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Studies of aquatic communities represent a unique method of monitoring the health of water resources. High Point City Lake small subunit 16S ribosomal DNA clone libraries were compared across small spatial and temporal scales. This study used polymerase chain reaction and shotgun cloning to isolate individual 16S rDNA sequences. A total of 437 operational taxonomic units were found in this study. Among all libraries 84 sequences demonstrated affiliation to the phylum Proteobacteria, including representatives from the classes α, β, δ, and γ. The next most abundant category of putatively identified 16S ribosomal DNA was 24 sequences affiliated with the class Actinobacteria. Seventeen sequences demonstrated similarity to clones previously isolated from Crater Lake, CA bacterium.

These findings suggest that variability among the replicate libraries may be a reflection of the small library size. S_{CHAO1} and S_{ACE} diversity estimators suggest larger libraries would be required to achieve a stable estimate of OTUs, but given the small size of each of the replicate libraries their similarity values were not unexpected. Variability among clone libraries from diurnal samples taken at the same location was low. Variability among clone libraries across short spatial scales showed suggested little difference among samples in well mixed areas of the lake, but one library may have reflected the influence of a tributary inflow. Overall, these data suggest that the 16S rDNA libraries were similar to one another.

A Comparison of Small Subunit 16S Ribosomal DNA Recombinant Plasmid Clone Libraries from High Point City Lake, NC

by

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Approved by

_____
Committee Chair

To Jennifer D. Alexander, Horace R. Alexander, Jeremey R. Alexander and Leroy Harrison for inspiring perseverance through difficulty.

APPROVAL PAGE

This thesis has been approved by the following committee of the Faculty of The Graduate School at The University of North Carolina at Greensboro.

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Date of Acceptance by Committee

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Date of Final Oral Examination

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TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER I

INTRODUCTION

An increased awareness of terrorist activities in 2001 led the US Government to initiate policies of proactive deterrence of terrorist attacks. In congressional testimony to the United States House of Representatives, Jeffrey Danneels of Sandia National Labs stated that, "the first component in the water system to be assessed for security risk is the sources or supplies of water, which include reservoirs, lakes, rivers, streams and ground water wells" (Danneels, 2001). The United States Environmental Protection Agency (US EPA) has taken the lead on critical infrastructure protection of domestic water resources. One of the US EPA's goals is to improve the security around water utilities (US EPA, 2002). A tactic to be employed includes the use of universities and the use of private sector businesses to identify and to respond to contamination events (US EPA, 2002). Methods to increase awareness of biological threats include two strategies that can help reduce vulnerabilities. One strategy involves the direct detection of specific pathogens. Another strategy involves the use of community assessments or bioindicators to detect environmental change. Molecular techniques have been used to investigate phylogeny for more than a quarter century. Advances in nucleic acid extraction, amplification and sequencing technologies have made these methods practical for important public uses, including forensic and national security analysis. The most effective approach may

involve a combination of both detecting individuals and identifying community profiles through the use of microarray technology.

The application of molecular biology to help solve the challenge of community assessment can be traced to works by Woese and Fox (1977) and Pace *et al.,* (1986). Researchers determined that 16S and 18S rDNA were optimal molecules to assess evolutionary relationships because they were found in all self-replicating organisms, easily isolated, and their molecular structure is stable over time. These data, quantified by association coefficients, demonstrated that three major classifications of life exist on earth; eubacteria; archaebacteria; and eukaryotes (Woese and Fox, 1977).

Pace *et al.,* (1986) explored the use of small subunit (SSU) 16SrDNA sequencing as a means to define natural microbial populations and improve understanding of uncultured organisms. Their approach involved shotgun cloning, a method to assess individual phylotypes from a mixed population of phylotypes, to categorize populations within a large microbial community. They found the use of 16S rDNA preferable to 5S rDNA because of the greater number of bases available for comparative analysis. They also noted that the greater number of variations within 16S rDNA allowed for more detailed phylogenetic mapping. This work supported the use of sequence comparisons as an effective tool to estimate phylogenic relationships (Pace *et al.*, 1986). Similarly, the use of SSU rDNA in the determination of phylogeny may represent an approach to the problem of assessing security needs and environmental health, when combined with emerging detection technologies.

The presence of highly conserved and variable regions within rDNA and the relative ease of finding extant information add to the appeal of rDNA as a target molecule. These regions allow researchers to differentiate between closely related strains (Pace *et al.*, 1986). There are extensive databases already in existence for the SSU rDNA (e.g. GenBank). Extant databases may aid in the identification of organisms clustered into operational taxonomic units (OTUs), groups of sequences exhibiting at least 97.5% identity (Marshall, 2002). Previous work by many authors (Marshall, 2002; Amos, 2002; Balser, 2003 and Yannarell *et al.*, 2004) demonstrates the utility of rDNA as an effective measure of comparison over space and time in aquatic systems.

Numerous variables can be used to make community assessments, including: physical parameters, relative species abundance, and diversity. Differing physical parameters have been shown to impact bacterioplankton communities. For example, Lindstrom *et al.* (2000) suggested that internal and external factors can have an impact on bacterioplankton communities. Internal processes are changes in lake physical parameters, predation and the abundance of autochthonous bacteria. External processes are inflow of allochthonous bacteria through runoff, nutrient load, sedimentary content, and removal of vegetation. They compared five lakes, noting little variation in water temperature and humic concentration, but differences in water color, lake size, and size of drainage area, and found differences in the bacterial communities.

Differences in the relative abundance may also provide insight to bacterial community makeup. Crump *et al.* (2003) described reasons for relatively high bacterial production in Toolik Lake, suggesting that the combination of phytoplankton organic and

terrestrial matter, result in high rates of bacterial growth. Investigators worked to differentiate allochthonous and autochthonous sources of organic matter and to describe the role of seasonal fluctuations on the quality and types of organic matter available. As a result of these efforts, persistent and transient populations were identified in Toolik Lake. Bacterial and phytoplankton production cycles were identified as components in the seasonal fluctuation of communities (Crump *et al.*, 2003). DNA fingerprint comparisons using denaturing gradient gel electrophoresis (DGGE) suggest communities shift with changes in environmental conditions. Researchers hypothesized that large seasonal changes in the physical parameters of a body of water would result in shifts in the species composition of the bacterioplankton community (Crump *et al.,* 2003). They found that relative abundance and advection of allochthonous bacteria resulted in shifts in species composition (Crump *et al.*, 2003). Bacterial production near the surface increased by 30 times, while production at 12-16 meters only increased by 2.5 times (Crump *et al.*, 2003). As a result of these findings researchers suggested that community shifts may occur as a result of succession or advection (Crump *et al.*, 2003).

Other studies have focused on wide distribution of common organism types found over extended spatial scales using multiple limnetic sources. Hiorns *et al.,* (1997) conducted a survey of fresh water rDNA sequence data using PCR and shotgun cloning. They were able to produce an overview of the range of phylotypes and diversity found in 7 Adirondack lakes (Hiorns *et al.*, 1997). The results of this study revealed that most members identified were from the class Proteobacteria, the phylum Cytophaga-Flavobacteria-Bacteroides and the order Actinomycetales, and suggested that at least

some taxa were widely distributed. This conclusion has been supported by additional studies that have suggested global distribution of at least some microbial taxa (Glöckner *et al.* 2000, Zwart, *et al.* 1998, Zwart, *et al.* 2002)

Yannarell *et al.,* (2004) conducted a study of within and between lake variability using multiple spatial scales and found that samples taken at spatial scales greater than or equal to 100m and between lakes showed significant variability, suggesting that within lake variability might be caused by geographic features of the lake that isolate communities in bays or narrow channels. However, different lakes having similar physical conditions tended to have similar bacterial communities (Yannarell *et al.*, 2004).

Liu *et al.,* (1997) were able to take advantage of 16S rDNA to differentiate all bacterial strains in a model bacterial community that had six differing strains. PCR was used to amplify target sequences in the model community followed by terminal restriction fragment length polymorphism analysis. Based on the length of the T-RFLP fragments, they were able to distinguish all six bacterial strains present (Liu *et al.,* 1997). They noted that the most critical parameters needed for describing the diversity and structure of a microbial community are species richness and species evenness (Liu *et al.,* 1997). Other studies have suggested that a combination of 16S rDNA analysis and microarray technology may represent a rapid tool to assess water quality (Marshall, 2002; Amos, 2002; Balser, 2003; Zhou and Thompson, 2002).

Microarrays are useful for identifying populations within natural environments (Zhou and Thompson, 2002). It is possible to identify populations using microarrays because common functional groups within different populations may demonstrate unique

gene sequences that can be used as probes. This information may then be used to monitor the presence or absence of populations and serve as an environmental indicator (Zhou and Thompson, 2002).

The application of the microarray approach in defense and public health include three primary detection objectives. These are early detection, quality assurance, and exposure reduction. Early detection of target oligonucleotide sequences offers a valuable tool for making decision makers aware of the presence of harmful microbes. Microarrays can offer an effective quality assurance protocol that will help ensure the control of undesirable microbes in water supplies by providing a reliable detection mechanism. The task of separating pathogens from consumers may be improved with the ability to determine viability and/or infectivity of target microbes (Straub and Chandler, 2003). Before a useful microarray can become a reality, the design and selection of specific complementary nucleic acid probe sequences must first be made (Stenger *et al.*, 2002). Rapid assessment of water bodies, using both the detection of specific pathogens and community profiles, is now possible using microarray technology. It is unfortunate that the infrastructure to support such an assessment for water quality on a multiphasic basis is not in place at present (Straub and Chandler, 2003). The proposed work involves a comparison among different clone libraries that will provide a foundation which might be useful for the development of a rapid community assessment method.

Increasing understanding of the extant natural communities of multiple limnetic systems may aid in developing aquatic analytic methods that might include a biodetection or environmental microarray. By building a better understanding of the existing

microbial communities across small and large scales and over time, suitable molecular markers may be identified. Work to understand extant microbial community composition within selected freshwater systems has already begun. Marshall (2002) and Amos (2002) addressed microbial community composition over large spatial and temporal scales. Marshall (2002) analyzed natural communities to characterize eukaryotic diversity in ecosystems. Three lakes were sampled, including three samples from one of the lakes, for a total of five samples. The study found that species abundance appeared to be more evenly distributed in temperate lakes as compared to an arctic lake. Each library contained 50 clones, which were grouped into operational taxonomic units (OTUs), defined as sequences with $\geq 97.5\%$ similarity. Sorenson coefficients, used to measure community similarity, ranged from 0.062 to 0.212, demonstrating low overlap of OTUs between libraries. One OTU, identified as *Cryptomonas ovata*, was found to be cosmopolitan among the libraries studied. Amos (2002) studied the identical samples for 16S diversity. Again, five libraries were created, and 50 clones were sequenced from each. Prokaryotic libraries were found to be more diverse than eukaryotic libraries. These data suggests temperate lakes surveyed were similar in microbial composition. OTUs common to all five libraries were not found.

Balser (2003) assessed the similarity of 18S rDNA (eukaryotic) clone libraries among replicate samples and across small spatial and temporal scales from City Lake, North Carolina. Each library contained between 78-95 clones. Replicates varied slightly. Spatial samples suggested increasing variation with distance. Temporal samples did not appear to present a pattern. The number of clones in each OTU varied among libraries,

giving Sorensen similarity coefficients ranging from 0.32 to 0.38, suggesting limited overlap of sequences between libraries. Persistent cool and cloudy conditions during Balser's (2003) sampling may have reduced diurnal variability. Community characteristics were similar, although 79% of the sequences were not found in GenBank. The most common OTU was putatively identified as *Cryptomonas ovata,* which was also found in this lake previously by Marshall (2002).

Overall, the results of Marshall (2002), Amos (2002), and Balser (2003) included several key observations: 1) BLASTn yielded results that included both previously known and novel rDNA sequence; the majority of sequences were novel; 2) some OTUs were unique to individual libraries while other OTUs were shared among multiple libraries; 3) the distribution of OTUs was found to be log normal. Because Marshall (2002) and Amos (2002) addressed intermediate to large scale variation within freshwater lakes, the logical next step was to examine small scale temporal and spatial variation. Balser (2003) completed such a study on small scale variation for eukaryotes, finding that nearly 400,000 sequences would be needed to obtain complete diversity of City Lake and that time of sampling had little effect on microbial communities sampled.

The objective of this study was to characterize the prokaryotic microbial community of City Lake (High Point, NC) on small temporal and spatial scales. This was accomplished by comparing different clone libraries using 16S rDNA sequences (Figure 1). While the study does not give an exhaustive detail of the populations present in City Lake, it provides a snap-shot of the ranges of phylotypes and diversity present in City Lake, in a fashion similar to Hiorns *et al.,* (1997).

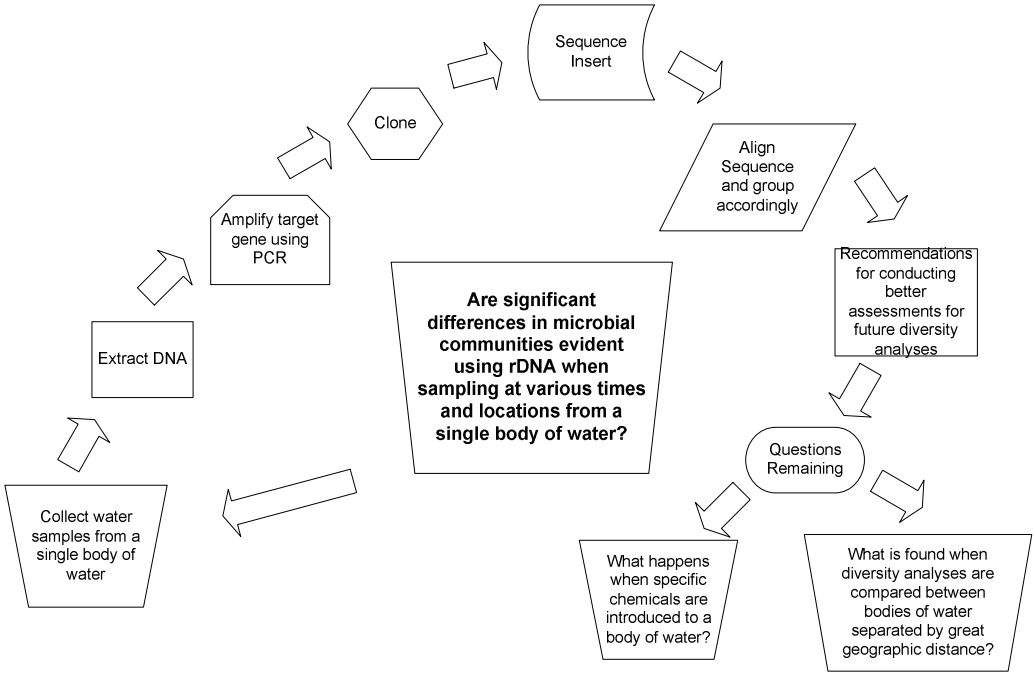


Figure 1: Overview of study approach

CHAPTER II

METHODS

Water samples were taken from six sites in City Lake, High Point, NC on 18 and 19 December 2002 (Figure 2). Collection points were selected along the axis of the lake to provide spatially distant samples within the lake. Triplicate 50 ml samples were passed through Whatman GF/F glass microfiber filters. Filters were placed in screw cap microcentrifuge tubes in 1 ml of cetyltrimethylammonium bromide (CTAB) buffer for storage at ambient temperature. Samples were also taken in triplicate for diurnal sampling on 18 December, at six hour intervals beginning at noon, 18 December 2002, for a 24-hour period at station 1-1300/3 (Figure 2) and stored at ambient temperature. DNA was extracted using a CTAB protocol described by Schaefer (1997). Briefly, the contents of the microcentrifuge tube were transferred to a 14 ml conical tube and 1 ml of 2x CTAB buffer was added to make a total of 2 ml CTAB buffer. The GF/F filters containing sample cells were ground separately using wooden applicator sticks. The sample was incubated at 60-65 °C for 1-hour with periodic vortexing. Two milliliters of a solution of 24: 1(v: v) chloroform to isoamyl alcohol was added to the CTAB/filter mixture, shaken and then centrifuged for 20-60 minutes at full speed in a clinical centrifuge, until the top aqueous layer separated from the organic and filter mixture. The aqueous layer was pipetted into a 1.5 ml microcentrifuge tube and a volume of 100% 2-propanol equivalent to 0.7 volume of the aqueous layer was added. The tubes were then

inverted to ensure mixing and immediately centrifuged at 16,000 X g for 25 minutes. The supernatant was then poured off and the precipitate was allowed to air dry. The precipitate was then resuspended in 25 µl sterile Tris-EDTA buffer (pH 7.4). Following genomic DNA extraction, target sequences were amplified using protocols described in TOPO Cloning Kits for Sequencing (Invitrogen, 2000). Primers 8 forward (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1541 reverse (5' AAG GAG GTG ATC CAG CCG CA-3') were used to target 16S rDNA for use in subsequent cloning reactions (Bruce *et al.*, 1992; Zwart *et al.,* 1998; Stein *et al.*, 2002). Each 50µl PCR reaction contained 2.5µl 16mM dNTP stock , 5µl Mg free10X PCR Buffer, 5µl 25mM $MgCl_2$, 5µl 100µM BSA, 1µl 10µM 8F primer, 1µl 10µM 1541R primer, 0.2µl Promega Taq DNA Polymerase, 30.2µl sdH_20 and 1µl template DNA.

The PCR protocol involved an initial denaturation at 94°C for 2 minutes, followed by 29 cycles of the following: 94°C denaturation 1 minute; 54°C anneal 1 minute;72°C extension 1 minute and 72°C final extension 2.5 minutes. An effort was made to avoid freezing or vortexing of the PCR product to preserve fragment quality. The PCR product was then run in a gel to verify the presence of the 1600 base pair (bp) fragment. DNA fragments were then extracted from the agarose gel using a Qiagen Gel Extraction Kit (Qiagen, 2000). When gel electrophoreses showed distinct band separation, the amplicon was directly purified using a Qiagen PCR Cleanup Kit

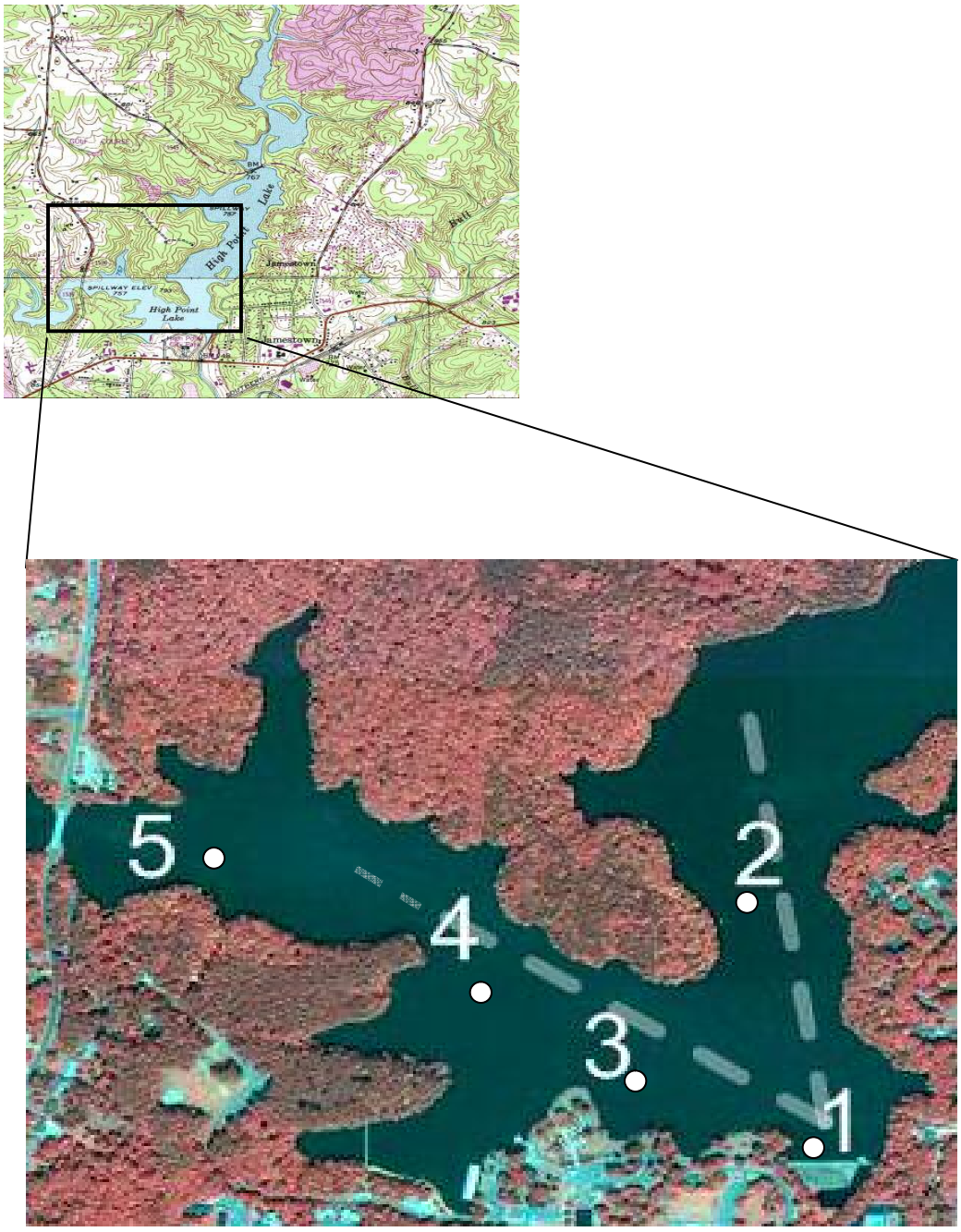


Figure 2: High Point City Lake Aerial Photo taken 1 Dec 1999 and map provided by the City of High Point, NC. The numbers indicate sample collection stations while the white lines represent bubble aerators in the lake. All replicate and diurnal samples were taken at station 1.

(Qiagen, 2000). Following a successful extraction, DNA was stored at 4°C or -20°C until ready for use in the cloning reaction.

The purified PCR product was then used in the cloning reaction. Each sample was inserted into a pCR 4 TOPO plasmid vector and *E.coli* cells transformed (Invitrogen, 2000). Cells were then plated on LB containing 50µg/ml ampicillin and incubated at 37°C overnight. Colonies with recombinant inserts were selected and inoculated into LB broth and incubated overnight in a shaking water bath at 37°C and 200 rpm. Aliquots of the culture were taken for storage and for sequencing. Storage preparation included using 0.930 ml culture broth and 0.070 ml dimethylsulfoxide (DMSO) for a total volume of 1 ml that was immediately placed in -80°C storage.

Cultured broth (1 ml) was prepared for sequencing reaction using the Qiaprep Miniprep kit for the purification of plasmid DNA following manufacturer's directions. Plasmid DNA was then placed in cold storage at -20°C prior to a restriction digest.

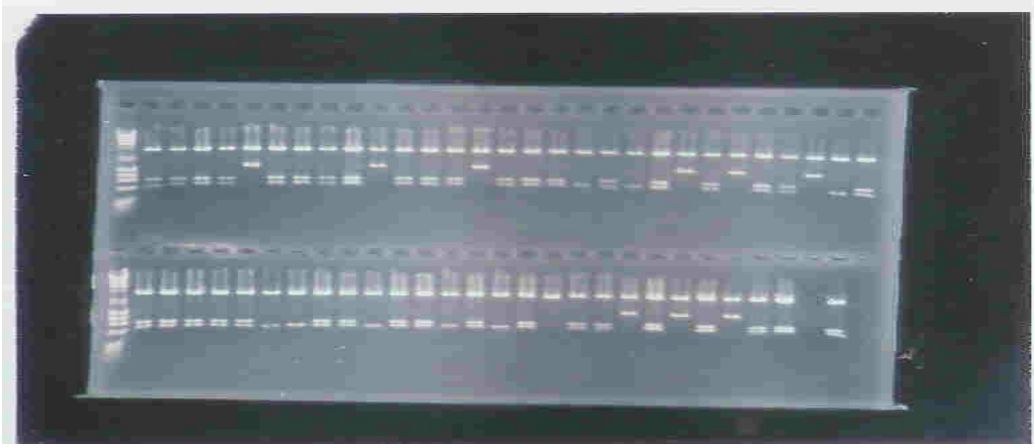


Figure 3: Agarose gel of restriction digest product. The passenger DNA or insert was separated from the vector plasmid using EcoR I restriction enzyme. The passenger insert can be visualized at 1600 bp while the plasmid is visible at 2300 bp. In many instances an EcoR I restriction site was present within the passenger DNA. This is visualized as bands at 900 bp and 700 bp.

The 1600 bp insert was cut from the plasmid (EcoR1, 1.5 hr at 37°C) and an aliquot of product run in an agarose gel to verify its presence. Recombinant plasmids were placed in cold storage at -20°C after the 1600 bp fragment was verified (Figure 3). 16S rDNA was amplified for sequencing using the M13 forward, M13 reverse, or 524 internal primer in a linear fashion following manufacturer directions with the DYEnamic ET Dye Terminator Cycle Sequencing Kit (Amersham Biosciences, 2002). A volume of template DNA solution was added so that each sequencing reaction contained between 300-600 ng/ml. The final extension time was modified to allow complete elongation of the emerging DNA strand. The amplified sequencing mix was removed from the thermocycler and an ethanol cleanup was performed to remove any unincorporated dye terminators. The amplified mixture was then sequenced on a MegaBACE DNA Analysis System (Amersham Biosciences, 2002).

The electropherogram of each sequence was assigned a quality score using the Phred/Phrapsoftware package (Ewing *et al.,* 1998). Sequences with scores below 20 were removed from further analysis. Following these analyses, sequence files were converted to .ACE files and compiled in Biolign's alignment viewer (Hall, 2000). Each sequence was then viewed to ensure correct base calls, proper orientation, and suitable quality for comparison. Sequences were aligned using CLUSTAL W, a software package designed to align DNA and proteins using a progressive multiple alignment method (Thompson, 1994). Alignment allowed the grouping of organisms having genes with similar functional groups or OTUs (Kumar, 2001). Marshall (2002) found that 500 bp were more than sufficient to distinguish unique operational taxonomic units. A sequence

identity matrix was computed using the Biolign software package by conducting a pairwise comparison of each sequence that provided the proportion of identical residuals in the alignment (Hall, 2000). Sequences showing identity ≥ 0.975 were grouped as members of a single OTU. A second pairwise comparison was made between each library in a similar fashion. Sorenson similarity coefficients were calculated as described by Fox *et al.* (1977), Woese and Fox (1977), and McCaig *et al.* (1999). Sequences from each library were submitted to NCBI's BLASTn to determine nearest matches (Altschul, *et al.* 1997). MEGA version 3.1 was used to generate dendrograms of phylotypes that were used to infer the phylogenetic relationships within each library (Kumar, 2004).

Richness and evenness were assessed using the Shannnon-Wiener and Simpson indices, respectively. The Shannon-Wiener index (H), where $H = \Sigma \rho_i \ln \rho_i$ and $\rho = (n/N)$ n = sequences in *i*th OTU and N = total sequences in library, provides a probability that a random individual selected will belong to a given category (McCaig *et al.,* 1999).

Richness and proportion of OTU was assessed using the Simpson Index. The Simpson Index (D), gives the probability that two randomly selected individuals from a sample belong to the same OTU. The equation is given as $D = \Sigma \rho^2_i$, where ρ is the proportion of individuals in the *i*th OTU (Dunbar *et al*., 1999; McCaig *et al*., 1999). An assessment of the completeness of subsampling was determined using Good's Coverage, calculated using $C = 1-(\rho_i) \times 100$, where ρ_i is the number of individuals in the *i*th OTU (McCaig *et al.*, 1999).

The Sorenson similarity index is an incidence-based evaluation that makes pairwise comparisons of libraries to determine if any common individuals exist in both. An index

of 1 would indicate that all individual types are found in both libraries, while a score of 0 would indicate no individual types in common. Sorenson similarity coefficients were calculated as 2C/(A+B) where A = the number of unique OTUs found in library A; B = the number of unique OTUs found in library B; C = the number of unique OTUs found in both library A and B.

Rarefaction analysis is one way to estimate total community diversity from a subsample and is an excellent starting point when beginning data set analysis (Hughes and Hellmann, 2005). Rarefaction was used to compare multiple sites having similar sample numbers or to evaluate differences in community diversity. Strongly curvilinear data plots may be evidence that diversity was adequately sampled (Kemp and Aller, 2004b). Rarefaction gives an estimate of a single sample's community diversity that can then be used to compare among samples.

S_{CHAO1} , an abundance based non-parametric estimator, was used to determine if libraries were of sufficient size to yield stable unbiased estimates of OTU richness (Kemp and Aller, 2004b). In addition, S_{ACE} , a coverage based estimator that correlates well with S_{CHAO1} when there are fewer than 10 sequences per OTU and the majority of OTUs occur only once or twice, was used as a reference for S_{CHAO1}. Equations for S_{CHAO1} and S_{ACE} can be found in Kemp and Aller (2004b).

CHAPTER III

RESULTS

Eleven libraries were constructed from samples collected from City Lake on 18 and 19 December 2002 (Table 1). Between 89 and 112 clones were sequenced for each library. Six sequences, identified as potential chimera by ChimeraCheck software RDP, were eliminated from the analysis (Maidak *et al.*, 2001). Additionally, 44 sequences were eliminated from Library 1-1300/3, 43 eliminated from Library 1-1300/1, 59 eliminated from Library 1-1300/3, 37 eliminated from Library 2, 37 eliminated from Library 3, 31 eliminated from Library 1-1816, 38 eliminated from Library 1-0300, 31 eliminated from Library 1-0617, and 22 eliminated from Library 1-0617 because quality scores were below 20 on the Phred/Phrap quality score scale. From these libraries, 437 OTUs were identified.

Among all libraries, 84 clone sequences demonstrated affiliation with the division Proteobacterium, including representatives from each class, α, β, δ and γ. Twenty-four clone sequences affiliated with division Actinobacterium represented the next most abundant putatively identified 16S rDNA. Seventeen clone sequences demonstrated close association with Crater Lake bacterium. The most abundant OTU was found in Library 5, CL33 OTU 10 (43 clones), having 96-97% identity to uncultured bacterium FukuS 110 or uncultured freshwater clone 965004f10x1 (Glöckner *et al.*, 2000; Horner-

Table 1: Physical and chemical characteristics of City Lake samples collected on 18 and 19 Dec 2002. Samples 1-1816 to 1-1200 are diurnal samples taken from station 1.

Sample (Library #)	Date 2002	Time	Latitude (N)	Longitude (W)	Temp (ºC)	*O_2 (mgl^{-1})	Conduct ($mScm^{-1}$)	TDS (mgl^{-1})	*Turb (%)	pH	*ChlA (μgl^{-1})
1-1300/1 1-1300/2 1-1300/3	12/18	1300	35.59.725	79.56.669	6.36	10.37	120.1	0.0768	14.7	7.78	6.2
2	12/18	1409	36.00.028	79.56.778	5.65	10.07	123.7	0.0791	18.3	7.81	11.3
3	12/18	1426	35.59.773	79.56.750	6.02	12.3	119.1	0.0762	13	7.91	9.7
4	12/18	1438	35.59.904	79.57.005	5.84	11.02	112.5	0.0719	9.4	7.91	9.4
5	12/18	1454	35.59.976	79.57.001	6.61	11.17	113.9	0.0729	7.2	8.05	15.3
1-1816	12/18	1816	35.59.725	79.56.669	6.14	10.94	119.2	0.0763	13.1	7.98	15.5
1- 0300	12/19	0300	35.59.725	79.56.669	5.83	10.79	119.4	0.0764	11.9	8.01	9.3
1- 0617	12/19	0617	35.59.725	79.56.669	5.86	10.76	118.1	0.0756	11.9	8.09	16.2
1- 1200	12/19	1200	35.59.725	79.56.669	6.17	11.2	117.1	0.0749	12.2	8.11	14

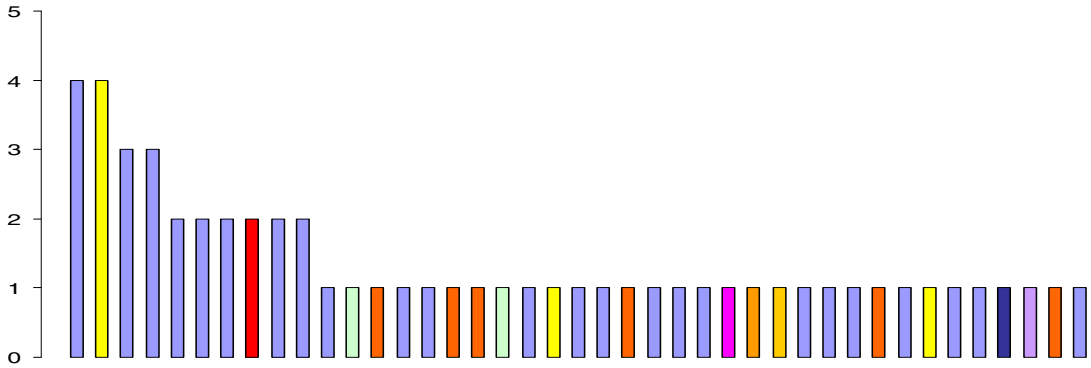
*O_2 = dissolved oxygen; ChlA = chlorophyll a; Turb = turbidity

closely resembling sequences of uncultured bacterium clone ES3-22 (unpublished), and Library 1-1816, CL38 OTU 3 (7 clones), consistent with uncultured bacterial clone ES3-27 (Burkert *et al.*, 2003).

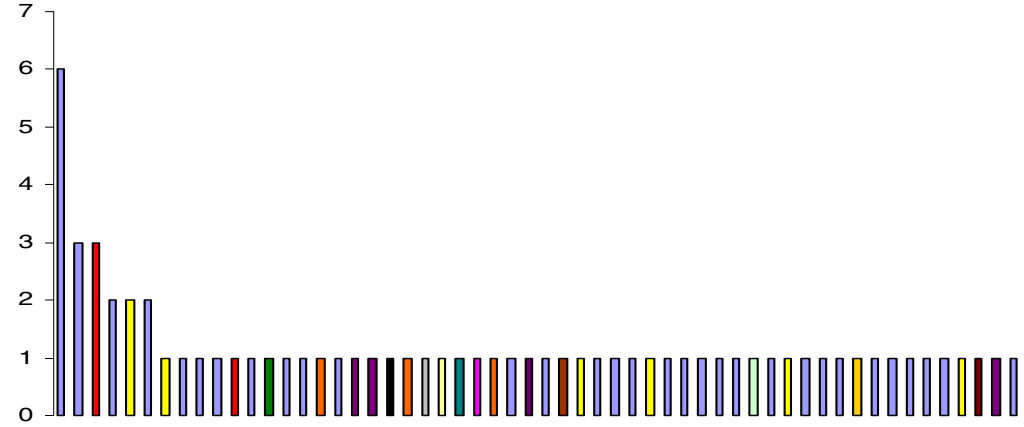
Rank abundance curves (Figure 4) make it practical to compare the richness of one OTU to others in a library. City Lake rank abundance curves are dominated by rare individuals (OTUs). The more steeply sloping curves indicate a more dominant OTU present in the library. The majority of OTUs have only one clone. The OTUs having the highest abundance were found in Library 5 OTU 10 (43 clones) followed by Library 2 OTU 3 (12 clones).

The overall range of observed Shannon-Wiener values was between 3.95 for Library 1-1300/1 to 1.86 for Library 5 (Table 2). The replicate Shannon-Wiener values were between 3.95 and 3.20. Spatial values ranged between 3.95 and 1.86. Shannon-Wiener scores from diurnal samples showed the least difference, ranging from 3.44 to 3.95. Library 5 was heavily weighted by a single OTU and represents the lowest recorded diversity in this study.

Overall Simpson Index values ranged from 0.0251 (1-1300/3) to 0.3751 (Library 5), with the larger value reflecting the large OTU and lower diversity in Library 5. Replicate Simpson Index values ranged slightly from 0.0251 to 0.0565. Spatial libraries demonstrated the greatest range in Simpson Index values, while diurnal values showed the least (Table 2). Coverage ranged from a high of 68% (Library 5) to a low of 18% (1-1300/3), with an overall average of 38%.



City Lake Library 1-1300/1 (Dec 2002) n=57



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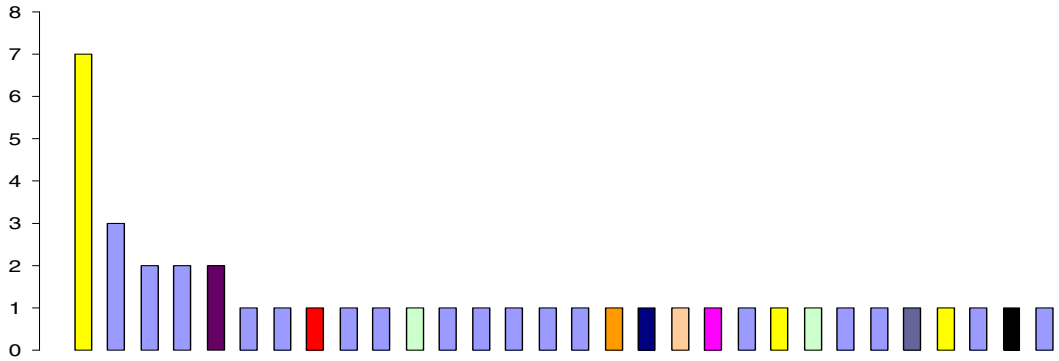
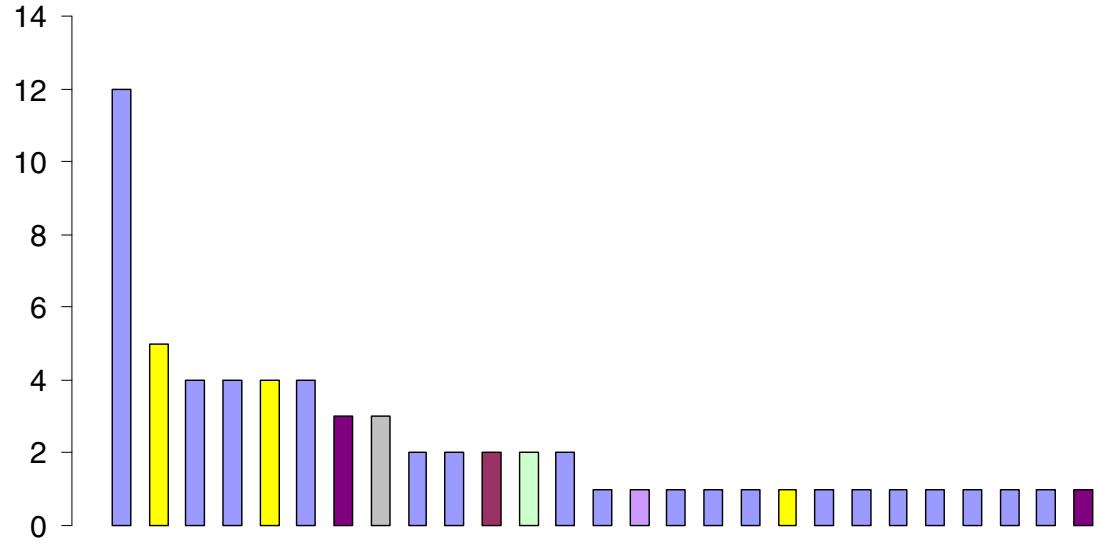
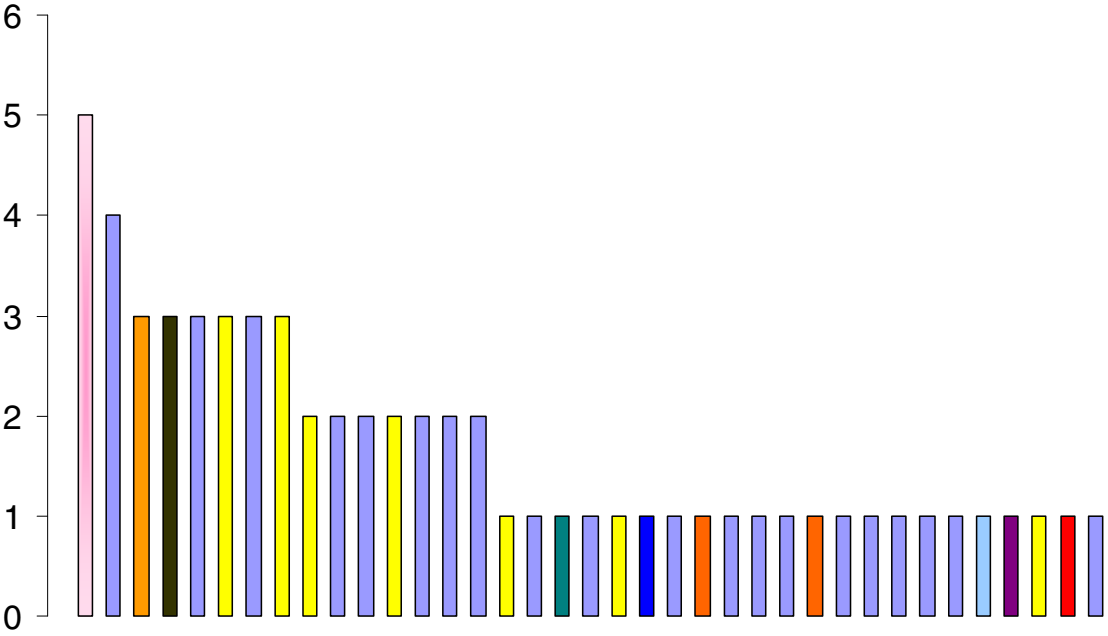


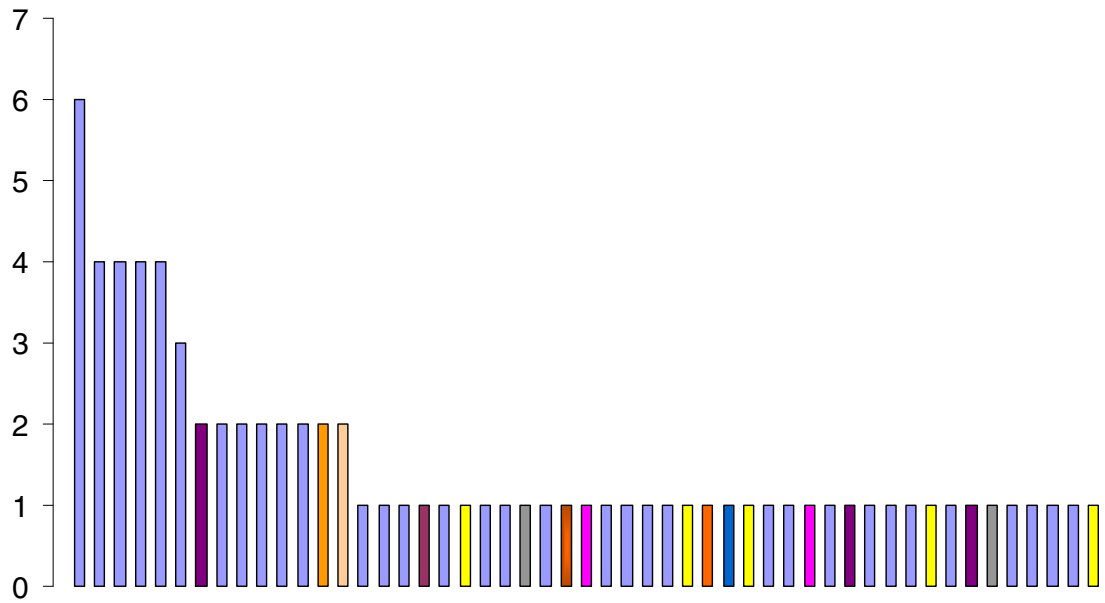
City Lake Library 1-1300/3 (Dec 2002) n=41



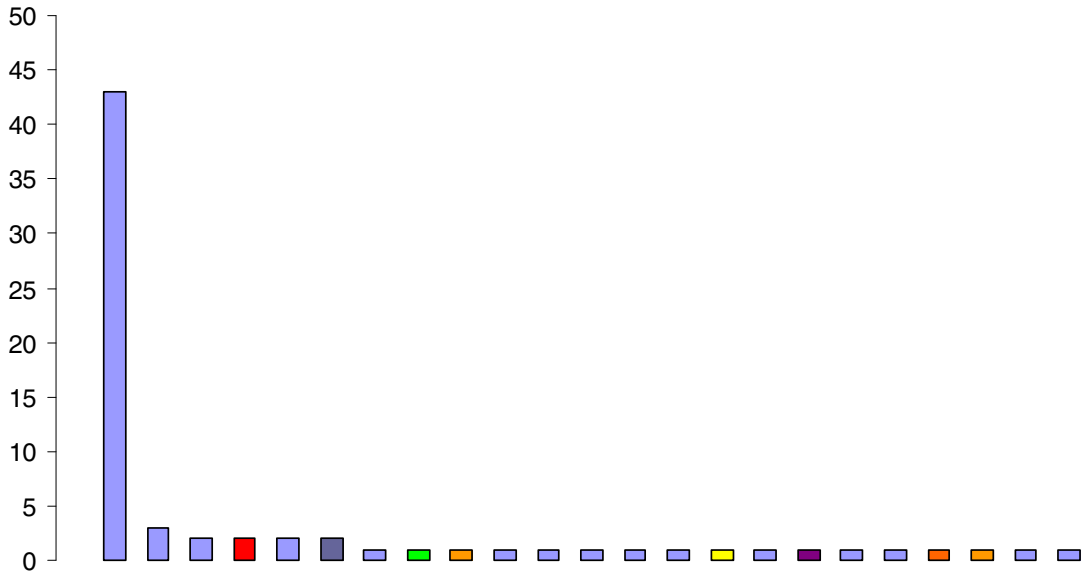
City Lake Library 2 (Dec 2002) n=63



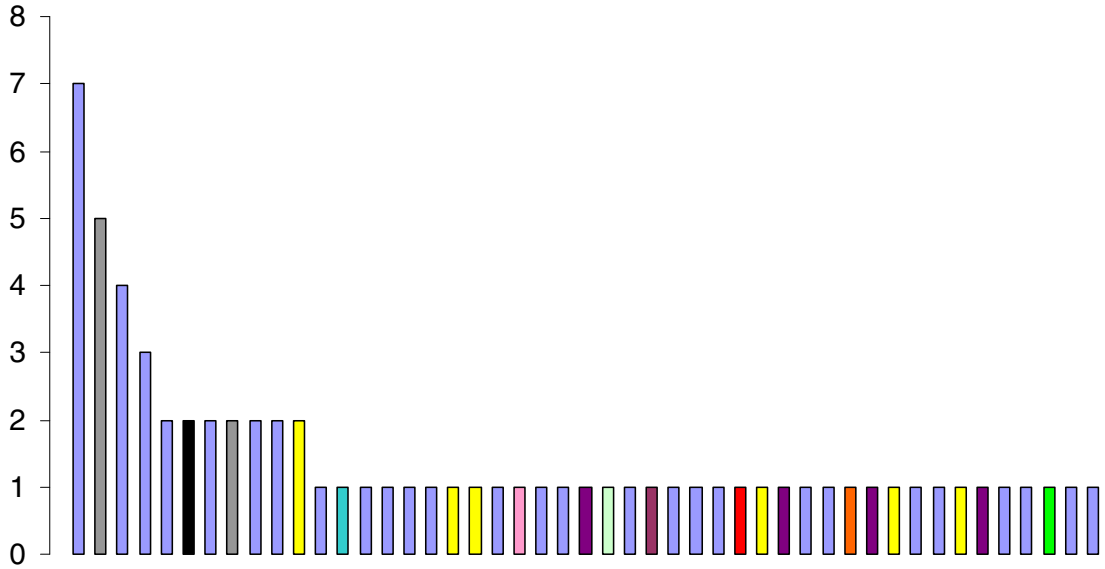
City Lake Library 3 (Dec 2002) n=63



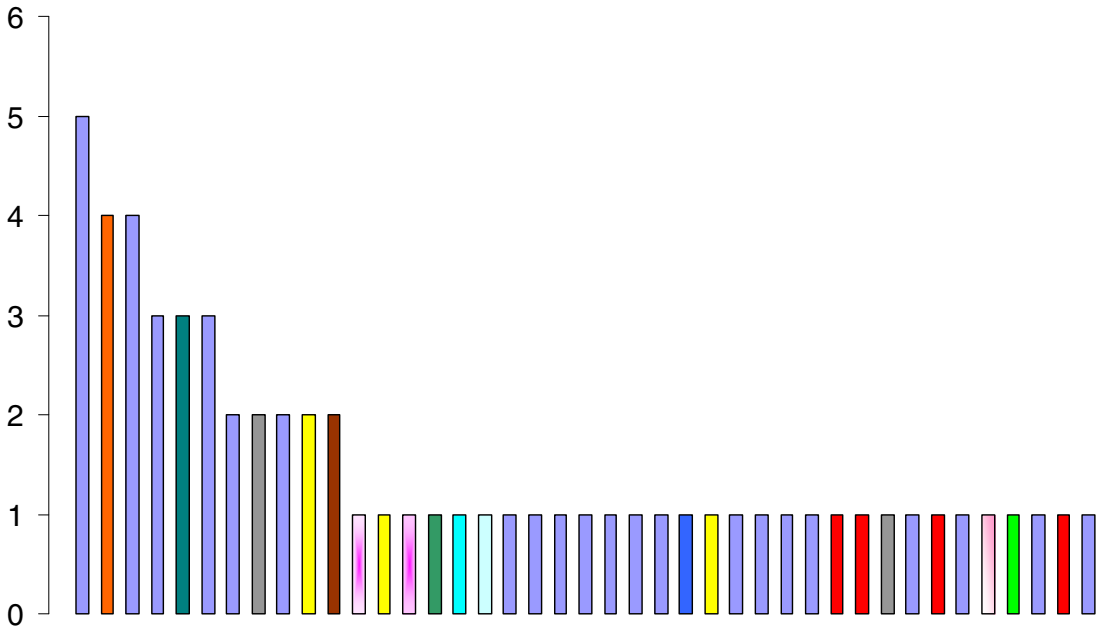
City Lake Library 4 (Dec 2002) n=78



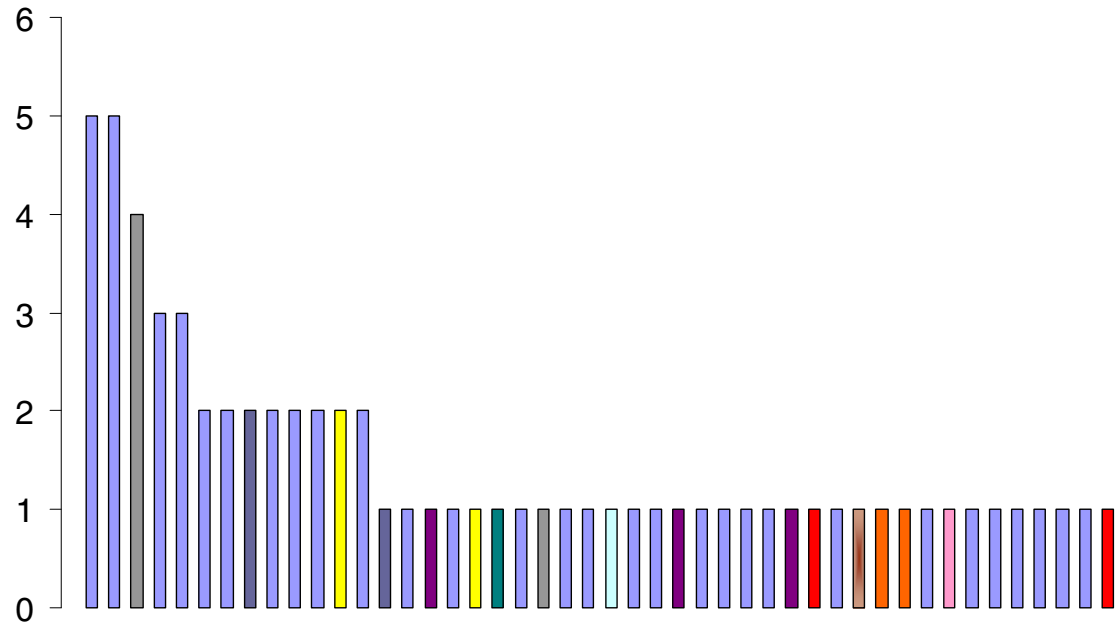
City Lake Library 5 (Dec 2002) n=71



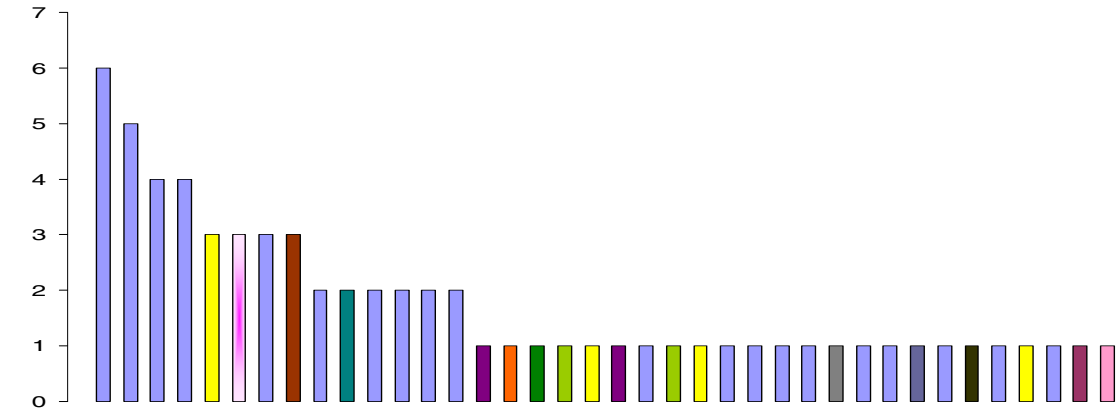
City Lake Library 1-1816 (Dec 2002) n=69



City Lake Library 1-0300 (Dec 2002) n=62



City Lake Library 1-0617 (Dec 2002) n=69



City Lake Library 1-1200 (Dec 2002) n=67

- Actinobacterium
- Aquamonas
- Aquatic Bacterium
- Bacteroidetes
- Burkholderias
- Crater Lake Bacterium
- Cyanobacterium
- Cytophagales
- Denitrifying bacterium
- Division TM6
- Feldmannia irregularis
- Firmicute
- Flavobacterium
- Flexibacteraceae
- Fucus vesiculosus chlorplast
- Gemmatimonadete
- Isochrysis
- Methlobacter
- Methylobacter psychrophilius
- Microbacterlaceae
- Phototropic eukaryote
- Plantomycete
- Polaromonas
- Pseudomonas
- Rhodobacteraceae
- Rhodoferax ferrireducens
- Rhodophyte
- Sphingobacteriales
- Verrucomicrobiales
- Zooglea
- Alpha proteobacteria
- Beta Proteobacteria
- Delta proteobacteria
- Gamma Proteobacteria
- Proteobacteria
- Chloroplast
- Emiliania huxleyi chloroplast
- Uncultured Bacterium

Figure 4: Rank abundance curves for each City Lake 16S rDNA clone library are shown depicting individual OTUs as single columns. Bar height indicates the number of individuals in the OTU. Bar color indicates the putative organism or putative groups of organisms that contains the closest matching 16S rDNA to the City Lake clone. When putatitive taxonomic identification was possible, it was limited to the information found in GenBank; therefore some affiliated 16S rDNA genes were putatively identified at the species level while others were putatively identified at other levels of taxonomic classification. The majority of clones matched the Uncultured Bacterium description in GenBank and are considered unidentified.

Table 2(A-C): Diversity, coverage, and richness characteristics of samples from this study.

A. Replicate Samples .

Sample	1-1300/1	1-1300/2	1-1300/3
Shannon Wiener Index	3.95	3.90	3.20
Simpson's Index	0.0321	0.0251	0.0565
% Coverage	29	18	27
Number of Sequences	57	68	41
OTU Richness	41	56	30

B. Samples taken along the axis of City Lake

Sample	1-1300/1	2	3	4	5
Shannon Wiener Index	3.95	2.98	3.46	3.75	1.86
Simpson Index	0.0321	0.0718	0.0365	0.0292	0.3751
Coverage	29	57	41	35	68
Number of Sequences	57	63	63	78	71
OTU Richness	41	27	37	51	23

C. Samples taken over diurnal period.

Sample	1-1300/1	1-1816	1-0300	1-0617	1-1200
Shannon Wiener Index	3.95	3.65	3.55	3.66	3.44
Simpson Index	0.0321	0.0342	0.0348	0.0313	0.0394
Coverage	29	32	34	33	43
Number of Sequences	57	69	62	69	67
OTU Richness	41	47	41	46	38

The Sorenson similarity coefficients for replicate samples (Table 3-A) were generally moderate, ranging from 0.2461 to 0.5538 in replicate libraries. Sorenson similarity coefficients were most variable across the spatial samples (Table 3-B), with the lowest and highest recorded for all sample groups, ranging from 0.0833 to 0.6622. Diurnal similarity coefficients (Table 3-C) ranged from 0.2286 to 0.5263.

Upweighted Pair Group Method with Arithmetic Mean (UPGMA) application was used to generate phyletic dendrograms for each library (Kumar, 2001). Sequences from each library were used to generate phylogenetic trees. The results depict the evolutionary relationship of the partial 16S rDNA sequences found in each library (Figures 5-15).

Table 3: Sorenson values for 16S rDNA samples collected in this study and for 18S rDNA samples from Balser (2003). Sorenson values for 16S samples collected in this study and for 18S samples from Balser (2003). A: Replicate samples. B: Spatial samples. C: Diurnal samples. A. Sorenson values showing replicate samples

	1-1300/2		1-1300/1		1-1300/3	
	16S	18S	16S	18S	16S	18S
1-1300/2	1	1	0.55	0.35	0.24	0.38
1-1300/1			1	1	0.50	0.32
1-1300/3					1	1

B. Sorenson values showing samples taken along the axis of City Lake.

	Time	Dist.	1-1300/1		2		3		4		5	
			16S	18S	16S	18S	16S	18S	16S	18S	16S	18S
1-1300/1	1300	0	1	1	0.46	0.34	0.39	0.24	0.40	0.34	0.39	0.18
2	1409	0.414			1	1	0.62	0.20	0.08	0.27	0.30	0.19
3	1426	0.235					1	1	0.25	.29	0.10	0.24
4	1438	0.547							1	1	0.21	0.23
5	1454	0.793									1	1

C. Sorenson values showing samples taken over a diurnal period.

	Time	1-1300/1		1-1816		1-0300		1-0617		1-0617	
		16S	18S	16S	18S	16S	18S	16S	18S	16S	18S
1-1300/1	1300	1	1	0.45	0.26	0.51	0.31	0.23	0.31	0.38	0.25
1-1816	1816			1	1	0.41	0.26	0.38	0.37	0.48	0.33
1-0300	0300					1	1	0.53	0.29	0.46	0.30
1-0617	0617							1	1	0.30	0.33
1-0617	1200									1	1

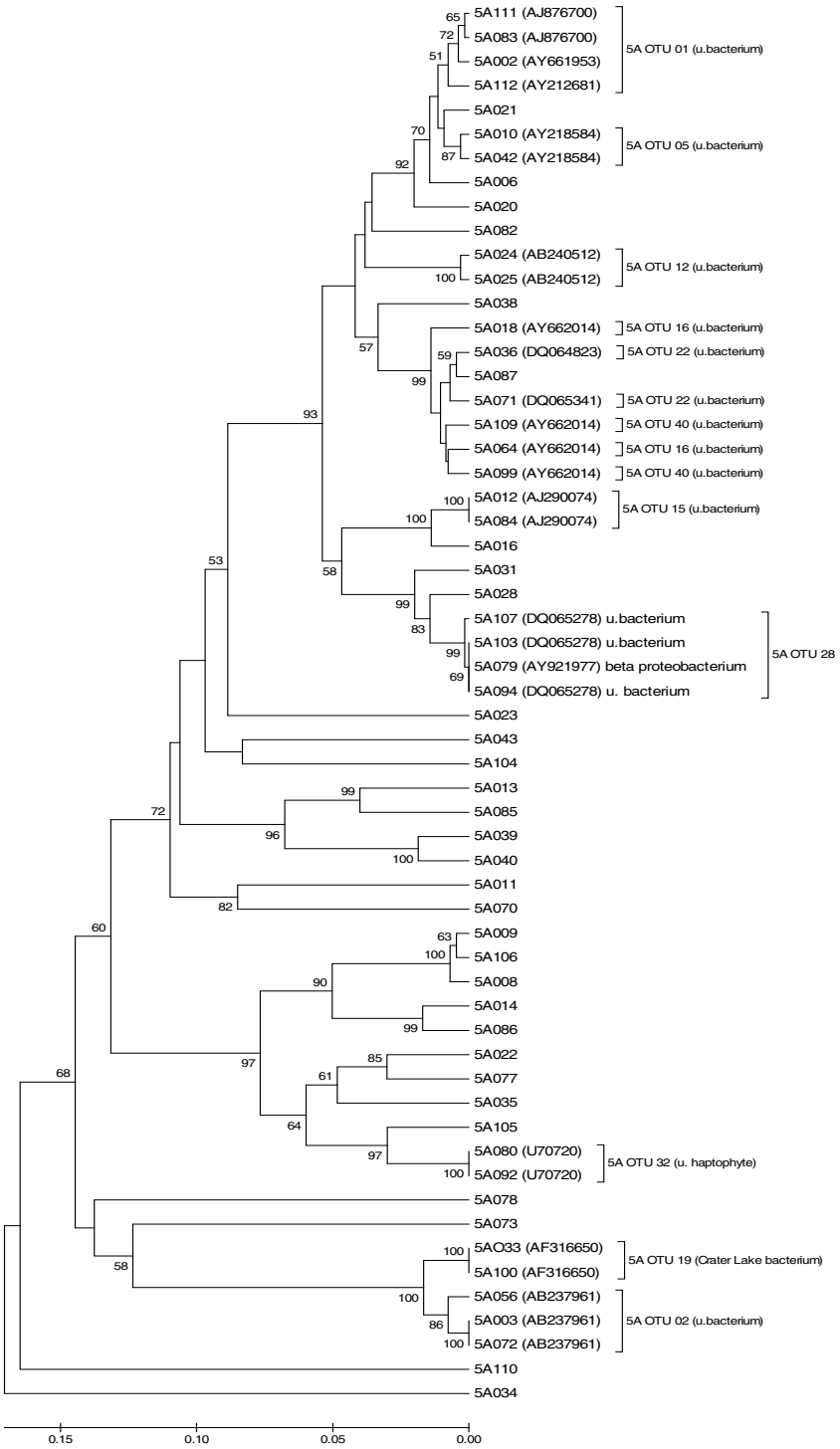


Figure 5: Bootstrap consensus tree for Library 1-1300/1 created using UPGMA Kimura 2-parameter with 500 replications. OTUs having two or more clones are grouped by brackets. GenBank Accession numbers are provided in parenthesis for OTUs with multiple clones. Bootstrap values below 50% are hidden.

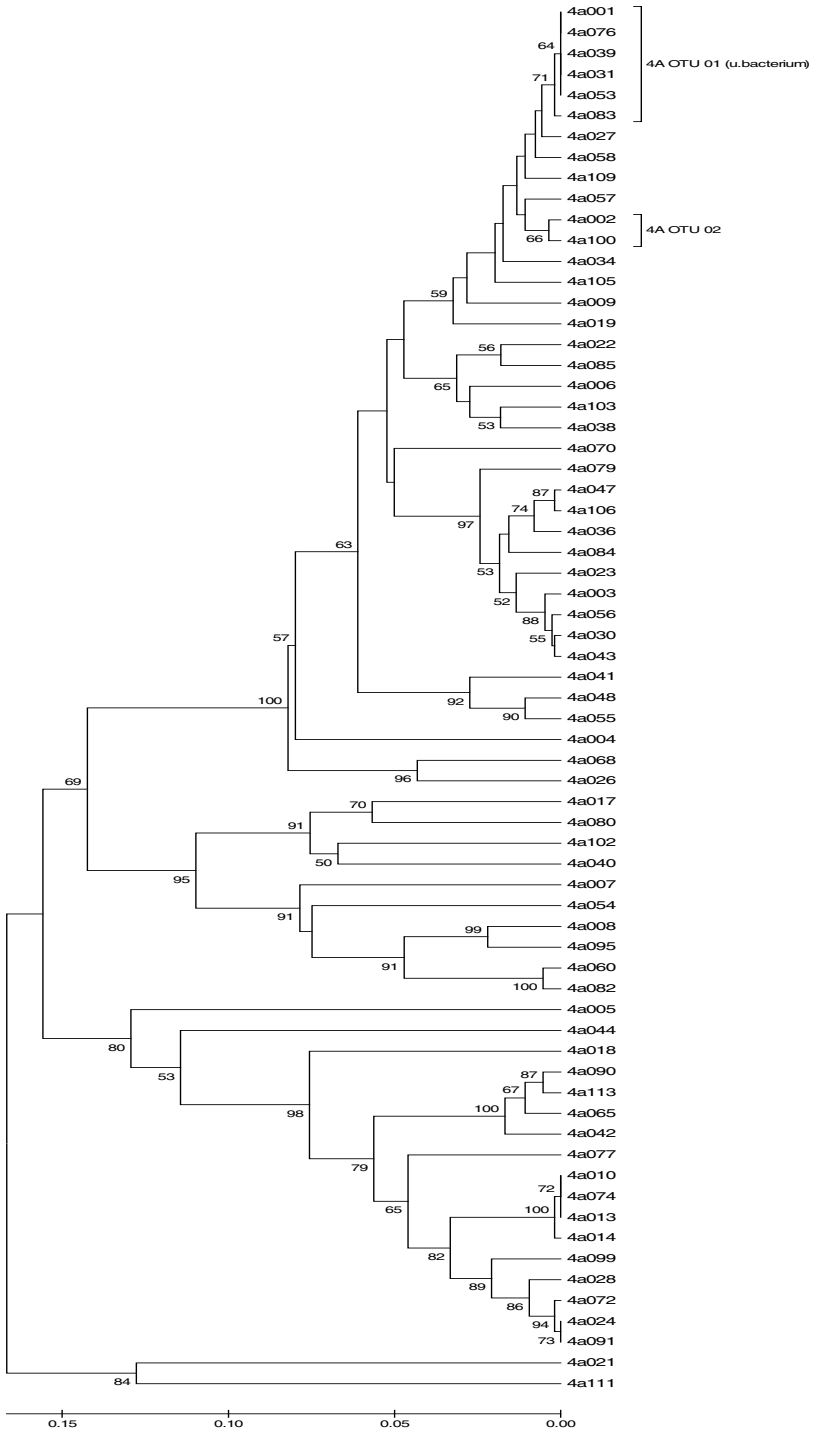


Figure 6: Bootstrap consensus tree for Library 1-1300 Rep1 created using UPGMA Kimura 2-parameter with 500 replications. OTUs having two or more clones are grouped by brackets. GenBank Accession numbers are provided in parenthesis for OTUs with multiple clones. Bootstrap values below 50% are hidden.

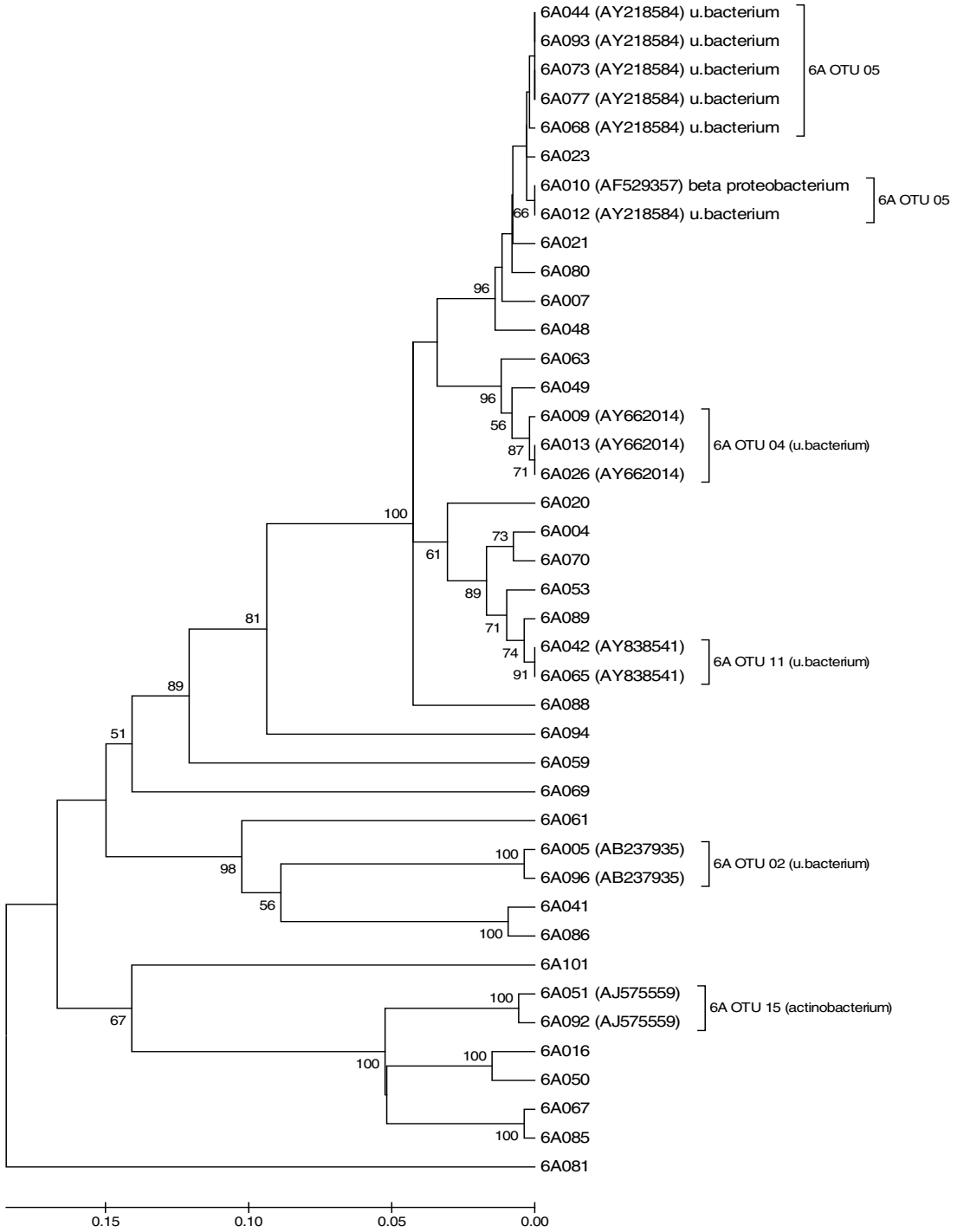


Figure 7: Bootstrap consensus tree for Library 1-1300 Rep2 created using UPGMA Kimura 2-parameter with 500 replications. OTUs having two or more clones are grouped by brackets. GenBank Accession numbers are provided in parenthesis for OTUs with multiple clones. Bootstrap values below 50% are hidden.

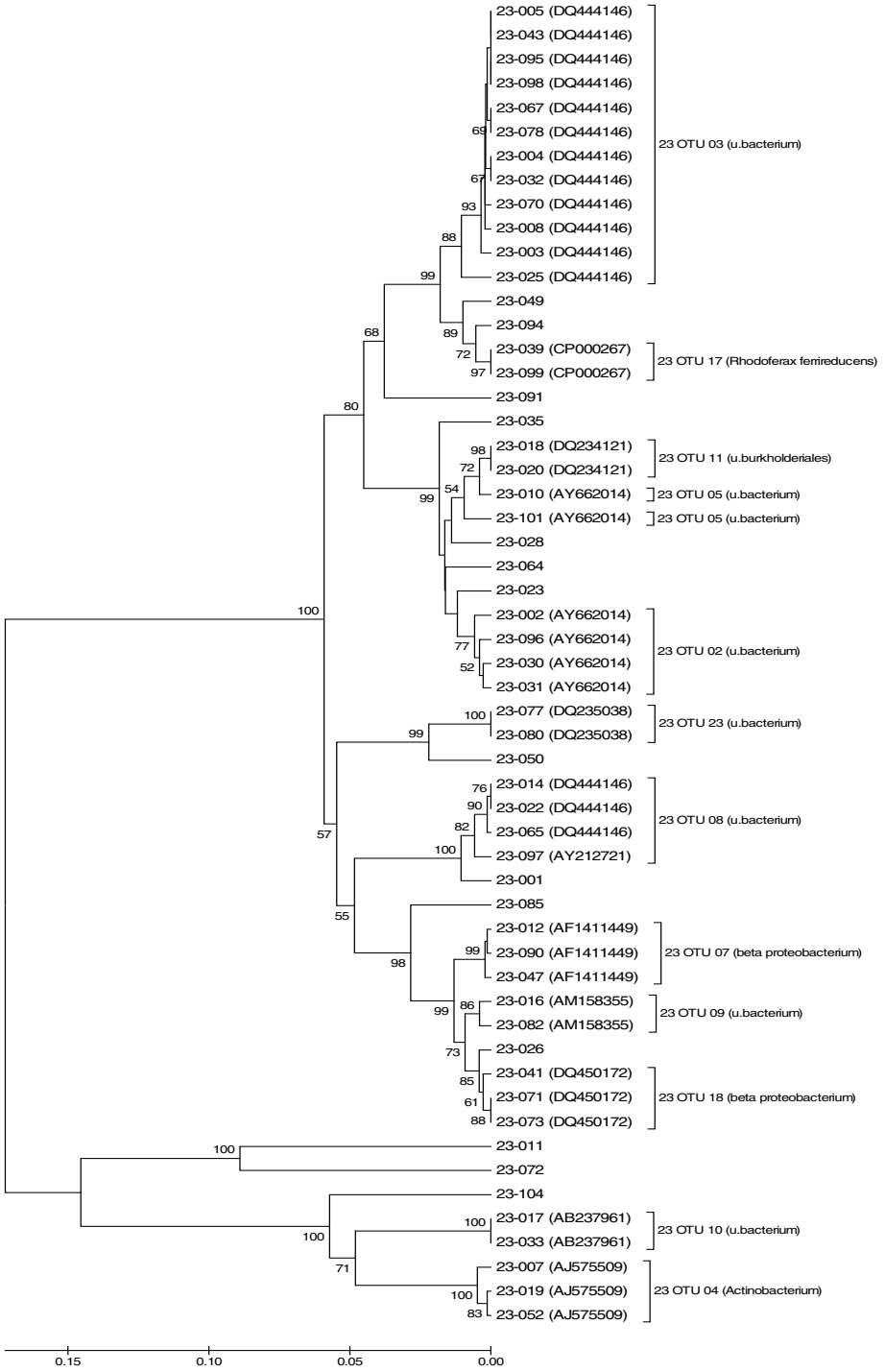


Figure 8: Bootstrap consensus tree for Library 2 created using UPGMA Kimura 2-parameter with 500 replications. OTUs having two or more clones are grouped by brackets. GenBank Accession numbers are provided in parenthesis for OTUs with multiple clones. Bootstrap values below 50% are hidden.

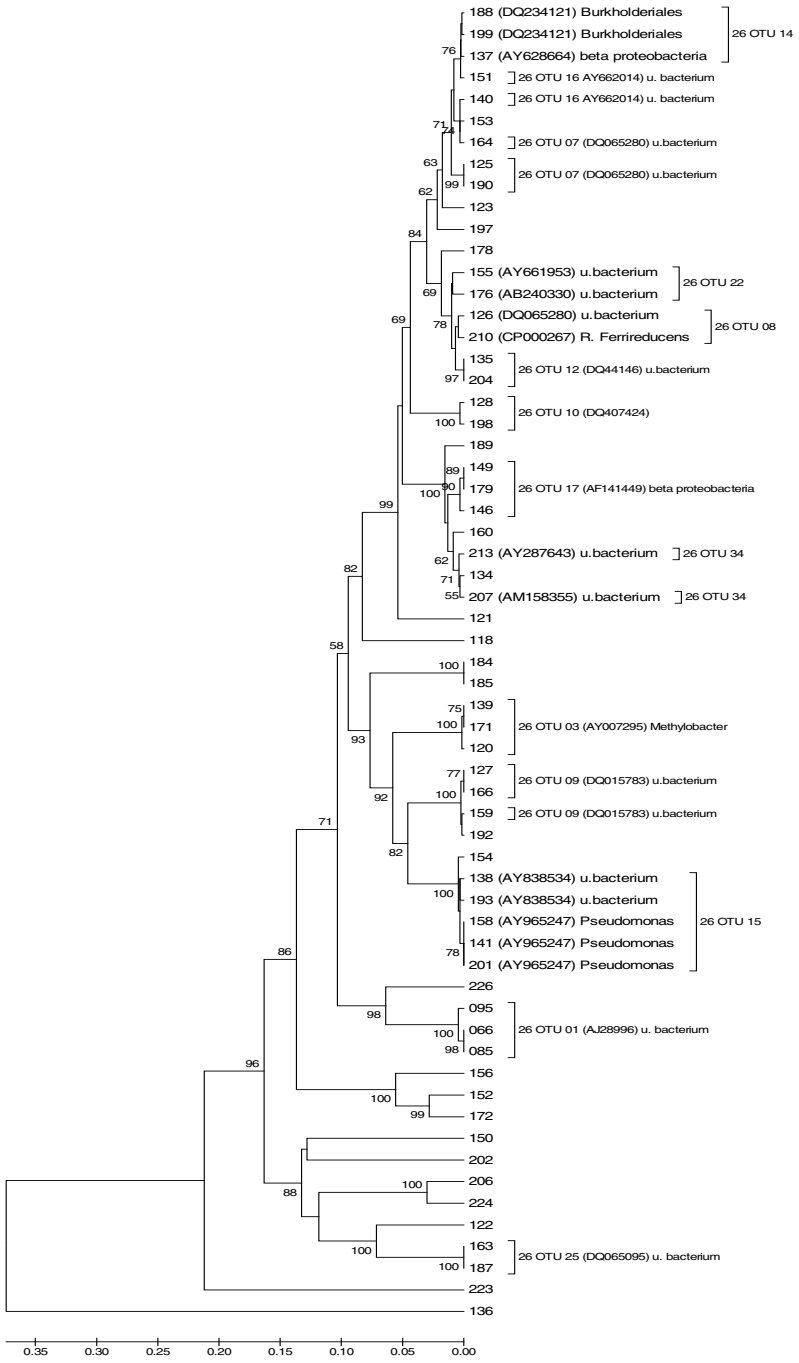


Figure 9: Bootstrap consensus tree for Library 3 created using UPGMA Kimura 2-parameter with 500 replications. OTUs having two or more clones are grouped by brackets. GenBank Accession numbers are provided in parenthesis for OTUs with multiple clones. Bootstrap values below 50% are hidden.

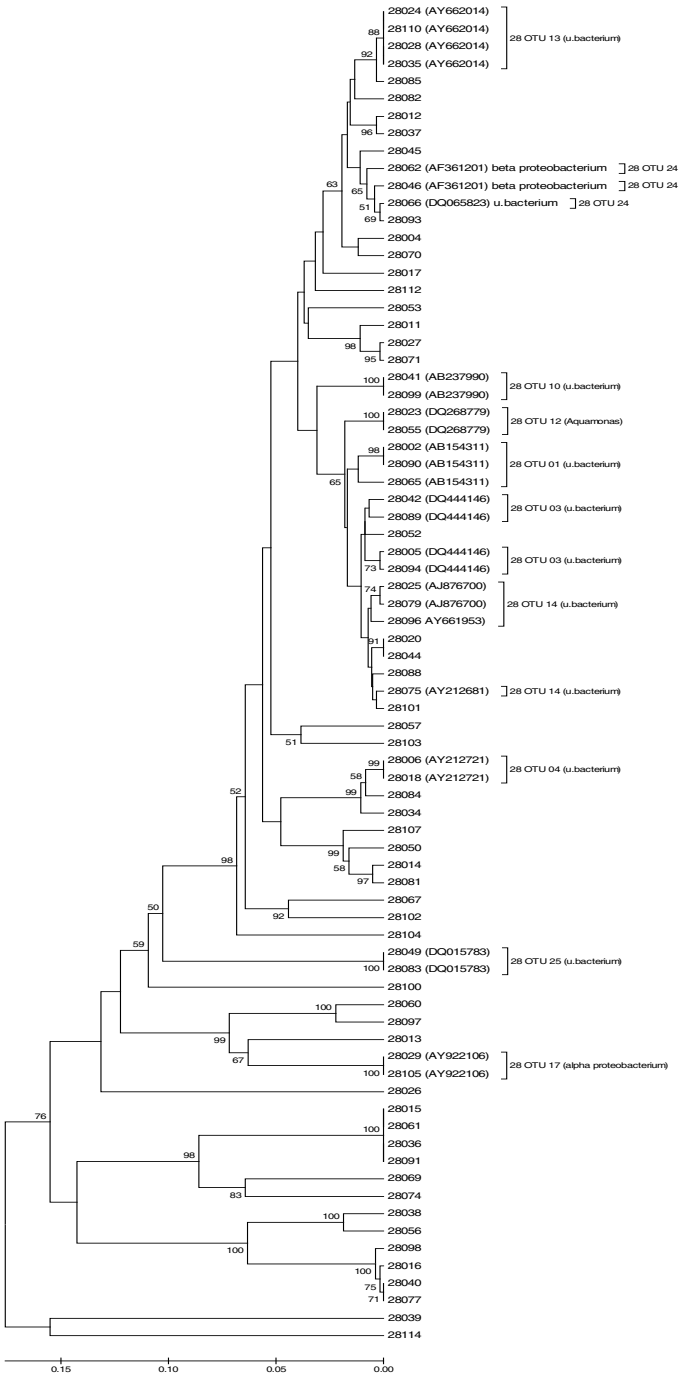


Figure 10: Bootstrap consensus tree for Library 4 created using UPGMA Kimura 2-parameter with 500 replications. OTUs having two or more clones are grouped by brackets. GenBank Accession numbers are provided in parenthesis for OTUs with multiple clones. Bootstrap values below 50% are hidden.

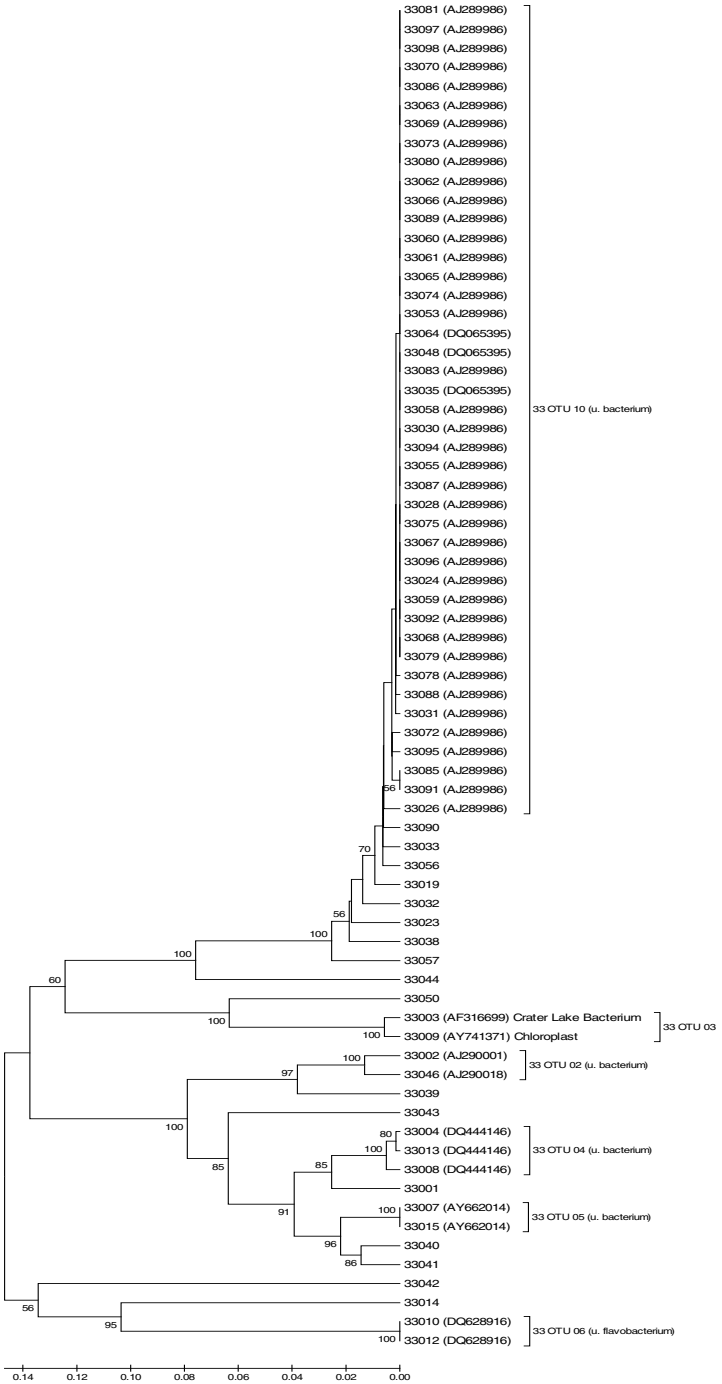


Figure 11: Bootstrap consensus tree for Library 5 created using UPGMA Kimura 2-parameter with 500 replications. OTUs having two or more clones are grouped by brackets. GenBank Accession numbers are provided in parenthesis for OTUs with multiple clones. Bootstrap values below 50% are hidden.

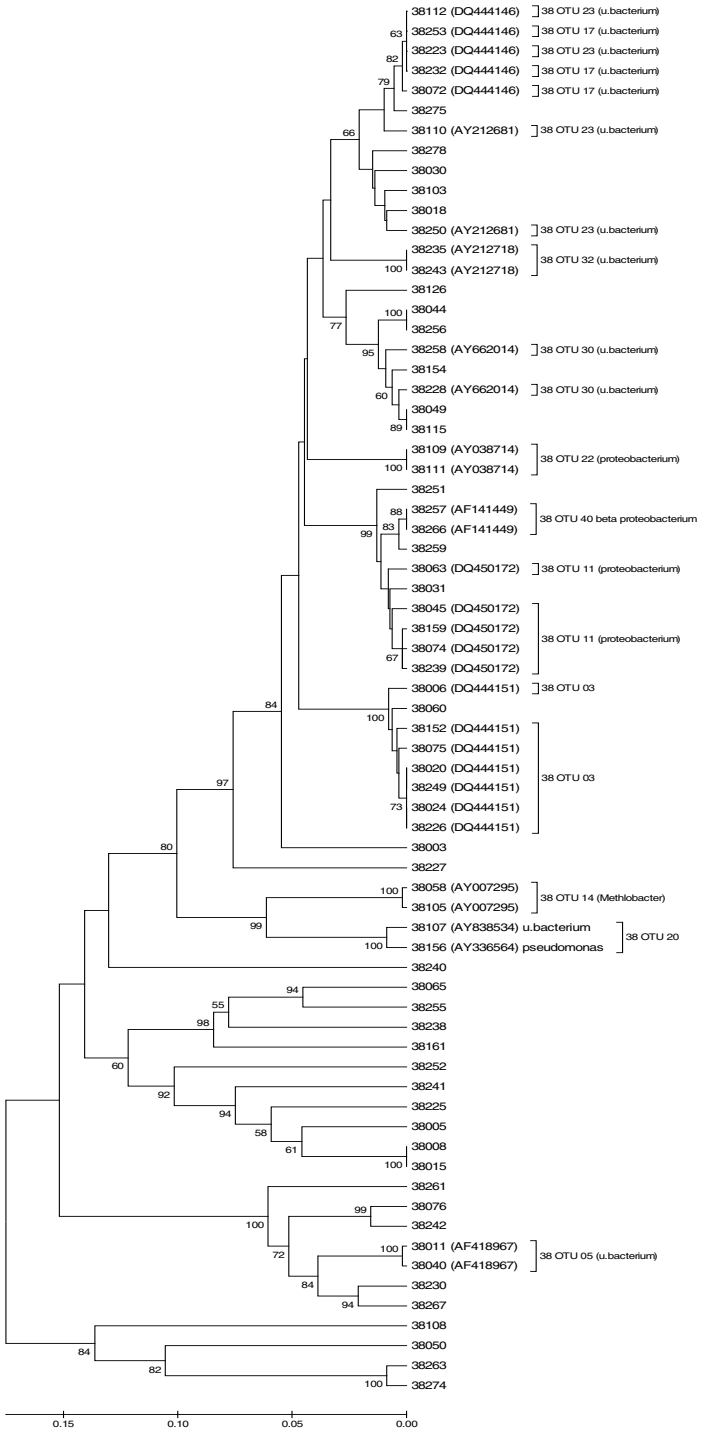


Figure 12: Bootstrap consensus tree for Library 1-1816 created using UPGMA Kimura 2-parameter with 500 replications. OTUs having two or more clones are grouped by brackets. GenBank Accession numbers are provided in parenthesis for OTUs with multiple clones. Bootstrap values below 50% are hidden.

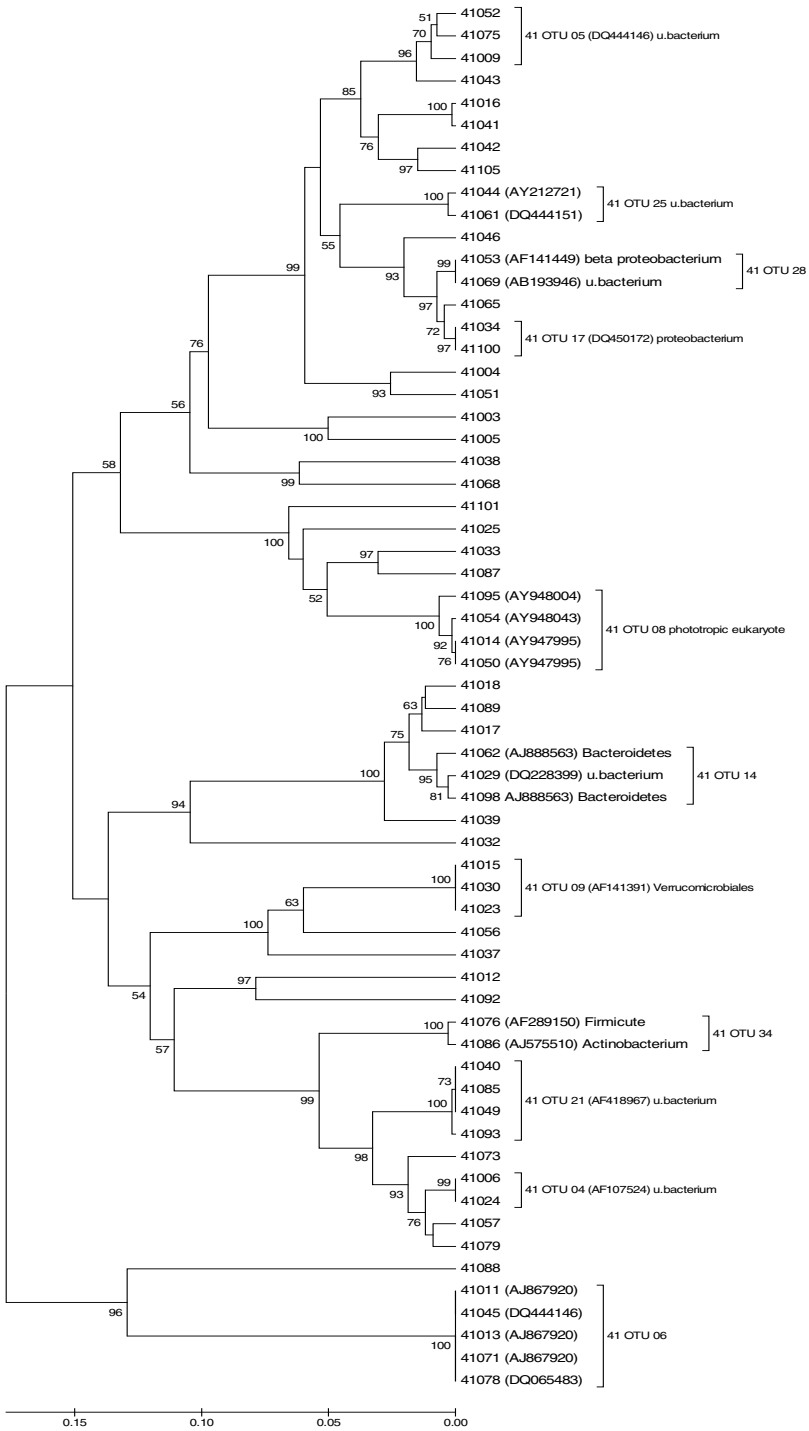


Figure 13: Bootstrap consensus tree for Library 1-0300 created using UPGMA Kimura 2-parameter with 500 replications. OTUs having two or more clones are grouped by brackets. GenBank Accession numbers are provided in parenthesis for OTUs with multiple clones. Bootstrap values below 50% are hidden.

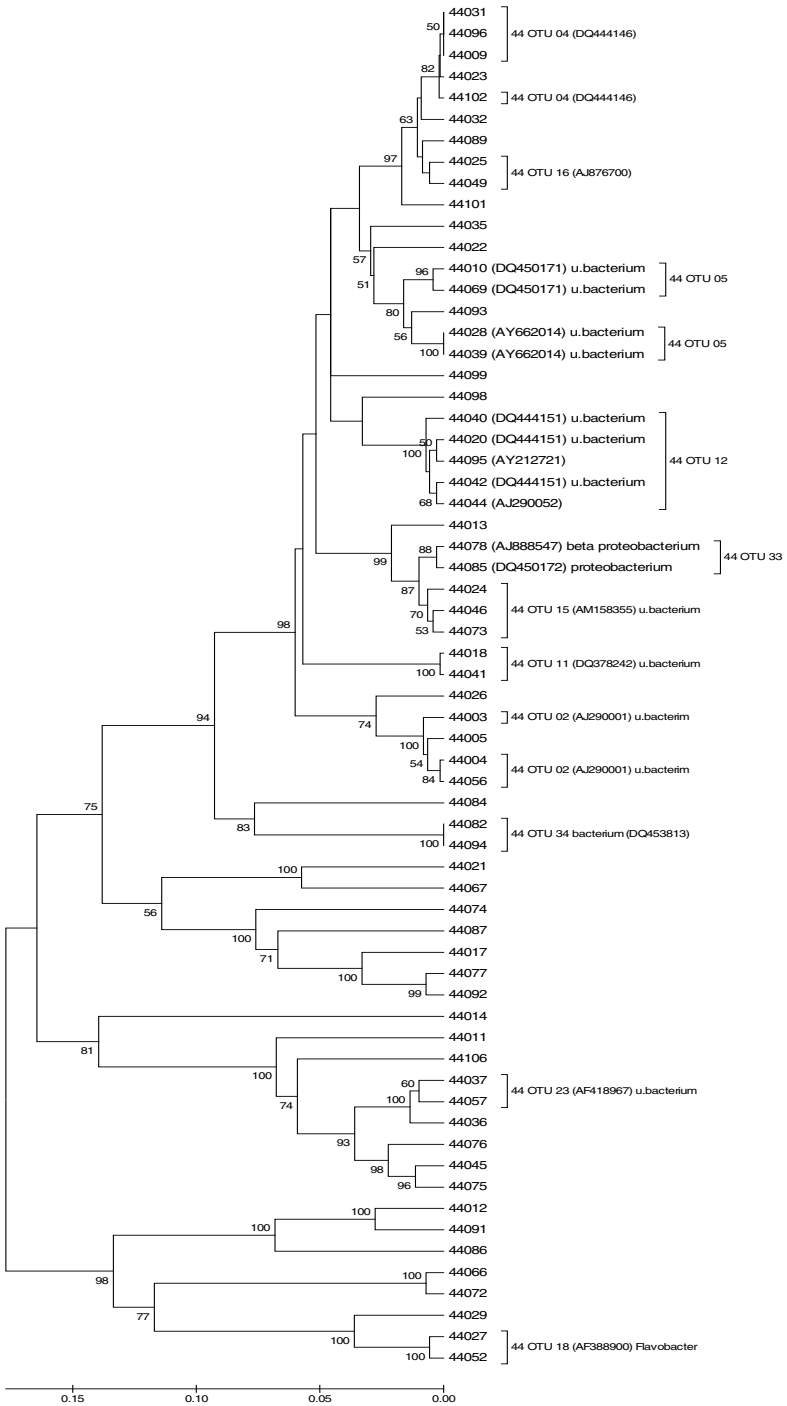


Figure 14: Bootstrap consensus tree for Library 1-0617 created using UPGMA Kimura 2-parameter with 500 replications. OTUs having two or more clones are grouped by brackets. GenBank Accession numbers are provided in parenthesis for OTUs with multiple clones. Bootstrap values below 50% are hidden.

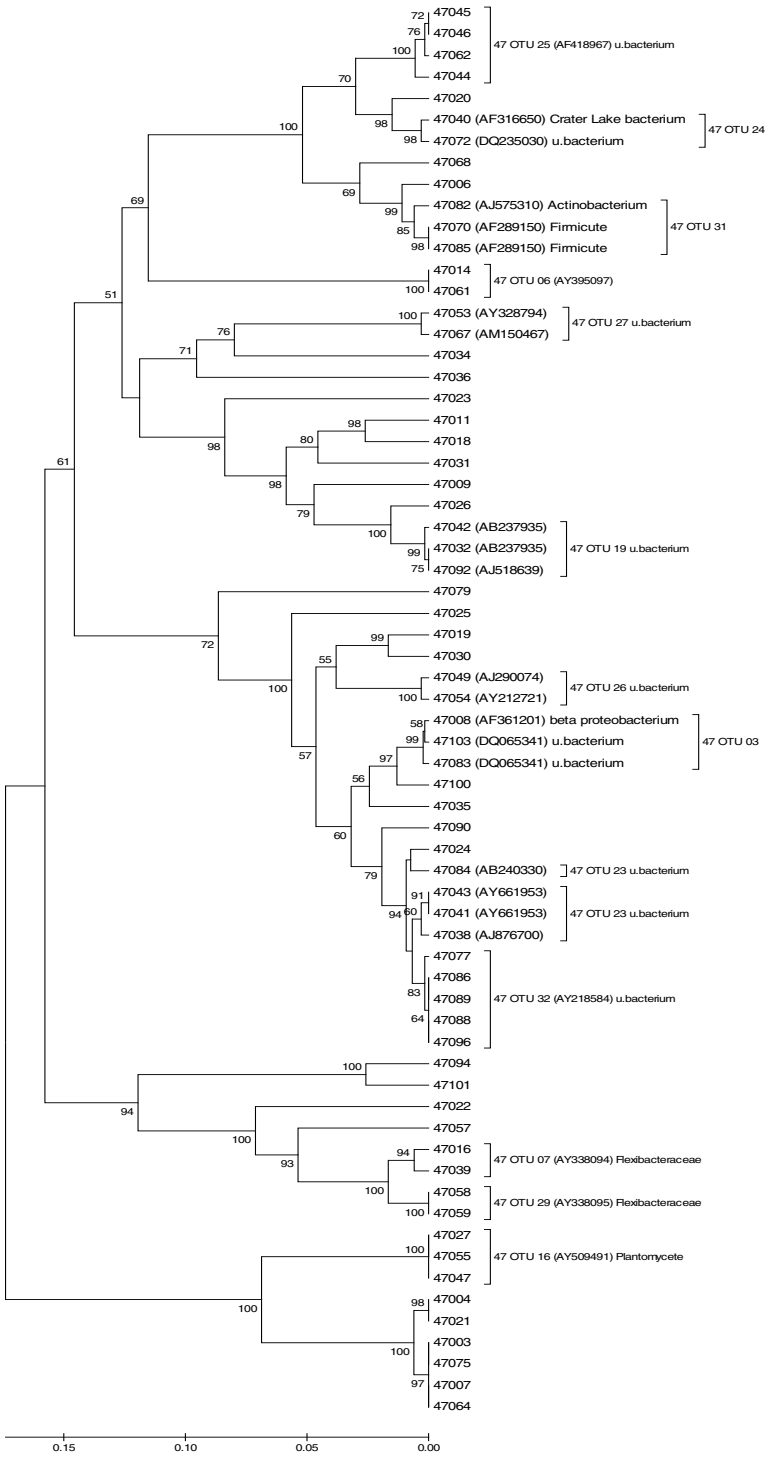


Figure 15: Bootstrap consensus tree for Library 1-1200 created using UPGMA Kimura 2-parameter with 500 replications. OTUs having two or more clones are grouped by brackets. GenBank Accession numbers are provided in parenthesis for OTUs with multiple clones. Bootstrap values below 50% are hidden.

CHAPTER IV

DISCUSSION

The identification of clones affiliated with Proteobacterium, Actinobacterium, and Crater Lake bacterium among others are consistent with Glöckner *et al.,* (2000), Burkert *et al.* (2003) and Amos (2002). Glöckner *et al.,* (2000) and Burkert *et al.*, (2003) found that Proteobacterium and Actinobacterium composed the most abundant and second most abundant putatively identified organisms in their studies. Amos (2003) and Burkert *et al.*, (2003) found Crater Lake bacterium, but in reduced numbers likely caused by elevated water temperatures. Shannon-Wiener and Simpson indices suggested that the City Lake clone libraries were well sampled overall. Generally, Sorenson similarity coefficients demonstrated considerable overlap among libraries, but less than the expected overlap for the replicate libraries. Replicate libraries demonstrated more variability than expected. This may be explained by the elimination of poor quality clones that may have represented the only incidence of prokaryotes that would not otherwise be documented in a given library. Another cause may have been an overestimation of the number of OTUs as a result of multiple single nucleotide polymorphisms within some clones.

The data reported in this study show patterns similar to Balser (2003). Representative 16S rDNA libraries sampled from City Lake on the basis of replicate, spatial and temporal scales reveal an expected distribution of representative OTUs

(Marshall, 2002; Amos, 2002; Balser, 2003). 16S rDNA library Shannon-Wiener values plot in a manner similar to Balser's (2003) 18S rDNA libraries (Fig 16).

Libraries 2 and 3 share the greatest number of OTUs as revealed by a similarity coefficient of 0.62. This suggests that over half of the OTUs identified in each of these libraries were found in both libraries. Similarity coefficients for 16S rDNA clone libraries in this study ranged from 0.08 to 0.62. The overall mean similarity coefficient was 0.35, which suggest good overlap for libraries of this size. Larger libraries would have yielded larger similarity coefficients.

Library 5 was problematic. Low diversity and one OTU with 43 members might be the result of sampling organic matter or floating debris present in the lake at the time of sampling. The presence of allochthonous materials might explain the large number of clones in a single OTU (Horner-Devine *et al.*, 2003). Alternatively unusually low

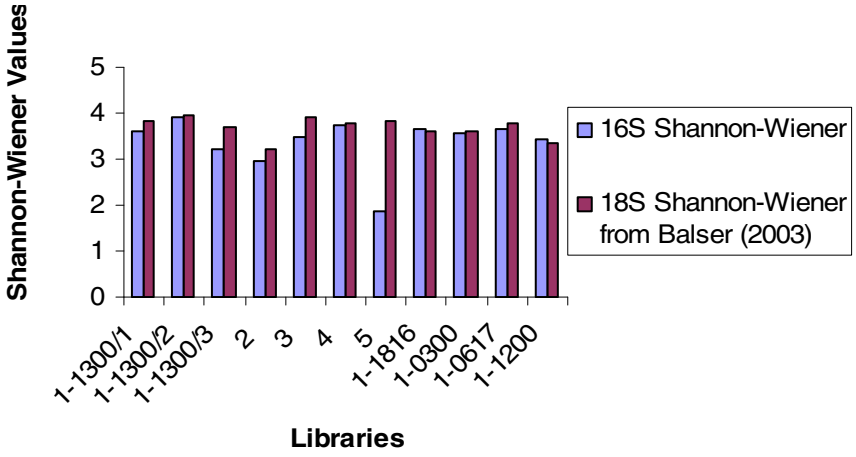


Figure 16: 18S and 16S Shannon-Wiener Values derived from City Lake clones

diversity may be due to preferential amplification, PCR artifacts, or heteroduplexes (Wang and Wang, 1997). Finally, it is possible that this sample reflects a strong influence of inflowing tributary water, though this seems unlikely..

Similarity coefficients among spatial samples do not demonstrate a discernible trend (Figure 17). Investigators have found that bacterial diversity may vary as primary productivity changes in a system (Horner-Devine *et al.*, 2003; Torsvik *et al.*, 1998). This writing may explain the aberrant distribution of similarity coefficients as related to distance from sites. The presence of bubble aerators (Figure 2) may have impacted similarity coefficients and relative diversity distributions as related to sampling site distance. Yannarell and Triplett (2004) demonstrated that geographic obstacles within a single body of water can impact community composition by acting as a physical barrier.

For example the site of collection for Library 2 was separated by a tract of land from the site of collection for Library 4 (Figure 2). The similarity coefficient for Libraries 2 and 4 represent the lowest pairwise similarity scores of all samples. Spatial similarity coefficients also reveal that libraries 2 and 3 share the greatest number of OTUs overall samples. These results are possibly due to the relatively large OTU (12 clones) found in Library 2. Alternatively, sampling bias or molecular aberrations may have caused the unexpected occurrence of similarity coefficients observed in relation to sample site distance. It is also possible that the observed mix of similarity coefficients may have been due to a combination of the bubble aerators and sampling bias. Further study is needed to evaluate the relationship between similarity coefficients and sample distance.

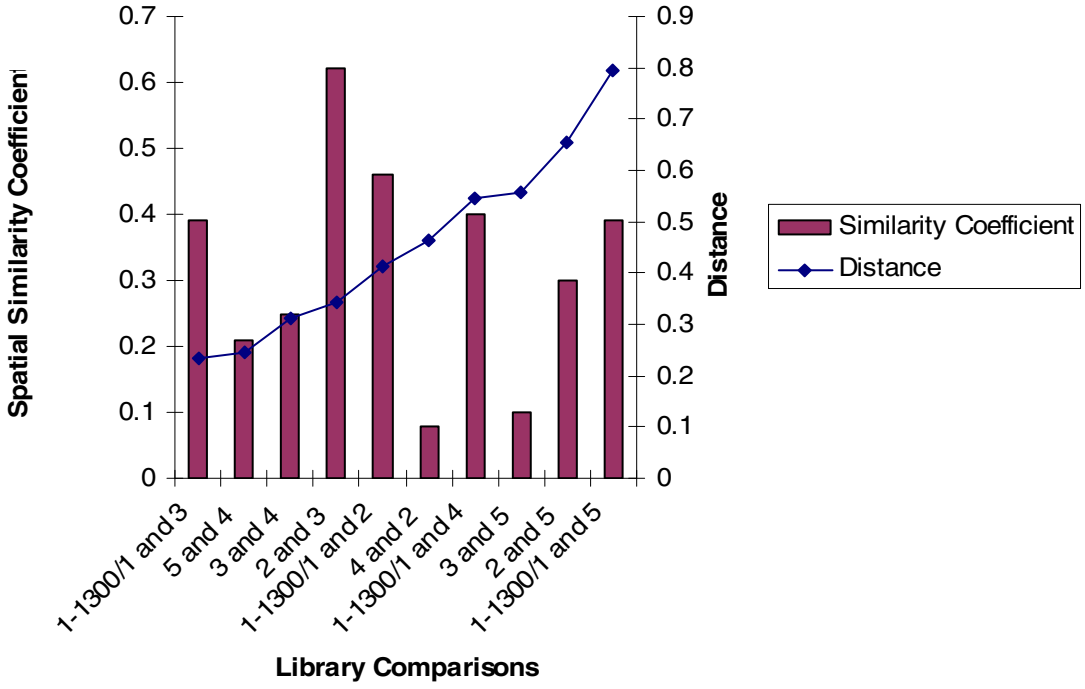


Figure 17: Similarity Coefficient versus Distance. The chart above shows spatial similarity coefficients with relation to distance from sampling site. Sites are arranged (left to right) from those separated by the least distance to those libraries separated by the greatest distance. For instance Libraries 1-1300/1 and 3 are the closest libraries, while Libraries 1-1300/1 and 5 are the most distant. No obvious pattern is observed.

Diurnal similarity coefficients revealed no obvious patterns. Libraries 1-0300 and 1-0617 were collected at 0300 and 0617 respectively on 19 December 2002 and shared the greatest number of OTUs for the diurnal samples. Sorenson coefficients indicate overlap, averaging 0.4654, and suggesting that the community may have been well sampled.

Coverage was calculated to evaluate how effectively the microbial communities were sampled (Ravenschlag *et al.*, 1999). Coverage values ranged from a high of 68% (Library 5) to a low of 18% (Library 1-1300/2). Balser (2003) found coverage values

ranging from 44% to 69% using the identical samples used in this study, but targeting 18S rDNA clone libraries. Amos (2002) reported coverage to be 58% for both prokaryotic and eukaryotic targets in City Lake. The values reported in this study are consistent with these reports

Rarefaction analysis for City Lake replicate libraries reveal weakly curvilinear plots, suggesting that libraries should have been larger to give a more accurate assessment of relative diversity. Replicate sampling effort approaching 100 OTUs appear to be adequate to begin approaching an asymptomatic maximum (Figure 19).

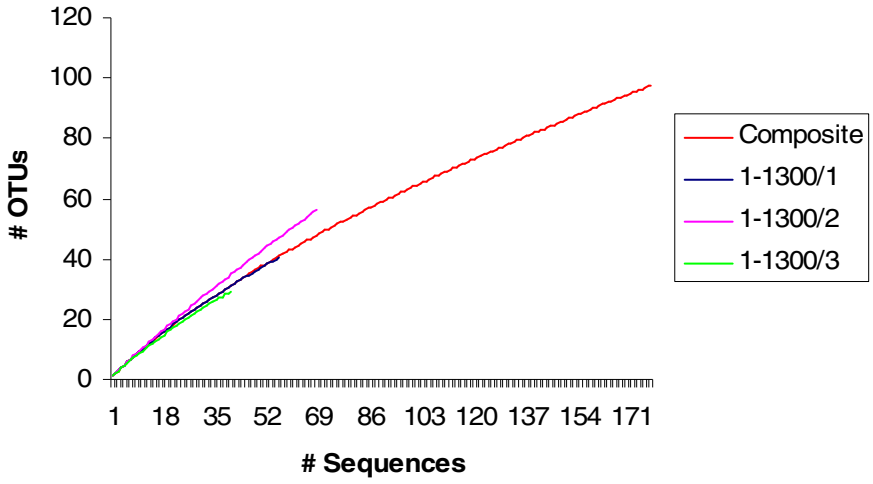


Figure 18: Rarefaction curves for individual replicates and a composite replicate sample. The curve suggests high diversity in the sample and it suggests more OTUs are needed to begin an approach to its asymptote. The expected number of OTUs is plotted versus the number of clone sequences.

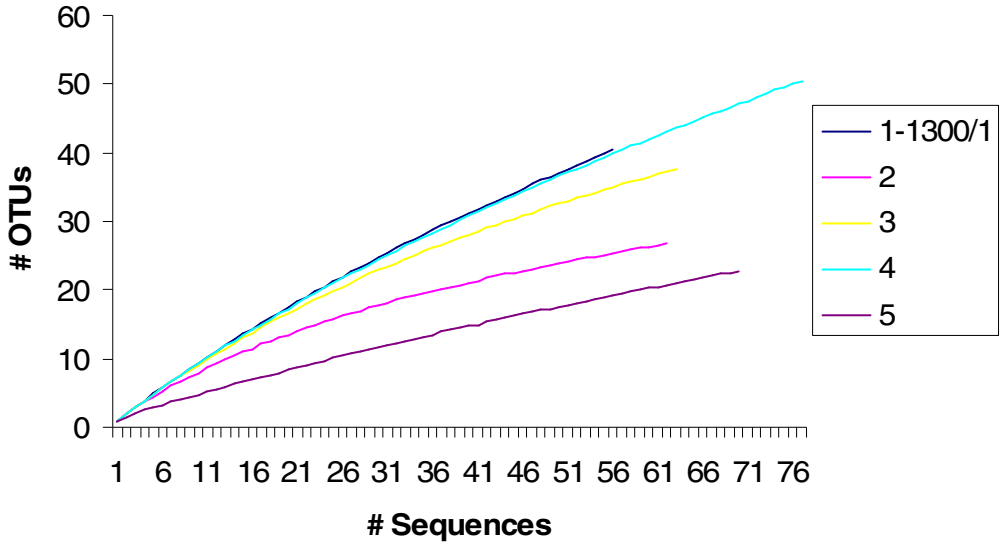


Figure 19: Rarefaction curves for spatial samples taken along a transect. The expected number of OTUs is plotted versus the number of clone sequences.

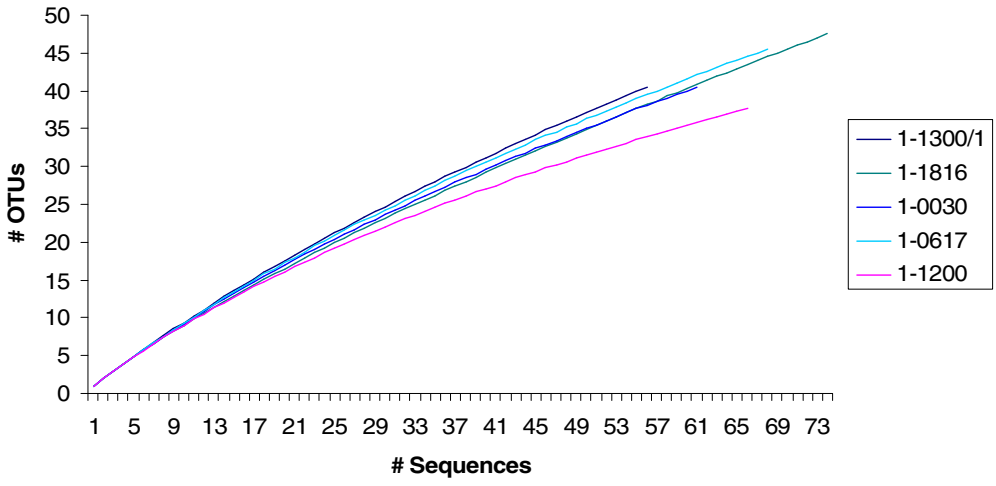


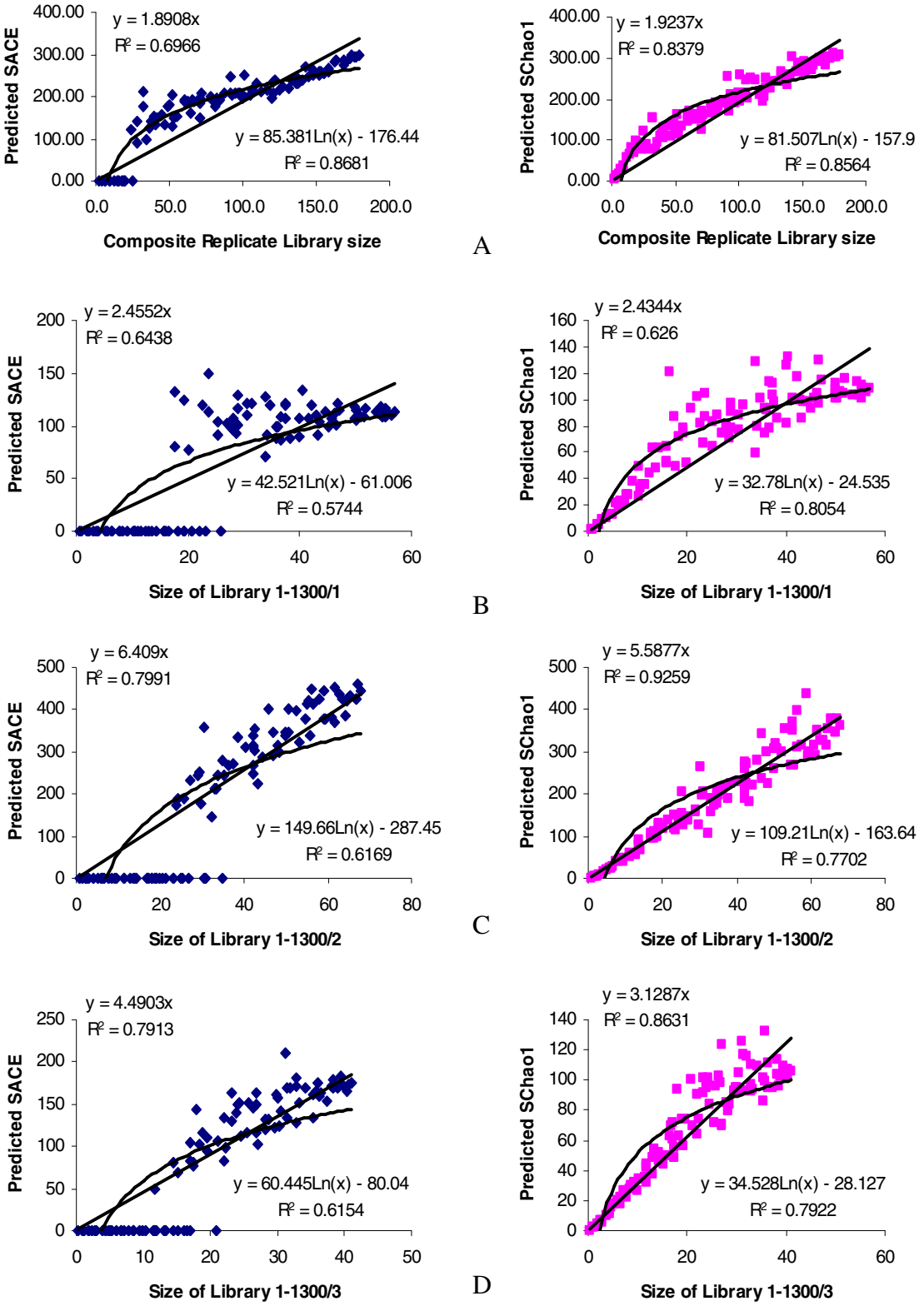
Figure 20: Rarefaction curves for diurnal samples. These data demonstrate the tightest clustering suggesting good replication. The expected number of OTUs is plotted versus the number of clone sequences.

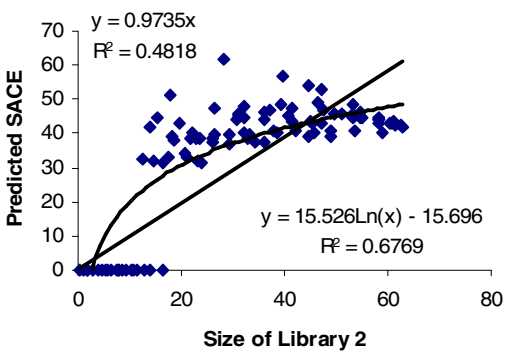
Weakly curvilinear plots are evident (Figures 19 – 20), indicating that OTU richness may have been underestimated (Kemp and Aller, 2004a). The plot for Library 2 (Figure 19) shows the most curvilinear plot, suggesting that the number of observed OTUs necessary for stable estimates was approximately thirty OTUs. Plots 4 and 1-1816 appear to be weakly curvilinear and suggest that more than 45 OTUs would be necessary to achieve adequate sampling of the community. In contrast, a strongly curvilinear plot would have suggested that diversity was exhaustively sampled (Kemp and Aller, 2004a).

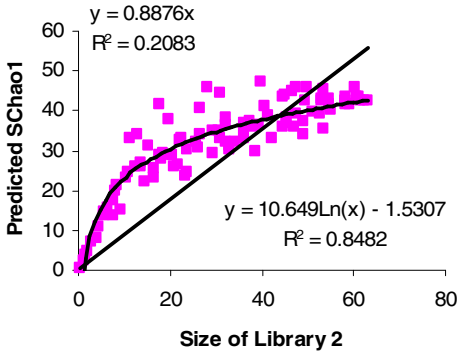
The composite replicate curves for S_{CHAO1} and S_{ACE} (Figure 21), predict that approximately 270 OTUs ,approximately 486 clones, would be needed for our library to be large enough to achieve maximum diversity coverage. Trend lines were calculated using least squares fit through subset points by using y = clnx + b, where c and b are constants and y = mx + b where b is a constant and m is the slope. Subset points were generated by using the average of 10 replicate subsets with replacement (Kemp and Aller, 2004a). The linear trend line was calculated to allow the evaluator to compare linear versus logarithmic fit. R squared values for both linear and logarithmic equations were included to allow a determination of the best fit (Cao *et al.*, 2002).

Near stable estimates of OTU richness were reached in 4 of the 11 S_{CHAO1} and S_{ACE} runs (Fig 21 C, E, F, L). There were 5 cases where S_{CHAO1} and S_{ACE} did not agree regarding the stability of OTU estimates (Figure 21 G, H, I, J, K). In these cases, S_{CHOU1} indicated an approach to an asymptote. Estimates for libraries 1-1300/2 and 1-1300/3 did not reach a near stable estimate or approach an asymptote for the predicted number of OTUs. Considering that the composite replicate curve suggests a near stable estimate,

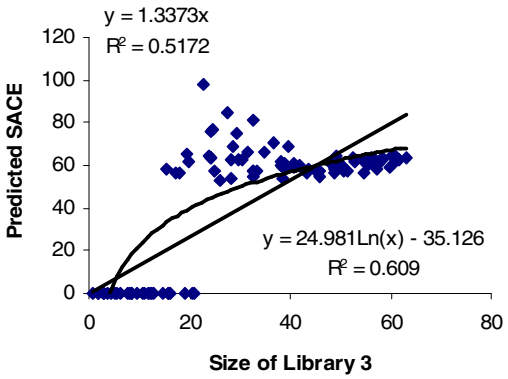
this likely means that libraries 1-1300/2 and 1-1300/3 were not large enough individually (Kemp and Aller, 2004a).

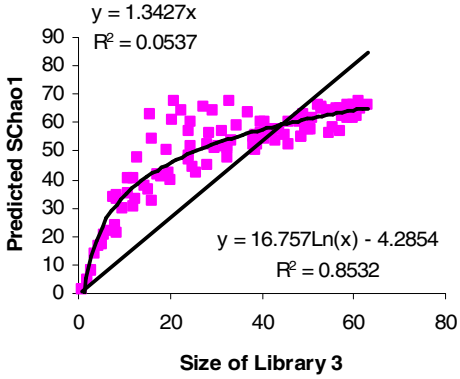

y = 1.8908x
R² = 0.6966
y = 85.381Ln(x) - 176.44
R² = 0.8681
Predicted SACE
Composite Replicate Library size
A
y = 1.9237x
R² = 0.8379
y = 81.507Ln(x) - 157.9
R² = 0.8564
Predicted SChao1
Composite Replicate Library size
y = 2.4552x
R² = 0.6438
y = 42.521Ln(x) - 61.006
R² = 0.5744
Predicted SACE
Size of Library 1-1300/1
B
y = 2.4344x
R² = 0.626
y = 32.78Ln(x) - 24.535
R² = 0.8054
Predicted SChao1
Size of Library 1-1300/1
y = 6.409x
R² = 0.7991
y = 149.66Ln(x) - 287.45
R² = 0.6169
Predicted SACE
Size of Library 1-1300/2
C
y = 5.5877x
R² = 0.9259
y = 109.21Ln(x) - 163.64
R² = 0.7702
Predicted SChao1
Size of Library 1-1300/2
y = 4.4903x
R² = 0.7913
y = 60.445Ln(x) - 80.04
R² = 0.6154
Predicted SACE
Size of Library 1-1300/3
D
y = 3.1287x
R² = 0.8631
y = 34.528Ln(x) - 28.127
R² = 0.7922
Predicted SChao1
Size of Library 1-1300/3


y = 0.9735x
R² = 0.4818
y = 15.526Ln(x) - 15.696
R² = 0.6769
Predicted SACE
Size of Library 2

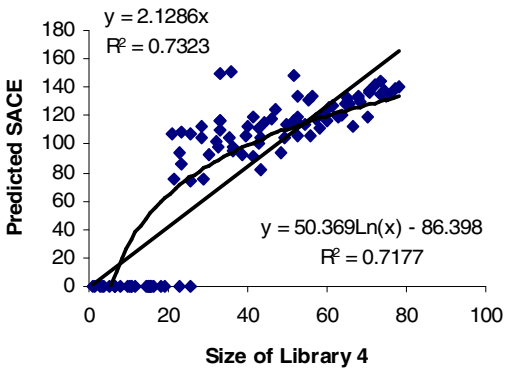

y = 0.8876x
R² = 0.2083
y = 10.649Ln(x) - 1.5307
R² = 0.8482
Predicted SChao1
Size of Library 2

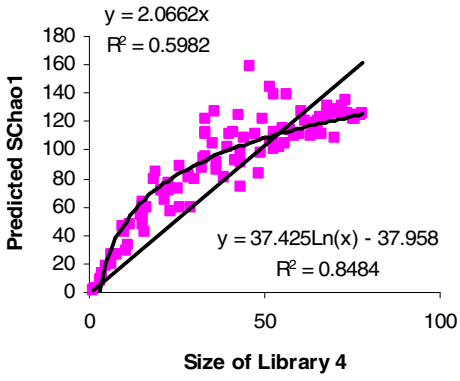
E


y = 1.3373x
R² = 0.5172
y = 24.981Ln(x) - 35.126
R² = 0.609
Predicted SACE
Size of Library 3

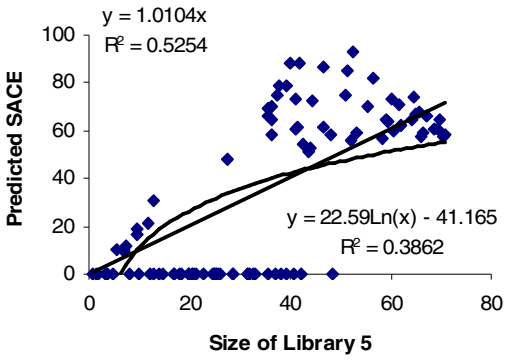

y = 1.3427x
R² = 0.0537
y = 16.757Ln(x) - 4.2854
R² = 0.8532
Predicted SChao1
Size of Library 3

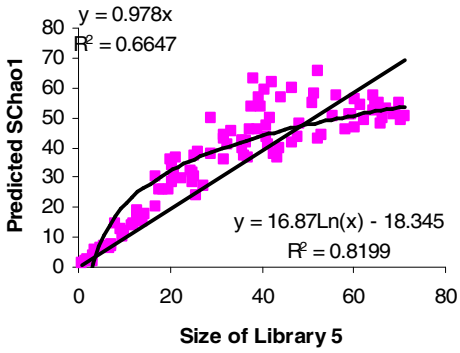
F


y = 2.1286x
R² = 0.7323
y = 50.369Ln(x) - 86.398
R² = 0.7177
Predicted SACE
Size of Library 4


y = 2.0662x
R² = 0.5982
y = 37.425Ln(x) - 37.958
R² = 0.8484
Predicted SChao1
Size of Library 4

G


y = 1.0104x
R² = 0.5254
y = 22.59Ln(x) - 41.165
R² = 0.3862
Predicted SACE
Size of Library 5


y = 0.978x
R² = 0.6647
y = 16.87Ln(x) - 18.345
R² = 0.8199
Predicted SChao1
Size of Library 5

H

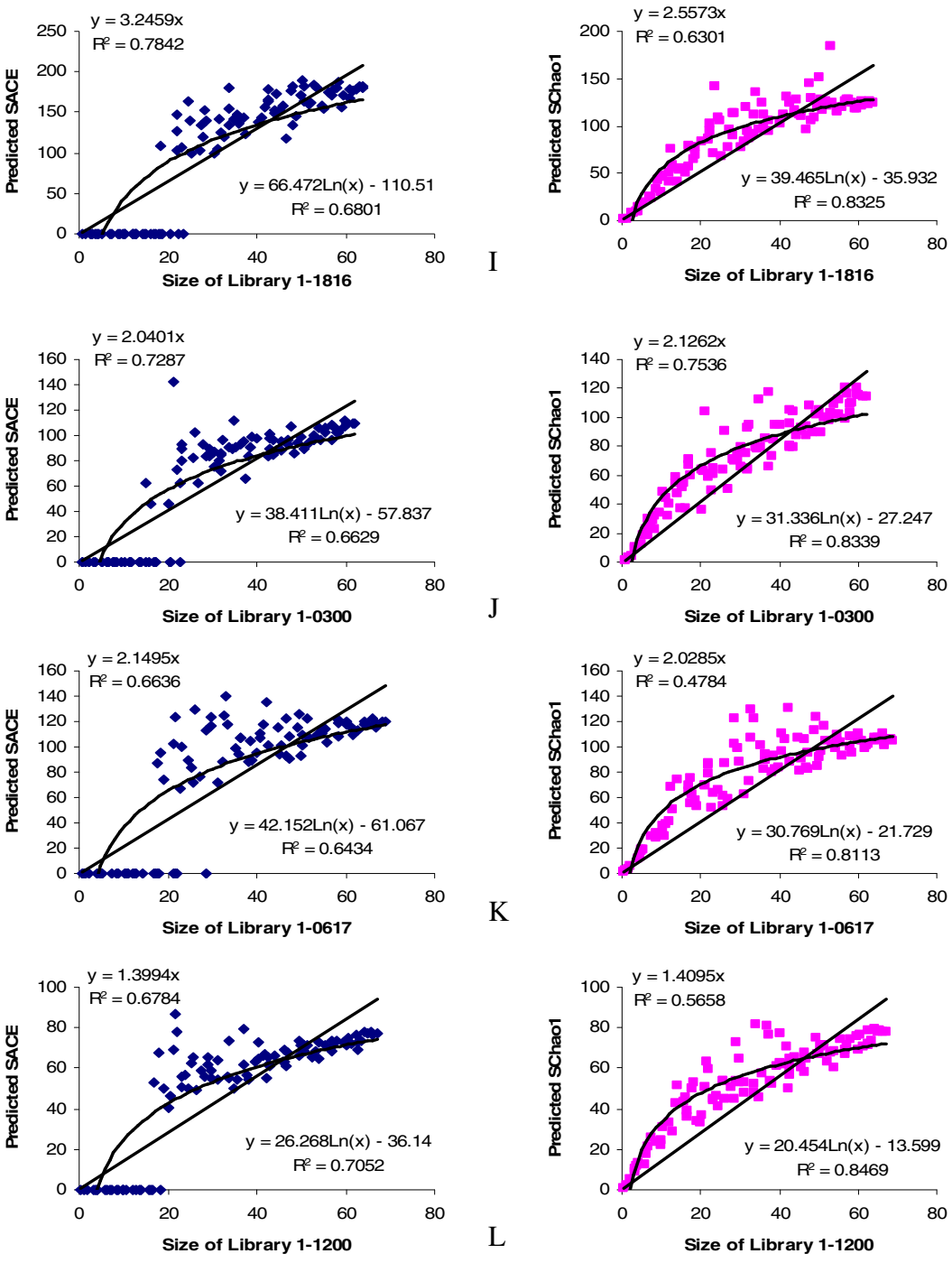


Figure 21(A-L): Predicted number of OTUs based on S_{CHAO1} and S_{ACE} estimators compared to library size. Each point is the average of 10 replica subsets. Undefined S_{ACE} values are shown as zero (Kemp and Aller, 2004a).

As reported earlier, library comparisons were made using the Sorenson similarity coefficient, values ranged from 0.0833 to 0.6222. While OTUs suggest a grouping for target genes, these are not definitive. Studies have revealed that seemingly identical genes can be found in distantly related organisms. Kemp and Aller (2005) recognized that OTU classification criteria have an impact on the number of OTUs observed. OTU overlap made definitive OTU grouping difficult. In some cases, a single clone was found in two different OTUs. For example, clone 4A100 was found in two OTUs. This and similar cases were resolved by adding the sequence to the OTU with the highest corresponding similarity score.

Another difficulty in OTU classification is the potential over estimation of OTUs. Researchers have documented that high similarity ($\geq 97.5\%$) does not indicate species identification, rather a functional similarity (Osorio et al., 2005; Drancourt *et al.*, 2000; Cilia *et al.*, 1996). For example, in Library 1-1300/2, 56 OTUs were identified. After conducting a BLASTn search for each of the sequences in Library 1-1300/2, 6 previously identified OTUs were found to the match the same GenBank entry with 98% identity or greater, suggesting an overestimation of OTUs for this library. In another instance, the dendrogram for Library 1-1300/1 revealed clones determined to be a single OTU were separated from their cluster of sequences by a cluster of different OTUs. For example 5AOTU 40 (Fig 5) is divided from its cluster members by 5AOTU 16. Specifically, clone 5A109 was 97.3% identical to clone 5A064 and 96.9% identical to clone 5A018: 5A109 nearly met the criteria to be included in 5AOTU 16, but instead qualified for inclusion in 5AOTU 40. The depiction found in the dendrogram for Library 1-1300/1

provides a more objective relationship of these clones to one another and as a result 5AOTU 40 appears to be separated by 5AOTU 16. Cilia *et al.,* (1996) encountered a similar problem differentiating a strain of *Escherichia* from a clade of *Shingella,* and determined that the lack of data point differences did not provide enough information to effectively build a representative dendrogram.

Attempts were made to minimize bias by treating all samples similarly. Extraction of genomic DNA and PCR run parameters were consistent for all libraries. Sources of bias outside of direct control may have included variation in the number of alleles present, G+C content, secondary structure, and the presence of chimeric templates (Kroes *et al.*, 1999). Preferential amplification of 16S rDNA templates may have been another source of bias that could have affected observed community structure (Reysehbach *et al.* 1992). Sample collection is another area of concern. For example, debris in City Lake may have contributed to the results of Library 5. In addition to procedural causes of bias, researchers have suggested that public databases are replete with chimeric sequences that have been identified as novel OTUs despite awareness of the problem of chimera (Hugenholtz and Huber, 2003). While this is a concern, every effort to eliminate the presence of chimeric sequences was made. Studies have found that as the number of PCR cycles increase, the chances of chimeric formation increase. PCR cycles in this study were limited to 29 cycles. The presence of multiple rRNA genes from the same organism can also induce chimeric formation as well (Wang and Wang, 1997). There is no definitive way to assure detection of all chimeras, but there are

generally accepted tools (Ribosomal Database Project Chimera_Check and Bellerophon) that can detect chimeric sequences (Hugenholtz and Huber, 2003).

This study explored a portion of the existing community composition of High Point City Lake based on small temporal and spatial scales. The general hypothesis predicted that replicate samples would be the most similar, spatial samples taken from closer sampling sites would be more similar than those taken from more distant sites, and diurnal samples might reflect day-night differences. These hypotheses were not clearly supported by this data. The findings of this study suggest that the variability among the replicate libraries may be a reflection of the small library size. S_{CHAO1} and S_{ACE} predictors suggested larger libraries would be required to achieve a stable estimate of OTUs, but given the small size of the replicate libraries their similarity values were not bad.

Spatial samples studied did not overlap as much as expected, perhaps due to low coverage for these samples. Spatial samples did not demonstrate more similarity among closer libraries. However Library 5 had the most different community composition. Library 5 was collected from a shallow part of the lake without aerators. In addition this part of the lake may have been influenced by a stream. It is unclear if the different community composition truly represents differences due to geographic differences or if the differences were due to an unusually large OTU that doesn't reflect biological reality. Examination of Library 5 replicates would be required to determine if the difference observed was real or not.

The diurnal samples suggested little difference among samples. The similarity of the diurnal libraries may have been caused by cool weather and cloud cover, the presence of bubble aerators in the lake or a combination of both. The diurnal libraries with the greatest overlap were generated from samples collected with only 3 hours difference in collection time, as compared to 6 hours for the other diurnal samples.

The range of overlap among 16S rDNA libraries was greater than the range Balser (2003) found among 18S rDNA libraries; however, the mean 16S rDNA library similarity coefficient was 0.37, while Balser's (2003) 18S rDNA library similarity coefficient mean was 0.28. These values suggest that the 16S rDNA libraries were similar to one another with limited variability among libraries.

Clone sequences affiliated with Proteobacteria,, Actinobacterium and Crater Lake bacterium represented the most common cross referenced sequences in GenBank for this study. Crater Lake bacterium and *Zooglea ramgeria* were the only categories of organisms shared among this study and Amos (2002). This study found 12 occurrences of clones having ≥ 0.975 similarity to Crater Lake bacterium. Amos (2002) found two occurrences of clones having ≥ 0.975 similarity to Crater Lake bacterium. In contrast, this study putatively identified only one clone as *Zoogloea ramigera*, having only 0.96 similarity to its nearest GenBank match. Amos (2002) found eleven 16S rDNA clones with similarity ≥0.975 to *Zooglea ramigera*. The differences in the occurrence of the Crater Lake bacterium and *Zooglea ramigera* may be attributed to sample site selection or the difference may be attributed to differences in surface temperatures. The average air surface temperature on the day of sample collection for this study was 42ºC. Water

temperatures in City Lake were not reported in Amos (2002); however, surface air temperatures at City Lake on the day of sample collection was an average of 72ºC as reported by weatherunderground.com.

Studies focused on multiple genes would facilitate the development of a catalogue of 16S diversity. The criteria for determining the number of gene targets must be evaluated to allow broad community coverage, yet specific enough to determine differences for reliable taxonomic purposes. Studies by Balser (2003), Amos (2002), Marshal (2002), Lindstrom (2002), Glöckner *et al.* (2003) and Hiorns et al. (2003) suggest the presence of 16S rDNA of various phylotypes, some common across geographic and climactic scales. These studies suggest a ubiquitous set of organisms that may be present regardless of lake trophic status, perhaps present in differing abundance.

The difficulty of determining the identity of rare organisms is further hindered because most prokaryotic organisms are not culturable (Zengler et al. 2002). The use of microarray technology may be a near term solution to this obstacle. In addition, microarray technology may afford a practical monitoring tool for local utilities and water resource managers. Improved molecular, taxonomic, and biochemical methods combined with a better understanding of nutrient poor culture techniques will advance the microbial ecological understanding of microbial communities in aquatic environments.

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APPENDIX A: Categories of Organisms Affiliated with City Lake Clone Libraries

CATEGORY	1-1300/1	1-1300/2	1-1300/3	2	3	4	5	1-1816	1-0300	1-0617	1-1200
Actinobacterium		(3)	2	4	1	1 (2)	1	3 (1)	1	1 (2)	1 (1)
Aquamonas			1			2					
Aquatic bacterium		1									
Bacteroidetes								(1)	2	1	1
Burkholderiales				2	2	(1)		1			1
Crater Lake Bacterium	2	4 (1)	1		1		(1)	1	1 (2)	1 (1)	1
Cyanobacterium											2
Cytophagales		1					1	1	1		
Denitrifying bacterium									1		
Division TM6									(1)		
Feldmannia irregularis virus strain Firr V					1						
Firmicute		(1)							1		2
Flavobacterium							2			2 (1)	(1)
Flexibacteraceae											3 (1)
Fucus vesiculosus chloroplast								(1)			

CATEGORY	1-1300/1	1-1300/2	1-1300/3	2	3	4	5	1-1816	1-0300	1-0617	1-1200
Gemmatimonadetes		(1)									
Isochrysis			(1)								
Methlobacter			(1)		(3)						
Methylobacter psychrophilius								(2)		(1)	(1)
Microbacterlaceae		1									
Phototropic eukaryote	2 (4)	2					1	(1)		2	(1)
Plantomycete					1				(1)		(3)
Polaromonas						1			1		
Pseudomonas					3			1	2		
Rhodobacteraceae	1		1			1					
Rhodoferax ferrireducens	(2)	1	(2)	2	1			1			
Rhodophyte	(1)		(1)								
Sphingobacteriales									1	1	
Verrucomicrobiales					1				3	(1)	
Zooglea						(1)					
Alpha Proteobacterium	1		1		1(1)	(2)	(2)				

CATEGORY	**1-1300/1**	**1-1300/2**	**1-1300/3**	**2**	**3**	**4**	**5**	**1-1816**	**1-0300**	**1-0617**	**1-1200**
Beta proteobacterium	2 (2)	4 (3)		6	8	7	1	5 (3)	2	(2)	3 (1)
Delta Proteobacterium											(1)
Gamma Proteobacter	1	(1)									
Proteobacterium		1	4	4		2		2 (5)	1 (2)	3 (1)	
Chloroplast					1				4		
Emiliania Huxleyi Chloroplast	1			(1)			(1)				
NUMBER OF CATEGORIES PRESENT IN LIBRARY	**9**	**12**	**10**	**6**	**12**	**9**	**8**	**12**	**15**	**10**	**13**

APPENDIX B: Library 1-1300/1

OTU	City Lake Clone ID	City Lake Clone % Identity	GenBank/Blast	Accession #	%BLASTn Identity	# match bp	Total # bp
5A OTU 01	5a-2		Uncultured bacterium clone 005C-F09 small subunit ribosomal RNA	AY661953	99	420	421
5A OTU 01	5a-83	0.992	Uncultured bacterium partial 16S rRNA gene, clone R2_14	AJ876700	99	419	421
5A OTU 01	5a-112	0.985	Uncultured bacterium clone 234ds5 16S ribosomal RNA gene, partial	AY212681	99	419	421
5A OTU 01	5a-111	0.99	Uncultured bacterium partial 16S rRNA gene, clone R2_14	AJ876700	99	418	421
5A OTU 02	5a-3		Uncultured bacterium gene for 16S rRNA, partial sequence, clone 16S-AK-W-53	AB237961	99	421	422
5A OTU 02	5a-56	0.99	Uncultured bacterium gene for 16S rRNA, partial sequence, clone 16S-AK-W-53	AB237961	99	421	423
5A OTU 02	5a-72	0.99	Uncultured bacterium gene for 16S rRNA, partial sequence, clone 16S-AK-W-54	AB237961	99	419	421
5A OTU 03	5a-6		Rhodoferax ferrireducens DSM 15236, complete genome	CP000267 AAJK01000000 AAJK01000001-AAJK01000113	97	412	421
5A OTU 04	5a-8		Uncultured phototrophic eukaryote clone JL-WNPG-T36 16S ribosomal Uncultured phototrophic eukaryote clone JL-WNPG-T36 16S ribosomal	AY664132	0.95	205	214
5A OTU 05	5a-10		Uncultured bacterium clone KD2-45 16S ribosomal RNA gene, partial	AY218584	97	414	423
5A OTU 05	5a-42	0.988	Uncultured bacterium clone KD2-45 16S ribosomal RNA gene, partial	AY218584	97	413	422

5A OTU 06	5a-11		Uncultured bacterium partial 16S rRNA gene, clone A1-94	AM180055	91	358	393
5A OTU 07	5a-13		Uncultured bacterium FukuN57 16S rRNA gene	AJ290000	97	406	
5A OTU 08	5a-14		Uncultured phototrophic eukaryote clone PRD18F11 16S ribosomal RNA gene, partial sequence; chloroplast	AY948053	97	391	401
5A OTU 09	5a-9		Uncultured phototrophic eukaryote clone JL-WNPG-T36 16S ribosomal RNA gene, partial sequence; chloroplast	AY664132	96	208	215
5A OTU 10	5a-21		Rhodoferax ferrireducens DSM 15236, complete genome	CP000267 AAJK01000000 AAJK01000001-AAJK01000113	95	414	421
5A OTU 11	5a-23		Uncultured bacterium clone C02R 16S ribosomal RNA gene, partial	AY395116	95	396	416
5A OTU 12	5a-24		Uncultured bacterium gene for 16S rRNA, partial sequence, clone: SRRB38	AB240512	99	420	421
5A OTU 12	5a-25	0.99	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: SRRB39	AB240512	99	419	421
5A OTU 13	5a-28		Uncultured beta proteobacterium clone CRE-FL22 16S ribosomal	AF141449	98	395	40
5A OTU 14	5a-31		Uncultured bacterium partial 16S rRNA gene, clone T119D	AM158397	98	415	420
5A OTU 15	5a-12		Uncultured bacterium FukuS36 partial 16S rRNA gene	AJ290074	100	421	421
5A OTU 15	5a-84	1	Uncultured bacterium FukuS36 partial 16S rRNA gene	AJ290074	100	421	421
5A OTU 16	5a-18		Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	98	415	420

5A OTU 16	5a-64	0.976	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	98	415	422
5A OTU 16	5a-99	0.983	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	99	419	421
5A OTU 17	5a-20		Uncultured bacterium clone KD2-45 16S ribosomal RNA gene, partial	AY218584	95	404	422
5A OTU 18	5a-22		Uncultured phototrophic eukaryote clone PRD18E02 16S ribosomal RNA gene, partial sequence; chloroplast	AY948033	99	399	401
5A OTU 19	5a-33		Uncultured Crater Lake bacterium CL0-64 16S ribosomal RNA gene	AF316650	99	418	422
5A OTU 19	5a-100	0.995	Uncultured Crater Lake bacterium CL0-64 16S ribosomal RNA gene	AF316650	99	419	421
5A OTU 20	5a-34		Uncultured bacterium partial 16S rRNA gene, clone c5LKS47	AM086132	100	401	401
5A OTU 21	5a-35		Uncultured freshwater bacterium clone 965018H09.x1 16S ribosomal	DQ065028	98	414	422
5A OTU 22	5a-36		Uncultured freshwater bacterium clone 965015B06.x1 16S ribosomal	DQ064823	99	417	419
5A OTU 22	5a-71	0.983	Uncultured freshwater bacterium clone 965004A02.x1 16S ribosomal	DQ065341	99	417	421
5A OTU 23	5a-38		Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	95	400	421
5A OTU 24	5a-39		Uncultured Rhodobacteraceae bacterium clone M13-52 16S ribosomal	AY338109	99	400	401
5A OTU 25	5a-40		Uncultured alpha proteobacterium clone IRD18D07 16S ribosomal	AY947933	99	399	401
5A OTU 26	5a-43		Uncultured gamma proteobacterium clone CRE-PA74 16S ribosomal	AF141542	98	317	322
5A OTU 27	5a-70		Uncultured bacterium FukuN9 16S rRNA gene	AJ290009	98	415	421

5A OTU 28	5a-79		Uncultured beta proteobacterium clone AKYG1724 16S ribosomal	AY921977	96	408	421
5A OTU 28	5a-94	0.995	Uncultured freshwater bacterium clone 965002G05.x1 16S ribosomal	DQ065278	96	410	423
5A OTU 28	5a-103	0.993	Uncultured freshwater bacterium clone 965002G05.x1 16S ribosomal	DQ065278	97	411	423
5A OTU 28	5a-107	0.992	Uncultured freshwater bacterium clone 965002G05.x1 16S ribosomal	DQ065278	96	410	423
5A OTU 29	5a-73		Uncultured bacterium gene for 16S rRNA, partial sequence, clone 16S-AK-W-53	AB237920	97	407	416
5A OTU 30	5a-110		bacterium partial 16S rRNA gene, clone C8	AJ867920	99	396	399
5A OTU 31	5a-77		Uncultured phototrophic eukaryote clone PRD18E12 16S ribosomal RNA gene, partial sequence; chloroplast	AY948043	97	387	395
5A OTU 32	5a-80		Unidentified haptophyte OM153 16S ribosomal RNA gene, partial	U70720	96	407	421
5A OTU 32	5a-92	0.995	Unidentified haptophyte OM153 16S ribosomal RNA gene, partial	U70720	96	407	420
5A OTU 33	5a-78		Uncultured bacterium partial 16S rRNA gene, clone c1LKS42	AM086084	98	395	403
5A OTU 34	5a-87		Uncultured beta proteobacterium clone 08 16S ribosomal RNA gene	AF361201	98	416	421
5A OTU 35	5a-82		Uncultured beta proteobacterium clone nsc122 16S ribosomal RNA	DQ211472	95	333	348
5A OTU 36	5a-85		Uncultured freshwater bacterium clone 965017H08.y1 16S ribosomal	DQ064985	99	419	421
5A OTU 37	5a-86		Unidentified rhodophyte PRD01a010B 16S ribosomal RNA gene, partial sequence	AF289158	95	401	418
5A OTU 38	5a-105		Emiliania huxleyi strain CCMP 373 chloroplast, complete genome	AY741371	95	402	421

5A OTU 39	5a-106		Uncultured phototrophic eukaryote clone JL-WNPG-T36 16S ribosomal RNA gene, partial sequence; chloroplast	AY664132	97	192	196
5A OTU 40	5a-99		Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	99	419	421
5A OTU 40	5a-109	0.981	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	99	415	422
5A OTU 41	5a-104		Uncultured bacterium partial 16S rRNA gene, clone c5LKS24	AM086117	96	389	404

APPENDIX C: Library 1-1300/2

OTU	City Lake Clone ID	City Lake Clone % Identity	GenBank/Blast ID	Accession #	Identity	# match bp	Total # bp
4A OTU 01	4a-001		Uncultured bacterium clone KD2-45 16S ribosomal RNA gene, partial; Ardley Island Antarctica; AY218584	AY218584	97	425	434
4A OTU 01	4a-031	0.99	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	428	433
4A OTU 01	4a-039	0.99	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	428	434
4A OTU 01	4a-053	0.993	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	429	434
4A OTU 01	4a-076	0.993	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	428	434
4A OTU 01	4a-083	0.99	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	428	434
4A OTU 02	4a-002		Uncultured bacterium clone 005C-F09 small subunit ribosomal RNA; groundwater contaminated with high levels of nitric acid-bearing uranium waste	AY661953	100	432	432
4A OTU 02	4a-100		Uncultured bacterium clone 40ds5 16S ribosomal RNA gene, partial	AY212735	99	430	432
4A OTU 03	4a-003		Uncultured beta proteobacterium clone AKYG1724 16S ribosomal gene, partial sequence	AY921977	96	416	431
4A OTU 04	4a-023		Uncultured freshwater bacterium clone 965002G05.x1 16S ribosomal	DQ065278	93	380	407

4A OTU 05	4a-004		Uncultured bacterium partial 16S rRNA gene, clone S306R	AM158355	95	249	260
4A OTU 06	4a-019		Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	93	392	420
4A OTU 07	4a-007		Uncultured Crater Lake bacterium CL0-6 16S ribosomal RNA gene	AF316682	99	429	432
4A OTU 08	4a-005		Uncultured bacterium clone mol25Chloro 16S ribosomal RNA gene	AY775462	98	404	412
4A OTU 09	4a-021		Uncultured Cytophagales clone PRD01b007B 16S ribosomal RNA gene, partial sequence	AF289167	98	425	430
4A OTU 10	4a-111		Uncultured bacterium clone DS3-56 16S ribosomal RNA gene, partial	DQ463235	97	423	432
4A OTU 11	4a-017		Uncultured freshwater bacterium clone 965004G06.y1 16S ribosomal	DQ065403	93	289	308
4A OTU 12	4a-008		Uncultured phototrophic eukaryote clone PRD18E12 16S ribosomal RNA gene, partial sequence; chloroplast	AY948043	100	412	412
4A OTU 13	4a-010		Uncultured bacterium clone HT2E3 16S ribosomal RNA gene, partial	AF418967	99	430	431
4A OTU 13	4a-013	0.99	Uncultured bacterium clone HT2E3 16S ribosomal RNA gene, partial	AF418967	99	431	432
4A OTU 13	4a-074	0.993	Uncultured bacterium clone HT2E3 16S ribosomal RNA gene, partial	AF418967	100	432	432
4A OTU 14	4a-014		Uncultured bacterium clone HT2E3 16S ribosomal RNA gene, partial sequence	AF418967	97	422	432
4A OTU 15	4a-024		Uncultured Crater Lake bacterium CL0-64 16S ribosomal RNA gene	AF316650	99	427	431
4A OTU 15	4a-072	0.988	Uncultured Crater Lake bacterium CL0-64 16S ribosomal RNA gene	AF316650	99	429	432

4A OTU 15	4a-091	0.99	Uncultured Crater Lake bacterium CL0-64 16S ribosomal RNA gene	AF316650	99	430	432
4A OTU 16	4a-018		Uncultured Actinobacterium partial 16S rRNA gene, clone R5.	AJ575501	97	283	290
4A OTU 17	4a-042		Uncultured clone IRD18A09 16S ribosomal RNA gene, partial seq	AY947900	97	402	412
4A OTU 18	4a-065		Uncultured Microbacteriaceae bacterium clone M13-99 16S ribosomal RNA gene, partial sequence	AY337957	98	407	412
4A OTU 19	4a-054		Uncultured phototrophic eukaryote clone PRD18F11 16S ribosomal RNA gene, partial sequence; chloroplast	AY948053	94	387	410
4A OTU 20	4a-044		Uncultured Gemmatimonadetes bacterium clone AKYG753 16S ribosomal	AY922060	97	421	431
4A OTU 21	4a-068		Uncultured proteobacterium clone R7C24 16S ribosomal RNA gene, partial sequence	DQ450180	96	419	434
4A OTU 22	4a-060		Unidentified haptophyte OM125 16S ribosomal RNA gene, partial	U70719	95	413	432
4A OTU 23	4a-082		Uncultured Crater Lake bacterium CL120-48 16S ribosomal RNA gene	AF316699	93	399	425
4A OTU 24	4a-095		Uncultured phototrophic eukaryote clone PRD18E02 16S ribosomal RNA gene, partial sequence; chloroplast	AY948033	99	410	413
4A OTU 25	4a-077		Uncultured bacterium clone ML-5-8 16S ribosomal RNA gene, partial sequence	DQ234995	99	428	432
4A OTU 26	4a-090		Uncultured Actinobacterium partial 16S rRNA gene, clone R2	AJ575498	99	431	432

4A OTU 27	4a-099		Uncultured bacterium gene for 16S rRNA, partial sequence, clone 16S-AK-W-53	AB237961	96	398	411
4A OTU 28	4a-113		Uncultured Firmicute clone PRD01a002B 16S ribosomal RNA gene	AF289150	97	420	430
4A OTU 29	4a-047		Uncultured beta proteobacterium clone CRE-FL22 16S ribosomal	AF141449	99	411	413
4A OTU 29	4a-106	0.99	Uncultured beta proteobacterium clone CRE-FL22 16S ribosomal	AF141449	99	411	412
4A OTU 30	4a-105		Uncultured beta proteobacterium clone nsc122 16S ribosomal RNA	DQ211472	98	385	391
4A OTU 31	4a-027		Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	418	426
4A OTU 32	4a-030		Uncultured bacterium partial 16S rRNA gene, clone S306R	AM158355	99	428	432
4A OTU 32	4a-056	0.988	Uncultured bacterium partial 16S rRNA gene, clone S306R	AM158355	98	420	426
4A OTU 33	4a-043		Uncultured bacterium partial 16S rRNA gene, clone S306R	AM158355	96	416	431
4A OTU 34	4a-034		Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	96	417	432
4A OTU 35	4a-036		Uncultured beta proteobacterium clone CRE-FL22 16S ribosomal	AF141449	95	392	412
4A OTU 36	4a-041		Uncultured bacterium clone 28ds5 16S ribosomal RNA gene, partial	AY212721	98	349	355
4A OTU 37	4a-048		Uncultured bacterium clone ES3-27 16S ribosomal RNA gene, partial	DQ444151	99	429	433
4A OTU 38	4a-053		Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444147	98	429	434
4A OTU 39	4a-055		Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 16S-AK-W-2	AB237914	100	432	432

4A OTU 40	4a-057	Uncultured bacterium gene for 16S rRNA, partial sequence, clone:S9F-53	AB154311	99	410	412
4A OTU 41	4a-058	Rhodoferax ferrireducens DSM 15236, complete genome	CP000267 AAJK01000000 AAJK01000001-AAJK01000113	99	429	432
4A OTU 42	4a-070	Uncultured bacterium FukuN65 16S rRNA gene	AJ290001	99	431	432
4A OTU 43	4a-084	Uncultured beta proteobacterium clone LiUU-3-4 16S ribosomal	AY509421	98	398	404
4A OTU 44	4a-109	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	97	416	427
4A OTU 45	4a-102	Uncultured bacterium 16S rRNA gene, clone RB90b-424	AM159467	98	409	416
4A OTU 46	4a-040	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	94	409	434
4A OTU 47	4a-026	Uncultured gamma proteobacterium clone CRE-PA74 16S ribosomal	AF141542	95	370	387
4A OTU 48	4a-080	Uncultured freshwater bacterium clone 965006H10.x1 16S ribosomal	DQ065573	97	423	433%
4A OTU 49	4a-006	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	95	349	367
4A OTU 50	4a-022	Uncultured freshwater bacterium clone 965004A02.x1 16S ribosomal	DQ065341	99	430	432
4A OTU 51	4a-085	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	96	409	422
4A OTU 52	4a-103	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	97	419	429
4A OTU 53	4a-079	Uncultured beta proteobacterium clone N11.130WL 16S ribosomal	AF431218	99	401	402
4A OTU 54	4a-009	Aquatic bacterium R1-B31 gene for 16S ribosomal RNA, partial	AB195763	98	410	417

4A OTU 55	4a-028	Uncultured Actinobacterium clone ST11-10 16S ribosomal RNA gene	DQ316356	97	404	414
4A OTU 56	4a-038	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	99	334	337

APPENDIX D: Library 1-1300/3

OTU	City Lake Clone ID	City Lake Clone % Identity	GenBank/Blast ID	Accession #	Blast % Identity	# match bp	Total # bp
6a OTU 01	6a-4		Uncultured beta proteobacterium clone CRE-FL22 16S ribosomal	AF141449	0.99	330	331
6a OTU 02	6a-5		Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 16S-AK-W-23	AB237935	0.97	344	352
6a OTU 02	6a-96	0.988	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 16S-AK-W-24	AB237935	0.98	346	351
6a OTU 03	6a-7		Uncultured bacterium clone KD2-45 16S ribosomal RNA gene, partial	AY218584	98	339	352
6a OTU 04	6a-9		Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	99	350	351
6a OTU 04	6a-13	0.997	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	100	351	351
6a OTU 04	6a-26	0.991	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	99	350	352
6a OTU 05	6a-10		Uncultured beta proteobacterium clone CLs95 16S ribosomal RNA	AF529357	99	325	327
6a OTU 05	6a-12	0.98	Uncultured bacterium clone KD2-45 16S ribosomal RNA gene, partial	AY218584	97	347	353
6a OTU 05	6a-44	0.977	Uncultured bacterium clone KD2-45 16S ribosomal RNA gene, partial	AY218584	98	346	353
6a OTU 05	6a-73	0.983	Uncultured bacterium clone KD2-45 16S ribosomal RNA gene, partial	AY218584	98	346	352
6a OTU 05	6a-77	0.983	Uncultured bacterium clone KD2-45 16S ribosomal RNA gene, partial	AY218584	98	346	352

6a OTU 05	6a-93	0.977	Uncultured bacterium clone KD2-45 16S ribosomal RNA gene, partial	AY218584	97	344	352
6a OTU 05	6a-68	0.981	Uncultured bacterium clone KD2-45 16S ribosomal RNA gene, partial	AY218584	97	343	353
6a OTU 06	6a-16		Uncultured Crater Lake bacterium CL0-64 16S ribosomal RNA gene	AF316650	99	348	351
6a OTU 07	6a-20		Uncultured bacterium clone 1790d-08 16S ribosomal RNA gene, partial	AY917536	96	341	352
6a OTU 08	6a-23		Uncultured bacterium clone KD2-45 16S ribosomal RNA gene, partial	AY218584	95	334	348
6a OTU 09	6a-21		Uncultured Rhodoferax sp. clone TD-46 16S ribosomal RNA gene	DQ076455	96	343	357
6a OTU 10	6a-41		Unidentified haptophyte OM153 16S ribosomal RNA gene,	U70720	95	336	351
6a OTU 11	6a-42		Uncultured bacterium clone LE06 16S ribosomal RNA gene, partial	AY838541	96	339	351
6a OTU 11	6a-65	0.997	Uncultured bacterium clone LE06 16S ribosomal RNA gene, partial	AY838541	96	338	351
6a OTU 12	6a-48		Uncultured bacterium clone HTA10 16S ribosomal RNA gene, partial sequence		97	342	352
6a OTU 13	6a-49		Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	98	326	330
6a OTU 14	6a-50		Uncultured bacterium SY4-1 16S ribosomal RNA gene, partial sequence	AF107524	98	346	351
6a OTU 15	6a-51		Uncultured Actinobacterium partial 16S rRNA gene, clone NO7	AJ575559	99	349	351
6a OTU 15	6a-92	0.985	Uncultured Actinobacterium partial 16S rRNA gene, clone NO7	AJ575559	98	348	352
6a OTU 16	6a-53		Uncultured bacterium partial 16S rRNA gene, clone S306R	AM158355	98	320	326

6a OTU 17	6a-59	Uncultured alpha proteobacterium clone LiUU-9-26 16S ribosomal	AY509403	99	321	322
6a OTU 18	6a-61	Unidentified rhodophyte PRD01a010B 16S ribosomal RNA gene, partial	AF289158	91	317	345
6a OTU 19	6a-63	Aquamonas sp. C5 16S ribosomal RNA gene, partial sequence	DQ268779	98	345	350
6a OTU 20	6a-69	Uncultured Rhodobacteraceae bacterium clone M13-107 16S ribosomal	AY338110	99	329	331
6a OTU 21	6a-67	Uncultured bacterium clone HT2E3 16S ribosomal RNA gene, partial	AF418967	100	351	351
6a OTU 22	6a-70	Uncultured beta proteobacterium clone CRE-FL22 16S ribosomal	AF141449	99	310	311
6a OTU 23	6a-80	Uncultured Rhodoferax sp. clone TD-46 16S ribosomal RNA gene,	DQ076455	98	346	353
6a OTU 24	6a-81	Uncultured freshwater bacterium clone 965005G08.x1 16S ribosomal	DQ065483	97	344	352
6a OTU 25	6a-85	Uncultured bacterium clone HT2E3 16S ribosomal RNA gene, partial	AF418967	96	338	352
6a OTU 26	6a-86	Isochrysis sp. plastid 16S rRNA gene	X75518	93	330	352
6a OTU 27	6a-88	Uncultured beta proteobacterium clone 06 16S ribosomal RNA gene	AF361192	99	350	351
6a OTU 28	6a-89	Uncultured bacterium partial 16S rRNA gene, clone S306R	AM158355	98	348	352
6a OTU 29	6a-94	Methylobacter psychrophilus 16S ribosomal RNA, complete sequence	AF152597	97	346	354
6a OTU 30	6a-101	Uncultured bacterium gene for 16S rRNA, partial sequence, clone:S9F-18	AB154307	98	327	331

APPENDIX E: Library 2

OTU	City Lake Clone ID	City Lake Clone % Identity	GenBank/Blast ID	Accession #	Identity	# match bp	Total # bp
23 OTU 01	23-001		Uncultured bacterium clone 28ds5 16S ribosomal RNA gene, partial sequence	AY212721	97	412	421
23 OTU 02	23-002		Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	98	416	421
23 OTU 02	23-030	0.983	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	99	420	421
23 OTU 02	23-031	0.983	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	99	420	421
23 OTU 02	23-096	0.978	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	99	418	421
23 OTU 03	23-003		Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial sequence	DQ444146	98	416	423
23 OTU 03	23-004	0.992	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	417	423
23 OTU 03	23-005	0.995	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	418	423
23 OTU 03	23-008	0.992	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	417	423
23 OTU 03	23-025	0.978	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	97	413	423
23 OTU 03	23-032	0.992	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	417	423
23 OTU 03	23-043	0.99	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	418	424
23 OTU 03	23-067	0.992	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	417	423

23 OTU 03	23-070	0.992	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	417	423
23 OTU 03	23-078	0.992	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	417	423
23 OTU 03	23-095	0.995	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	418	423
23 OTU 03	23-098	0.995	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	418	423
23 OTU 04	23-007		Uncultured Actinobacterium partial 16S rRNA gene, clone S8	AJ575509	98	413	420
23 OTU 04	23-019	0.981	Uncultured Actinobacterium partial 16S rRNA gene, clone S8	AJ575509	99	421	419
23 OTU 04	23-052	0.978	Uncultured Actinobacterium partial 16S rRNA gene, clone S8	AJ575509	99	421	418
23 OTU 05	23-010		Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA gene, partial sequence	AY662014	98	421	416
23 OTU 05	23-030	0.976	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA gene, partial sequence	AY662014	99	421	420
23 OTU 05	23-031	0.976	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA gene, partial sequence	AY662014	99	421	420
23 OTU 05	23-101	0.983	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA gene, partial sequence	AY662014	97	421	410
23 OTU 06	23-011		Emiliania huxleyi strain CCMP 373 chloroplast, complete genome	AY741371	95	421	402
23 OTU 07	23-012		Uncultured beta proteobacterium clone CRE-FL22 16S ribosomal RNA	AF141449	99	401	400

23 OTU 07	23-044	0.992	Uncultured beta proteobacterium clone CRE-FL22 16S ribosomal RNA	AF141449	99	401	399
23 OTU 07	23-047	0.995	Uncultured beta proteobacterium clone CRE-FL22 16S ribosomal RNA	AF141449	99	401	400
23 OTU 07	23-090	0.997	Uncultured beta proteobacterium clone CRE-FL22 16S ribosomal RNA	AF141449	100	401	401
23 OTU 08	23-14		Uncultured bacterium clone ES3-27 16S ribosomal RNA gene, partial	DQ444151	100	421	421
23 OTU 08	23-22	1	Uncultured bacterium clone ES3-27 16S ribosomal RNA gene, partial	DQ444151	100	421	421
23 OTU 08	23-65	0.997	Uncultured bacterium clone ES3-27 16S ribosomal RNA gene, partial	DQ444151	99	421	420
23 OTU 08	23-97	0.95	Uncultured bacterium clone 28ds5 16S ribosomal RNA gene, partial	AY212721	99	421	418
23 OTU 09	23-16		Uncultured bacterium partial 16S rRNA gene, clone S306R.	AM158355	97	422	412
23 OTU 09	23-82	0.981	Uncultured bacterium partial 16S rRNA gene, clone S306R	AM158355	98	421	416
23 OTU 10	23-17		Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 16S-AK-W-53	AB237961	98	422	414
23 OTU 10	23-33	0.995	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 16S-AK-W-54	AB237961	98	414	421
23 OTU 11	23-18		Uncultured Burkholderiales bacterium clone DS037 16S ribosomal RNA	DQ234121	99	419	421
23 OTU 11	23-20	1	Uncultured Burkholderiales bacterium clone DS037 16S ribosomal RNA	DQ234121	99	419	421

23 OTU 12	23-23		Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA gene, partial sequence	AY662014	97	409	421
23 OTU 13	23-26		Uncultured yard-trimming-compost bacterium clone S-27 16S ribosomal	AY095398	98	339	343
23 OTU 14	23-28		Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	96	407	421
23 OTU 15	23-35		Uncultured proteobacterium clone R7C62 16S ribosomal RNA gene	DQ450171	99	418	421
23 OTU 16	23-36		Uncultured beta proteobacterium clone 08 16S ribosomal RNA gene, partial sequence	AF361201	99	419	421
23 OTU 16	23-57	0.985	Uncultured freshwater bacterium clone 965004A02.x1 16S ribosomal	DQ065341	99	418	421
23 OTU 16	23-76	0.985	Uncultured freshwater bacterium clone 965004A02.x1 16S ribosomal	DQ065341	99	418	421
23 OTU 16	23-83	0.988	Uncultured freshwater bacterium clone 965015B06.x1 16S ribosomal	DQ064823	100	421	421
23 OTU 16	23-84	0.985	Uncultured freshwater bacterium clone 965015B06.x1 16S ribosomal	DQ064823	99	420	421
23 OTU 17	23-39		Rhodoferax ferrireducens DSM 15236, complete genome	CP000267 REGION: 4118284..4119814	98	414	421
23 OTU 17	23-99	0.978	Rhodoferax ferrireducens DSM 15236, complete genome	CP000267 REGION: 4118284..4119814	99	417	421
23 OTU 18	23-41		Uncultured proteobacterium clone R7C71 16S ribosomal RNA gene, partial sequence	DQ450172	96	410	423
23 OTU 18	23-71	0.995	Uncultured proteobacterium clone R7C71 16S ribosomal RNA gene,	DQ450172	96	410	423
23 OTU 18	23-73	0.995	Uncultured proteobacterium clone R7C71 16S ribosomal RNA gene,	DQ450172	96	410	423

23 OTU 19	23-49		Uncultured bacterium gene for 16S rRNA, partial sequence, clone: RB308	AB240330	99	418	421
23 OTU 20	23-50		Uncultured beta proteobacterium clone PRD01b012B 16S ribosomal RNA	AF289172	99	416	418
23 OTU 21	23-64		Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	95	404	421
23 OTU 22	23-72		Uncultured bacterium gene for 16S rRNA, partial sequence, clone 16S-AK-W-40	AB237948	99	417	421
23 OTU 23	23-77		Uncultured bacterium clone ML-7-19 16S ribosomal RNA gene, partial	DQ235038	100	421	421
23 OTU 23	23-80	1	Uncultured bacterium clone ML-7-19 16S ribosomal RNA gene, partial	DQ235038	100	421	421
23 OTU 24	23-85		Uncultured bacterium clone 92 16S ribosomal RNA gene, partial	DQ165180	94	391	414
23 OTU 25	23-91		Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 16S-AK-W-22	AB237934	99	418	421
23 OTU 26	23-94		Uncultured bacterium clone HTA10 16S ribosomal RNA gene, partial	AF418942	98	415	421
23 OTU 27	23-104		Uncultured Actinobacterium partial 16S rRNA gene, clone NO7.	AJ575559	97	412	421

APPENDIX F: Library 3

OTU	City Lake Clone ID	City Lake Clone % Identity	GenBank/Blast ID	Accession #	Blast % Identity	# match bp	Total # bp
CL 26 OTU 01	26-066		Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	416	427
CL 26 OTU 01	26-085	0.997	Uncultured bacterium FukuS110 16S rRNA gene.	AJ289986	97	415	427
CL 26 OTU 01	26-095	0.988	Uncultured alpha proteobacterium clone CrystalBog021H4 16S	AY792286	95	407	424
CL 26 OTU 02	26-118		Uncultured beta proteobacterium clone nsc122 16S ribosomal RNA gene, partial sequence	DQ211472	91	343	374
CL 26 OTU 03	26-120		Methylobacter sp. LW12 16S ribosomal RNA gene, partial sequence.	AY007295	96	410	423
CL 26 OTU 03	26-139	0.992	Methylobacter sp. LW12 16S ribosomal RNA gene, partial sequence	AY007295	96	409	424
CL 26 OTU 03	26-171	0.995	Methylobacter sp. LW12 16S ribosomal RNA gene, partial sequence	AY007295	96	408	423
CL 26 OTU 04	26-121		Uncultured bacterium clone ML-7-19 16S ribosomal RNA gene, partial	DQ235038	99	421	423
CL 26 OTU 05	26-122		Uncultured Verrucomicrobiales clone CR-FL5 16S ribosomal RNA gene,	AF141391	99	399	403
CL 26 OTU 06	26-123		Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	96	410	424
CL 26 OTU 07	26-125		Uncultured freshwater bacterium clone 965002G07.x1 16S ribosomal RNA gene, partial sequence	DQ065280	99	422	423

CL 26 OTU 07	26-164	0.978	Uncultured freshwater bacterium clone 965002G07.x1 16S ribosomal RNA gene, partial sequence	DQ065280	99	422	423
CL 26 OTU 07	26-190	1	Uncultured freshwater bacterium clone 965002G07.x1 16S ribosomal RNA gene, partial sequence	DQ065280	99	422	423
CL 26 OTU 08	26-126		Uncultured bacterium clone BG.d1 16S ribosomal RNA gene, partial	DQ228371	98	418	423
CL 26 OTU 08	26-210	0.981	Rhodoferax ferrireducens DSM 15236, complete genome	CP000267 AAJK01000000 AAJK01000001-AAJK01000113	99	419	423
CL 26 OTU 09	26-127		Uncultured bacterium clone ELB19-080 16S ribosomal RNA gene	DQ015783	98	418	423
CL 26 OTU 09	26-159	0.99	Uncultured bacterium clone ELB19-080 16S ribosomal RNA gene,	DQ015783	99	422	423
CL 26 OTU 09	26-166	0.995	Uncultured bacterium clone ELB19-080 16S ribosomal RNA gene,	DQ015783	99	422	423
CL 26 OTU 09	26-192	0.988	Uncultured bacterium clone ELB19-080 16S ribosomal RNA gene,	DQ015783	99	421	423
CL 26 OTU 10	26-128		Uncultured bacterium clone tpb-16-234-D05 small subunit ribosomal	DQ407424	99	420	423
CL 26 OTU 10	26-198	0.988	Uncultured bacterium clone tpb-16-234-D05 small subunit ribosomal	DQ407424	99	423	424
CL 26 OTU 11	26-134		Uncultured beta proteobacteria partial 16S rRNA gene, clone TH480	AJ888547	96	410	425
CL 26 OTU 12	26-135		Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	420	425
CL 26 OTU 12	26-204	1	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	420	425
CL 26 OTU 13	26-136		Feldmannia irregularis virus a strain FirrV-1 contig C, partial	AY225135	99	186	186

CL 26 OTU 14	26-137		Uncultured beta proteobacterium clone LS-E5 16S ribosomal RNA gene,	AY628664	99	421	424
CL 26 OTU 14	26-188	0.99	Uncultured Burkholderiales bacterium clone DS037 16S ribosomal RNA	DQ234121	99	421	423
CL 26 OTU 14	26-199	0.99	Uncultured Burkholderiales bacterium clone DS037 16S ribosomal RNA	DQ234121	99	421	424
CL 26 OTU 15	26-138		Uncultured bacterium clone PO20 16S ribosomal RNA gene, partial	AY838534	99	422	423
CL 26 OTU 15	26-141	0.995	Pseudomonas sp. WT OTU2 small subunit ribosomal RNA gene, partial	AY965247	100	423	423
CL 26 OTU 15	26-158	0.995	Pseudomonas sp. WT OTU2 small subunit ribosomal RNA gene, partial	AY965247	100	423	423
CL 26 OTU 15	26-193	0.992	Uncultured bacterium clone PO20 16S ribosomal RNA gene, partial	AY838534	99	421	423
CL 26 OTU 15	26-201	0.995	Pseudomonas sp. WT OTU2 small subunit ribosomal RNA gene, partial	AY965247	100	423	423
CL 26 OTU 16	26-140		Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA gene, partial sequence	AY662014	100	423	423
CL 26 OTU 16	26-151	0.981	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA gene, partial sequence	AY662014	98	416	422
CL 26 OTU 16	26-164	0.976	Uncultured freshwater bacterium clone 965002G07.x1 16S ribosomal	DQ065280	98	415	423
CL 26 OTU 17	26-146		Uncultured beta proteobacterium clone CRE-FL22 16S ribosomal RNA	AF141449	99	401	405
CL 26 OTU 17	26-149	0.983	Uncultured beta proteobacterium clone CRE-FL22 16S ribosomal RNA	AF141449	99	400	402

CL 26 OTU 17	26-179	0.983	Uncultured beta proteobacterium clone CRE-FL22 16S ribosomal RNA	AF141449	99	401	403
CL 26 OTU 18	26-150		Uncultured bacterium clone CRC4 16S ribosomal RNA gene, partial	AF392683	93	331	354
CL 26 OTU 19	26-152		Uncultured phototrophic eukaryote clone PRD18H11 16S ribosomal RNA	AY948073	95	378	395
CL 26 OTU 20	26-153		Uncultured beta proteobacterium clone IRD18E09 16S ribosomal RNA	AY947947	99	396	399
CL 26 OTU 20	26-164	0.981	Uncultured freshwater bacterium clone 965002G07.x1 16S ribosomal	DQ065280	98	415	423
CL 26 OTU 21	26-154		Uncultured bacterium clone PO20 16S ribosomal RNA gene, partial	AY838534	99	404	406
CL 26 OTU 22	26-155		Uncultured bacterium clone 005C-F09 small subunit ribosomal RNA	AY661953	99	422	423
CL 26 OTU 22	26-176	0.985	Uncultured bacterium gene for 16S rRNA, partial sequence, clone	AB240330	99	420	423
CL 26 OTU 23	26-156		Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 16S-AK-W-23	AB237935	97	410	419
CL 26 OTU 24	26-160		Uncultured bacterium clone KD6-99 16S ribosomal RNA gene, partial	AY218774	97	409	419
CL 26 OTU 25	26-163		Uncultured freshwater bacterium clone 965020C09.y1 16S ribosomal	DQ065095	99	421	423
CL 26 OTU 25	26-187	0.995	Uncultured freshwater bacterium clone 965020C09.y1 16S ribosomal	DQ065095	99	422	423
CL 26 OTU 26	26-172		Uncultured phototrophic eukaryote clone PRD18E02 16S ribosomal RNA gene, partial sequence; chloroplast	AY948033	99	401	404

CL 26 OTU 27	26-178		Uncultured bacterium clone 010A-D01 small subunit ribosomal RNA gene, partial sequence	AY661958	95	405	423
CL 26 OTU 28	26-184		Bacterium H4 16S ribosomal RNA gene, partial sequence	AY345546	99	415	417
CL 26 OTU 29	26-185		Bacterium H4 16S ribosomal RNA gene, partial sequence.	AY345546	99	415	417
CL 26 OTU 30	26-189		Uncultured bacterium partial 16S rRNA gene, clone T319D.	AM158425	97	412	421
CL 26 OTU 31	26-197		Uncultured bacterium clone 220ds20 16S ribosomal RNA gene, partial	AY212668	97	412	424
CL 26 OTU 32	26-202		Uncultured planctomycete clone JY9 16S ribosomal RNA gene, partial	DQ393188	96	371	386
CL 26 OTU 33	26-206		Uncultured Actinobacterium partial 16S rRNA gene, clone S8.	AJ575509	99	421	423
CL 26 OTU 34	26-207		Uncultured bacterium partial 16S rRNA gene, clone S306R	AM158355	98	419	424
CL 26 OTU 34	26-213	0.976	Uncultured bacterium clone KD6-99 16S ribosomal RNA gene, partial	AY218774	98	416	423
CL 26 OTU 35	26-223		Uncultured beta proteobacterium 341ev partial 16S rRNA gene	AJ287643	98	250	253
CL 26 OTU 36	26-224		Uncultured Crater Lake bacterium CL0-64 16S ribosomal RNA gene	AF316650	99	420	423
CL 26 OTU 37	26-226		Uncultured freshwater bacterium clone 965017H08.y1 16S ribosomal	DQ064985	99	422	423

APPENDIX G: Library 4

OTU	City Lake Clone ID	City Lake Clone % Identity	GenBank/Blast ID	Accession #	Identity	# match bp	Total # bp
28 OTU 01	28-002		Uncultured bacterium gene for 16S rRNA, partial sequence, clone:S9F-53	AB154311	98	395	403
28 OTU 01	28-065	0.983	Uncultured bacterium gene for 16S rRNA, partial sequence, clone:S9F-53	AB154311	99	400	403
28 OTU 01	28-090	1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone:S9F-53	AB154311	98	395	403
28 OTU 02	28-004		Uncultured proteobacterium clone R7C62 16S ribosomal RNA gene,	DQ450171	99	419	423
28 OTU 03	28-005		Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial sequence	DQ444146	98	420	425
28 OTU 03	28-020	0.988	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial sequence	DQ444146	98	420	425
28 OTU 03	28-042	0.983	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial sequence	DQ444146	97	420	425
28 OTU 03	28-044	0.978	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial sequence	DQ444146	97	414	424
28 OTU 03	28-089	0.985	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial sequence	DQ444146	98	417	425
28 OTU 03	28-094	0.988	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial sequence	DQ444146	97	417	426

28 OTU 04	28-006		Uncultured bacterium clone 28ds5 16S ribosomal RNA gene, partial sequence AY212721	AY212721	100	423	423
28 OTU 04	28-018	0.99	Uncultured bacterium clone 28ds5 16S ribosomal RNA gene, partial sequence AY212721	AY212721	99	421	424
28 OTU 05	28-011		Uncultured bacterium clone ORCA-17N106 16S ribosomal RNA gene partial sequence	DQ823213	96	411	425
28 OTU 06	28-012		Uncultured Freshwater bacterium clone 965002G07.x1 16S ribosomal RNA gene partial sequence	DQ065280	98	399	407
28 OTU 07	28-013		Uncultured Freshwater bacterium clone 965017H08.y1 16S ribosomal RNA gene, partial sequence	DQ064985	98	418	423
28 OTU 08	28-038		Uncultured Actinobacterium partial 16S rRNA gene, clone S9	AJ575510	94	375	395
28 OTU 09	28-039		Uncultured bacterium clone HTA2 16S ribosomal RNA gene, partial sequence	AF418943	99	419	423
28 OTU 10	28-041		Uncultured bacterium gene for 16S rRNA, partial sequence, clone 16S-AK-B-33	AB237990	95	403	423
28 OTU 10	28-099	0.992	Uncultured bacterium gene for 16S rRNA, partial sequence, clone 16S-AK-B-33	AB237990	94	402	424
28 OTU 11	28-045		Uncultured beta proteobacterium partial 16S rRNA gene, clone B8	AJ867911	99	421	424
28 OTU 12	28-023		Aquamonas sp. C5 16S ribosomal RNA gene, partial sequence	DQ268779	98	418	423
28 OTU 12	28-055	1	Aquamonas sp. C5 16S ribosomal RNA gene, partial sequence	DQ268779	98	418	423

28 OTU 13	28-024		Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	99	423	424
28 OTU 13	28-028	0.995	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	99	423	424
28 OTU 13	28-035	0.976	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	98	418	423
28 OTU 13	28-110	0.992	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	99	422	424
28 OTU 14	28-025		Uncultured bacterium partial 16S rRNA gene, clone R2_14	AJ876700	99	421	424
28 OTU 14	28-075	0.981	Uncultured bacterium clone 234ds5 16S ribosomal RNA gene, partial	AY212681	99	418	422
28 OTU 14	28-079	0.99	Uncultured bacterium partial 16S rRNA gene, clone R2_14	AJ876700	99	421	423
28 OTU 14	28-096	0.985	Uncultured bacterium clone 005C-F09 small subunit ribosomal RNA	AY661953	100	423	423
28 OTU 15	28-026		Uncultured bacterium clone S22.53WL 16S ribosomal RNA gene, partial	AF432731	97	383	393
28 OTU 16	28-007						
28 OTU 17	28-029		Uncultured alpha proteobacterium clone AKYH1402 16S ribosomal RNA	AY922106	97	412	422
28 OTU 17	28-105	0.978	Uncultured alpha proteobacterium clone AKYH1402 16S ribosomal RNA	AY922106	97	415	424
28 OTU 18	28-034		Uncultured bacterium clone ES3-27 16S ribosomal RNA gene, partial	DQ444151	99	375	378
28 OTU 19	28-037		Uncultured Fresh water bacterium clone 965002G07.x1	DQ065280	99	421	423
28 OTU 20	28-038		Uncultured Actinobacterium partial 16S rRNA gene, clone S9.	AJ575510	94	375	395

28 OTU 21	28-039		Uncultured bacterium clone HTA2 16S ribosomal RNA gene, partial	AF418943	99	419	423
28 OTU 22	28-041		Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 16S-AK-B-33.	AB237990	95	403	423
28 OTU 22	28-099	0.992	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 16S-AK-B-33.	AB237990	94	402	424
28 OTU 23	28-045		Uncultured beta proteobacterium partial 16S rRNA gene, clone B8	AJ867911	99	421	424
28 OTU 24	28-046		Uncultured beta proteobacterium clone 08 16S ribosomal RNA gene, partial sequence	AF361201	98	419	425
28 OTU 24	28-062	0.978	Uncultured beta proteobacterium clone 08 16S ribosomal RNA gene, partial sequence	AF361201	98	416	424
28 OTU 24	28-066	0.981	Uncultured Freshwater bacterium clone 965015B06.x1 16S ribosomal	DQ064823	99	422	423
28 OTU 25	28-049		Uncultured bacterium clone ELB19-080 16S ribosomal RNA gene, complete sequence	DQ015783	99	421	423
28 OTU 25	28-083	1	Uncultured bacterium clone ELB19-080 16S ribosomal RNA gene, complete sequence	DQ015783	99	421	423
28 OTU 26	28-050		Uncultured bacterium clone TBc-14 16S ribosomal RNA gene, partial	DQ791390	99	421	424
28 OTU 27	28-052		Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	96	411	425
28 OTU 28	28-053		Uncultured bacterium clone nsc066 16S ribosomal RNA gene, part sequence	DQ211421	97	411	422
28 OTU 29	28-056		Uncultured Actinobacterium partial 16S rRNA gene, clone R2	AJ575498	99	422	423

28 OTU 30	28-057		Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	95	340	355
28 OTU 31	28-060		Rhodobacter sp. TCRI2 and TCRI4 gene for 16S rRNA, partial	AB017798	97	408	420
28 OTU 32	28-066		Uncultured Freshwater bacterium clone 965015B06.x1 16S ribosomal	DQ064823	99	419	423
28 OTU 32	28-093	0.981	Uncultured Freshwater bacterium clone 965015B06.x1 16S ribosomal	DQ064823	98	416	423
28 OTU 33	28-067		Uncultured soil bacterium clone M22_Pitesti 16S ribosomal RNA gene complete sequence	DQ378242	97	414	424
28 OTU 34	28-069		Unidentified haptophyte OM125 16S ribosomal RNA gene, partial	U70719	96	408	424
28 OTU 35	28-070		Uncultured beta proteobacterium clone 13 16S ribosomal RNA gene	AF361202	99	422	424
28 OTU 36	28-071		Polaromonas sp. 'hydrogenovorans' 16S ribosomal RNA gene, partial	DQ094183	99	422	423
28 OTU 37	28-074		Uncultured phototrophic eukaryote clone PRD18E02 16S ribosomal RNA	AY948033	99	400	403
28 OTU 38	28-082		Uncultured beta proteobacterium clone IRD18E09 16S ribosomal RNA	AY947947	95	384	404
28 OTU 39	28-083		Uncultured bacterium clone ELB19-080 16S ribosomal RNA gene,	DQ015783	99	421	423
28 OTU 40	28-084		Uncultured bacterium clone ES3-27 16S ribosomal RNA gene, partial	DQ444151	97	406	415
28 OTU 41	28-085		Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	97	417	426
28 OTU 42	28-088		Uncultured bacterium clone 234ds5 16S ribosomal RNA gene, partial	AY212681	98	409	417
28 OTU 43	28-097		Uncultured Rhodobacteraceae bacterium clone M13-52 16S ribosomal	AY338109	99	399	402

28 OTU 44	28-100	Uncultured Zoogloea sp. clone F8 16S ribosomal RNA gene, partial	AF375832	96	259	268
28 OTU 45	28-101	Uncultured bacterium clone 234ds5 16S ribosomal RNA gene, partial	AY212681	98	407	415
28 OTU 46	28-102	Uncultured proteobacterium gene for 16S ribosomal RNA, partial	AB161079	99	423	424
28 OTU 47	28-103	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	91	376	412
28 OTU 48	28-104	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: SRRB38.	AB240512	93	393	419
28 OTU 49	28-107	Uncultured beta proteobacterium clone LiUU-5-225 16S ribosomal RNA gene, partial sequence	AY509449	98	381	386
28 OTU 50	28-112	Uncultured Burkholderiales bacterium clone DS037 16S ribosomal RNA gene, partial sequence	DQ234121	95	319	333
28 OTU 51	28-114	Uncultured bacterium partial 16S rRNA gene, clone C8	AJ867920	99	397	401

APPENDIX H: Library 5

OTU	City Lake ID	City Lake % Identity	GenBank/Blast ID	Accession #	Identity	# match bp	Total # bp
33 OTU 01	33-001		Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	96%	357	371
33 OTU 02	33-002		Uncultured bacterium FukuN65 16S rRNA gene	AJ290001	99	419	422
33 OTU 02	33-046	0.976	Uncultured bacterium FukuS93 16S rRNA gene	AJ290018	99	420	422
33 OTU 03	33-003		Uncultured Crater Lake bacterium CL120-48 16S ribosomal RNA gene	AF316699	95	401	422
33 OTU 03	33-009	0.985	Emiliania huxleyi strain CCMP 373 chloroplast, complete genome	AY741371	95	405	422
33 OTU 04	33-004		Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	99	420	424
33 OTU 04	33-008	0.992	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	417	424
33 OTU 04	33-013	0.997	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	419	424
33 OTU 05	33-007		Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	99%	421	424
33 OTU 05	33-015	0.985	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	99	420	422
33 OTU 06	33-010		Uncultured Flavobacterium sp. clone BFA 6E 16S ribosomal RNA gene	DQ628916	98	393	401
33 OTU 06	33-012	0.995	Uncultured Flavobacterium sp. clone BFA 6E 16S ribosomal RNA gene	DQ628916	98	394	401
33 OTU 07	33-014		Uncultured Cytophagales clone PRD01a001B 16S ribosomal RNA gene	AF289149	99%	417	419
33 OTU 08	33-019		Uncultured alpha proteobacterium clone CrystalBog021H4 16S ribosomal	AY792286	93%	387	413

33 OTU 09	33-023		Uncultured freshwater bacterium clone 965004F10.x1 16S ribosomal	DQ065395	95	322	336
33 OTU 10	33-024		Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-026	0.983	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	96	409	426
33 OTU 10	33-028	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-030	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-031	0.992	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	96	413	426
33 OTU 10	33-035	0.981	Uncultured freshwater bacterium clone 965004F10.x1 16S ribosomal	DQ065395	96	411	425
33 OTU 10	33-048	0.978	Uncultured freshwater bacterium clone 965004F10.x1 16S ribosomal	DQ065395	96	391	401
33 OTU 10	33-053	0.997	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	414	426
33 OTU 10	33-055	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-058	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-059	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-060	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-061	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-062	0.997	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	414	426
33 OTU 10	33-063	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426

33 OTU 10	33-064	0.983	Uncultured freshwater bacterium clone 965004F10.x1 16S ribosomal	DQ065395	96	410	426
33 OTU 10	33-065	0.985	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	96	413	426
33 OTU 10	33-066	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-067	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-068	0.995	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	414	426
33 OTU 10	33-069	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-070	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-072	0.985	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	96	410	426
33 OTU 10	33-073	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-074	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-075	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-078	0.995	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	96	413	426
33 OTU 10	33-079	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-80	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-081	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-083	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426

33 OTU 10	33-085	0.997	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	414	426
33 OTU 10	33-086	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-087	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-088	0.997	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	414	426
33 OTU 10	33-089	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-091	0.997	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	414	426
33 OTU 10	33-092	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-094	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-095	992	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	96	412	426
33 OTU 10	33-096	1	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	415	426
33 OTU 10	33-097	0.985	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	96	409	422
33 OTU 10	33-098	0.997	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	97	414	426
33 OTU 11	33-032		Uncultured freshwater bacterium clone 965004F10.x1 16S ribosomal	DQ065395	95%	380	397
33 OTU 12	33-033		Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	95%	402	422
33 OTU 13	33-038		Uncultured freshwater bacterium clone 965004F10.x1 16S ribosomal	DQ065395	93%	377	403
33 OTU 14	33-039		Uncultured bacterium FukuN65 16S rRNA gene	AJ290001	98%	321	327

33 OTU 15	33-040	Uncultured beta proteobacterium clone 08 16S ribosomal RNA gene	AF361201	98	415	422
33 OTU 16	33-041	U bacterium clone 220ds20 16S ribosomal RNA gene, partial	AY212668	99	419	422%
33 OTU 17	33-042	Uncultured Actinobacterium clone ST11-10 16S ribosomal RNA gene	DQ316356	99%	420	422
33 OTU 18	33-043	Uncultured bacterium clone 28ds5 16S ribosomal RNA gene, partial	AY212721	99%	419	422
33 OTU 19	33-044	Uncultured bacterium clone HTH6 16S ribosomal RNA gene, partial	AF418965	99%	421	422
33 OTU 20	33-050	Uncultured phototrophic eukaryote clone PRD18E02 16S ribosomal RNA gene, partial sequence; chloroplast	AY948033	98	395	402
33 OTU 21	33-056	Uncultured alpha proteobacterium clone CrystalBog021H4 16S ribosomal	AY792286	94	400	423
33 OTU 22	33-057	Uncultured freshwater bacterium clone 965004F10.x1 16S ribosomal	DQ065395	95%	335	349
33 OTU 23	33-090	Uncultured bacterium FukuS110 16S rRNA gene	AJ289986	94%	394	415

APPENDIX I: Library 1-1816

OTU	City Lake Clone ID	City Lake Clone % Identity	GenBank/Blast	Accession #	Identity	# match bp	Total # bp
CL38 OTU 01	38-003		Uncultured bacterium clone ML-7-19 16S ribosomal RNA gene, partial	DQ235038	98	415	422
CL38 OTU 02	38-005		Fucus vesiculosus 16S ribosomal RNA gene, partial sequence chloroplast	DQ307678	96	191	198
CL38 OTU 03	38-006		Uncultured bacterium clone ES3-27 16S ribosomal RNA gene, partial	DQ444151	98	416	422
CL38 OTU 03	38-020	0.983	Uncultured bacterium clone 28ds5 16S ribosomal RNA gene, partial	AY212721	100	422	422
CL38 OTU 03	38-024	0.985	Uncultured bacterium clone ES3-27 16S ribosomal RNA gene, partial	DQ444151	100	422	422
CL38 OTU 03	38-075	0.978	Uncultured bacterium clone ES3-27 16S ribosomal RNA gene, partial	DQ444151	99	419	422
CL38 OTU 03	38-152	0.978	Uncultured beta proteobacterium partial 16S rRNA gene, clone NE58	AJ575687	99	415	419
CL38 OTU 03	38-226	0.981	Uncultured bacterium clone ES3-27 16S ribosomal RNA gene, partial	DQ444151	99	420	422
CL38 OTU 03	38-249	0.981	Uncultured bacterium clone ES3-27 16S ribosomal RNA gene, partial	DQ444151	99	422	423
CL38 OTU 04	38-008		Uncultured bacterium gene for 16S rRNA, partial sequence, clone:16S-AK-W-23	AB237935	97	408	419
CL38 OTU 05	38-011		Uncultured bacterium clone HT2E3 16S ribosomal RNA gene, partial	AF418967	99	420	422
CL38 OTU 05	38-040	0.992	Uncultured bacterium clone HT2E3 16S ribosomal RNA gene, partial	AF418967	99	421	422
CL38 OTU 06	38-015		Uncultured bacterium gene for 16S rRNA, partial sequence, clone:	AB237935	97	409	419

CL38 OTU 07	38-018		Uncultured bacterium clone 234ds5 16S ribosomal RNA gene, partial	AY212681	97	402	412
CL38 OTU 08	38-030		Uncultured bacterium clone 40ds5 16S ribosomal RNA gene, partial	AY212735	96	400	413
CL38 OTU 09	38-031		Uncultured beta proteobacterium clone AKYG1724 16S ribosomal RNA	AY921977	94	388	409
CL38 OTU 10	38-044		Uncultured beta proteobacterium clone 13 16S ribosomal RNA gene	AF361202	99	420	421
CL38 OTU 11	38-045		Uncultured proteobacterium clone R7C71 16S ribosomal RNA gene	DQ450172	95	406	423
CL38 OTU 11	38-063	0.978	Uncultured proteobacterium clone R7C71 16S ribosomal RNA gene,	DQ450172	96	405	420
CL38 OTU 11	38-074	0.983	Uncultured proteobacterium clone R7C71 16S ribosomal RNA gene	DQ450172	96	409	423
CL38 OTU 11	38-159	0.988	Uncultured proteobacterium clone R7C71 16S ribosomal RNA gene,	DQ450172	97	411	423
CL38 OTU 11	38-239	0.985	Uncultured proteobacterium clone R7C71 16S ribosomal RNA gene	DQ450172	96	410	423
CL38 OTU 12	38-049		Uncultured freshwater bacterium clone 965004A02.x1 16S ribosomal	DQ065341	99	421	422
CL38 OTU 13	38-050		Uncultured Bacteroidetes partial 16S rRNA gene, clone TH419	AJ888563	97	338	347
CL38 OTU 14	38-058		Methylobacter sp. LW12 16S ribosomal RNA gene, partial sequence.	AY007295	96	407	422
CL38 OTU 14	38-105	0.997	Methylobacter sp. LW12 16S ribosomal RNA gene, partial sequence	AY007295	96	406	422
CL38 OTU 15	38-060		Uncultured bacterium clone 28ds5 16S ribosomal RNA gene, partial	AY212721	97	408	418

CL38 OTU 16	38-065		Soil bacterium TWE165 16S ribosomal RNA gene, partial sequence	DQ493433	96	411	424
CL38 OTU 17	38-072		Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	417	424
CL38 OTU 17	38-232	0.992	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	418	424
CL38 OTU 17	38-253	0.99	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	417	424
CL38 OTU 18	38-076		Uncultured Actinobacterium clone IRD18E05 16S ribosomal RNA gene	AY947943	95	386	403
CL38 OTU 19	38-103		Rhodoferax ferrireducens partial 16S rRNA gene, clone EB01	AM265401	98	416	422
CL38 OTU 20	38-107		Uncultured bacterium clone PO20 16S ribosomal RNA gene, partial	AY838534	99	421	422
CL38 OTU 20	38-156	0.983	Pseudomonas sp. pfB35 16S ribosomal RNA gene, partial sequence	AY336564	99	419	423
CL38 OTU 21	38-108		Uncultured bacterium clone DS3-56 16S ribosomal RNA gene, partial	DQ463235	97	411	422
CL38 OTU 22	38-109		Uncultured proteobacterium clone TAF-B73 16S ribosomal RNA gene	AY038714	99	419	422
CL38 OTU 22	38-111	0.99	Uncultured proteobacterium clone TAF-B73 16S ribosomal RNA gene,	AY038714	99	419	422
CL38 OTU 23	38-110		Uncultured bacterium clone 234ds5 16S ribosomal RNA gene, partial	AY212681	99	416	420
CL38 OTU 23	38-112	0.985	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	419	424
CL38 OTU 23	38-223	0.985	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	419	424
CL38 OTU 23	38-250	0.981	Uncultured bacterium clone 234ds5 16S ribosomal RNA gene, partial	AY212681	99	416	420

CL38 OTU 24	38-115		Uncultured Burkholderiales bacterium	DQ234121	99	420	422
CL38 OTU 25	38-126		Uncultured bacterium clone RA13C6 16S ribosomal RNA gene, partial	AF407405	98	417	422
CL38 OTU 26	38-154		Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	98	405	413
CL38 OTU 27	38-161		Uncultured bacterium clone HTH6 16S ribosomal RNA gene, partial	AF418965	98	417	422
CL38 OTU 28	38-225		Uncultured Crater Lake bacterium CL0-6 16S ribosomal RNA gene,	AF316682	98	299	305
CL38 OTU 29	38-227		Uncultured beta proteobacterium gene for 16S rRNA, partial	AB076869	95	406	423
CL38 OTU 30	38-228		Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	98	415	422
CL38 OTU 30	38-258	0.981	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	99	421	422
CL38 OTU 31	38-230		Uncultured Actinobacterium partial 16S rRNA gene, clone S7.	AJ575508	99	419	422
CL38 OTU 32	38-235		Uncultured bacterium clone 268ds10 16S ribosomal RNA gene, partial	AY212718	100	422	422
CL38 OTU 32	38-243	1	Uncultured bacterium clone 268ds10 16S ribosomal RNA gene, partial	AY212718	100	422	422
CL38 OTU 33	38-238		Uncultured bacterium clone 300C-G07 small subunit ribosomal RNA	AY662019	91	286	311
CL38 OTU 34	38-240		Uncultured bacterium clone SX3-41 16S ribosomal RNA gene, partial	DQ469230	97	411	422
CL38 OTU 35	38-241		Uncultured phototrophic eukaryote clone PRD18F11 16S ribosomal RNA, chloroplast	AY948053	97	391	402
CL38 OTU 36	38-242		Uncultured Actinobacterium clone IRD18A09 16S ribosomal RNA gene,	AY947900	99	400	402

CL38 OTU 37	38-251		Uncultured beta proteobacterium clone LiUU-3-4 16S ribosomal RNA	AY509421	99	392	394
CL38 OTU 38	38-252		Unidentified haptophyte OM153 16S ribosomal RNA gene, partial	U70720	96	406	421
CL38 OTU 39	38-256		Uncultured bacterium clone C3 16S ribosomal RNA gene, partial	AY962276	99	421	425
CL38 OTU 40	38-257		Uncultured beta proteobacterium clone CRE-FL22 16S ribosomal RNA	AF141449	100	402	402
CL38 OTU 40	38-266	0.995	Uncultured beta proteobacterium clone CRE-FL22 16S ribosomal RNA	AF141449	99	402	403
CL38 OTU 41	38-259		Uncultured beta proteobacterium clone CRE-FL22 16S ribosomal RNA	AF141449	97	392	401
CL38 OTU 42	38-261		Uncultured Actinobacterium clone ST11-4 16S ribosomal RNA gene	DQ316366	98	416	422
CL38 OTU 43	38-263		Uncultured bacterium clone 207ds20 16S ribosomal RNA gene, partial	AY212653	99	418	422
CL38 OTU 44	38-267		Uncultured bacterium clone ML-7-6 16S ribosomal RNA gene, partial	DQ235030	98	416	422
CL38 OTU 45	38-274		Uncultured Cytophagales clone PRD01a001B 16S ribosomal RNA gene	AF289149	100	419	419
CL38 OTU 46	38-275		Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	97	398	408
CL38 OTU 47	38-278		Uncultured bacterium gene for 16S rRNA, partial sequence	AB154311	99	398	402

APPENDIX J: Library 1-0300

OTU	City Lake Clone ID	City Lake Clone % Identity	GenBank/Blast	Accession #	Identity	# match bp	Total # bp
41 OTU 01	41-003		Pseudomonas sp. CPC20 small subunit ribosomal RNA gene, partial	DQ013850	99	419	422
41 OTU 02	41-004		Uncultured beta proteobacterium clone PRD01b012B 16S ribosomal RNA	AF289172	99	416	419
41 OTU 03	41-005		Uncultured Pseudomonas sp. clone M12-82 16S ribosomal RNA gene,	AY338163	98	398	403
41 OTU 04	41-006		Uncultured bacterium SY4-1 16S ribosomal RNA gene, partial	AF107524	99	419	422
41 OTU 04	41-024	1	Uncultured bacterium SY4-1 16S ribosomal RNA gene, partial	AF107524	99	419	422
41 OTU 05	41-009		Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	418	424
41 OTU 05	41-052	1	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	418	424
41 OTU 05	41-075	0.978	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	418	424
41 OTU 06	41-011		Uncultured bacterium partial 16S rRNA gene, clone C8	AJ867920	99	397	400
41 OTU 06	41-013	1	Uncultured bacterium partial 16S rRNA gene, clone C8	AJ867920	99	397	400
41 OTU 06	41-045	0.997	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	418	424
41 OTU 06	41-071	1	Uncultured bacterium partial 16S rRNA gene, clone C8	AJ867920	99	397	400
41 OTU 06	41-078	0.983	Uncultured freshwater bacterium clone 965005G08.x1 16S ribosomal	DQ065483	96	409	422

41 OTU 07	41-012		Uncultured division TM6 bacterium clone NMS8.130WL 16S ribosomal	AY043961	97	384	392
41 OTU 08	41-014		Uncultured phototrophic eukaryote clone PRD18A10 16S ribosomal RNA gene, partial sequence; chloroplast	AY947995	99	401	402
41 OTU 08	41-050	1	Uncultured phototrophic eukaryote clone PRD18A10 16S ribosomal RNA gene, partial sequence; chloroplast	AY947995	99	401	402
41 OTU 08	41-054	0.995	Uncultured phototrophic eukaryote clone PRD18E12 16S ribosomal RN	AY948043	100	402	402
41 OTU 08	41-095	0.981	Uncultured phototrophic eukaryote clone PRD18B08 16S ribosomal RNA	AY948004	98	396	402
41 OTU 09	41-015		Uncultured Verrucomicrobiales clone CR-FL5 16S ribosomal RNA gene,	AF141391	99	398	402
41 OTU 09	41-023	0.995	Uncultured Verrucomicrobiales clone CR-FL5 16S ribosomal RNA gene,	AF141391	98	397	402
41 OTU 09	41-030	1	Uncultured Verrucomicrobiales clone CR-FL5 16S ribosomal RNA gene,	AF141391	98	397	402
41 OTU 10	41-016		Denitrifying bacterium W128b gene for 16S rRNA, partial sequence	AB162100	98	413	418
41 OTU 11	41-017		Uncultured Sphingobacteriales bacterium partial 16S rRNA gene	AJ697703	98	416	422
41 OTU 12	41-018		Uncultured bacterium clone SX2-53 16S ribosomal RNA gene, partial	DQ469224	96	410	425
41 OTU 13	41-025		Uncultured bacterium gene for 16S rRNA, partial sequence, clone:	AB237935	97	410	419

41 OTU 14	41-029		Uncultured bacterium clone JEG.d11 16S ribosomal RNA gene, partial	DQ228399	98	399	404
41 OTU 14	41-062	0.985	Uncultured Bacteroidetes partial 16S rRNA gene, clone TH419	AJ888563	98	417	422
41 OTU 14	41-098	0.992	Uncultured Bacteroidetes partial 16S rRNA gene, clone TH419	AJ888563	99	420	422
41 OTU 15	41-032		Uncultured freshwater bacterium clone 965003E04.x1 16S ribosomal	DQ065321	97	385	396
41 OTU 16	41-033		Unidentified haptophyte OM153 16S ribosomal RNA gene, partial	U70720	96	407	421
41 OTU 17	41-034		Uncultured proteobacterium clone R7C71 16S ribosomal RNA gene,	DQ450172	97	411	423
41 OTU 17	41-100	1	Uncultured proteobacterium clone R7C71 16S ribosomal RNA gene,	DQ450172	97	411	423
41 OTU 18	41-037		Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 16S-AK-W-19	AB237931	97	406	415
41 OTU 19	41-038		Uncultured freshwater bacterium clone 965002A08.y1 16S ribosomal	DQ065225	97	413	422
41 OTU 20	41-039		Uncultured freshwater bacterium clone 965006C01.x1 16S ribosomal	DQ065519	94	400	422
41 OTU 21	41-040		Uncultured bacterium clone HT2E3 16S ribosomal RNA gene, partial	AF418967	100	422	422
41 OTU 21	41-049	0.997	Uncultured bacterium clone HT2E3 16S ribosomal RNA gene, partial	AF418967	100	420	420
41 OTU 21	41-085	1	Uncultured bacterium clone HT2E3 16S ribosomal RNA gene, partial	AF418967	100	422	422
41 OTU 21	41-093	0.997	Uncultured bacterium clone HT2E3 16S ribosomal RNA gene, partial	AF418967	99	421	422

41 OTU 22	41-041		Polaromonas sp. JS666, complete genome	CP000316 AAFQ02000000 AAFQ02000001-AAFQ02000045	100	422	422
41 OTU 23	41-042		Uncultured beta proteobacterium clone 13 16S ribosomal RNA gene,	AF361202	99	419	422
41 OTU 24	41-043		Uncultured bacterium clone 40ds5 16S ribosomal RNA gene, partial	AY212735	99	418	422
41 OTU 25	41-044		Uncultured bacterium clone 28ds5 16S ribosomal RNA gene, partial	AY212721	99	420	422
41 OTU 25	41-061	0.992	Uncultured bacterium clone ES3-27 16S ribosomal RNA gene, partial	DQ444151	100	422	422
41 OTU 26	41-046		Uncultured bacterium gene for 16S rRNA, partial sequence, clone: Sc-EB06	AB193946	95	405	423
41 OTU 27	41-051		Uncultured bacterium clone ML-7-19 16S ribosomal RNA gene, partial	DQ235038	99	421	423
41 OTU 28	41-053		Uncultured beta proteobacterium clone CRE-FL22 16S ribosomal RNA	AF141449	99	401	402
41 OTU 28	41-069	0.99	Uncultured bacterium gene for 16S rRNA, partial sequence, clone:	AB193946	95	405	423
41 OTU 29	41-056		Uncultured bacterium clone Ac90 16S ribosomal RNA gene, partial	AF388352	95	403	423
41 OTU 30	41-057		Uncultured Crater Lake bacterium CL0-64 16S ribosomal RNA gene	AF316650	97	402	413
41 OTU 31	41-065		Uncultured bacterium clone KD6-99 16S ribosomal RNA gene, partial	AY218774	99	419	422
41 OTU 32	41-068		Uncultured proteobacterium clone R7C64 16S ribosomal RNA gene,	DQ450166	98	416	422
41 OTU 33	41-073		Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 16S-AK-W-53	AB237961	100	422	422

41 OTU 34	41-076		Uncultured Firmicute clone PRD01a002B 16S ribosomal RNA gene	AF289150	98	412	419
41 OTU 34	41-086	0.995	Uncultured Actinobacterium partial 16S rRNA gene, clone S9	AJ575510	98	416	422
41 OTU 35	41-079		Uncultured Crater Lake bacterium CL0-64 16S ribosomal RNA gene,	AF316650	99	420	422
41 OTU 36	41-087		Uncultured bacterium clone FS140-151B-02 16S ribosomal RNA gene	DQ513011	95	404	422
41 OTU 37	41-088		Uncultured planctomycete clone 17 16S ribosomal RNA gene, partial	AY902692	96	349	362
41 OTU 38	41-089		Uncultured Cytophagales clone PRD01b007B 16S ribosomal RNA gene,	AF289167	99	416	420
41 OTU 39	41-092		Uncultured bacterium clone EV818SWSAP86 16S ribosomal RNA gene	DQ337097	96	410	423
41 OTU 40	41-101		Uncultured Crater Lake bacterium CL0-93 16S ribosomal RNA gene,	AF316708	95	405	423
41 OTU 41	41-105		Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	100	422	422

APPENDIX K: Library 1-0617

OTU	City Lake Clone ID	City Lake Clone % Identity	GenBank/Blast	Accession #	Identity	# match bp	Total # bp
44 OTU 01	44-1		Flavobacterium sp. GH1-10 16S ribosomal RNA gene, partial sequence	DQ222427	96	394	410
44 OTU 02	44-3		Uncultured bacterium FukuN65 16S rRNA gene.	AJ290001	100	423	423
44 OTU 02	44-4	0.988	Uncultured bacterium FukuS93 16S rRNA gene.	AJ290018	99	421	423
44 OTU 02	44-56	0.99	Uncultured bacterium FukuS93 16S rRNA gene.	AJ290018	99	422	423
44 OTU 03	44-5		Uncultured bacterium partial 16S rRNA gene, clone 54	AM049206	99	310	312
44 OTU 04	44-9		Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	419	427
44 OTU 04	44-23	0.988	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	419	426
44 OTU 04	44-31	0.988	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	419	425
44 OTU 04	44-96	0.99	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	420	425
44 OTU 04	44-102	0.992	Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	98	421	426
44 OTU 05	44-10		Uncultured proteobacterium clone R7C62 16S ribosomal RNA gene,	DQ450171	99	422	423
44 OTU 05	44-28	0.978	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	100	423	423
44 OTU 05	44-39	0.978	Uncultured bacterium clone 300I-B10 small subunit ribosomal RNA	AY662014	100	423	423
44 OTU 05	44-69	0.983	Uncultured proteobacterium clone R7C62 16S ribosomal RNA gene	DQ450171	98	409	414

44 OTU 06	44-11		Uncultured Actinobacterium clone IRD18E05 16S ribosomal RNA gene,	AY947943	97	390	400
44 OTU 07	44-12		Uncultured bacterium clone CEA1 16S ribosomal RNA gene, partial	AF392705	94	401	423
44 OTU 08	44-13		Uncultured beta proteobacteria partial 16S rRNA gene, clone TH480.	AJ888547	95	401	421
44 OTU 09	44-14		Uncultured Verrucomicrobiales bacterium clone TAF-B46 16S ribosomal	AY038743	97	413	423
44 OTU 10	44-17		Uncultured bacterium gene for 16S rRNA, partial sequence, clone 16S-AK-W-23	AB237935	93	378	405
44 OTU 11	44-18		Uncultured soil bacterium clone M22_Pitesti 16S ribosomal RNA gene,	DQ378242	98	418	423
44 OTU 11	44-41	0.992	Uncultured soil bacterium clone M22_Pitesti 16S ribosomal RNA gene,	DQ378242	98	418	423
44 OTU 12	44-20		Uncultured bacterium clone ES3-27 16S ribosomal RNA gene, partial	DQ444151	99	416	418
44 OTU 12	44-40		Uncultured bacterium clone ES3-27 16S ribosomal RNA gene, partial	DQ444151	99	420	423
44 OTU 12	44-42		Uncultured bacterium clone ES3-27 16S ribosomal RNA gene, partial	DQ444151	99	420	423
44 OTU 12	44-44		Uncultured bacterium FukuS183 partial 16S rRNA gene.	AJ290052	99	421	423
44 OTU 12	44-95		Uncultured bacterium clone 28ds5 16S ribosomal RNA gene, partial	AY212721	99	422	423
44 OTU 13	44-21		Uncultured proteobacterium clone R7C54 16S ribosomal RNA gene,	DQ450165	99	338	341
44 OTU 14	44-22		Uncultured freshwater bacterium clone 965004A02.x1 16S ribosomal	DQ065341	95	405	422

44 OTU 15	44-24		Uncultured bacterium partial 16S rRNA gene, clone S306R	AM158355	98	416	423
44 OTU 15	44-46	0.985	Uncultured bacterium partial 16S rRNA gene, clone S306R.	AM158355	98	416	423
44 OTU 15	44-73	0.983	Uncultured bacterium partial 16S rRNA gene, clone S306R.	AM158355	98	416	423
44 OTU 16	44-25		Uncultured bacterium partial 16S rRNA gene, clone R2_14	AJ876700	99	422	423
44 OTU 16	44-49	0.976	Uncultured bacterium partial 16S rRNA gene, clone R2_14	AJ876700	98	416	423
44 OTU 17	44-26		Uncultured bacterium FukuS93 16S rRNA gene	AJ290018	95	399	416
44 OTU 18	44-27		Uncultured Flavobacterium sp. clone GOBB3-CL208 16S ribosomal RNA	AF388900	98	372	377
44 OTU 18	44-52	0.983	Uncultured Flavobacterium sp. clone GOBB3-CL208 16S ribosomal RNA	AF388900	98	371	376
44 OTU 19	44-29		Uncultured Sphingobacteriales bacterium partial 16S rRNA gene	AJ697703	98	416	424
44 OTU 20	44-32		Uncultured bacterium clone ES3-22 16S ribosomal RNA gene, partial	DQ444146	96	378	393
44 OTU 21	44-35		Uncultured bacterium clone RA13C6 16S ribosomal RNA gene, partial	AF407405	99	419	423
44 OTU 22	44-36		Uncultured bacterium clone HT2E3 16S ribosomal RNA gene, partial	AF418967	98	412	419
44 OTU 22	44-37	0.976	Uncultured bacterium clone HT2E3 16S ribosomal RNA gene, partial	AF418967	99	421	423
44 OTU 23	44-37		Uncultured bacterium clone HT2E3 16S ribosomal RNA gene, partial	AF418967	99	421	423
44 OTU 23	44-57	0.978	Uncultured bacterium clone HT2E3 16S ribosomal RNA gene, partial	AF418967	98	416	423
44 OTU 24	44-45		Uncultured Actinobacterium partial 16S rRNA gene, clone R5.	AJ575501	99	421	423

44 OTU 25	44-56		Uncultured bacterium FukuS93 16S rRNA gene	AJ290018	99	422	423
44 OTU 26	44-66		Uncultured freshwater bacterium clone 965003E04.x1 16S ribosomal	DQ065321	99	421	423
44 OTU 27	44-67		Uncultured eubacterium WR878 partial 16S rRNA, clone WR878.	AJ292846	96	407	422
44 OTU 28	44-73		Uncultured bacterium partial 16S rRNA gene, clone S306R	AM158355	98	416	423
44 OTU 29	44-74		Uncultured bacterium clone HF770_E7_P1 16S ribosomal RNA gene	DQ300918	92	363	392
44 OTU 30	44-75		Uncultured Actinobacterium partial 16S rRNA gene, clone S7	AJ575508	97	409	420
44 OTU 31	44-76		Uncultured Crater Lake bacterium CL0-64 16S ribosomal RNA gene	AF316650	99	420	423
44 OTU 32	44-77		Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 16S-AK-W-23	AB237935	97	405	414
44 OTU 33	44-78		Uncultured beta proteobacteria partial 16S rRNA gene, clone TH480	AJ888547	96	412	425
44 OTU 33	44-85	0.995	Uncultured proteobacterium clone R7C71 16S ribosomal RNA gene	DQ450172	97	411	423
44 OTU 34	44-82		Bacterium m4 16S ribosomal RNA gene, partial sequence	DQ453813	100	423	423
44 OTU 34	44-94	0.995	Bacterium m4 16S ribosomal RNA gene, partial sequence	DQ453813	99	423	424
44 OTU 35	44-84		Methylobacter psychrophilus 16S ribosomal RNA, complete sequence	AF152597	95	403	423
44 OTU 36	44-87		Uncultured phototrophic eukaryote clone PRD18E12 16S ribosomal RNA	AY948043	98	395	403

44 OTU 37	44-88	Uncultured phototrophic eukaryote clone PRD18E12 16S ribosomal RNA gene, partial sequence; chloroplast.	AY948043	98	424	432
44 OTU 38	44-89	Uncultured bacterium clone BG.d1 16S ribosomal RNA gene, partial	DQ228371	98	418	423
44 OTU 39	44-91	Uncultured Bacteroidetes bacterium clone M13-97 16S ribosomal RNA	AY338069	99	401	404
44 OTU 40	44-92	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 16S-AK-W-23	AB237935	97	408	417
44 OTU 41	44-93	Uncultured freshwater bacterium clone 965002G07.x1 16S ribosomal	DQ065280	99	421	423
44 OTU 42	44-95	Uncultured bacterium clone 28ds5 16S ribosomal RNA gene, partial	AY212721	99	422	423
44 OTU 43	44-98	Uncultured bacterium clone 44ds5 16S ribosomal RNA gene, partial	AY212739	94	400	423
44 OTU 44	44-99	Uncultured bacterium clone PH10-20 16S ribosomal RNA gene, partial	DQ444057	98	418	423
44 OTU 45	44-101	Uncultured bacterium gene for 16S rRNA, partial sequence, clone	AB240330	98	411	419
44 OTU 46	44-106	Uncultured Crater Lake bacterium CL500-17 16S ribosomal RNA gene	AF316660	92	387	419

APPENDIX L: Library 1-1200

OTU	City Lake Clone ID	City Lake Clone % Identity	GenBank/Blast	Accession #	Identity	# match bp	Total # bp
47 OTU 01	47-3		Uncultured bacterium partial 16S rRNA gene, clone C8	AJ867920	98	394	400
47 OTU 01	47-4	0.99	Uncultured bacterium partial 16S rRNA gene, clone C8	AJ867920	99	396	400
47 OTU 01	47-7	1	Uncultured bacterium partial 16S rRNA gene, clone C8	AJ867920	98	394	400
47 OTU 01	47-21	0.99	Uncultured bacterium partial 16S rRNA gene, clone C8	AJ867920	99	396	400
47 OTU 01	47-64	1	Uncultured bacterium partial 16S rRNA gene, clone C8	AJ867920	98	394	400
47 OTU 01	47-75	1	Uncultured bacterium partial 16S rRNA gene, clone C8	AJ867920	98	394	400
47 OTU 02	47-6		Uncultured Actinobacterium partial 16S rRNA gene, clone NO7	AJ575559	98	412	419
47 OTU 03	47-8		Uncultured beta proteobacterium clone 08 16S ribosomal RNA gene,	AF361201	99	420%	422
47 OTU 03	47-83	0.983	Uncultured freshwater bacterium clone 965004A02.x1 16S ribosomal	DQ065341	99	420%	422
47 OTU 03	47-103	0.985	Uncultured freshwater bacterium clone 965015B06.x1 16S ribosomal	DQ064823	99	420	422
47 OTU 04	47-9		Uncultured phototrophic eukaryote clone JL-WNPG-T36 16S ribosomal RNA gene, partial sequence; chloroplast	AY664132	96	209	216
47 OTU 05	47-11		Environmental clone OCS20 small subunit ribosomal RNA gene chloroplast gene for chloroplast RNA, complete sequence	AF001654	98	416	422
47 OTU 06	47-14		Uncultured bacterium clone B01R 16S ribosomal RNA gene, partial	AY395097	95	401%	422

47 OTU 06	47-61	1	Uncultured bacterium clone B01R 16S ribosomal RNA gene, partial	AY395097	95	401	422
47 OTU 07	47-16		Uncultured Flexibacteraceae bacterium clone M13-16 16S ribosomal	AY338094	97	393	403
47 OTU 07	47-39	0.99	Uncultured Flexibacteraceae bacterium clone M13-16 16S ribosomal	AY338094	98	397	403
47 OTU 08	47-18		Uncultured Cyanobacterium clone M13-140 16S ribosomal RNA gene	AY338151	98	388	395
47 OTU 09	47-19		Uncultured beta proteobacterium clone CRE-FL22 16S ribosomal RNA	AF141449	99	398	402
47 OTU 10	47-20		Uncultured Actinobacterium clone ST11-10 16S ribosomal RNA gene	DQ316356	99	419	422
47 OTU 11	47-22		Uncultured bacterium gene for 16S rRNA, partial sequence, clone: RB423	AB240370	96	406	422
47 OTU 12	47-23		Cyanobacterium sp. IW11 partial 16S rRNA gene	AJ565868	98	397	402
47 OTU 13	47-24		Uncultured beta proteobacterium clone CRE-FL50 16S ribosomal	AF141469	99	401	402
47 OTU 14	47-25		Uncultured freshwater bacterium clone 965002G07.x1 16S ribosomal	DQ065280	95	324	340
47 OTU 15	47-26		Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 16S-AK-W-40	AB237948	99	419	422
47 OTU 16	47-27		Uncultured planctomycete clone LiUU-5-456 16S ribosomal RNA gene,	AY509491	91	349	380
47 OTU 16	47-47	1	Uncultured planctomycete clone LiUU-5-456 16S ribosomal RNA gene	AY509491	91	349	380
47 OTU 16	47-55	1	Uncultured planctomycete clone LiUU-5-456 16S ribosomal RNA gene	AY509491	91	349	380

47 OTU 17	47-30		Uncultured bacterium partial 16S rRNA gene, clone S306R	AM158355	97	413	422
47 OTU 18	47-31		Unidentified haptophyte OM153 16S ribosomal RNA gene, partial	U70720	96	405	421
47 OTU 19	47-32		Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 16S-AK-W-23	AB237935	97	409	418
47 OTU 19	47-42	0.99	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 16S-AK-W-23	AB237935	97	410	419
47 OTU 19	47-92	0.985	Unidentified bacterium partial 16S rRNA gene, clone Sai1P1-86	AJ518639	99	384	383
47 OTU 20	47-34		Uncultured delta proteobacterium clone AL19 16S ribosomal RNA	AY455847	96	406	422
47 OTU 21	47-35		Uncultured bacterium gene for 16S rRNA, partial sequence, clone: RB028	AB240288	96	407	422
47 OTU 22	47-36		Uncultured bacterium clone HTH6 16S ribosomal RNA gene, partial sequenceAF418965	AF418965	98	417	422
47 OTU 23	47-38		Uncultured bacterium partial 16S rRNA gene, clone R2_14	AJ876700	99	421	422
47 OTU 23	47-41	0.99	Uncultured bacterium clone 005C-F09 small subunit ribosomal RNA	AY661953	99	421	423
47 OTU 23	47-43	0.995	Uncultured bacterium clone 005C-F09 small subunit ribosomal RNA	AY661953	99	421	422
47 OTU 23	47-84	0.983					
47 OTU 24	47-40		Uncultured Crater Lake bacterium CL0-64 16S ribosomal RNA gene	AF316650	99	420	422
47 OTU 24	47-72	0.99	Uncultured bacterium clone ML-7-6 16S ribosomal RNA gene, partial	DQ235030	98	416	422
47 OTU 25	47-44		Uncultured bacterium clone HT2E3 16S ribosomal RNA gene, partial	AF418967	98	416	422
47 OTU 25	47-45	0.983	Uncultured bacterium clone HT2E3 16S ribosomal RNA gene, partial	AF418967	100	422	422

47 OTU 25	47-46	0.983	Uncultured bacterium clone HT2E3 16S ribosomal RNA gene, partial	AF418967	100	422	422
47 OTU 25	47-62	0.981	Uncultured bacterium clone HT2E3 16S ribosomal RNA gene, partial	AF418967	99	421	423
47 OTU 26	47-49		Uncultured bacterium FukuS36 partial 16S rRNA gene	AJ290074	99	421	423
47 OTU 26	47-54	0.985	Uncultured bacterium clone 28ds5 16S ribosomal RNA gene	AY212721	99	421	422
47 OTU 27	47-53		Uncultured bacterium DSSD97 16S ribosomal RNA gene, partial sequence	AY328794	96	407	422
47 OTU 27	47-67	0.983	Uncultured bacterium 16S rRNA gene, clone RB90b-424	AM159467	97	393	404
47 OTU 28	47-57		Flavobacterium succinicans partial 16S rRNA gene, strain DSM 4003	AM230493	97	405	417
47 OTU 29	47-58		Uncultured Flexibacteraceae bacterium clone M13-141 16S ribosomal	AY338095	99	401	402
47 OTU 29	47-59	1	Uncultured Flexibacteraceae bacterium clone M13-141 16S ribosomal	AY338095	99	401	402
47 OTU 30	47-68		Uncultured bacterium clone HT2E3 16S ribosomal RNA gene,	AF418967	95	402	422
47 OTU 31	47-70		Uncultured Firmicute clone PRD01a002B 16S ribosomal RNA gene	AF289150	98	412	419
47 OTU 31	47-82	0.985	Uncultured Actinobacterium partial 16S rRNA gene, clone S9	AJ575510	97	412	422
47 OTU 31	47-85	1	Uncultured Firmicute clone PRD01a002B 16S ribosomal RNA gene,	AF289150	98	412	419
47 OTU 32	47-77		Uncultured bacterium clone KD2-45 16S ribosomal RNA gene, partial	AY218584	98	412	420
47 OTU 32	47-86	0.988	Uncultured bacterium clone KD2-45 16S ribosomal RNA gene, partial	AY218584	98	415	423
47 OTU 32	47-88	0.99	Uncultured bacterium clone KD2-45 16S ribosomal RNA gene, partial	AY218584	98	416	423

47 OTU 32	47-89	0.983	Uncultured bacterium clone KD2-45 16S ribosomal RNA gene, partial	AY218584	97	415	424
47 OTU 32	47-96	0.99	Uncultured bacterium clone KD2-45 16S ribosomal RNA gene	AY218584	98	416	423
47 OTU 33	47-79		Methylobacter sp. LW12 16S ribosomal RNA gene, partial sequence	AY007295	96	409	422
47 OTU 34	47-84		Uncultured bacterium gene for 16S rRNA, partial sequence, clone RB308	AB240330	99	420	422
47 OTU 35	47-90		Uncultured beta proteobacterium clone BRIC47 16S ribosomal RNA	DQ294023	96	407	423
47 OTU 36	47-94		Uncultured bacterium clone D01ST 16S ribosomal RNA gene	AY395128	94	397	422
47 OTU 37	47-100		Uncultured Burkholderiales bacterium clone DS037 16S ribosomal	DQ234121	98	417	422
47 OTU 38	47-101		Uncultured Bacteroidetes bacterium clone M13-97 16S ribosomal	AY338069	98	394	400

Appendices B-L list City Lake clones and their % similarity. GenBank's BLASTn was used to find the nearest matching sequence to the City Lake clone and that match was included. GenBank accession numbers were also included as a reference aid. The last three columns provide BLASTn % identity to City Lake clones and the number of matching nucleotides.