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ORIGINAL ARTICLE

Phenology of high-elevation pelagic bacteria: the roles of meteorologic variability, catchment inputs and thermal stratification in structuring communities

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Many eukaryotic communities exhibit predictable seasonality in species composition, but such phenological patterns are not well-documented in bacterial communities. This study quantified seasonal variation in the community composition of bacterioplankton in a high-elevation lake in the Sierra Nevada of California over a 3-year period of 2004–2006. Bacterioplankton exhibited consistent phenological patterns, with distinct, interannually recurring community types characteristic of the spring snowmelt, ice-off and fall-overturn periods in the lake. Thermal stratification was associated with the emergence of specific communities each summer and increased community heterogeneity throughout the water column. Two key environmental variables modulated by regional meteorologic variation, lake residence time and thermal stability, predicted the timing of occurrence of community types each year with 75% accuracy, and each corresponded with different aspects of variation in community composition (orthogonal ordination axes). Seasonal variation in dissolved organic matter source was characterized fluorometrically in 2005 and was highly correlated with overall variation in bacterial community structure ($r_{Mantel} = 0.75$, $P < 0.001$) and with the relative contributions of specific phylotypes within the Cyanobacteria, Actinobacteria and β-Proteobacteria. The seasonal dynamics of bacterial clades (tracked through coupling of randomized clone sequence libraries to restriction fragment length polymorphism fingerprints) matched previous results from alpine lakes and were variously related to solute inputs, thermal stability and temperature. Taken together, these results describe a phenology of high-elevation bacterioplankton communities linked to climate-driven physical and chemical lake characteristics already known to regulate eukaryotic plankton community structure.
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Introduction

Predicting the impacts of environmental change on ecosystem processes requires a theoretical and practical understanding of relationships among the structure of ecological communities, ecosystem processes and the abiotic environment. Prokaryotes and other microorganisms have an important function in many fundamental biogeochemical processes (Fuhrman *et al.*, 1989; Conrad, 1996; Cotner and Biddanda, 2002), and microbial community structure is linked to the rates and dynamics of these processes (Cottrell and Kirchman, 2000; Covert and Moran, 2001; Carlson *et al.*, 2002). Ecological theory guides research into the relationships between community structure and function, but the overwhelming genotypic and phenotypic diversity of microorganisms in natural environments confounds efforts to ascribe macroorganismal concepts of community assembly to the microbial world (Finlay, 2002; Prosser *et al.*, 2007).

Phenology, the timing of periodic biological phenomena generally linked to abiotic or climatic factors, is a trait characteristic of many natural communities. Although seasonal changes in environmental factors are likely to generate predictable seasonality in microbial community structure, there have been few studies examining the nature and drivers of microbial phenology. Furthermore, anthropogenic changes in climate and land use may alter seasonal changes in abiotic conditions and microbial communities with consequences for

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biodiversity and ecosystem function, making an understanding of microbial phenology relevant to the evaluation of human impacts on ecosystems (Chapin *et al.*, 2000; Walther *et al.*, 2002).

The enormous diversity, small size, large populations, rapid generation times, high probability of dispersal, functional redundancy and genomic/phenotypic plasticity of bacteria can produce a remarkable degree of intra- and interannual variability in community composition (Ochman *et al.*, 2000; Curtis and Sloan, 2004). Most studies that have examined the seasonal dynamics of prokaryotic communities have reported significant temporal trajectories across seasons, but incomplete or inconsistent interannual cycles (Lindstrom, 1998; Crump *et al.*, 2003; Stepanauskas *et al.*, 2003; Yannarell *et al.*, 2003; Kent *et al.*, 2004). Perhaps unsurprisingly, recent efforts to quantify synchronous temporal variation in spatially segregated microbial communities from similar environments have met with some success, likely due, in part, to the homogenization of regional climatic variability among study sites (Crump and Hobbie, 2005; Kent *et al.*, 2007). A suite of more recent studies of lake, stream, estuarine, coastal and open ocean environments has shown recurring interannual patterns in bacterioplankton community structure by relying on high-frequency, multi-year data sets (Morris *et al.*, 2005; Fuhrman *et al.*, 2006; Hullar *et al.*, 2006; Kan *et al.*, 2006; Shade *et al.*, 2007).

Examining the temporal dynamics of bacterial community composition in isolated resource-poor environments with strong seasonality can limit many of the potential sources of variability in prokaryotic community structure, allowing a clear and detailed understanding of microbial phenology. High-elevation lakes are ideal systems for this approach because they are well-constrained, dilute, unproductive environments remote from human influence. In addition, these systems have large and relatively predictable seasonal variations in nutrient availability, primary production, external inputs and physical properties (Melack *et al.*, 1997). High-elevation and high-latitude ecosystems are recognized as early-warning systems for detecting ecological responses to global environmental changes because of their sensitivity to anthropogenic disturbance and regional climatic shifts (Beniston *et al.*, 1997; Melack *et al.*, 1997; Carrera *et al.*, 2002; Fenn *et al.*, 2003; Sickman *et al.*, 2003b; Morales-Baquero *et al.*, 2006b). Montane lakes worldwide have become increasingly affected by the atmospheric deposition of nutrients and pollutants (Psenner, 1999), factors that directly influence the metabolism and community structure of bacteria (Cotner *et al.*, 1997; Carlson *et al.*, 2002; Haukka *et al.*, 2006). Biogeochemical and food web processes in oligotrophic aquatic environments such as these are predicted to be dominated by microbial metabolism (Biddanda *et al.*, 2001), indicating that understanding the role of prokaryotes in these environments is relevant to predicting system-level ecological responses to environmental change. Finally, high-elevation and high-latitude systems display large temporal changes in the source and composition of dissolved organic matter, a fundamental link between ecosystem function and the community structure of heterotrophic bacteria, making them ideal systems in which to examine this key relationship (Crump *et al.*, 2003; Hood *et al.*, 2003; Judd *et al.*, 2006; Perez and Sommaruga, 2006).

The study detailed here examined bacterial community composition over a 3-year period throughout the water column of a high-elevation cirque lake in the Sierra Nevada of California (USA). PCR-based community fingerprinting methods were combined with the construction of multiple randomized clone-sequence libraries to provide estimates of the relative abundance of specific bacterial phylotypes through time and space, as well as to relate bacterial community composition to a variety of measured physical and biogeochemical variables. The results demonstrate a predictable phenology of bacterial community structure in Emerald Lake related to lake physical and chemical parameters. Clone library analysis permitted estimation of the diversity of, and phylogenetic differences in, the bacterial community across seasons. The temporal dynamics of bacterial community structure were linked to interannual variation in weather as well as seasonal transitions in catchment inputs and lake thermal structure, supporting evidence for fundamental linkages among organic matter composition, nutrient availability, climatic forcing and the structure of bacterial communities.

Materials and methods

Research location

This research was conducted at Emerald Lake, a headwater cirque lake located within Sequoia National Park on the western slope of the south central Sierra Nevada (California, CA, USA, 36°35′49″N, 118°40′30″W). The lake, situated at 2800 m above sea level in a sparsely forested granitic catchment, is representative of the >4000 high-elevation (>2500 m above sea level) lakes scattered throughout the Sierra Nevada in size (2.7 ha, 10.5 m z_{max}), solute chemistry and catchment composition (median slope 31%; surface area 75% exposed granitic and granodioritic rock, 19% montane meadow, 3% sparse conifer, 3% water; Melack and Stoddard, 1991). The lake has been studied for several decades, and its hydrology, biogeochemistry and ecology are well characterized (Sickman *et al.*, 2003b). Emerald Lake is remote, accessible only by an 8 km hike (with 1 km elevation gain) from the nearest road. The lake has a single main inlet and several smaller inlets all of which carry terrestrial runoff. Emerald Lake is dimictic and typically snow-

covered from November to June, with the timing of ice-off ranging from mid-May to late July depending on the size of the snowpack and the timing of spring thaw. Spring mixing is brief, lasting about a week following ice-off, whereas fall mixing may continue for up to 2 months before the lake ices over. Metalimnetic temperatures range from 4 to 15 °C and epilimnetic temperatures range from 0 to 22 °C. During summer stratification, the metalimnion and thermocline generally begin between 4 and 7 m depth and extend to the bottom of the lake. Snowmelt flushing of nitrate is characteristic of catchments in the Sierra Nevada (Sickman *et al.*, 2003a), and concentrations within Emerald Lake may range over two orders of magnitude (0.1–10 μmol l^{-1}) throughout the growing season as snowmelt progresses.

Sample collection and processing

The lake was sampled approximately biweekly throughout the ice-free periods of 2004 and 2005 and three times in 2006. Several samples were collected midwinter 2004–2005 and during the ice-covered snowmelt period in 2005 and 2006. Midday on each sampling date I collected 3–6 l of water for chemical analyses from four depths in the center of the lake and from the inlet through a hand-cranked peristaltic pump through platinum-cured silicone tubing into collapsible 10 l low-density polyethylene carboys (Reliance Products 250013). Samples collected and parameters measured included the following: particulate carbon, nitrogen and reactive phosphorus; total dissolved nitrogen (TDN) and phosphorus; dissolved organic carbon (DOC); dissolved inorganic nitrate/nitrite (DIN); soluble reactive phosphorus (SRP); dissolved fluorescence (fluorescence index (FI), detailed below); and concentrations of chlorophyll a. I collected an additional water sample from each depth and location in polycarbonate bottles for DNA analysis. Samples were filtered and preserved on site and stored in the dark at ambient temperature (<15 °C) for 24 h during transport to cold storage facilities. All plasticware were acid-washed and rinsed between sampling events and triple-rinsed with sample water before collection.

I collected particulates by gentle in-line gravity filtration of ~450 ml of sample water onto combusted (2 h, 450 °C) 47-mm Whatman GF/F filters (nominal pore size 0.7 μm) held in polycarbonate filter cartridges. One filter was used for chlorophyll analysis, a second for particulate carbon and particulate nitrogen, and a third for particulate reactive phosphorus, each folded closed, wrapped in combusted aluminium foil and frozen (−20 °C) upon return to the laboratory. Dissolved nutrients (TDN, total dissolved phosphorus, DIN and SRP) were analyzed from GF/F filtrate collected in new sample-rinsed HDPE bottles (Nalgene brand, Thermo Fisher Scientific, Rochester, NY, USA). DOC and dissolved fluorescence were analyzed from GF/F filtrate collected in acid-washed, combusted (12 h at 450 °C), amber glass EPA vials (I-Chem brand, Thermo Fisher Scientific, Rochester, NY, USA) with Teflon-coated septa immediately amended with concentrated acid (to ~20 μmol l^{-1} HCl) and stored frozen (−20 °C) upon return to the laboratory. I collected DNA samples by filtering ~400 ml of whole water sample onto a 0.22-μm pore size polyethersulfone filter cartridge (Millipore Sterivex SVGP01050). After filtering, the cartridge was immediately emptied of excess water, filled with sucrose lysis buffer (40 mmol l^{-1} EDTA, 50 mmol l^{-1} Tris-HCl, 750 mmol l^{-1} sucrose, pH adjusted to 8.0), sealed with parafilm, stored at an ambient temperature overnight and frozen (−80 °C) on return to the laboratory (tests comparing terminal restriction fragment length polymorphism (TRFLP) fingerprints between samples immediately frozen and those held at an ambient temperature for 1–4 days were not significantly different and exhibited Sorensen distances of <0.05).

At each sampling time, I measured vertical profiles of dissolved oxygen and temperature at 50 cm intervals with an elevation-calibrated, battery-operated, handheld meter (YSI 55). Magnitude and temperature of discharge from the lake were monitored continuously using a calibrated weir and pressure transducer with temperature sensor, and hydraulic residence time on each day was calculated as the number of summed preceding discharge days ($m^3\,d^{-1}$) required to equal the lake volume ($1.8 \times 10^5\,m^3$). Lake thermal stability was calculated from thermal profiles according to Schmidt (1928) following the modifications of Idso (1973).

Chemical analytical procedures

Particulate carbon and particulate nitrogen were analyzed through double-drop GF/F combustion on an organic elemental analyzer (Control Equipment Corporation Model 440HA) by the UCSB Marine Science Institute Analytical Laboratory. I measured concentrations of particulate chlorophyll a by dark-digesting filters for 24–48 h in 90% acetone at −20 °C followed by 10 m dark centrifugation and immediate fluorescence analysis on a Turner AU-10 fluorometer (Smith *et al.*, 1981). DIN and SRP were measured by the UCSB Marine Science Institute Analytical Laboratory on a Lachat Instruments Quickchem 8000 flow injection autoanalyzer. TDN and total dissolved phosphorus were measured as DIN and SRP from GF/F filtrate following digestion (10 ml sample amended with oxidation reagent and autoclaved according to Valderrama, 1981), neutralization with NaOH and subtraction of digested double-distilled water blanks. Particulate reactive phosphorus was analyzed by digesting GF/F filters in 30 ml double-distilled water amended as for TDN/P above and measuring SRP after filter-blank subtraction to correct for silicate interference. Dissolved organic nitrogen (DON) was calculated

as the difference between TDN and DIN, and total phosphorus (TP) was calculated as the sum of particulate reactive phosphorus and total dissolved phosphorus. I measured DOC concentrations on a Shimadzu TOC-V adapted to increase precision in the range of 5–100 μmol l^{-1} (Carlson *et al.*, 2004).

Fluorescence index was used to determine seasonal variation in the relative influence of terrestrial and lake-derived sources of dissolved organic matter (DOM) (McKnight *et al.*, 2001). The index was calculated as the ratio of emission intensity at 450 nm to emission intensity at 500 nm under excitation at 370 nm after subtraction of a double-distilled water water blank measured on a Shimadzu RF-1501 spectrofluorometer in the laboratory of JO Sickman at the University of Florida, Gainesville, FL, USA. This metric estimates the relative contribution of allochthonous (terrestrially derived) and autochthonous (phytoplankton-derived) fulvic acids to the dissolved organic pool of surface waters and has been employed successfully in high-elevation lakes and streams in the Rocky Mountains, USA (Hood *et al.*, 2003).

Bacterial community fingerprinting

TRFLP was used as a fingerprinting method for comparison of bacterial community composition among DNA samples (Liu *et al.*, 1997). Sterivex capsules were thawed and amended with proteinase K (0.2 mg ml^{-1}), sodium dodecyl sulfate (1% w/v) and lysozyme (0.5 mg ml^{-1}) at 37 °C for 30 min followed by 55 °C for 2–12 h and extracted immediately. Genomic DNA extraction was performed using Qiagen DNEasy centrifuge columns. Extracted DNA was amplified by PCR as follows: a reaction mix consisting of 2.5 mmol l^{-1} $MgCl_2$, 200 μmol l^{-1} dNTP mix, 5% Acetamide, 4–14% template (~10 ng), 10 μl mineral oil, 1 U Taq DNA Polymerase in buffer and 200 nmol l^{-1} each of primers 27F (AGAGTTTGATCMTGGCT, FAM-labeled) and 519R (GWATTACCGCGGCKGCTG, unlabeled) was subjected to thermocycling consisting of 3 min initial denaturation at 94 °C, 30 cycles of 1 min 94 °C denaturation followed by 1 min 58 °C annealing followed by 2 min 72 °C extension and 10 min final extension at 72 °C before being held at 4 °C until further processing. PCR products were cleansed of small-fragment DNA using the Qiagen QiaQuick DNA Cleanup Kit and then digested for 4 h at 37 °C with restriction enzyme *Hae*III (New England Biolabs, Ipswich, MA, USA) followed by 20 min enzyme inactivation at 80 °C and storage at 4 °C.

Digested products were analyzed on an Applied Biosystems ABI 310 sequencer using GeneScan Software. A size standard (MapMarker 1000) was run with every sample to define the standard curve between 25 and 600 bp, and the Global Southern curve-fitting algorithm was used to calculate bp lengths of identified fluorescence peaks. The peak identification cutoff for relative fluorescence units (rfu) was typically 50 rfu, the minimum peak half-width was three data points and the typical range of quantified peaks was 300–3000 rfu. Samples were completely reprocessed if the bulk of the peaks in the electropherogram were below 500 rfu.

Terminal restriction fragment length polymorphism data from all samples were aligned using a novel frequency-based alignment method. This method avoids the inaccuracies associated with common approaches to 'binning' fragment lengths using integer rounding or truncation methods (Figure 1, see also Hewson and Fuhrman, 2006). In RFLP analyses, GeneScan fragment lengths are calculated using size calculation algorithms derived from simultaneous electrophoresis of a size standard and do not exhibit consistent rounded integer lengths either in theory or in practice. The frequency-based alignment method employed here relies on comparison of samples among a large environmental data set to identify and group electropherogram peaks based on frequency distributions among samples (Figure 1). Briefly, fragment-length data from the entire data set were pooled and sorted by length. Fragments within the length ranges where multiple samples exhibit dominant electrophoretic fluorescence peaks were assigned integer lengths based on the mean and variance of each group and the size of the gap between regions of higher sample density. Distributional gaps between named groups, which differ by <1 bp, were further delineated using samples that contain both fragments under the assumption that individual samples are unlikely to display two peaks 1 bp apart if only one fragment is present (the phenomenon of 'shouldering' rarely extends a full 1-bp distance). In the case of the present data set, fragments were defined by delineating frequency distributions by 0.05 bp gaps from a data set of all peaks comprising >1% total within-sample electropherogram area (Figure 1). Smaller peaks of 0.5% or greater sample electropherogram area, which fell within 1 s.d. of a group mean, were added *post facto*.

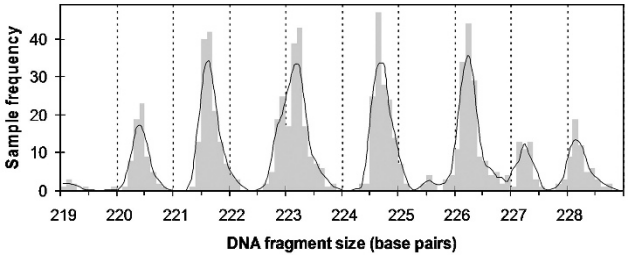

Figure 1 Frequency histogram of representative GeneScan-calculated fragment lengths derived from TRFLP analysis of 16S rDNA collected from Emerald Lake, 2004–2006. The line is a three-point moving average. Note that binning fragments through integer rounding or truncation (integers denoted by dashed lines) will produce innaccurate groupings, whereas the binning method employed in this study can clearly group similar fragments into phylotypes using frequency distributions derived from comparing samples within a larger data set. TRFLP, terminal restriction fragment length polymorphism.

Bacterial clone library construction

To ascribe terminal restriction fragment lengths to specific bacterial phylotypes, five clone libraries (96 clones each) were constructed from environmental samples drawn from the lake in 2005. Libraries were randomly generated from 2-m depth samples on three dates (6 June 2005, 31 July 2005 and 18 September 2005) to capture the three interannually recurring TRFLP community types, and one library was generated from the inlet on 6 June 2005. Duplicate libraries were generated by separate cloning procedures from the 2-m depth DNA sample on 31 July 2005 to allow statistical reference comparison of identical libraries (see Supplementary Information).

DNA from these environmental samples was extracted using identical methods as above and duplicate 100 μl PCRs were carried out as above except without an FAM tag on the 27F primer. PCR products were concentrated twofold using QiaQuick columns, then gel-extracted and further concentrated to 30 μl with a second QiaQuick column. PCR products were then ligated into the pGEM-t plasmid cloning vector (Promega, Madison, WI, USA) and transformed into competent *Escherichia coli* DH5α according to the vendor's guidelines. Transformants were plated onto LB/Amp (100 μg ml^{-1})/X-Gal/IPTG plates, randomly selected for sequencing by white/blue screening, and sequenced by unidirectional rolling-circle PCR by the University of Washington High Throughput Sequencing Facility.

Sequences were collated and trimmed to the primer region using the BioEdit program (Hall, 1999), aligned using the NAST aligner (DeSantis *et al.*, 2006a), classified using maximum likelihood algorithms to the GreenGenes 16S rDNA database (DeSantis *et al.*, 2006b) and checked for chimeras using Bellerophon 1.3 (Huber *et al.*, 2004). Putative chimeras and sequences classified as plastids were excluded from further analyses, the library was dereplicated into unique phylotypes using FASTGROUPII (Yu *et al.*, 2006) at a level of 97 percent sequence identity, and the remaining sequences were digested *in silico* using the Sequence Manipulation Suite software (Stothard, 2000).

As *in silico* TRF lengths frequently differ from measured GeneScan TRF links because of minor instrument and PCR error (Morris *et al.*, 2005), a subset of 25 clones representing unique *in silico* restriction lengths was analyzed by TRFLP (as above except plasmid DNA was extracted using Qiagen Miniprep columns rather than DNEasy) using the duplicate pooling approach detailed by Grant and Ogilvie (2004). A linear regression ((GeneScan bp length) = 1.018 (*in silico* bp length) − 7.583; $r^2 = 0.99$; range 39–517 bp) was used to correct GeneScan-estimated TRF lengths from experimental samples and representative clones were ascribed to each matching TRF length (Stepanauskas *et al.*, 2003; Brown *et al.*, 2005; Morris *et al.*, 2005).

Data analysis

Bacterial community composition data for all TRFLP samples were collated using relative abundance data (estimated as within-sample relativized eletropherogram peak area for each fragment; Yannarell and Triplett, 2005) after removal of all the fragments less than 150 bp (due to size-calling algorithm inaccuracies associated with smaller fragment lengths). Multivariate community distances were calculated as the Sørenson dissimilarity index (aka Bray-Curtis or Percent Dissimilarity, calculated $1-(2W/(A+B))$ where W is the sum of shared relative abundances) A and B are the sums of relative abundances in individual sample units; (Sørensen, 1948)) using PC-ORD software (McCune and Mefford, 2006). Samples were clustered using the flexible-β algorithm (Figure 2; $\beta = -0.25$) in PC-ORD and divided into groups using a distance cutoff selected to minimize number of groups, minimize within-group distances and maximize variance explained (estimated using the 'elbow criteria' in a graph relating variance explained to the number of groups). Nonmetric multidimensional scaling (NMS) was employed to ordinate data in a lower dimensional space using PC-ORD software. The ordination was forced to a two-dimensional solution to enhance interpretability, and bacterial community data from terrestrial inlet samples were excluded from the ordination because their relatively high dissimilarity from lake samples across years concealed variability among lake samples.

A phylogenetic tree was constructed from the pooled clone library sequences to characterize the taxonomic relatedness of bacterioplankton across seasons. Aligned sequences of the three most similar cultured and uncultured sequences to each Emerald Lake clone and in the GreenGenes prokMSA database (along with a single Archaeal outgroup sequence) were combined into a complete alignment (800 sequences altogether, 351 clones and 449 GenBank/prokMSA sequences) to allow parsimonious tree construction in the context of existing phylogenies. DNADist and NEIGHBOR algorithms in the software package PHYLIP (Felsenstein, 2005) were used to generate a neighbor-joining tree from a distance matrix of the aligned sequences and the resulting tree was uploaded to the Interactive Tree of Life (Letunic and Bork, 2007) and pruned to remove sequences not derived from Emerald Lake samples. Nodes were assigned taxonomic clade identities when possible based on the lowest defined classification level of the sequences within a cluster displaying a maximum likelihood similarity of >0.85 to classified GreenGenes prokMSA sequences (Hugenholtz taxonomy).

Relationships between bacterial community structure and environmental measurements were examined using both linear and multivariate modeling approaches. All environmental measurements and relative abundance data for individual bacterial phylotypes were transformed to meet the

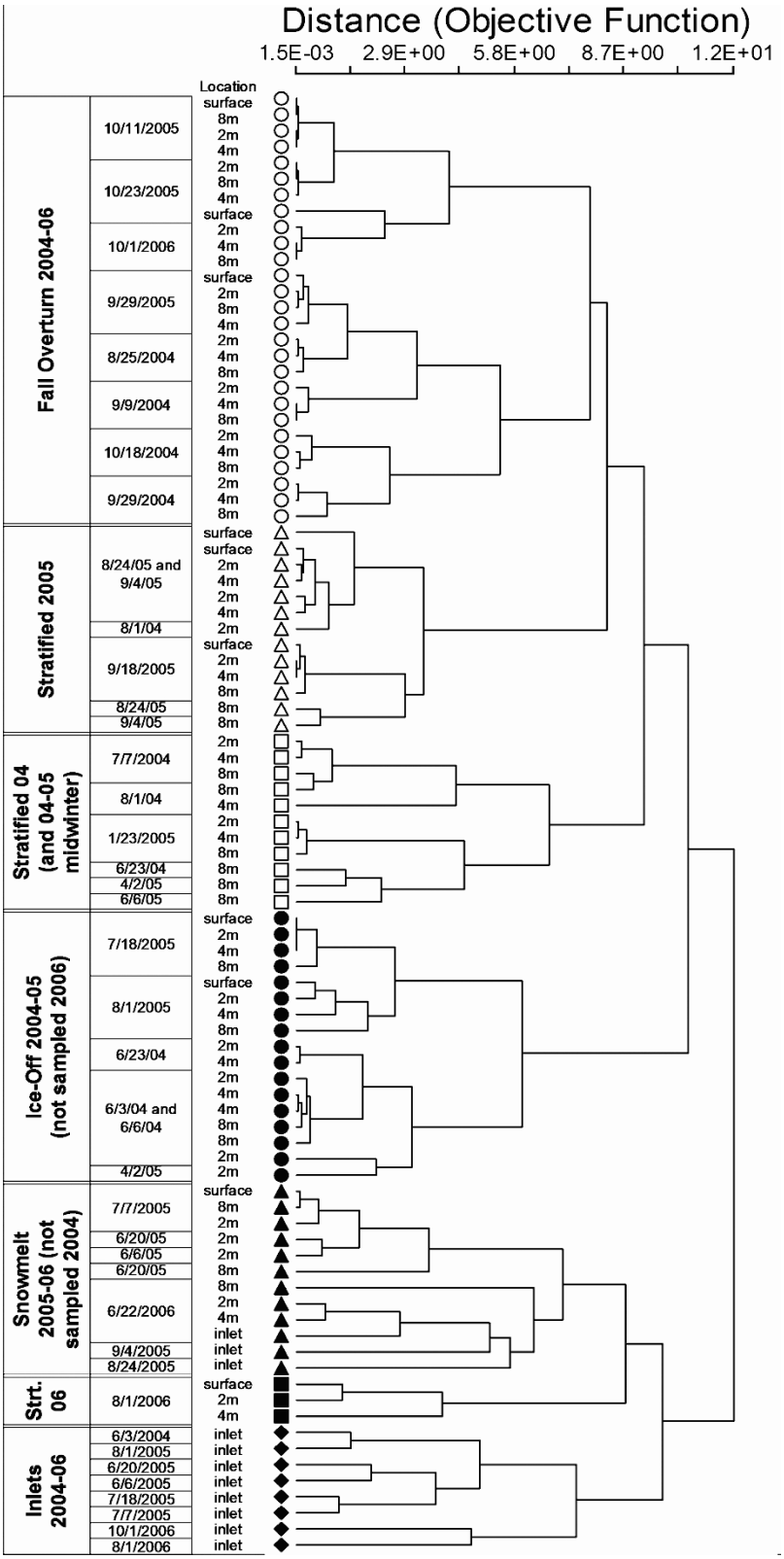

Figure 2 Cluster dendrogram of TRFLP fingerprint data from DNA samples collected from Emerald Lake and its inlet, 2004–2006. The dendrogram was constructed from Sørensen distances between TRFLP samples clustered using the flexible-β method ($\beta = -0.25$) and scaled according to Wishart's objective function. Samples are coded by seven groups selected to minimize within-group distances and maximize the proportion of the overall variance explained in the data set. Groups are named according to similarity between samples with respect to lake mixing status. All groups are significantly different according to the Multiresponse Permutation Procedure (MRPP; McCune and Mefford (2006): $A = 0.28$, all pairwise $P \ll 0.001$, within-group Sørensen dissimilarity <50 except inlets $= 0.62$). TRFLP, terminal restriction fragment length polymorphism.

assumptions of parametric statistical analyses (JMP V.7; SAS-Institute, 2007). Twenty lake samples were excluded from multivariate environmental analyses (final $n=60$) because of incomplete environmental characterization: January and April (midwinter) 2005 (five samples), third and sixth June 2004 (six samples), and all of 2006 (nine samples). Relationships between NMS ordination axes of bacterial community structure and environmental variables were assessed using least squares linear regression analyses. Forward stepwise multiple regression was used to examine relationships between environmental variables and the relative abundances of summed fragments within clades as follows: environmental variables were added to the model sequentially and additive increases in the coefficient of determination were examined, with non-significant variables being dropped from the final model. One-way analysis of variance and Tukey multiple comparisons tests were used to evaluate if various environmental or phylotype variables differed significantly among TRFLP clusters defined in Figure 2. Stepwise discriminant function analysis (a form of multivariate analysis of variance) was used to evaluate which independent variables (environmental parameters or sequence-based phylotypes) have significantly different means among TRFLP clusters and can successfully discriminate between the cluster groupings. Finally, the BIO-ENV routine was employed in PRIMER-E software v5 (Clarke and Ainsworth, 1993) to identify the environmental and biogeochemical variables most highly correlated with TRFLP variation between samples. This routine generates Euclidean distance matrices of samples based on environmental variables individually or in combination and tests the correlation between each matrix and the Sørensen dissimilarity matrix of bacterial community composition generated from the TRFLP fingerprint data (analogous to the Mantel test).

Results

Interannual variation in lake properties

The sampling period, 2004–2006, spanned a wide range of regional meteorologic patterns, with 2004 being a relatively dry year and both 2005 and 2006 exhibiting near-record winter precipitation and snowpack duration (Figure 3). Peak snowmelt discharge in 2004 was $<10\,000\,m^3\,d^{-1}$, snowmelt extended from late April to mid-June and the ice cover on the lake melted in late May. In contrast, 2005 and 2006 both exhibited peak discharge values $>40\,000\,m^3\,d^{-1}$, with snowmelt extending from mid-May to late August and the lake remaining fully ice-covered until mid-July. The duration and magnitude of summer stratification (measured as thermal stability) were larger in 2004 than in 2005 and 2006, and the length of the growing season was considerably curtailed in the latter years. Average integrated stocks of both dissolved and particulate organic carbon in 2004 were nearly double those of 2005 and 2006, and rates of primary production and concentrations of chlorophyll a were higher on average in 2004 than in the latter 2 years (data not shown). A steady decline in lake nitrate concentrations throughout the summer period with declining snowmelt inputs was consistent in all 3 years as has been observed previously in many high-elevation environments (for example, Sickman *et al.*, 2003a).

Temporal patterns in bacterial community structure

Seven distinct bacterial community types were defined from multivariate clustering of samples according to similarity in TRFLP fingerprints (Figure 2). Inflowing terrestrial runoff communities were distinct from lake communities across all 3 years, although during snowmelt periods, inlet communities were more similar to lake communities than at other times of the year (Figures 2 and 3). Lake communities were more similar within sampling dates than between dates when the water column was well mixed (Figure 3), but community distance throughout the water column on a given date was positively related to both water-column distance and degree of stratification (Figure 4). During strongly stratified periods, epilimnetic and hypolimnetic communities were sufficiently distinct to be grouped as separate community types, with hypolimnetic communities generally grouping with samples from the previous sampling date (Figure 3).

Three of the six lake community types were observed to recur in multiple samples across years, whereas the remaining types were specific for a given year and observed only during stratified periods (Figures 2 and 3). Communities were most similar interannually during fall following convective overturn across all 3 years (Fall Overturn Group). Lake communities during peak snowmelt periods (before ice-off) were similar in 2005 and 2006 (Snowmelt Group; this period was not sampled in 2004) as were lake communities found during early stratification following ice-off in 2004 and 2005 (Ice-Off Group—this period was not sampled in 2006; Figures 2 and 3). Lake community types differed significantly in mean ordinal date, residence time and thermal stability, and discriminant analysis using residence time and thermal stability alone successfully delineated bacterial community type for 75% of the samples (Table 1).

Correlation between community structure and environmental parameters

Bacterial rDNA samples were ordinated into two dimensions by NMS of TRFLP community similarity (Figure 5c), with lake thermal stability and hydraulic residence time strongly correlated

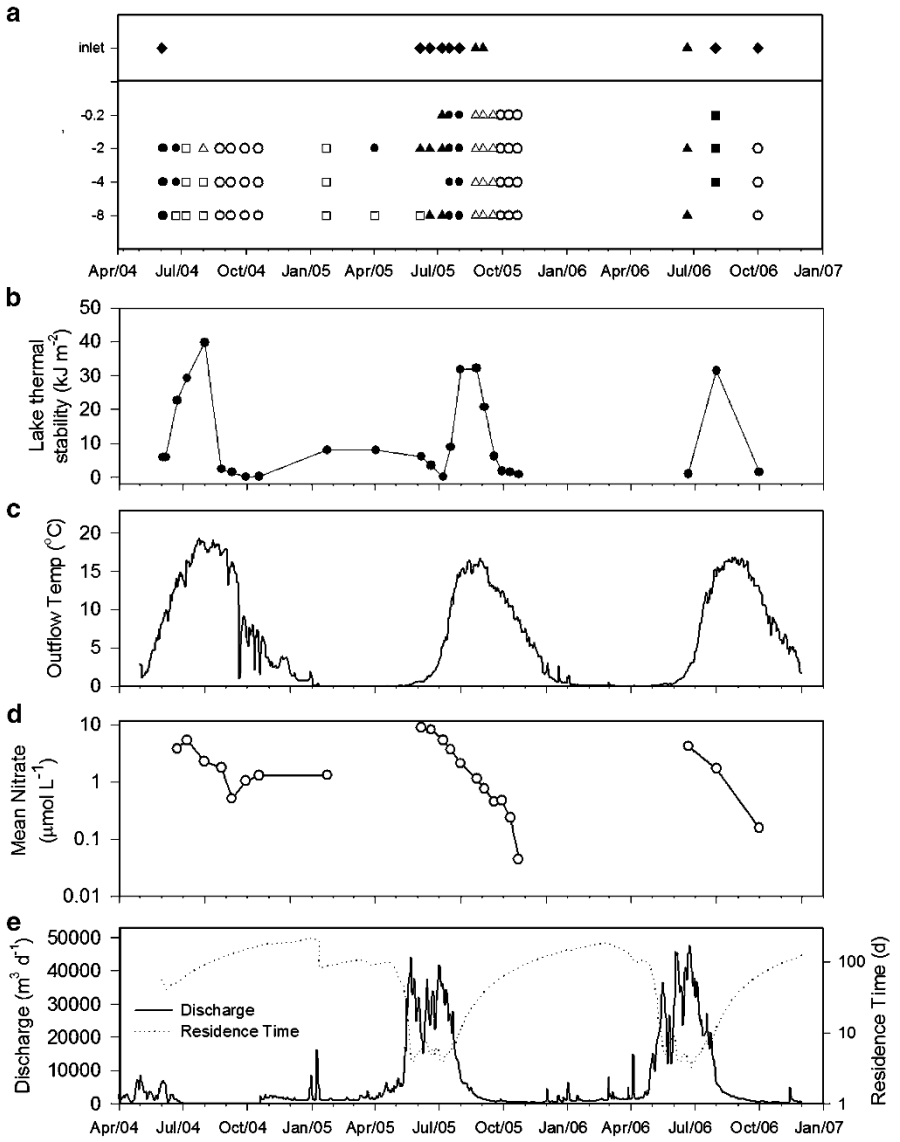

Figure 3 Bacterial community types and values of physical variables in Emerald Lake, 2004–2006. (**a**) shows the distribution of TRFLP community fingerprint types (defined in Figure 1) through time at different depths in the water column as well as the inlet stream. (**b**) shows the thermal stability of the lake at each sampling time based on temperature profiles. (**c**) shows the temperature in the outflow stream. (**d**) shows depth-averaged water-column nitrate concentration and (**e**) displays the discharge in the outlet stream, as measured using a pressure transducer at the outlet weir and calculated theoretical lake hydraulic residence time. TRFLP, terminal restriction fragment length polymorphism.

with the respective NMS axes (Figures 5b and d). Although measured only in 2005, the DOM FI was strongly related to NMS Axis 2 and was more strongly correlated with NMS axis values than any other variable in that year (Figure 5a). Additional variables significantly related to NMS axes scores across the 3-year data set were temperature, nitrate, particulate carbon, particulate nitrogen, DOC, DON and chlorophyll a ($P<0.01$, $r^2=0.15$–0.27). Correlations between TRFLP Sørensen distances and environmental distances using the BIO-ENV algorithm supported the strong correlation between community structure and hydraulic residence time ($r_{\mathrm{Mantel}}=0.64$), with the addition of thermal stability, temperature, nitrate and DON marginally strengthening the relationship ($r_{\mathrm{Mantel}}=0.73$ between TRFLP community distance and a Euclidean distance matrix generated from all five parameters; Table 2). In 2005, a distance matrix generated from DOM FI values alone had nearly as strong a correlation to TRFLP community distance as did the environmental distance matrix generated from the five variables used in the final model outlined above (Table 2; $r_{\mathrm{Mantel}}=0.75$ and 0.81 for correlation between TRFLP distances and matrices generated either from FI or from the five variables, respectively, using only 2005 data).

Temporal dynamics and environmental correlates of bacterial phylotypes

To better understand seasonal dynamics of specific bacterioplankton taxa, I calculated relative abundances for each of the eight dominant bacterial clades by summing the relative areas of TRFLP peaks belonging to a given clade (Figure 6 and Supplementary Information). Most clades were comprised of clones exhibiting one to four TRFLP fragment lengths, although the Burkholderiales group within the β-Proteobacteria contained 12 fragment lengths (Table 3 and Figure 6). Statistically, variation in the relative abundances of these eight clades dominated total community structure variation, successfully classifying 95% of TRFLP samples into the community types defined in Figure 2 (Wilk's Lambda, $P<0.0001$).

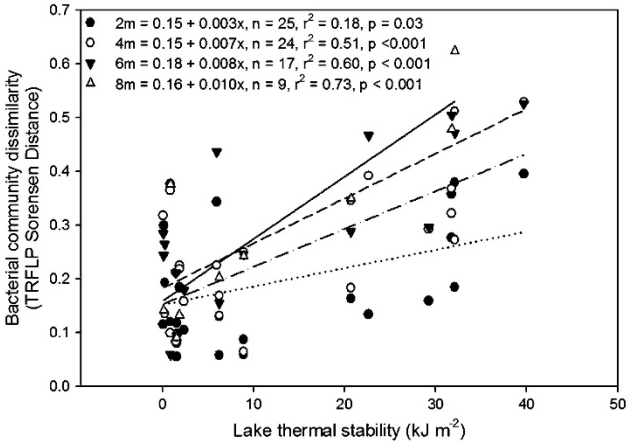

Figure 4 Relationship between lake thermal stability and bacterial community dissimilarity (measured as the Sorensen distance between TRFLP samples) for pairs of samples separated by given water-column distances (depth differences). Each data point represents the community distance between two samples within the water column of Emerald Lake during the ice-free periods of 2004 and 2005 coded by symbols according to the pairwise difference in intersample depth. Specifically, 2 m distances include the surface: 2 m and 2:4 m sample pairs, 4 m distance includes the surface:4 m and 4:8 m pairs, the 6 m distance is the 2:8 m pair and the 8 m distance is the surface:8 m pair for individual sampling dates. Surface samples were collected only in 2005. During stratified periods, the thermocline lies between 4 and 6 m. Note that community dissimilarity increases with an increasing intensity of stratification and the slope of this relationship increases with increased distance (depth differences) between samples in the water column. Community differences in epilimnetic samples 2 m apart were not related to the intensity of stratification, likely due to diurnal and wind-driven mixing in the epilimnion. TRFLP, terminal restriction fragment length polymorphism.

In general, clades were unevenly represented among seasons within the lake, with early-season samples dominated by Flavobacteria and β-Proteobacteria and late-season samples dominated by Actinobacteria and Sphingobacteria. The proportion of the total relative abundances of fragment lengths contributed by the eight dominant clades was generally higher during the growing season (late July through September), whereas less common phylotypes showed greater representation in the community, resulting in higher evenness, during midwinter and snowmelt periods (Table 3, Figure 7). Flavobacteria comprised a larger proportion of the community during ice-off and 2006 stratification and Actinobacteria became dominant during stratified periods. Burkholderiales generally comprised a large proportion of the community in all seasons, whereas Verrucomicrobia were generally a small proportion of the community with modest peaks in relative abundance during ice-off and stratification 2005. The Sphingobacteria and Methylophilales composed a greater proportion of the community during snowmelt and ice-off, with the Sphingobacteria showing another relative abundance peak during fall overturn, whereas the Cyanobacteria showed peak relative abundances during stratification (2005) and fall overturn, periods of peak primary production in the lake (data not shown). The inlet community was dominated by the Microbacteriaceae and Burkholderiales of the Actinobacteria and β-Proteobacteria.

The DOM FI, which was the environmental variable with the strongest relationship to community structure in 2005 (Table 2), was positively

Table 1 Mean values and results of ANOVA comparing residence time and thermal stability for each lake TRFLP cluster defined in Figure 1

Group	n	*Residence time* (d)		*Thermal stability* ($kJ\,m^{-2}$)	
Snowmelt	9	5	E	1.77	C
Ice-off	17	21	CD	14.79	B
Stratified 2004/midwinter 2005	11	61	AB	20.72	AB
Stratified 2005	13	41	BC	21.21	AB
Stratified 2006	3	12	DE	31.44	A
Overturn	27	96	A	1.18	C
ANOVA r^2 (F ratio)	80	0.74 (41)		0.70 (35)	
DA step (# misclassified)	80	1 (44)		2 (20)	

Abbreviations: ANOVA, analysis of variance; TRFLP, terminal restriction fragment length polymorphism.
Inlets are excluded. Letters denote significant differences using Tukey multiple comparisons test at $P<0.01$. Columns are ordered according to results of stepwise Discriminant Analysis, with residence time the first variable entered and thermal stability the second and the number of misclassified samples ($n=80$) in parentheses.

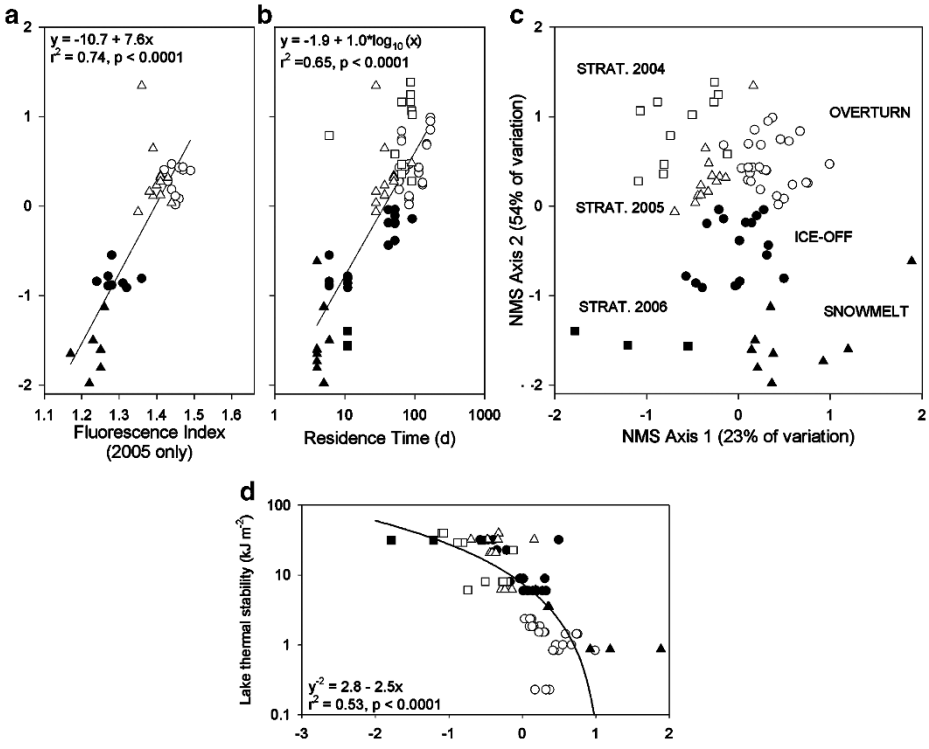

Figure 5 NMS ordination of lake TRFLP fingerprint samples in relation to environmental descriptors. (**c**) is the two-dimensional NMS solution with symbols coded according to groups defined in Figure 1 (final stress = 18.7, cumulative r^2 between ordination distances and original distances = 0.77, axes 99.2% orthogonal, $n = 80$). Panels (**a**, **b** and **d**) display relationships between ordination axes and the strongest univariate independent variables for each. Note that increased FI values are associated with an increased relative contribution of autochthonous DOM. DOM, dissolved organic matter; FI, fluorescence index; NMS, nonmetric multidimensional scaling; TRFLP, terminal restriction fragment length polymorphism.

correlated with the relative abundances of Cyanobacteria and both Actinobacteria clades and negatively correlated with both clades of the β-Proteobacteria. Stepwise multiple regressions were used to determine the proportion of variation in the relative abundances of dominant clades explained by various environmental factors (Table 3). Coefficients of determination for the multivariate models ranged from 0.14 (Verrucomicrobia) to 0.84 (Methylophilales) with an average of 0.41. Lake residence time and thermal stability were significantly related to the relative contribution of every clade, save the Burkholderiales and Cellulomonadaceae, both of which were related to nitrogen concentration and temperature. Temperature was also significantly related to the relative contributions of Sphingobacteria and Methylophilales. DOC was weakly but significantly related to Cyanobacterial relative abundance, and TP was significantly related to Flavobacteria relative abundance.

Discussion

Characterizing microbial phenology

The establishment of recurring interannual patterns in bacterioplankton communities highlighted here and elsewhere, as well as correlations between these patterns and physical and chemical variables, provides important new information on the description and possible drivers of microbial community structure (Morris *et al.*, 2005; Fuhrman *et al.*, 2006; Hullar *et al.*, 2006; Kan *et al.*, 2006; Martiny *et al.*, 2006; Shade *et al.*, 2007). As predictable phenological patterns have been observed widely in plant and animal communities, delineating such patterns in bacterioplankton communities lends support to the concept that general ecological principles may apply to both macro- and microorganisms. In one of the first studies providing a high-frequency, multiyear data set of bacterioplankton community composition, Morris *et al.* (2005) demonstrated that specific dominant phylotypes showed recurring population patterns associated with convective overturn and stratification at the Bermuda Atlantic Time Series station in the Sargasso Sea. More recently, Fuhrman *et al.* (2006) and Shade *et al.* (2007) used similar 6-year data sets to demonstrate consistent phenological patterns in bacterial communities in coastal and lake ecosystems, respectively, with both studies identifying a suite of physical, chemical and biological variables, including water temperature, mixing regime, nutrient availability and phytoplankton growth, which were correlated with bacterial community structure. The results of this study extend these findings,

Table 2 Results of BIO-ENV algorithm comparing Sørensen dissimilarity matrix of lake TRFLP samples with multiple Euclidean distance matrices generated from all possible combinations of selected environmental variables

Environmental variables	*r*
Matrices generated from multiple variables	
Residence time, thermal stability	0.68
Residence time, thermal stability, local temperature	0.71
Residence time, thermal stability, local temperature, DON	0.72
Residence time, thermal stability, local temperature, DON, nitrate	0.73
Matrices generated from individual variables	
DOM fluorescence index (only measured in 2005, $n=39$)	0.75
Residence time	0.64
Thermal stability	0.26
Local temperature	0.41
DON	0.37
Nitrate	0.33
PC	0.37
PN	0.34
DOC	0.23
TP	0.19

Abbreviations: DOC, dissolved organic carbon; DOM, dissolved organic matter; PC, particulate carbon; PN, particulate nitrogen; TRFLP, terminal restriction fragment length polymorphism.
With the exception of the DOM fluorescence index noted above, all depths and years were included in the analysis ($n=80$). The correlation statistic *r* may be interpreted as a stepwise Mantel test of correlation between the TRFLP Sørensen bacterial community distance matrix and a Euclidean distance matrix generated from the specified environmental variables.

demonstrating predictable seasonal patterns in both overall bacterioplankton community composition and the relative abundance dynamics of specific bacterial phylotypes, and relating each to interannual patterns of specific physical and chemical environmental variables.

The timing of limnetic transitions related to water-column stability and hydraulic residence time, which are in turn related to climatic conditions, is highly correlated with bacterioplankton phenology in Emerald Lake. Phytoplankton seasonality has been observed and studied extensively throughout the past century, and many studies in small dimictic lakes have categorized the emergence of predictable community types according to seasonal physical transitions similar to those defined for bacterioplankton phenology in this study (Sommer *et al.*, 1986; Reynolds, 2006). In the ocean, the emergence of defined phytoplankton community types is broadly predictable in part based on interacting gradients of nutrient availability and mixing regime (Margalef, 1978; Smayda and Reynolds, 2001). These axes of physicochemical variability may be analogous to those observed in this study (residence time and thermal stability), suggesting that the phenology of both eukaryotic and bacterial microbial communities in pelagic ecosystems is regulated by similar processes. In Emerald Lake, nutrient availability is strongly correlated with hydraulic residence time by two key nutrient delivery processes associated with snowmelt: the pulse of nitrate from catchment snowpack melting (for example, Sickman *et al.*, 2003a) and the flushing of terrigenous DOM from catchment soils and vegetation (Boyer *et al.*, 1997; Hood *et al.*, 2005).

Linking DOM source to bacterial community structure

The source and composition of the organic matter pool in aquatic environments has been shown repeatedly to be related to bacterial community structure and function (Cottrell and Kirchman, 2000; Covert and Moran, 2001; Carlson *et al.*, 2002; Judd *et al.*, 2006; Langenheder *et al.*, 2006; Perez and Sommaruga, 2006; Kritzberg *et al.*, 2006b). This study demonstrates a strong relationship between bacterioplankton community structure and seasonal shifts in the source of DOM (allochthonous vs autochthonous, measured by FI; Figure 5, Table 3). Crump *et al.* (2003) noted that shifts in bacterial community structure and production over 1 year in an Arctic lake were associated with the influx of terrestrially derived organic matter during snowmelt and a subsequent period of *in situ* production of DOM derived from phytoplankton. A suite of recent experimental studies examining the ability of DOM from different sources to alter the structure and function of the bacterioplankton community has demonstrated that communities generally shift to match DOM substrates from different sources across the landscape or among lakes (Judd *et al.*, 2006; Kritzberg *et al.*, 2006a), although these patterns are not consistent across all systems (Langenheder *et al.*, 2005, 2006).

Recent experimental studies in high-elevation lakes have shown that the representation of Actinobacterial groups in bacterioplankton communities increases with the addition of terrigenous DOM and that bacterial communities become rapidly dominated by β-Proteobacteria with the addition of algal lysate (Perez and Sommaruga, 2006). In this study, correlations between FI and the relative abundances of Actinobacteria and β-Proteobacteria (Table 3), as well as temporal patterns in the relative abundances of both clones (Figure 6) and TRFLP peaks (Figure 7), suggest that Actinobacteria are a larger component of the bacterioplankton community when phytoplankton-derived organic matter is dominant, whereas β-Proteobacteria are dominant during snowmelt and ice-off when DOM is primarily derived from terrigenous sources. Although these results and those of Perez and Sommaruga (2006) appear to conflict, it is important to remember the limitations of relative abundance estimates and note that the same key bacterial groups were highlighted as responding to shifts in DOM composition and source in both studies. Moreover, the algal lysate enrichments employed by Perez and Sommaruga (2006) contained 10-fold more phosphorus than soil

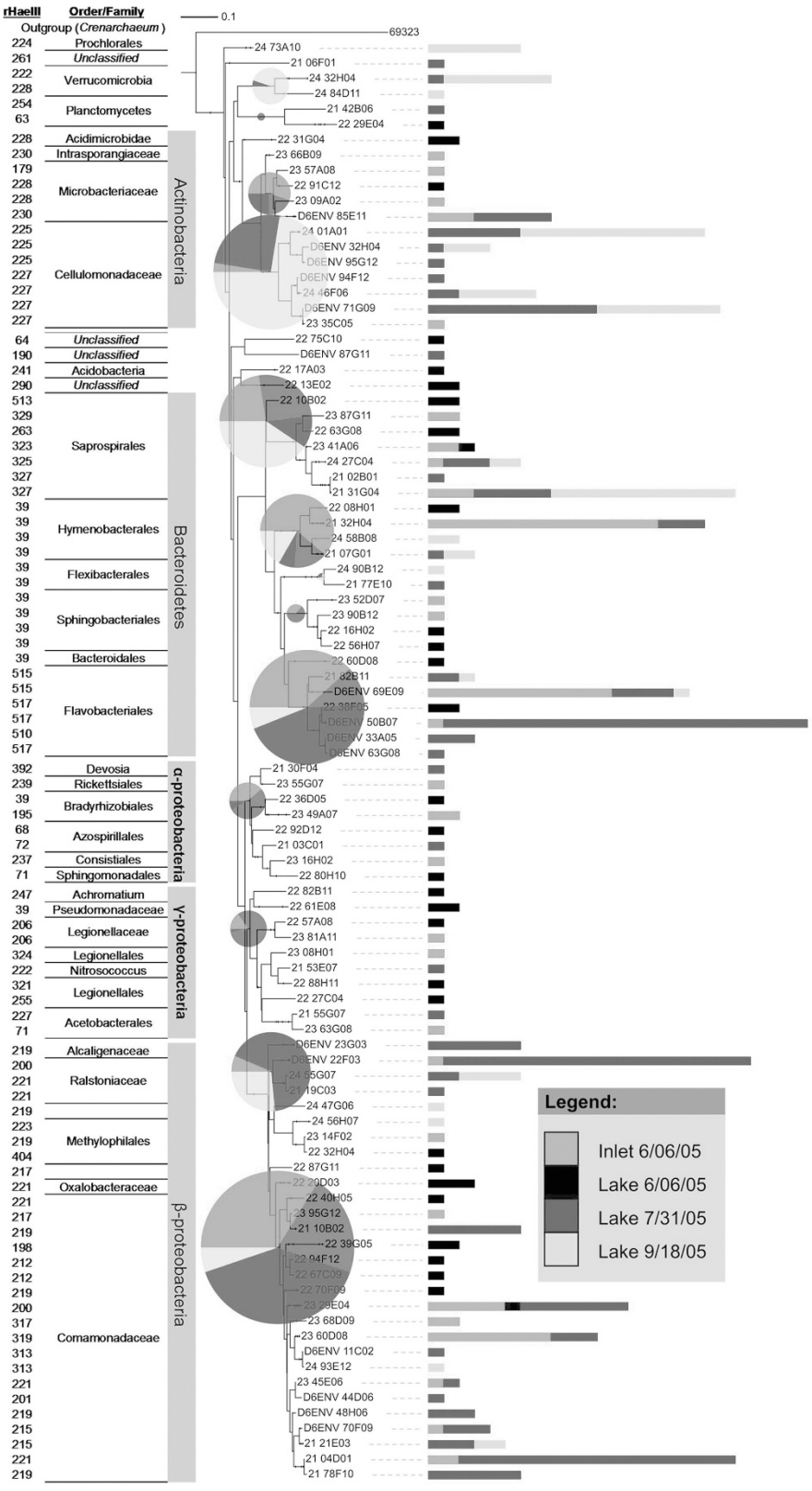

Figure 6 Phylogenetic tree of unique clones from Emerald Lake. Each clone is labeled with estimated terminal restriction length after *in silico Hae*III digestion. Colored bars extending to the right of each clone ID represent the number of duplicate clones found in each environmental sample (scaled with the shortest bars equal to one clone). Pie charts centered at the base of dominant clades represent clone frequency distribution among the four sampled times in the lake: the size of the pie chart indicates the % of total clones belonging to that clade, whereas the colors in the pie chart indicate the relative frequency of the clade in clone libraries from each of the four samples (for example, α-Proteobacteria were a larger fraction of 6 June 2005 lake clones than 31 July 2005 lake clones).

Table 3 Statistical analyses of clade composition and seasonality, including clone and TRFLP fragment composition, relative abundances in different community types and relationships with environmental variables

Clade	*Cyanobacteria: Prochlorales*	*β-Proteobacteria: Methylophilales*	*Verrucomicrobia: Verrucomicrobiales*	*Bacteroidetes: Flavobactera*	*Bacteroidetes: Sphingobacteria*	*β-Proteobacteria: Burkholderiales*	*Actinobacteria: Microbacteriaceae*	*Actinobacteria: Cellulomonadaceae*
No. of unique clones	1	3	2	6	7	19	6	7
Fragment lengths in clade	224	404	222	515,517	323,325, 327,329	198–221, 313–19*	228, 230	225, 227
The mean relative abundance of each clade in the seven community types identified in Cluster Analyses (ANOVA and Tukey multiple comparison tests)								
ANOVA r^2	0.58	0.84	0.46	0.69	0.30	0.20	0.40	0.78
Inlet	0.00 D	0.04 B	0.01 C	0.02 BC	0.05 C	**0.29 A**	**0.21 AB**	0.01 D
Snowmelt	0.00 D	**0.05 A**	0.02 C	0.01 BC	**0.12 AB**	**0.23 AB**	0.10 C	0.02 D
Ice-off	0.00 D	0.02 C	**0.07 A**	**0.13 A**	**0.11 AB**	0.20 B	0.08 C	0.10 C
Strat. 2004	0.01 BC	0.01 DE	0.04 BC	0.02 BC	0.08 BC	0.19 B	**0.25 A**	0.08 C
Strat. 2005	**0.02 A**	0.00 E	**0.05 AB**	0.00 C	0.07 BC	0.19 B	0.14 BC	**0.28 A**
Strat. 2006	0.00 CD	0.02 BCD	0.01 BC	**0.11 A**	**0.09 ABC**	**0.22 AB**	0.09 BC	0.03 CD
Overturn	**0.02 AB**	0.00 E	0.02 C	0.04 B	**0.14 A**	0.18 B	0.11 C	0.14 B
Correlation r vs FI (2005 only)	0.85	−0.87	NS	NS	NS	−0.57	0.45	0.61
Results of stepwise regression of environmental variables (X) versus clade relative abundance (Y) across samples (Variables included: residence time (RT), thermal stability (TS), TP, nitrate, Chlorophyll, DOC, DON, PC, PN, temp)								
Adjusted r^2	0.44	0.84	0.14	0.28	0.27	0.40	0.45	0.42
Variable 1	RT	RT	RT	TP	TS	Nitrate	DON	nitrate
Variable 2	DOC	Temp	TS	RT	Temp	DON	TS	temp
Variable 3		Nitrate		PC		Temp	RT	

Abbreviations: ANOVA, analysis of variance; DOC, dissolved organic carbon; FI, fluorescence index; PC, particulate carbon; PN, particulate nitrogen; NS, not significant; TRFLP, terminal restriction fragment length polymorphism.
The ANOVA r^2 indicates the proportion of the total variation in the relative abundances for each clade attributable to community type, with data on the mean relative abundance of each clade in each community type including the results of analyses (Tukey multiple comparisons test, $P<0.05$) showing significant differences in relative abundances for each clade across community types (community types with the same letter are not significantly different; bold values denote community types where each clade had highest relative abundance). The middle of the table shows significant ($P<0.01$) Pearson's correlation coefficients for relationships between the relative abundances of individual clades and the fluorescence index and the bottom of the table shows the results of multiple stepwise regression analyses of the relationships between the relative abundances of individual clades and specific environmental variables, including the overall coefficient of determination for each regression model and the environmental variables significantly ($P<0.05$) related to each clade's relative abundance, ordered by importance. ANOVA $n=91$, stepwise regression $n=60$ (inlets and samples with incomplete environmental data excluded as detailed in Materials and methods) and FI correlations, $n=39$ (inlets excluded and FI only measured in 2005). *Included fragment lengths 198, 200, 201, 206, 212, 215, 217, 219, 221, 313, 317 and 319.

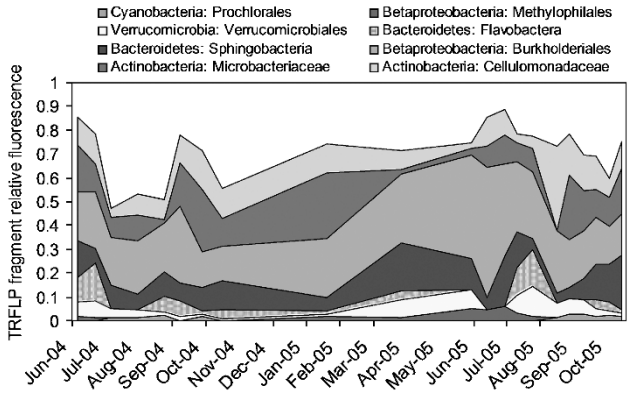

Figure 7 Temporal dynamics of dominant bacterioplankton clades at 2 m depth in Emerald Lake from June 2004 to October 2005. Each color represents the summed relative contribution of TRFLP peaks with fragment lengths belonging to a given phylogenetic clade to the total TRFLP peak area for samples collected at 2 m depth. TRFLP, terminal restriction fragment length polymorphism.

DOM enrichments. Experimental nutrient enrichment studies carried out in Emerald Lake have demonstrated that phosphorus enrichment tends to decrease the relative abundance of Actinobacteria and increase that of β-Proteobacteria, results akin to those of Perez and Sommaruga (2006), suggesting that nutrient enrichment and DOM source may interact to influence the relative abundances of these key groups (CE Nelson and CA Carlson, in review).

Phenology of bacteria in high-elevation aquatic ecosystems

In their seminal study of the population dynamics of bacterioplankton over a 1-year period in a high-elevation alpine lake, Pernthaler *et al.* (1998) associated major shifts in the morphological and phylogenetic structure of bacterial communities with limnetic physical transitions, including ice-off, summer stratification and autumn overturn. In combination with a companion study conducted in the same lake (Glockner *et al.*, 2000), their work demonstrated strong and predictable seasonal changes in the relative abundances of dominant bacterial groups (Actinobacteria, β-Proteobacteria and Bacteroidetes; aka CFB) similar to those detailed here for Emerald Lake. In both studies, a maximum in the relative abundance of the β-Proteobacteria was associated with snowmelt, ice-off and spring mixing, followed by a midsummer peak in Actinobacteria, with peaks in the relative abundances of the Bacteroidetes occurring during periods of mixing, including fall overturn and ice-off (Table 3). Further work has since demonstrated that the β-Proteobacteria and Actinobacteria constitute dominant proportions of the metabolically active bacterial community in high-elevation lakes (Perez and Sommaruga, 2006) with Actinobacteria adapted to high ultraviolet light levels (Warnecke *et al.*, 2005) and both groups hypothesized to inhabit contrasting niches with respect to the seasonal availability of allochthonous material. Similarly, both groups were dominant members of the Emerald Lake community throughout the year, with their population dynamics linked to temporal variation in nitrate, DON, temperature and DOM source (Table 3), all factors associated with variation in snowmelt inputs.

Defining phenological changes in microbial communities is of prime importance because changes in microbial community structure are linked to shifts in fundamental ecosystem properties, including pelagic metabolism and organic matter export (for example, Carlson *et al.*, 1998; Steinberg *et al.*, 2001; Winder and Schindler, 2004; Morales-Baquero *et al.*, 2006a). Recent climate shifts have led to an increasingly earlier onset of spring in the western United States, and some models predict that warming may lead to increasing variation in the magnitude and timing of winter precipitation in the mountainous regions (Beniston *et al.*, 1997; Cayan *et al.*, 2001). As this study encompassed disparate extremes of winter snowpack size and duration in the Sierra Nevada, these results highlight the significance of interannual climatic variation to the phenology of bacteria. In addition to demonstrating predictable prokaryotic phenology, recent work has contrasted temporal and spatial patterns of bacterioplankton community structure within morphologically complex lake basins, providing evidence that temporal patterns in bacterial community composition are related more to regional meteorologic patterns than to spatial environmental heterogeneity (Stepanauskas *et al.*, 2003; Kan *et al.*, 2006) and that unconnected aquatic ecosystems within the same region exhibit synchronous bacterial community dynamics (Crump and Hobbie, 2005; Kent *et al.*, 2007). More evidence for the control of bacterial communities by regional climate processes may be found in linkages between the community structure of the bacterioplankton and that of the phytoplankton (Kent *et al.*, 2004; Pinhassi *et al.*, 2004).

Stratification and bacterioplankton community differentiation

Dissimilarity between bacterioplankton communities at different depths in Emerald Lake increased as thermal stratification increased, with increasing differences in communities separated by greater depth distances (Figure 4). Measurements of temperature profiles in Emerald Lake showed that thermal stability increases steadily during spring warming (Figures 3b and c), indicating that communities at different depths during this period experience extended periods of isolation. These results therefore lend support to the idea that microbial community differentiation scales with the duration and distance of physical isolation (Papke and Ward,

2004). Summer lake stratification led to differences in bacterioplankton community types at different depths, as well as the emergence of communities that exhibited low interannual similarity (Figure 3). During stratified periods, including both summer and midwinter, TRFLP fingerprint patterns from the hypolimnion often clustered more closely with samples from adjacent dates and were generally distinct from epilimnetic samples.

Studies of temporal variation in bacterioplankton community structure in Wisconsin lakes have revealed similar patterns of decreased interannual similarity during summer stratification (Yannarell *et al.*, 2003; Shade *et al.*, 2007). During highly stratified periods, sporadic climatic events (for example, passing storms, wind-driven mixing, cloud cover) can have a larger impact on water-column density structure and concomitant biogeochemical dynamics than during mixed periods, with subsequent spatiotemporal resource heterogeneity possibly translating into unique environmental conditions that regulate the microbial community (MacIntyre *et al.*, 2006). As microbial community dissimilarity increased with increasing distance of physical (depth) separation during stratified periods (Figure 4), these results suggest sensitive community responses to depth differences in environmental conditions, such as water temperature or light. Although not measured in this study, previous research in high-elevation lakes has emphasized the important function of solar radiation in structuring bacterial communities, with particular phylotypes adapted to the high flux of ultraviolet radiation found in shallow environments (Carrillo *et al.*, 2002; Warnecke *et al.*, 2005).

Conclusions

This study presents evidence that bacterioplankton community structure in high-elevation lakes exhibits predictable seasonal patterns across years. These phenological patterns are largely predictable from inter- and intraannual variation in snowmelt inputs and lake thermal stability, suggesting that the physicochemical factors (nutrient availability and mixing regime) driving bacterioplankton seasonality are similar to those understood to regulate phytoplankton community structure in both lakes and the oceans. However, multivariate models incorporating these variables explained less than half of the variation in the relative abundances of specific bacterial clades, suggesting that other factors have an important function in driving microbial population dynamics. In Emerald Lake, a headwater lake receiving primarily terrigenous snowmelt inputs and temporal variation in dissolved organic matter source was highly correlated with shifts in community composition and with the relative abundances of several dominant taxa, supporting the idea that variation in the composition of this heterogeneous resource may be an important driver of bacterioplankton community dynamics. Bacterial communities in the lake exhibited greater variation throughout the water column during periods of increasing thermal stratification, suggesting that extended physical isolation and decreased community mixing may enhance phylogenetic differences in bacteria at different depths. Together, these results provide a clear framework for bacterioplankton phenology linked to regional meteorologic patterns, landscape processes and seasonal lake transitions, enhancing our understanding of how microbial communities respond to environmental factors at multiple scales.

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