

Synchrony and seasonality in bacterioplankton communities of two temperate rivers

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Abstract

The bacterioplankton community composition (measured with denaturing gradient gel electrophoresis of 16S ribosomal DNA [rDNA]) of two nonintersecting temperate rivers was nearly identical and changed synchronously over 2.5 yr, suggesting that intrinsic controls on bacteria were similar in the two rivers and that seasonal changes were driven by extrinsic factors such as climate. Most potential controls on community composition also exhibited synchrony; these included bacterial production rate (leucine incorporation), water temperature, river flow rate, and a suite of chemical measurements. Temperature and river flow rate were the best predictors of temporal patterns in diversity. However, diversity patterns also correlated with bacterial production and concentrations of dissolved organic nitrogen and nitrate, suggesting that diversity is directly or indirectly influenced by complex seasonal shifts in environmental conditions. Winter and summer communities were somewhat predictable over 3 yr, although these communities were not identical. Two polymerase chain reaction (PCR)-amplified clone libraries of 16S rDNA, constructed with summer samples from each river, were not significantly different and contained typical freshwater bacterioplankton of the beta-*Proteobacteria*, *Bacteroidetes*, and *Actinobacteria*, including members of five new freshwater bacterioplankton clusters. However, libraries also included several phylotypes related to bacteria from soil and sediment, indicating the potential importance of allochthonous organisms in river diversity.

Rivers and lakes within a geographic region share climatic drivers such as temperature and precipitation that directly or indirectly influence the activity and diversity of bacterioplankton and other aquatic organisms. Signals of this influence are often detected as synchronous changes in time series of physical, chemical, and biological data (Magnuson et al. 1990; Benson et al. 2000; Kling et al. 2000). Synchrony, defined as the concurrent variation of time-series data among ecosystems (Pace and Cole 2002), is best described for variation among lakes in which extrinsic climatic forces such as precipitation and insolation are evenly applied across lakes and catchments. Deviations from synchrony, and even asynchrony, among lakes result from variations in intrinsic, site-specific controls.

Nonreactive variables such as temperature and alkalinity are often synchronous across river basins and lake regions (Magnuson et al. 1990; Soranno et al. 1999; Clair et al. 2001) but can show less synchronous behavior in regions with more complex landscapes and hydrogeology (Meybeck 1993; Kling et al. 2000; Webster et al. 2000). Reactive chemical variables, such as nutrients and dissolved organic carbon (DOC), and biological variables, such as chlorophyll *a* (Chl *a*) and zooplankton, which hold greater significance to bac-

terioplankton communities, also show synchronous behavior (Baines et al. 2000; Miller et al. 2003; Clark et al. 2004). These chemical and biological variables are generally thought to be more subject to variability in local food web structure and limnological features and thus farther removed from the direct influence of climate.

There is mounting evidence of a numerically limited and globally distributed set of freshwater bacterioplankton types (Glockner et al. 2000; Zwart et al. 2002). Despite this, bacterial community composition is often highly variable among freshwater systems (Hiorns et al. 1997; Konopka et al. 1999) and within systems over time (Yannarell et al. 2003; Zwislner et al. 2003). Several studies link this variability to differences in temperature (Yannarell and Triplett 2004); water chemistry, including pH, alkalinity, DOC, and nutrient concentration (Methe and Zehr 1999; Crump et al. 2003); and food web structure, including phytoplankton community composition, grazing rate, and viral abundance (Simek et al. 2002; Matz and Jurgens 2003).

But very few studies use the strategy of comparing temporal patterns of bacterioplankton community change among systems to evaluate the influence of extrinsic factors such as climate (Lindstrom 2000; Van der Gucht et al. 2001), and only one demonstrated seasonally synchronous shifts in community composition (Yannarell et al. 2003). In each of these studies, factors influencing bacterial community composition were identified by comparing temporal shifts in community composition among systems and correlating those patterns with spatial and temporal conditions of systems.

This strategy of comparisons among systems holds great potential for understanding controls on bacterial community composition. But before this approach can be applied, it is

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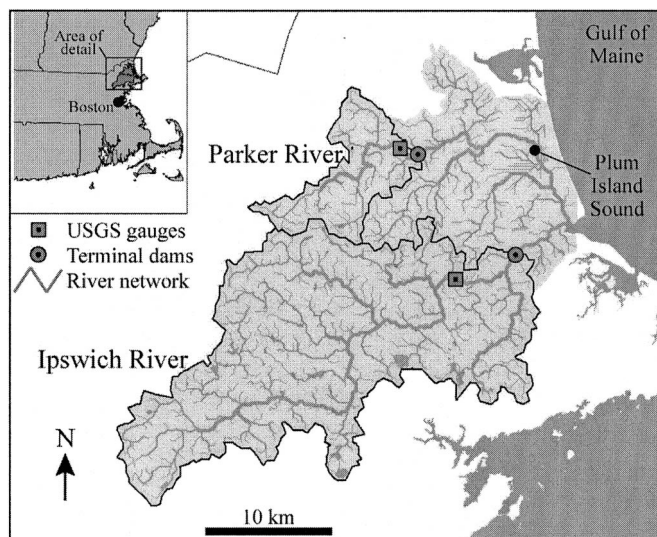


Fig. 1. Map of the Ipswich and Parker River watersheds.

necessary to show that systems affected by very similar or identical extrinsic factors will have similar bacterial community composition and will change synchronously. To test this, we analyzed patterns of bacterial community composition of two neighboring temperate rivers for 2.5 yr and compared these patterns with physical, chemical, and biological measurements. Not only were the communities in the two rivers very similar, but they also changed synchronously. Correlation analyses suggest that shifts in community composition are related to climatically driven changes in temperature and river flow rate. We also found that the communities in these two rivers show a seasonal cycle, forming stable and somewhat predictable summer and winter communities.

Materials and methods

The Ipswich and Parker Rivers (Fig. 1) flow into Plum Island Sound, an estuary located on the Gulf of Maine in northeastern Massachusetts. The Ipswich River has a mean discharge of $5.59 \text{ m}^3 \text{ s}^{-1}$ (since 1990) and is impounded by three dams along its main channel, including a terminal dam. Land use in its watershed (404 km^2) is largely forest (49%) and urban/suburban-residential (35%) but includes agricultural land (7%). About 9% of the watershed is covered with lakes, ponds, and marshes. The watershed has a low average stream gradient (0.47 m km^{-1}), and much of the river and its tributaries flow through flanking wetlands. Human population in the watershed was approximately 130,000, or 322 people km^{-2} , in 2001 (Williams et al. 2004). The Parker River has a mean discharge of $1.05 \text{ m}^3 \text{ s}^{-1}$ (since 1990) and is impounded by eight dams, including a terminal dam where it enters the estuary. Its watershed upstream of the terminal dam (64.7 km^2) is composed of forests (54.5%) and residential development (25.4%) and undeveloped (presumed agricultural) land (5.1%) (Tomczyk 2002). About 15% of the watershed is covered with lakes, ponds, and marshes (Simcox 1992). Like the Ipswich, the Parker River watershed has

a low average stream gradient (1.14 m km^{-1}), and much of the river flows through flanking wetlands. Human population upstream of the terminal dam was approximately 11,866, or 183 people km^{-2} (Tomczyk 2002).

All chemical and biological measurements were made at impoundments located near the river mouths (Ipswich River Dam: $42^\circ 68' \text{N}$, $70^\circ 84' \text{W}$; Parker River Dam: $42^\circ 75' \text{N}$, $70^\circ 93' \text{W}$). Chemical measurements, provided by the Plum Island Ecosystem Long-Term Ecological Research program, included nitrate (NO_3^-), ammonium (NH_4^+), dissolved inorganic nitrogen (DIN), dissolved organic nitrogen (DON), particulate organic nitrogen (PON), phosphate (PO_4^{3-}), dissolved organic phosphorous (DOP), particulate phosphorous (PP), DOC, particulate organic carbon (POC), and suspended sediment (SS). River flow data were collected by the U.S. Geological Survey at gauging stations near the river mouths (Fig. 1). Subsurface water samples were collected just upstream of the terminal dams with clean and sample-rinsed 1-liter bottles and were immediately processed for bacterial production and filtered for DNA samples. Bacterial production was measured monthly from July 2000 to October 2001 and then again in February 2002. Duplicate DNA samples of 250–500 mL were collected monthly from February 2000 to February 2002 and then again in June and July 2002. DNA sampling and extraction procedures followed those described elsewhere (Crump et al. 2004).

All data were tested for normality using Kolmogorov–Smirnov tests and were log transformed when distributions were significantly different from normal. One anomalously high measurement of NO_3^- collected on 2 June 2000 from the Parker River was excluded from statistical analyses.

Bacterial production was measured as the rate of incorporation of L- ^3H leucine (50 nmol L^{-1} final concentration) using methods described elsewhere (Crump et al. 2004). Carbon production rate was calculated with the conversion factor $257.6 \text{ mol carbon mol leucine}^{-1}$ (Kirchman 1993).

Bacterioplankton community composition was compared on the basis of the presence or absence of bands in banding patterns created with denaturing gradient gel electrophoresis (DGGE) separation of polymerase chain reaction (PCR)-amplified 16S ribosomal DNA (rDNA), which is a phylogenetically informative gene. DGGE procedures followed those previously described (Muyzer et al. 1993; Crump et al. 2004) with the following modifications: touchdown PCR conditions consisted of an initial 5 min at 94°C , followed by 30 cycles of 1 min at 94°C ; 1 min at $65\text{--}55^\circ \text{C}$ (reducing temperature by 1° per cycle for 10 cycles plus 20 cycles at 55°C); and 1 min at 72°C , followed by 5 min at 72°C . Steps involving a temperature reduction were carried out at a rate of 0.3°C per second. PCR products were separated into bands by electrophoresis (CBS Scientific) for 19 h at 75 V on acrylamide (8%) gels prepared with 30% acrylamide/bis-acrylamide (37.5:1, Bio-Rad), $0.5\times$ TAE buffer ($1\times$ TAE is 40 mmol L^{-1} Tris, pH 8.0; 20 mmol L^{-1} acetic acid; and 1 mmol L^{-1} ethylenediamine-tetraacetic acid), and gradients of 35–50% denaturants (urea and formamide). One sample per gel was used as a ladder and was run in five different lanes in order to accurately determine the position (height) of bands across the gel.

DGGE gels were photographed with a Chemi-Doc gel

documentation system (Bio-Rad) with exposure times set such that the photographs were undersaturated. The bands, defined as those having a pixel density of at least 5% of the most dense band in the sample, were scored as present or absent at each position in the gel using the GelcomparII software package (Applied Maths). Comparison of banding profiles for different samples identified matching bands. Pairwise similarity matrices were calculated from these binary data sets using the Dice coefficient $S_d = 2a/(b + c)$ in which a is the number of shared bands, and b and c are the numbers of bands in each sample. Similarity matrices were then visualized using multidimensional scaling (MDS) (van Hanneken et al. 1999; Bernhard et al. 2005), calculated with the SPSS software package. MDS is an ordination method that uses an iterative optimization procedure to plot samples such that distances between the points indicate the degree of similarity among DGGE banding patterns.

Techniques that use phylogenetically informative genes such as 16S rDNA to compare the composition of bacterial communities range greatly in phylogenetic resolution such that one technique may detect similarities that another does not. DGGE of 16S rDNA provides phylogenetic resolution that is similar to highly optimized applications of terminal restriction fragment length polymorphism analysis (Ritchie et al. 2000) and provides resolution that is greater than that of techniques based on length variation within the rDNA (Fisher and Triplett 1999). It provides arguably less resolution than analyses of the intergenic spacer region between 16S and 23S rDNA, such as the automated approach for ribosomal intergenic spacer analysis (ARISA) (Fisher and Triplett 1999; Yannarell et al. 2003), as the sequence of this region is less conserved than that of rDNA.

Clone libraries of nearly full-length 16S rDNA were constructed with DNA collected on 28 June 2001 following previously described methods (Crump et al. 2003), except that PCR conditions were modified to include a "reconditioning PCR" step. After initial amplification with 20 PCR cycles, products were diluted 1 : 10 in fresh PCR cocktail and subjected to two more PCR cycles (Thompson et al. 2002). DNA sequences were determined with an ABI 3730 automated sequencer according to the manufacturers' instructions, aligned using the ARB sequence alignment program (www.arb-home.de/), and screened for chimera formation using CHIMERA-CHECK (Cole et al. 2005), BELLEROPHON (Huber and Peduzzi 2004), and visual inspection of secondary structure base pair matches. Five chimeric sequences were identified and excluded from further analysis.

Diversity of bacteria represented by clone library sequences was estimated as Operational taxonomic unit (OTU) richness using rarefaction analysis and the Chao 1 nonparametric species-richness estimator. Sequences were initially grouped into 97% and 99% sequence similarity groups, followed by rarefaction (Simberloff 1972) and calculation of the Chao 1 estimator (Chao 1984). DNA sequences from plastids were excluded from this analysis.

The two clone libraries were compared statistically using the LIBSHUFF computer program (Singleton et al. 2001), which calculates coverage (Good 1953) at various levels of evolutionary distance determined using the Jukes-Cantor model for nucleic acid substitution. Homologous coverage

is calculated using $C_x = 1 - (N_x/n)$, where N_x is the number of unique sequences in a library, and n is the total number of sequences. Similarly, heterologous coverage is calculated using $C_{xy} = 1 - (N_{xy}/n)$, where N_{xy} is the number of sequences in library X that are not found in library Y , and n is the number of sequences in library X . Both of these values increase to 1 as the criteria for uniqueness, evolutionary distance (D) calculated from sequence similarity, are made less stringent or higher. A test for differences between these coverage curves serves as a test for differences between clone libraries. The program calculates the distance between these curves according to the Cramer-von Mises test statistic (i.e., the distribution of $(C_x - C_{xy})^2$ with D) (Pettitt 1982) and tests to determine if that distance is significantly different from the distance created after randomly reshuffling the sequences in the two libraries.

The DNA sequences are available in the GenBank database under accession numbers AY947894 to AY948073.

Results

Physical, chemical, and biological measurements made at the river mouths followed very similar trajectories during the 32 months of this study (Fig. 2). Temperature was almost exactly the same in the two rivers. Nutrient concentrations, DOC, DON, and bacterial production were also very similar but occasionally diverged, particularly during the summer of 2001, when river flow was very low. Daily discharge rate was always lower in the smaller Parker River, but monthly changes in the rates tracked each other closely. In fact, all measurements made in the two rivers were strongly correlated, except for the SS and PP concentration (Table 1).

Correlation analyses among physical and chemical measurements for each river revealed several relationships common to the two rivers. All particle-related parameters (SS, PP, POC, and PON) were positively correlated. Temperature correlated negatively with nitrate and positively with ammonia and DON. In the Ipswich River, temperature was positively correlated with phosphate and DOC, but this was not the case in the Parker River, where temperature was correlated with PP, POC, and PON. Discharge correlated with PON and temperature in the Parker River; there was no correlation with discharge in the Ipswich River. A table of correlations can be found in the associated Web Appendix 1 (<http://www.aslo.org/lo/toc/vol50/issue-6/1718a1.pdf>).

Bacterial community composition, measured with DGGE, followed similar patterns in the Ipswich and Parker Rivers. In the Ipswich River, bacterioplankton alternated between a winter and a summer community from February 2000 to July 2002 (Fig. 3A). The Parker River had a similarly predictable winter community but with a more variable summer community, perhaps due to the much lower river discharge rates during the summers of 2001 and 2002 (Fig. 3B). When select samples from the two rivers were analyzed on the same DGGE gel, samples from each date were nearly identical and were grouped together on the MDS diagram (Fig. 3C). However, paired samples showed somewhat lower similarity in the summers compared to the winters, reflecting the variability among Parker River summer communities.

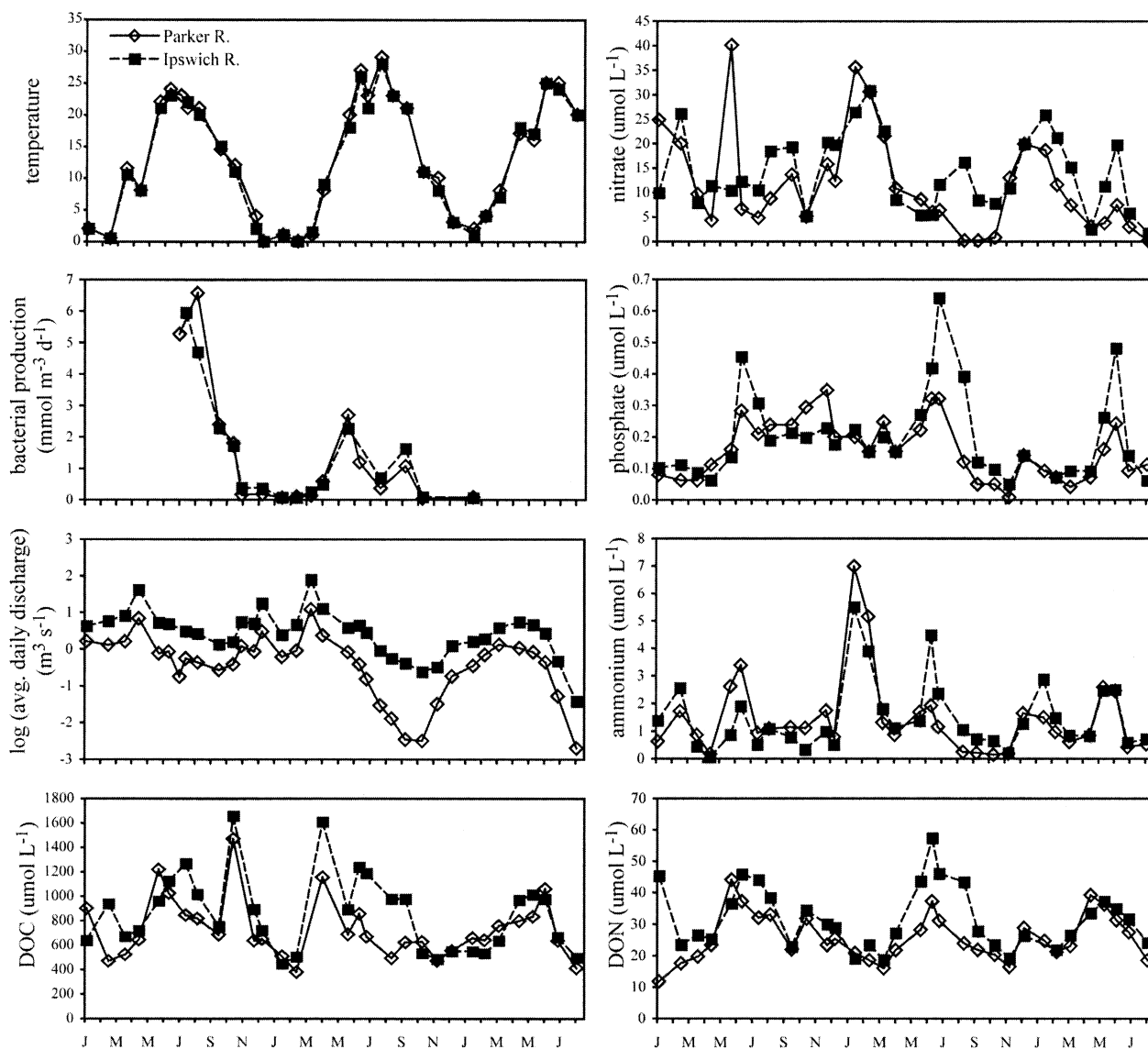


Fig. 2. Measurements of chemical constituents, bacterial production rate, and river discharge for the Ipswich and Parker Rivers from January 2000 to May 2002.

Bacterial production correlated most closely with temperature and DON in both the Ipswich and Parker Rivers (Table 2), but these relationships were not highly significant, primarily because of the large difference in production rates during the summers of 2000 and 2001. The reason for this difference is unclear, but it might be related to interannual differences in river flow.

To identify factors influencing bacterial community composition, the two dimensions of the MDS ordinations of relationships among DGGE banding patterns were compared to all measurements. Dimension 1 was most strongly correlated with temperature in both the Ipswich and Parker Rivers (Fig. 4A) but was also correlated with several chemical measurements that co-varied with temperature (Table 2). Dimension 2 was correlated, though not as strongly, with the discharge rate in each river (Fig. 4B; Table 2). Outliers from this pattern included samples collected on 26 March 2001,

which was 2 d after a very large storm that caused the rivers to overflow their banks and achieve one of the three highest discharge rates of the past 80 yr. Also, the Parker River discharge and MDS dimension 2 were not correlated if the three samples collected during very low flow in 2001 were omitted.

Clone libraries were dominated by beta-*Proteobacteria*, *Bacteroidetes*, and *Actinobacteria*, although the Parker River library also included many plastid sequences (Table 3). Rarefaction trajectories for the two clone libraries, calculated using bacterial sequences, overlapped at both the 97% and 99% sequence similarity OTU cutoffs, suggesting a similar level of diversity. However, these trajectories did not appear to be approaching an asymptote (Fig. 5A), indicating limited coverage of sequence diversity in the two clone libraries. Calculated Chao 1 estimators of sequence diversity were similar at 97% identity, but the Ipswich River contained po-

Table 1. Pearson correlation coefficients, significance values, and number of samples for comparisons of Ipswich and Parker Rivers. Correlations significant at $p < 0.02$ are in bold type.

Measurement	Pearson correlation	p (two-tailed)	n
BP	0.981	<0.001	14
Log discharge (day)	0.935	<0.001	35
Log discharge (week)	0.930	<0.001	35
Temperature	0.996	<0.001	33
NH ₄ ⁺	0.800	<0.001	32
NO ₃ ⁻	0.732	<0.001	31
DON	0.599	<0.001	31
PO ₄ ³⁻	0.719	<0.001	32
DOP	0.550	0.002	30
DOC	0.732	0.002	30
SS	0.215	0.238	32
PP	0.375	0.034	32
POC	0.607	<0.001	32
PON	0.632	<0.001	32

tentially greater diversity at 99% identity. Combined analysis of the two libraries estimated 211 OTUs (99% identity) and 111 OTUs (97% identity) (Table 4), but these estimates should be considered minimums, because the Chao 1 calculation generally underestimates diversity when libraries are undersampled (Hughes et al. 2001, 2002).

Despite our incomplete coverage of sequence diversity, there was tremendous overlap in sequence types between the Ipswich and Parker River clone libraries. Of the total 148 DNA sequences from bacteria, 62 belonged to shared clusters (i.e., clusters containing clones from both rivers) at 99% identity, and 99 belonged to shared clusters at 97% identity. Moreover, at both levels of sequence identity, most clusters contained sequences from both rivers (12 of 22 clusters at 99% identity and 13 of 17 clusters at 97% identity).

Statistical comparison of homologous and heterologous coverage curves for the two clone libraries suggests that the two microbial communities are not significantly different. This is visually represented in Fig. 5 in which the homologous coverage curve for the Parker River (C_x) is very similar to the heterologous coverage curve for the comparison of C_x to the Ipswich River library (C_{xy}). Also, the distribution of $(C_x - C_{xy})^2$ with D shows that the actual values of $(C_x - C_{xy})^2$ are much lower than the values predicted for coverage curves that are significantly different at $p = 0.05$ at all levels of D . This indicates that the overlap in the two libraries is close to the value expected from the levels of coverage and suggests that most taxa are present in both libraries. We found that when the smaller Parker River clone library ($n = 58$) was used as the homologous library, the resulting p value was much larger (0.769) than when the larger Ipswich River clone library ($n = 87$) was used as the homologous library ($p = 0.087$). However, in neither case was the p value low enough to suggest that the libraries were significantly different.

Most of the full-length DNA sequences from bacteria in the Ipswich and Parker Rivers were most closely related to PCR-amplified clones or cultivated isolates from other freshwater planktonic systems. Also, many clones exactly

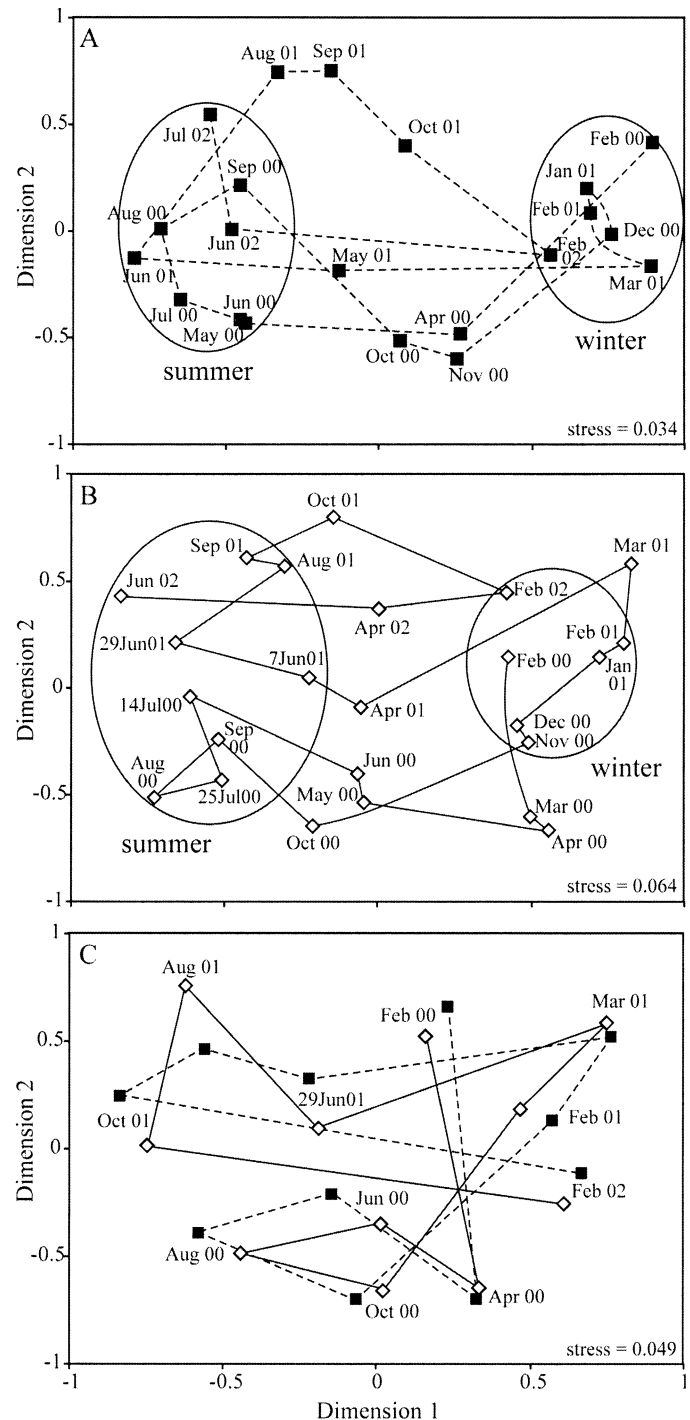


Fig. 3. Multidimensional scaling ordinations (with values of normalized stress) of pairwise estimates of similarity (Dice) calculated from DGGE banding patterns of DNA samples collected between February 2000 and July 2002 from (A) the Ipswich River, (B) the Parker River, and (C) selected samples from the two rivers. Points are connected by lines according to the progression of time. Clusters of summer and winter samples are circled.

Table 2. Pearson correlation coefficients, significance values (*italic*), and number of samples (in parentheses) for comparisons of measured values with bacterial production (BP) and values of the two dimensions of MDS analyses for the Ipswich and Parker Rivers. Correlations significant at $p < 0.02$ are in bold type.

	Ipswich River			Parker River		
	BP	Dimension 1	Dimension 2	BP	Dimension 1	Dimension 2
Dimension 1	-0.806 <i>0.001</i> (14)			-0.68 <i>0.004</i> (16)		
Dimension 2	-0.196 <i>0.503</i> (14)	0.075 <i>0.754</i> (20)		0.513 <i>0.042</i> (16)	0.001 <i>0.997</i> (23)	
Log daily discharge	-0.118 <i>0.676</i> (15)	0.378 <i>0.100</i> (20)	-0.544 <i>0.013</i> (20)	-0.034 <i>0.901</i> (16)	0.499 <i>0.015</i> (23)	-0.507 <i>0.013</i> (23)
Log weekly discharge	-0.09 <i>0.749</i> (15)	0.311 <i>0.181</i> (20)	-0.592 <i>0.006</i> (20)	-0.046 <i>0.867</i> (16)	0.465 <i>0.025</i> (23)	-0.494 <i>0.017</i> (23)
Temperature	0.632 <i>0.015</i> (14)	-0.937 <i>0.000</i> (19)	0.029 <i>0.906</i> (19)	0.529 <i>0.043</i> (15)	-0.846 <i>0.000</i> (22)	0.074 <i>0.744</i> (22)
NH ₄ ⁺	-0.44 <i>0.133</i> (13)	0.224 <i>0.371</i> (18)	0.192 <i>0.446</i> (18)	-0.235 <i>0.439</i> (13)	0.33 <i>0.156</i> (20)	-0.08 <i>0.737</i> (20)
NO ₃ ⁻	-0.342 <i>0.253</i> (13)	0.616 <i>0.007</i> (18)	0.285 <i>0.251</i> (18)	-0.322 <i>0.283</i> (13)	0.665 <i>0.002</i> (19)	-0.189 <i>0.439</i> (19)
PO ₄ ³⁻	0.61 <i>0.035</i> (12)	-0.526 <i>0.030</i> (17)	-0.288 <i>0.262</i> (17)	0.366 <i>0.219</i> (13)	-0.37 <i>0.108</i> (20)	0.255 <i>0.279</i> (20)
DON	0.791 <i>0.002</i> (12)	-0.752 <i>0.001</i> (17)	-0.45 <i>0.07</i> (17)	0.568 <i>0.043</i> (13)	-0.582 <i>0.007</i> (20)	0.432 <i>0.057</i> (20)
DOP	0.204 <i>0.525</i> (12)	-0.408 <i>0.104</i> (17)	0.304 <i>0.236</i> (17)	-0.329 <i>0.272</i> (13)	-0.185 <i>0.436</i> (20)	-0.094 <i>0.593</i> (20)
DOC	0.425 <i>0.169</i> (12)	-0.559 <i>0.020</i> (17)	-0.426 <i>0.088</i> (17)	0.229 <i>0.474</i> (12)	-0.508 <i>0.026</i> (19)	0.374 <i>0.115</i> (19)
SS	-0.023 <i>0.941</i> (13)	0.065 <i>0.799</i> (18)	-0.285 <i>0.251</i> (18)	0.232 <i>0.445</i> (13)	-0.173 <i>0.466</i> (20)	0.076 <i>0.751</i> (20)
PP	0.264 <i>0.383</i> (13)	-0.215 <i>0.392</i> (18)	-0.248 <i>0.321</i> (18)	0.359 <i>0.228</i> (13)	-0.48 <i>0.032</i> (20)	-0.098 <i>0.681</i> (20)
POC	0.162 <i>0.597</i> (13)	-0.264 <i>0.290</i> (18)	-0.331 <i>0.180</i> (18)	0.416 <i>0.157</i> (13)	-0.553 <i>0.011</i> (20)	-0.01 <i>0.967</i> (20)
PON	0.014 <i>0.965</i> (13)	-0.119 <i>0.638</i> (18)	-0.171 <i>0.499</i> (18)	0.171 <i>0.578</i> (13)	-0.498 <i>0.025</i> (20)	-0.222 <i>0.346</i> (20)

matched the sequences of eight different DGGE bands collected from the Parker River in September 2000 (Crump et al. 2004). Beta-*Proteobacteria* sequences fell into several phylogenetic clusters, including three well-recognized and globally distributed freshwater clusters: *Rhodospirillum rubrum* sp. Bal47, LD28, and *Polynucleobacter necessarius* (Zwart et al. 2002). *Bacteroidetes* clones were highly diverse, but 31 of 41 fell into five groups, two of which are well-recognized and globally distributed freshwater clusters: FukuN47 and

PRD01a001B (Zwart et al. 2002). All but two *Actinobacteria* clones fell into three previously identified freshwater clusters: ACK-M1 (Zwart et al. 2002), Luna-1, and Luna-2 (Hahn et al. 2003). These clusters were recently supported by a more comprehensive analysis of freshwater *Actinobacteria* diversity in which they were given the names acI-A, acII-A, and acII-B, respectively (Warnecke et al. 2004). Newly identified freshwater phylogenetic clusters were given the name of the longest associated 16S rDNA sequence. Phy-

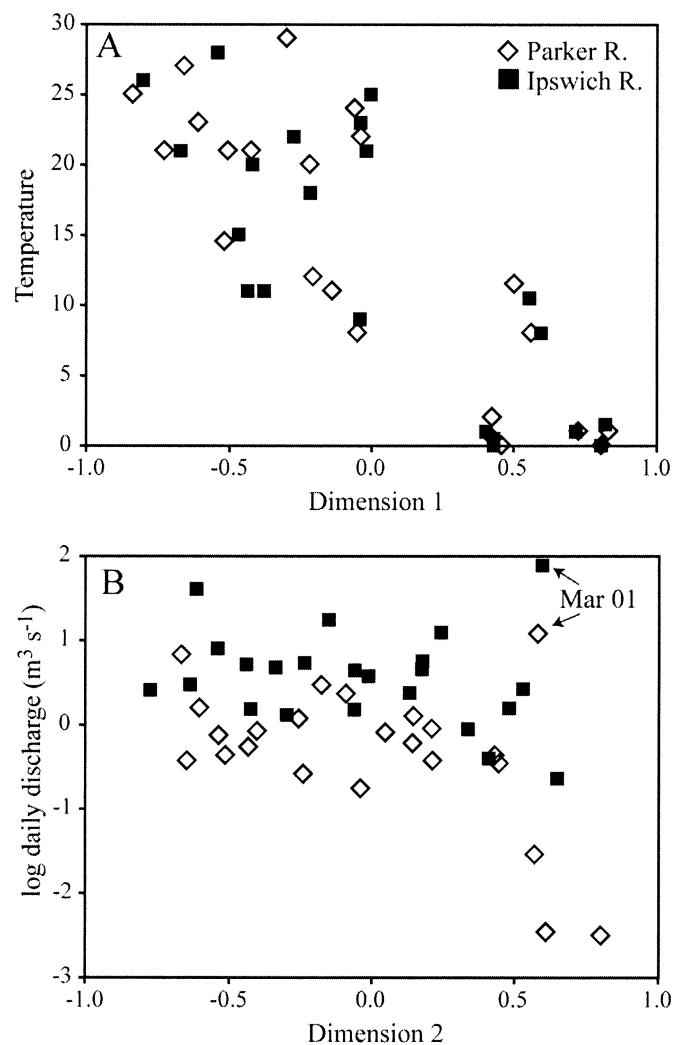


Fig. 4. Scatterplots of (A) MDS dimension 1 versus temperature and (B) MDS dimension 2 versus log values of the daily discharge rates of the Parker and Ipswich Rivers. MDS dimension values match those in Fig. 3A,B.

logenetic trees and an expanded description of the DNA sequence data can be found in the associated Web Appendix 2 (http://www.aslo.org/lo/toc/vol_50/issue_6/1718a2.pdf).

Discussion

Bacteria suspended in river water originate from many different sources. Autochthonous bacterioplankton populations that develop in the water column mix with allochthonous populations from forest soils, urbanized land, farm fields, and wetlands as well as from populations sloughed from surface biofilms and hyporheic sediments in the river. One small study in the Ipswich River basin found bacterial communities to be highly variable among first-order streams, perhaps reflecting differences among source populations and stream chemistry (Levine and Crump 2002). Many populations (i.e., DGGE bands) from these streams were also detected in the main stem, forming a community that was fairly constant along much of the length of the river. Given the

Table 3. Classification of organisms identified in environmental clone libraries.

Phylum of subphylum	No. of clones	
	Ipswich River	Parker River
Alpha-Proteobacteria	2	3
Beta-Proteobacteria	45	31
Gamma-Proteobacteria	2	0
Delta-Proteobacteria	3	0
Epsilon-Proteobacteria	1	0
Candidate division OP10	0	3
Bacteroidetes	28	13
Actinobacteria	7	7
Spirochaetes	1	0
Unknown	1	1
Plastid	2	30

variability in community composition among first-order streams and the apparent influence of these streams on main stem diversity, it is rather surprising that the Ipswich and Parker Rivers contain such similar microbial communities. Our results suggest that intrinsic controls on bacterioplankton community composition are the same in both river systems, resulting in remarkably similar main stem microbial communities that respond synchronously to extrinsic seasonal changes in temperature and river flow rates.

Intersystem variability—Bacterioplankton community composition changed synchronously in the Ipswich and Parker Rivers on a monthly basis during the 2.5 yr of this study, and the bacterial production rate changed synchronously over 1.5 yr. Synchrony in time-series data from two or more independent systems suggests shared influence of extrinsic factors such as climate, and deviations from synchrony or even asynchrony indicate the dominance of intrinsic, site-specific controls. For example, Pace and Cole (2002) observed synchronous changes in DOC and water color among 20 lakes in northern Michigan related to the ice-out date and spring and summer precipitation, but they found that deviations from synchrony appeared to correlate with the absence of an outlet stream. Such patterns in physical and chemical variables are often reflected in the composition of aquatic biological communities. In one example, Rusak et al. (1999) found weak synchrony in the abundance patterns of different cladoceran species among eight lakes in Ontario, Canada, but found stronger synchrony among “homogeneous” subsets of lakes, suggesting that broad-scale extrinsic controls were coupled with lake-specific intrinsic variability to control zooplankton abundance.

The strategy of using observations of synchrony to identify intrinsic and extrinsic ecological controls is potentially useful for understanding controls on bacterial community composition. But before this approach can be applied, it is necessary to identify and describe systems in which bacterial community composition is similar and changes synchronously. The small number of studies that apply this approach to bacterioplankton populations make the assumption that systems with identical controls will contain the same communities, but most do not identify systems in which the com-

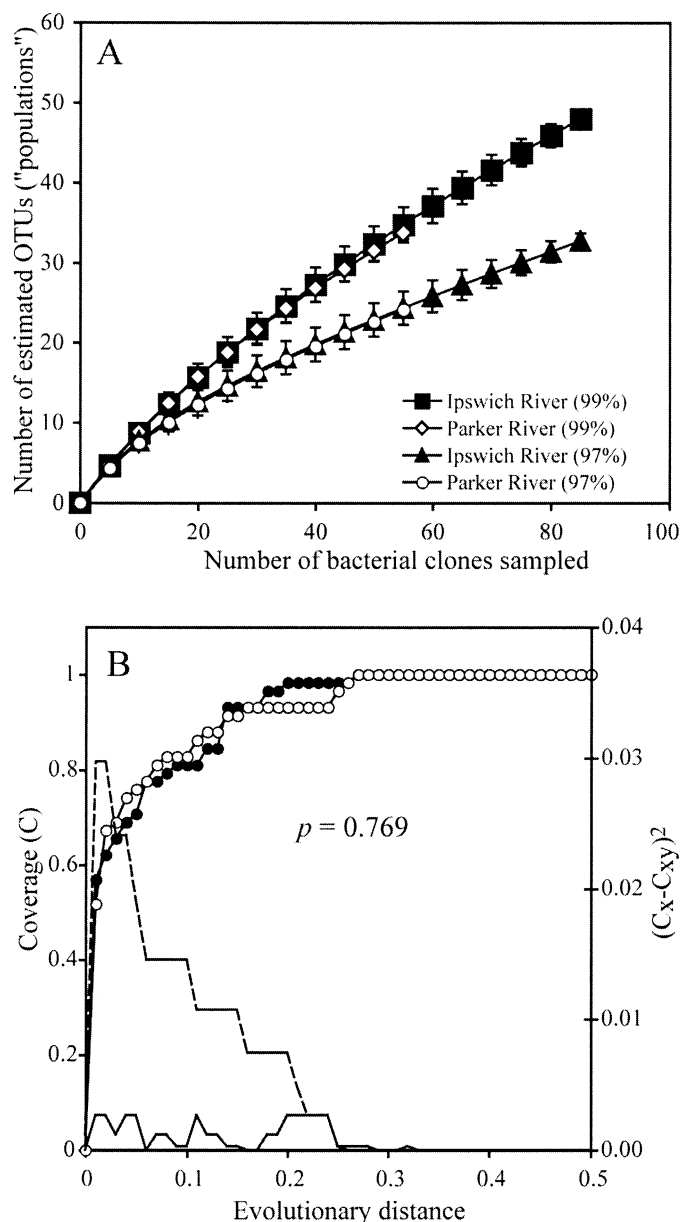


Fig. 5. (A) Rarefaction analysis of similarity groups within the Ipswich and Parker River clone libraries. Operational taxonomic units (OTUs) are defined as clusters having 99% sequence identity and 95% sequence identity. Bars indicate standard deviation of the statistical resampling process. (B) LIBSHUFF comparison of the homologous (open circles; Parker River only) and heterologous (closed circles; Parker and Ipswich Rivers) coverage curves for bacterial sequences in clone libraries. Solid line indicates the value of $(C_x - C_{xy})^2$ for samples at each value of evolutionary distance (D). D is equal to the Jukes–Cantor evolutionary distance determined by the DNADIST program of PHYLIP. Dashed line indicates the $p = 0.05$ value for $(C_x - C_{xy})^2$ of randomized samples. Libraries are considered significantly different when $p < 0.05$.

communities are the same (Lindstrom 2000; Van der Gucht et al. 2001; Yannarell et al. 2003). Is it reasonable to expect similar but unconnected systems to contain similar bacterioplankton communities? One large-scale study of lake bac-

Table 4. Estimates of bacterial diversity (OTU richness) in Parker and Ipswich River clone libraries separately and combined. OTU defined at 99% and 97% sequenced identity.

Clone library	Chao-I estimator of number of OTUs			
	99%	SD	97%	SD
Ipswich River	221	58	178	61
Parker River	113	32	129	46
Ipswich and Parker	211	41	111	27

terioplankton diversity using samples collected during June 2001 showed that lakes with similar characteristics contained similar communities (Yannarell and Triplett 2004), although many of these lakes were connected with streams. Our study also shows similarity in the bacterioplankton communities of two unconnected systems, but in addition, it shows how these communities change synchronously over time and how changes relate to synchronous changes in several physical, chemical, and biological variables.

Bacterial production was also synchronous between the two rivers, probably as a result of synchrony in the environmental factors thought to influence aquatic bacterial growth. Across a range of systems, bacterial production can be predicted with measurements of organic carbon supply or concentration (Cole et al. 1988) and is influenced by phytoplankton production, allochthonous organic matter (Findlay et al. 1991), temperature (Findlay et al. 1991), and nutrient concentration (Mohamed and Roberts 2003). In our study, temperature and all measurements of organic matter and nutrients were correlated between the two rivers, and bacterial production responded to these factors in the same way in both systems. According to the theory of synchronous behavior, this suggests that factors that differ between the two rivers do not influence bacterial production, including SS and PP concentration as well as larger-scale differences in river flow rate, watershed size, and land use.

Very few studies compare freshwater bacterioplankton communities in two or more unconnected rivers. Stepanauskas et al. (2003) characterized bacterioplankton communities in the Sacramento–San Joaquin river delta, United States, including samples from each river upstream of the delta. As with our study, they found these rivers contained similar communities that shifted consistently with the seasons. In contrast, two rivers in the same part of the Czech Republic contained statistically different communities on the basis of cell counts using in situ hybridization of phylum- and sub-phylum-specific probes (Masin et al. 2003). Comparing the communities in the Ipswich and Parker Rivers at any particular time point provides little information on factors influencing community composition because the communities are so similar. Therefore, we must look at how temporal changes in community composition relate to changes in environmental variables in order to identify variables that might influence community composition (and bacterial production) the same way in both rivers.

Temporal variability—Within each river, we detected large seasonal differences in bacterioplankton community composition and bacterial production. Community compo-

sition correlated most strongly with temperature and river flow rate (Table 2; Fig. 4), both of which are driven mainly by climate. Temporal shifts in community structure have been related to seasonal forces in several single-system studies (Hofle et al. 1999; Van der Gucht et al. 2001; Zwisler et al. 2003), and in one study, these forces had a similar effect on communities in three different lakes (Yannarell et al. 2003). In this final study, seasonal changes in water temperature and lake stratification state were fairly good predictors of bacterioplankton community composition in an oligotrophic and a eutrophic lake but failed to predict community fluctuations in a shallow humic lake, particularly during the summer when temperatures were high and communities were highly variable. Water temperature was the strongest predictor of changes in communities of the Ipswich and Parker Rivers, demonstrating the strong seasonality of river bacterioplankton communities.

Water temperature ranged from 0°C in winter when ice formed on the rivers to as high as 29°C in summer. Samples collected by Yannarell et al. (2003) covered a similar range of temperatures, and these researchers observed that season-specific bacterioplankton communities were most stable in spring and fall but more variable in summer. This contrasts with our results, which show relatively stable communities in winter and summer and more variable communities during fall and spring. In both cases, within-season variability indicates the influence of environmental factors other than temperature. Yannarell et al. (2003) suggest that intense biological activity during summer causes fluctuating bacterioplankton communities. Variability during spring and fall in the Parker and Ipswich Rivers is caused, in part, by the gradual shifting between winter and summer communities but could also reflect several other influences. Besides temperature, the first MDS dimension also correlated with nitrate and DON in both systems and with river flow rate in the Parker River. But when we restricted our analyses to results from spring (March, April, and early June) and fall (September, October, and November) of 2000–2001, the first MDS dimension correlated only with temperature and river flow rate. So, although temperature is the best predictor of community changes, these changes are probably influenced by several different physical and chemical factors, the relative importance of which varies with the season.

River flow rate, which was the only factor correlated with the second MDS dimension, should not directly influence the growth rates of bacterioplankton populations but could influence community composition indirectly through several mechanisms. Bacterial community composition in rivers generally shifts slowly, if at all, along river lengths (Levine and Crump 2002; Sekiguchi et al. 2002; Stepanauskas et al. 2003), but studies of reservoirs identify large longitudinal shifts in community composition (Dumestre et al. 2002; Masin et al. 2003). These shifts are potentially driven by many factors, including increased phytoplankton production (Masin et al. 2003), increased grazing pressure (Masin et al. 2003), and the development of an anoxic deep-water layer (Dumestre et al. 2002), but ultimately, they are caused by the extended residence time of water and planktonic communities behind dams. This same phenomenon could explain the relationship between bacterioplankton community com-

position and river flow rate in the Ipswich and Parker Rivers. As river flow rate decreases, the in-stream residence time increases, especially behind dams, permitting a greater shift in bacterioplankton community composition.

Variable river flow rate could also be linked to differences in the relative inputs of bacteria from different catchments in the watershed. During low river flow, some tributaries dry up, and during some summers, reaches of the main stem Ipswich River occasionally stop flowing (Armstrong et al. 2001). At the other extreme, very high flows result in flooding and the likely input of bacteria from land surfaces that are only rarely underwater. We witnessed major flooding near our sampling sites in March 2001 and found that bacterioplankton communities on that date were somewhat unusual (Figs. 3, 4b). Such shifts in the source communities of bacteria influence the composition of bacterioplankton communities either by changing the types of bacteria that are advected through the system or by changing the source of inocula for active bacterioplankton populations.

The first dimension of the MDS diagrams correlated with the bacterial production rate, suggesting a link between bacterial growth and community composition. These factors also correlated with temperature in both rivers, but we found that the relationship between growth and composition remained after partial correlation analysis in which we held temperature constant. Thus, it appears that river bacterioplankton vary between phylogenetically distinct fast-growing and slow-growing communities. Our DNA sequencing indicates that the fast-growing communities are composed of typical bacterioplankton, but it remains unclear whether slow-growing communities are composed of typical bacterioplankton or organisms advected from land.

Our approach of sampling monthly throughout the year probably emphasizes the influence of temperature, but we also identified other potentially important correlations. Among chemical measurements, DON correlated most strongly with bacterial production, but DON was also strongly correlated with temperature. Partial correlation analyses holding temperature constant supported this relationship for the Ipswich River (coefficient = 0.653, $p = 0.029$) but not for the Parker River (coefficient = 0.356, $p = 0.256$). DON is a proxy for labile organic matter or DOC with a low C:N ratio (Stepanauskas et al. 2002; Kaushal and Lewis 2003). A correlation of DON with bacterial production suggests substrate limitation of bacterial growth.

Interannual variability—Community composition in the summer and winter appeared to repeat from year to year on the basis of the MDS diagrams in Fig. 3. Similarity values among February samples for the 3 yr of this study averaged 0.69 for both rivers. Similarity values among June samples were also high, averaging 0.66 in the Ipswich River and 0.60 in the Parker River. Thus, more than half of the winter and summer bacterial communities return year after year or are present year-round. Similar results were found over two consecutive winter seasons in two Belgian lakes (Van der Gucht et al. 2001) and an Arctic tundra lake (Crump et al. 2003) and over two consecutive summer seasons in three temperate lakes in the northern United States (Yannarell et al. 2003). These studies contrast with a 2-yr study of a boreal forest

lake in which the bacterioplankton community showed no seasonality, but rather, a slow gradual change (Lindstrom 1998). Seasonality in bacterioplankton communities is clearly variable among freshwater systems, but in none of the cases does the exact community reappear. The degree of recurrence of individual phylotypes probably depends on the interannual variability of controlling factors but may also depend on the phylogenetic diversity of functionally suitable bacteria and the random substitution of functionally identical populations (Curtis and Sloan 2004). In our study, summer communities in the Parker River were more variable inter-annually than in the Ipswich River, but the cause of this variability could not be identified.

Variables not tested—Several potential controls on bacterioplankton production and community composition were not measured in this study. First among these is primary production or chlorophyll concentration. The connection between bacterial production and primary production is very well described in aquatic systems (Cole 1982), and shifts in bacterioplankton community composition have been connected to phytoplankton growth and diversity (Lindstrom 2000; Crump et al. 2003; Stepanauskas et al. 2003). Chl *a*, measured in eight pairs of samples collected between 2 June and 6 December 2000, was four to eight times higher in the Parker River during the summer but was about the same in the two systems during the winter (data not shown). Also, the Parker River clone library, prepared with a summer sample from 2001, contained many more chloroplasts than the Ipswich library. Thus, the Parker River probably has more phytoplankton cells and may host more primary productivity during the summer, but this difference is not reflected in the bacterioplankton community compositions.

A second variable not tested in our study is the abundance and activity of bacterioplankton grazers. Protist grazing influences bacterioplankton production (Sanders et al. 1992) and bacterioplankton community composition (Simek et al. 2002). We did not measure grazer abundance or activity and thus can only assume either that grazing pressure in the two rivers was equal or that it simply was not an important control on bacterial production and community composition.

Summer diversity—Clone libraries prepared from summer samples were dominated by beta-*Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* (Table 3), most of which (48 of 76 for betas, 31 of 41 of *Cytophaga*, and 11 of 14 of *Actinobacteria*) fell into eight known freshwater bacterioplankton clusters and five newly identified clusters. None of the sequences were closely related to organisms associated with riverine biofilms (Brummer et al. 2003; Lawrence et al. 2004). However, several beta-*Proteobacteria* sequences (Fig. 2A) were related to clones from groundwater (IRD18H05 and PRD18A12); *Ferribacter limneticum*, an isolate from mining-affected lake sediments (IRD18B11); and a clone from “water-logged archaeological wood” (PRD18B04). Also, two clones from the Ipswich River clustered with the delta-*Proteobacteria geobacter* sp. and *Pelobacter propionicus*, which are fermentative organisms isolated from freshwater sediments and other belowground environments (data not shown). On the basis of these results, it seems clear that

some of the organisms in these rivers wash in from the watershed. In summer, when our clone libraries were prepared, allochthonous organisms were only a small fraction of the total population, but they could come to dominate the bacterioplankton in winter when low bacterial production may not allow the development of an autochthonous bacterioplankton community.

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