persicobacter*, found in both the river and Garças Lagoon.

The particle-attached and free-living bacterial communities shared many taxa; however, some differences were apparent

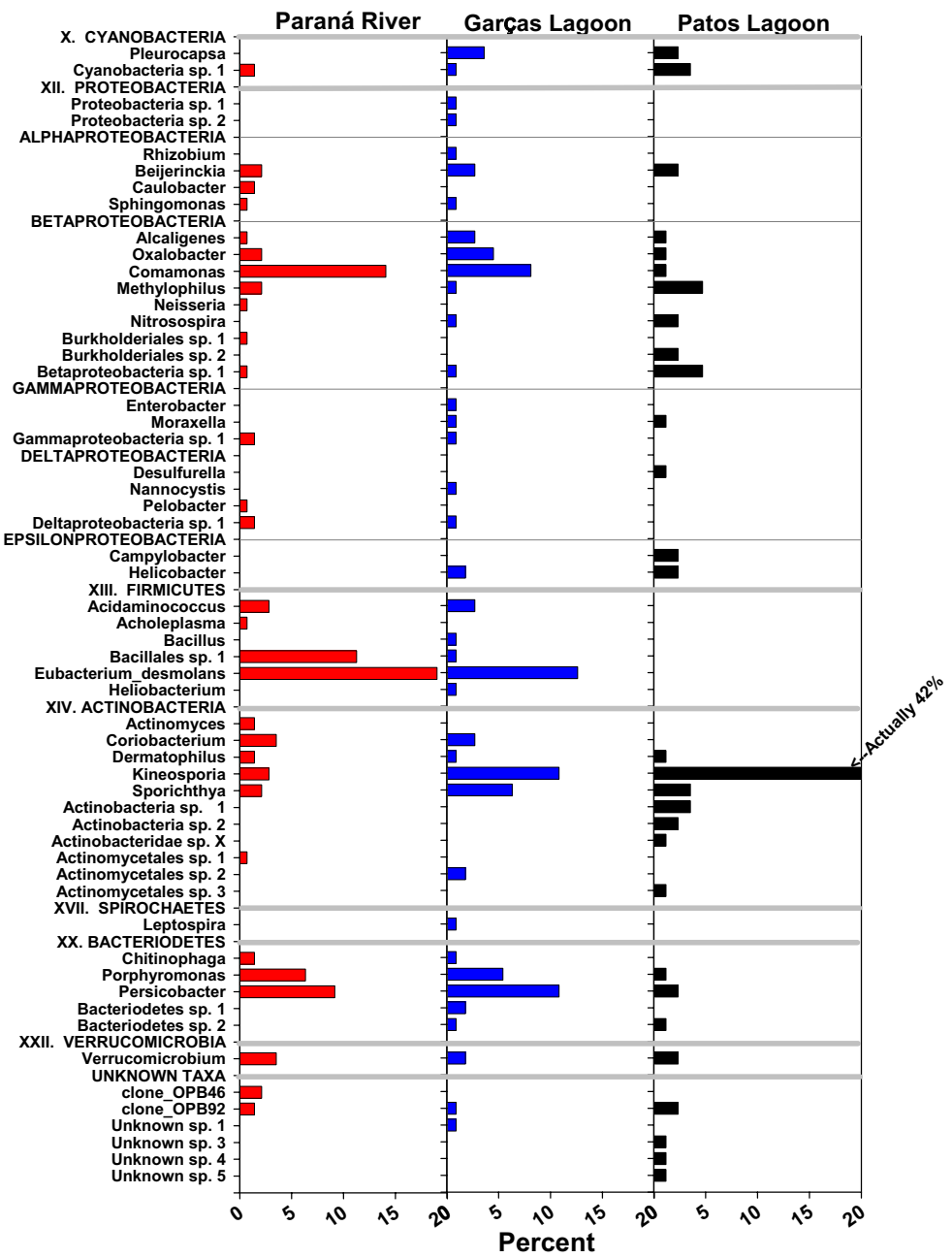

Figure 3 Percent abundance of different OTUs reported on the genera level or the closest relevant taxanomic grouping by sampling site: Paraná River, Garças Lagoon, and Patos Lagoon

(Fig. 4). The *Verrucomicrobia* were only detected in the free-living assemblage. Members of the *Firmicutes*, when present, had less than 1/2 of the relative abundance in free-living than particle-attached communities (i.e., Paraná River and Garças Lagoon). With reference to the clone libraries, there was more than twice the abundance of free-living *Actinobacteria* in Patos Lagoon than on the particles at the same location. Also, in Patos Lagoon, 50% or more of the free-living and particle-attached communities were *Actinobacteria*, while there was about three-fold less *β-Proteobacteria* in the free-living vs. the particle-attached assemblage.

Discussion

Use of culture-independent, molecular techniques to identify aquatic bacteria has begun to lay the foundation for a more in-depth understanding of aquatic microbial ecology. Freshwater bacteria commonly appear in the phyla *Cyanobacteria*, *Bacteroidetes*, *α-*, *β-*, and *γ-Proteobacteria*, *Actinobacteria*, and *Verrucomicrobia* [52] with the groups in the *α-* and *β-Proteobacteria*, *Cytophaga–Flavobacteria–Bacteroides*, *Actinobacteria*, *Verrucomicrobiales*, and gram-positives, now referred to as "globally distributed" freshwater phylogenetic clusters [4, 15, 16, 18, 25, 34, 53, 54]. Yet, globally

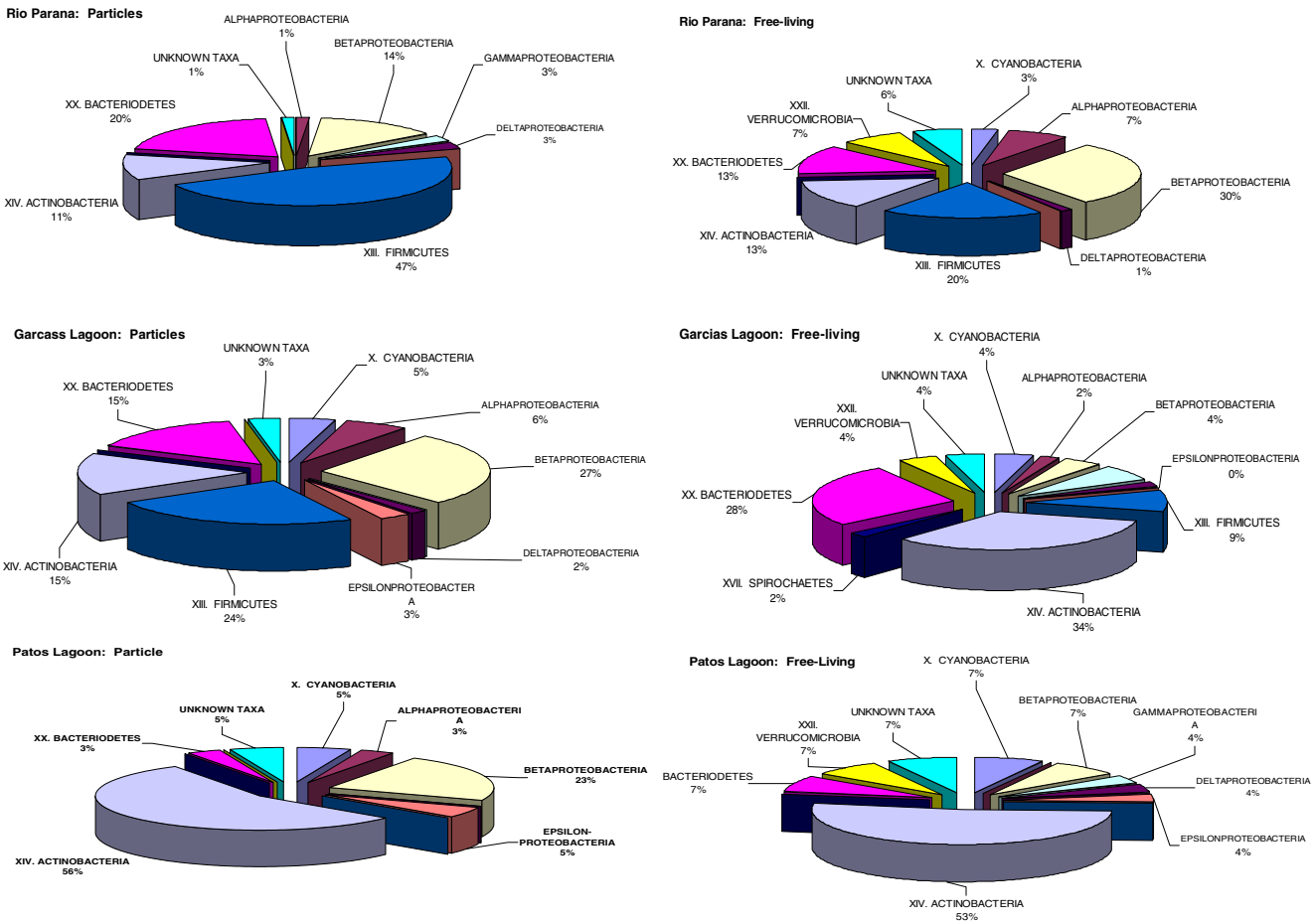

Figure 4 Comparison of the free-living and particle-attached microbial community as percentage of clones affiliated with different phyla and sub-phyla in three aquatic habitats from the Upper Paraná River: Paraná River, Garças Lagoon, and Patos Lagoon. Clones not associated with any known sequence are included as *Unknown Taxa*

distributed freshwater bacteria are not ubiquitous to all aquatic habitats. For instance, comparisons of 16S rRNA clone libraries and in situ hybridization reveals that the *β-Proteobacteria* subdivision common to freshwater lake and river habitats does not exist, or exists to a minor degree, in marine habitats (i.e., [22, 35, 52]).

Bacterial communities from the Upper Paraná River did not show unusually high biodiversity (56 OTUs) when compared to other freshwater microbial assemblage studies based on 16S rRNA clone libraries, FISH, and DGGE; other studies typically have <75 bacterioplankton OTUs in a given freshwater environment (e.g., [6, 15, 18, 30]. We expected higher species diversity because floodplain systems are characterized by disturbance, which can lead to increased species diversity. In floodplain water bodies, nitrogen and phosphorus usually increase during the beginning of the seasonal flood due to leaching of organic matter from the floodplain, as well as macrophyte decomposition. However, decomposition leads to strong oxygen decrease in the lagoons [44]; thus, shallow conditions in the lagoons during dry periods tend to correspond with peak chlorophyll and high daytime oxygen levels, as well as increased nitrogen and phosphorus concentrations in the water column [45]. No doubt higher diversity associated with these seasonal variations in nutrient and oxygen conditions would be observed if sampling were extended throughout the year.

Analysis of the OTUs showed that many of the designated globally distributed freshwater groups were present in this study, yet unique OTUs were also detected among the habitats and relative to particles. Over one quarter of the clones in the river and lagoon habitats belonged to the *Proteobacteria* phylum, with the most dominant sub-phylum being the beta group. The *β-Proteobacteria* has been found in most studies completed on freshwater environments, which has been primarily northern hemisphere lakes [1, 4, 8, 18, 21, 25, 34, 35, 49]. Interestingly, this sub-phylum has typically been absent from marine aquatic habitats (see review in [21]).

All other *Proteobacteria* (α, β, γ, δ, ε) sub-phylums were present in the Upper Paraná River system samples.

The α-, γ-, and δ-*Proteobacteria* groups were found in all habitats in much lower percentages (≤5%) than the beta subgroup. Both α- and γ-*Proteobacteria* have been commonly found in other freshwater lakes but varied greatly in abundance [18] with estimates as high as 19% and 9%, respectively, in Adirondack Lakes, NY [25]. Because many of the members of the γ-*Proteobacteria* are copitrophic and such conditions are supported sporadically in natural freshwaters, the relative abundance of members of this group was as variable as nutrient conditions [51]. The δ-*Proteobacteria* represented in this study were also present in microbial communities of six other Adirondack Lakes [34]. Interestingly, the ε-*Proteobacteria* have not been reported as typical members of other freshwater habitats yet existed in the lagoon sites in the Upper Paraná River.

Members of the *Bacteroidetes* phylum were present in all habitats, especially in the Paraná River and connected Garças Lagoon (17–19%). Distribution of *Bacteroidetes* in both habitats could be due to water exchange, especially during flooding, between Garças Lagoon and the Paraná River, or if the OTUs were simply adapted to both habitats. *Bacteroides*, in the *Bacteroidetes* phylum, have frequently been found in lakes in various locations (e.g., Austria, Germany, Russia, USA; [22, 25]. Patos Lagoon, with its high humic content (~50 ppm total organic carbon) and separate tributary water source, had about 1/3 less abundance of *Bacteroidetes*.

Another globally distributed freshwater group is the *Actinobacteria*, which dominated 54% of the microbial flora in Patos Lagoon. In the other two habitats, it made up less than a quarter of the assemblage. Other researchers report that about 60% of bacterioplankton communities can be from this group and note that numbers likely depend on trophic status, geographic location, and DOC content [22, 48].

The relationship between *Actinobacteria* abundance and dissolved organic carbon and/or humic content appears unresolved. Glockner et al. [22] speculated that β-*Proteobacteria* may rapidly utilize the main carbon input to alpine Lake Gossenkollesse, but the *Actinobacteria* were more effective at consuming lower levels of organic carbon thus able to maintain a presence and become dominant when other substrates had become exhausted. In contrast, Bukert et al. [9] noticed increased lake DOC correlated with decreased *Actinobacteria* abundance. This might suggest that *Actinobacteria* are outcompeted by other species when DOC levels are high, with low DOC lending them a competitive advantage. Haukka et al. [24] found *Actinobacteria* and *Verrucomicrobia* to be the first and second most dominant taxa in Finnish forest lakes of varying humic content and concluded that humics did not directly affect community composition. Finally, Methé and Zehr [35] found a positive correlation between DOC content and γ- and β-*Proteobacteria* abundance yet no correlation with *Actinobacteria* abundance further indicating that while lake water chemistry could have a significant influence on bacterial diversity and abundance in general, it seems to not affect the *Actinobacteria*. *Planctomycetales*, though often found in freshwater habitats [18, 22, 47], were not found in the Upper Paraná River floodpulse system.

It may be just as likely that the type of DOC affects *Actinobacteria* and *Bacteroidetes* abundance in Patos Lagoon. Oliverira et al. [38] explained that during high water periods, as was the case during this study, dissolved humic substances in Patos Lagoon were allochthonous in origin. Their spectroscopic results showed that fulvic acids found in Patos Lagoon water were similar to soils in area, suggesting that the source was pedogenic. However, Barreto et al. [5] provided evidence that while fulvic acids predominate in the DOC of the area (i.e., Lake Ipê, Curutuba Channel, part of the Upper Paraná River; Fig. 1), the organic acids were probably from plant leachate.

Differences in community composition associated with particles were documented in another large river, the Columbia, and, therefore, we wanted to see if differences could be detected in the Paraná River. In the Columbia River (USA), particles have been shown to maintain 90% of the heterotrophic bacterial activity (10–100% more active than free-living bacteria), be responsible for most of the organic matter degradation [12, 14], and support part of the detritivorous food web [40]. The operational process of filtering water in any study in an attempt to separate free-living bacteria from particle-attached has limitations due to inherent particle build up on the filter causing change in pore-size filtrate and potential collection of otherwise free-living bacteria on the particles. Therefore, we expected to have a relatively high amount of OTU overlap due to filtration artifact and the fact that the same globally distributed taxa would likely be attached to particles as found to be free living. Even so, we noted that the *Verrucomicrobia* were only found in the free-living community, just as shown by Crump et al. [15]. These data do not seem to correlate to other studies in which *Verrucomicrobiales* were frequently found in pelagic zones of lakes where particle suspension would not necessarily be likely as in the fluvial load of rivers [4, 25, 34, 53, 54].

Notes on species preference to habitat are necessary to increase our understanding of the ecology of microbes. Studies conducted in lakes have shown that bacteria communities can vary substantially, likely due to water turbidity, primary production, nutrient dynamics, macrophyte presence, and humic content [29, 46]. In this study, some OTU-specific differences existed between lotic and lentic habitats.

About 9% of the OTUs were only found in both lentic habitats (*Pleurocapsa*, *Alcaligenes*, *Moraxella*, *Helicobacter*, a *Bacteroidetes* genera). While members of the *Alcaligenes* are known to be strict aerobes, some members

have been isolated under denitrifying conditions; a physiological trait described in this genus. Presence of *Moraxella* and *Helicobacter* was more perplexing as members of these genera are typically isolated from human mucosal surfaces; perhaps similar habitats (i.e., exudates in rhizospheres) represented in these stagnant waters provide a similar ecological niche.

About 14% of the OTUs were only found in the lotic habitat (*Caulobacter*, *Neisseria*, *Pelobacter*, *Acholeplasma*, *Actinomyces*, clone OPB46, and a genera each of *Burkholderales, γ-Proteobacteria*, and *Actinomyceles*). The dimorphic prosthecate *Caulobacter* is ubiquitous in natural freshwaters, responding to spring nutrients and persisting through summer [17]. *Pelobacter* is described as making up a significant part of the anaerobic microbial community of sediments and sewage sludge, *Acholeplasma* belongs to the *Firmicutes* (Class *Mollicutes*) that include the smallest of bacteria lacking a cell wall, and the high G-C, gram positive filamentous *Actinomyceles* are primarily aerobic soil organisms known for their diverse production of extracellular enzymes and diverse production of metabolic products [17]. Large rivers, like the Paraná River, have many water sources, many affected by land use (i.e., livestock, untreated sewage, agriculture) and non-point source water input. This may possibly explain the presence of *Neisseria*, which are primarily commensal microorganisms most often found in the mucous membrane of mammals [27].

This study presented preliminary data on the composition of aquatic microbial communities in three habitats of the Upper Paraná River floodpulse system. The results showed (1) similar biodiversity as communities documented in other freshwater habitats, (2) the presence of globally distributed phyla, (3) differences in composition between habitats, and (4) taxa unique to habitat and particle attachment.

Acknowledgments We thank Sidinei M. Thomas and the Universidade Estadual de Maringá, Núcleo de Pesquisas em Limnologia, Ictiologia e Aqüicultura, Maringá—PR—Brazil. We also thank A. M. Lemke, J. Bartletti, K. Kiehl, and A. K. da Silva Nakamura for field assistance, S. Paver for improvements to the manuscript, and boatmen A. Soares and Roberto. Brazil samples were collected under CNPq permit Portaria MCT no. 260/03, and work was partially supported by CNPq (Long Term Ecological Program, Brazilian site 6). Work at the American Museum of Natural History was supported by the Lewis and Dorothy Cullman Program in Molecular Systematics, with funding from the Sackler Institute of Comparative Genomics. E. K. Lienau and J. Rothe acknowledge financial assistance from the MacCracken Fellowship (NYU) and Hudson River Foundation, NY, respectively.

References

1. Alfreider A, Pernthaler J, Amann R, Sattler B, Glockner F, Wille A, Psenner R (1996) Community analysis of the bacterial assemblages in the winter cover and pelagic layers of a high mountain lake by in situ hybridization. Appl Environ Microbiol 62:2138–2144
2. Agostinho AA, Thomaz SM, Minte-Vera CV, Winemiller KO (2000) Biodiversity in the High River Paraná floodplain. In: Gopal B, Junk WJ, Davis JA (eds) Biodiversity in wetlands: assessment, function and conservation. Backhuys, Leiden, pp 89–118
3. Agostinho AA, Zalewski M (1996) A planicie alagavel do alto rio Paraná: Importancia e preservacao. EDUEM, Maringa
4. Bahr M, Hobbie JE, Sogin ML (1996) Bacterial diversity in an arctic lake—a freshwater SAR1 cluster. Aquat Microb Ecol 11:271–277
5. Barreto SRG, Nozaki J, Barreto WJ (2003) Origin of dissolved organic carbon studied by UV–vis spectroscopy. Acta Hydrochimmica et Hydrobiologica 31:513–518
6. Besemer KM, Moeseneder M, Arrieta JM, Herndl GJ, Peduzzi P (2005) Complexity of bacterial communities in a river-floodplain system (Danube, Austria). Appl Environ Microbiol 71:609–620
7. Borneman J, Skroch PW, O'Sullivan KM, Palus JA, Rumjanek NG, Jansen JL, Nienhuis J, Triplett EW (1996) Molecular microbial diversity of an agricultural soil in Wisconsin. Appl Environ Microbiol 62:1935–1943
8. Bosshard PP, Santini Y, Grütter D, Stettler R, Bachofen R (2000) Bacterial diversity and community composition in the chemocline of the meromictic alpine Lake Cadagno as revealed by 16S rDNA analysis. FEMS Microbial Ecol 31:173–182
9. Bukert U, Warnecke F, Babenzien D, Zwirnmann E, Pernthaler J (2003) Members of a readily enriched *β-proteobacteria*l clade are common in surface waters of a humic lake. Appl Environ Microbio. 69:6550–6559
10. Carvalho P, Thomaz SM, Bini LM (2003) Effects of water level, abiotic and biotic factors on bacterioplankton abundance in lagoons of a tropical flooplain (Paraná River, Brazil). Hydrobiologia 510:67–74
11. Chao A (1984) Nonparametric estimation of the number of classes in a population. Scand J Stat 11:265–270
12. Crump BC, Baross JA (1996) Particle-attached bacteria and heterotrophic plankton in the Columbia River estuary. Mar Ecol Prog Ser 138:265–273
13. Crump BC, Baross JA (2000) Characterization of the bacterially-active particle fraction in the Columbia River estuary. Mar Ecol Prog Ser 206:12–22
14. Crump BC, Imenstad CA, Baross JA (1998) Particle-attached bacteria dominant the Columbia River estuary. Aquat Microb Ecol 14:7–18
15. Crump BC, Armbrust EV, Baross JA (1999) Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia River, its estuary, and the adjacent coastal ocean. Appl Environ Microbiol 65:3192–3204
16. Crump BC, Kling GW, Bahr M, Hobbie JE (2003) Bacterioplankton community shifts in an arctic lake correlate with seasonal changes in organic matter source. Appl Environ Microbiol 69:2253–2268
17. Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (Eds) (2006) The prokaryotes, 3rd ed. Springer, NY
18. Eiler A, Bertilsson S (2004) Composition of freshwater bacterial communities associated with cyanobacterial blooms in four Swedish lakes. Environ Microbiol 6:1228–1243
19. Felsenstein J (1978) Cases in which parsimony or compatibility methods will be positively misleading. Syst Zool 27:401–410
20. Gine MF, Bergamin H, Zaggato EAG, Reis BF (1980) Simultaneous determination of nitrate and nitrite by flow-injection analysis. Analytica Chimica Acta 114:191–197
21. Glöckner FO, Fuchs BM, Amann R (1999) Bacterioplankton compositions of lakes and oceans: A first comparison based on fluorescence in situ hybridization. Appl Environ Microbiol 65:3721–3726

22. Glöckner FO, Zaichikov E, Belkova N, Denissova L, Pernthaler J, Pernthaler A, Amann R (2000) Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of *Actinobacteria*. Appl Environ Microbiol 66:5053–5065
23. Golterman HL, Clymo RS, Ohmstad MAM (1978) Methods for physical and chemical analysis of fresh waters. Blackwell Scientific, Oxford, p 214
24. Haukka K, Heikkinen E, Kairesalo T, Karjaiainen H, Sivoneh K (2005) Effect of humic material on the bacterioplankton community composition in boreal lakes and mesocosms. Environ Microbiol 7:620–630
25. Hiorns WD, Methe BA, Nierzwicki-Bauer SA, Zehr JP (1997) Bacterial diversity in Adirondack mountain lakes as reveled by 16S rRNA gene sequences. Appl Environ Microiol 63:2957–2960
26. Junk WJ, Bayley PB, Sparks RE (1989) The floodpluse concept in river-floodplain systems. In: Dodge DP (ed) Proceedings of the International Large River Symposium, pp 110–127. Can. Spec. Publ. Fish. Aquat. Sci. 106
27. Knapp JS (1988) Historical perspectives and identification of *Neisseria* and related species. Clin Microbiol Rev 1:415–431
28. Lane DJ (1991) 16S/23S rRNA Sequencing. In: Stackebrandt E, Goofellow M (eds) Nucleic acid techniques in bacterial systematics. Wiley, New York, pp 115–175
29. Lindström ES (2000) Bacterioplankton community composition in a boreal forest lake. FEMS Microbiol Ecol 27:163–174
30. Lyautey E, Lacoste B, Ten-Hage L, Rols J-L, Garabetian F (2005) Analysis of bacterial diversity in river biofilms using 16S rDNA PCR-DGGE: Methodological settings and fingerprints interpretation. Water Res 39:380–388
31. Mackereth FYH, Heron J, Talling JJ (1978) Water analysis: some revised methods for limnologists. Freshwater Biological Association. Scientific Publication No. 36, Cumbria, p 120
32. Maddison DR, Maddison WP (2001) MacClade analysis of phylogeny and character evolution, Ver. 4. Wayne P. Sinauer, Sunderland, MA
33. Maidak BL, Cole JR, Liburn TG, Parker CT, Saxman PR, Farris RJ, Garrity GM, Olsen GJ, Schmidt TM, Tiedje JM (2001) The RDP-II (Ribosomal Database Project). Nucleic Acid Res 29:173–174
34. Methé BA, Hiorns WD, Zehr JP (1998) Contrasts between marine and freshwater bacterial community composition—analyses of communities in Lake George and six other Adirondack lakes. Limnol Oceanogr 43:368–374
35. Methé BA, Zehr JP (1999) Diversity of bacterial communities in Adirondack lakes: do species assemblages reflect lake water chemistry? Hydrobiologia 401:77–96
36. Muyzer G, De Waal ED, Uitterlindiden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59:695–700
37. Nakatsu CH, Torsvik V, Øveås L (2000) Soil community analysis using DGGE of 16S rDNA polymerase chain reaction products. Soil Sci Soc Am J 64:1382–1388
38. Oliveira JL, Boroski M, Azevedo JCR, Nozaki J (2006) Spectroscopic investigation of humic substances in a tropical lake during a complete hydrological cycle. Acta Hydrochimmica et Hydrobiologica 34:608–617
39. Rodrigues LC, Train S, do C, Roberto M, Pagioro TA (2002) Seasonal fluctuation of some limnological variables on a floodplain lake (Patos lagoon) of the Upper Paraná River, Mato Grosso do Sul State, Brazil. Brazilian Arch of Biol Tech 45:499–513
40. Simestad CA, Morgan CA, Cordell JR, Baross JA (1994) Flux, passive retention, and active residence of zooplankton in Columbia River estuarine turbidity maxima. In: Dyer KR, Orth BJ (eds) Changes in fluxes in estuaries: implications from science to management (ECSA22/ERF symposium, Plymouth, September 1992). Olsen and Olsen, Fredensborg, Denmark, pp 47–484
41. Sarkar N, Thornton JW, Planet PJ, Figurski DH, Schierwater D, DeSalle R (2002) An automated phylogenetic key for classifying homeoboxes. Mol Phylogenet Evol 24:388–399
42. Souza FEE, Stevaux JC (1997) Geolgia e geomorfologia do complex rio Baia, Corutuba, Ivinhema. In: Vazzoler AEAM, Agostinho AA, Hahn NS (eds) A Planicie de inundacao do alto rio Paraná: Aspectos Fisico Biologicos e socioeconomicos. EDUEM, Maringa, pp 3–43
43. Swofford DL (2002) Phylogenetic analysis using parsimony* version 4. Wayne P. Sinauer, Sunderland, MA
44. Thomaz SM, Pagioro TA, Bini LM, do Roberto CM, de Araújo Rocha RR (2004) Chapter 4: Limnological characterization of the aquatic environments and the influence of hydrometric levels. In: Thomaz SM, Agostinho AA, Hahn NS (eds) The upper Paraná River and its flooplain: physical aspects, ecology and conservation. Backhuys, Leiden, The Netherlands, pp 75–102
45. Thomaz S, Bini L, Bozelli R (2007) Floods increase similarity among aquatic habitats in river-flooplain systems. Hydrobiologia 579:1–13
46. Van der Gucht K, Sabbe K, De Meester L, Vloemans N, Zwart G, Gillis M, Vyverman W (2001) Contrasting bacterioplankton community composition and seasonal dynamics in two neighbouring hypertrophic freshwater lakes. Environ Microbiol 3:680–690
47. Ward N, Rainey FA, Stackebrandt E, Schlesner H (1995) Unraveling the extent of diversity within the order *Planctomycetales*. Appl Environ Microbiol 61:2270–2275
48. Warnecke R, Amann R, Pernthaler J (2004) Actinobacterial 16S rRNA genes from freshwater habitats cluster in four distinct lineages. Environ Microbiol 6:242–253
49. Weiss P, Schweitzer B, Amann R, Simon M (1996) Identification in situ and dynamics of bacteria on limnetic organic aggregates (lake snow). Appl Envion Microbiol 62:1998–2005
50. Winter C, Hein T, Kavka G, Mach RL, Farnleitner AH (2007) Longitudinal changes in the bacterial community composition of the Danube River: a whole-river approach. Appl Environ Microbiol 73:421–431
51. Zavarzin GA, Stackebrandt E, Murray RG (1991) A correlation of phylogenetic diversity in *Proteobacteria* with the influences of ecological forces. Can J Microbiol 37:1–6
52. Zwart G, Crump BC, Kamst-van Agterveld M, Hagen F, Han S-K (2002) Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. Aquat Microbial Ecol 28:141–155
53. Zwart G, Hiorns WD, Methe BA, van Agterveld MP, Huismans R, Nold SC, Zehr JP, Laanbroek HJ (1998) Nearly identical 16S rRNA sequences recovered from lakes in North America and Europe indicate the existence of clades of globally distributed freshwater bacteria. Syst Appl Microbiol 21:546–556
54. Zwart G, Huismans R, van Agterveld MP, Van de Peer Y, De Rijk P, Eenhorrn H, Muyzer G, van Hannen EJ, Gons HJ, Laanbroek HJ (1998) Divergent members of the bacterial division *Verrucomicrobiales* in a temperate freshwater lake. FEMS Microbiol Ecol 25:159–169