

Temporal Patterns in Bacterial Communities in Three Temperate Lakes of Different Trophic Status

A.C. Yannarell,¹ A.D. Kent,¹ G.H. Lauster,¹ T.K. Kratz,² E.W. Triplett³

¹ Center for Limnology, University of Wisconsin–Madison, Madison, WI 53706, USA

² Trout Lake Station, Center for Limnology, University of Wisconsin, 10810 County Highway N, Boulder Junction, WI 54512, USA

³ Department of Agronomy, University of Wisconsin–Madison, Madison, WI 53706, USA

Received: 16 January 2003; Accepted: 21 March 2003; Online publication: 14 August 2003

ABSTRACT

Despite considerable attention in recent years, the composition and dynamics of lake bacterial communities over annual time scales are poorly understood. This study used automated ribosomal intergenic spacer analysis (ARISA) to explore the patterns of change in lake bacterial communities in three temperate lakes over 2 consecutive years. The study lakes included a humic lake, an oligotrophic lake, and a eutrophic lake, and the epilimnetic bacterial communities were sampled every 2 weeks. The patterns of change in bacterial communities indicated that seasonal forces were important in structuring the behavior of the bacterial communities in each lake. All three lakes had relatively stable community composition in spring and fall, but summer changes were dramatic. Summertime variability was often characterized by recurrent drops in bacterial diversity. Specific ARISA fragments derived from these lakes were not constant among lakes or from year to year, and those fragments that did recur in lakes in different years did not exhibit the same seasonal pattern of recurrence. Nonetheless, seasonal patterns observed in 2000 were fairly successful predictors of the rate of change in bacterial communities and in the degree of autocorrelation of bacterial communities in 2001. Thus, seasonal forces may be important structuring elements of these systems as a whole even if they are uncoupled from the dynamics of the individual system components.

Introduction

Lake bacterial communities have long been regarded as “black boxes” in ecological studies, although the importance of bacteria in processes such as nutrient cycling is

well recognized [8, 13, 32]. Bacterial populations can undergo rapid turnover in both time [19, 26, 61] and space [7, 41, 61, 64], but the composition of bacterial communities and the dynamics of their changes over longer time scales are not well understood. This manuscript presents the results of a 2-year, culture-independent study of bacterioplankton community dynamics in three dimictic lakes

with differing physical, chemical, and biological characteristics. The patterns of change in bacterioplankton community composition (BCC) in these lakes provide novel information about the response of aquatic communities to seasonal changes in lakes. They also provide insights into the repeatability of the cycles of change in BCC.

Culture-independent studies of bacterial communities allow rapid assessment of changes in community structure over time and space. Bacterial strains that are readily culturable with current technology are not necessarily representative of either the composition or diversity of natural bacterial communities [5]. In addition, culture-independent studies provide the only means of obtaining information about the dynamics of communities in a natural setting. 16S rDNA-based community fingerprinting techniques such as automated ribosomal intergenic spacer analysis (ARISA), denaturing gradient gel electrophoresis (DGGE), and terminal restriction fragment length polymorphism (T-RFLP) generate a unique bacterial community signal from samples of bulk DNA [21, 33, 43, 50]. These community fingerprinting techniques allow researchers to ask questions about the similarities of different bacterial communities based on the underlying community composition.

Using a technique similar to ARISA but based on the 5S r-RNA gene, Höfle and others [26] demonstrated that the BCC of a hypereutrophic lake changed rather dramatically over a period of several months. Several multilake studies have also shown variation in BCC between different lakes, even those of similar trophic status [22, 23, 41, 48, 49]. BCC is sensitive to water chemistry parameters that may remain stable within lakes such as pH, alkalinity, and major cation concentrations [49]. However, bacterial communities can respond rapidly to changes that occur on seasonal scales, such as grazing pressure [24, 28, 31, 40], viral count [54, 61], algal abundance [30, 55], and nutrient concentrations [6, 15, 20, 58]. In addition, studies of bulk community measurements such as bacterial respiration and secondary production suggest that the activity of the bacterial community can be strongly dependent on temperature [16, 17, 65], light levels [62, 63], and gross primary productivity [14, 16]. Thus, patterns of change in BCC should reflect the effects of stable, lake-specific characteristics as well as the effects of factors that fluctuate on seasonal cycles.

Understanding temporal and spatial variability of bacterial communities requires frequent sampling from diverse locations. The present work examines patterns of

change in BCC derived from biweekly samples from three lakes of contrasting trophic status. Specifically, this work examines whether (1) changes in community composition in lakes are correlated to seasonal changes in limnological conditions, such as mixing and thermal dynamics; (2) the patterns of change are the same in lakes with different characteristics; (3) the seasonal patterns of change are the same from one year to the next; (4) the same organisms or sets of organisms account for these patterns from year to year or from lake to lake.

Methods

Study Sites and Sample Collection

Three lakes with contrasting chemical, morphometric, and trophic parameters (Table 1) were selected for study. Lake Mendota (ME; 89° 24' W long, 43° 06' N lat) is an anthropogenically eutrophied lake located in a primarily agricultural watershed in Southern Wisconsin. Crystal Lake (CR; 89° 37' W long, 46° N lat) and Crystal Bog Lake (CB; 89° 36' W long, 46° N lat) are situated in Wisconsin's Northern Highland State Forest in Vilas County, Wisconsin. CR is an oligotrophic lake with a sandy bottom, and CB is a shallow humic lake surrounded by an extensive *Sphagnum* mat. The oligotrophic and eutrophic lakes are dimictic lakes, but the humic lake is too shallow to stratify (Table 1). Each of the lakes is a primary study lake of the North Temperate Lakes Long-Term Ecological Research (NTL-LTER) program [44].

Water samples for bacterial analyses were collected from each lake biweekly throughout the ice-free periods of the years 2000 and 2001 and once through the ice in January 2001. Integrated water samples were collected at the deepest point using a sealable (via a ball-joint valve) length of ¼-inch PVC pipe. The oligotrophic and eutrophic lakes were sampled to a depth equal to the bottom of the epilimnion or 12 m (whichever was smaller), and the humic lake was sampled over the full 2-m water column. Water samples for community analyses were screened through a 10-µm nylon mesh (Spectrum) to exclude the larger eukaryotes; therefore, all bacterial cells passing through this mesh, including members of the cyanobacteria, were included in this study. All samples were cooled on ice for transport back to the laboratory. In the lab, DNA from 250–500 mL of lake water was concentrated onto 0.2-µm filters (Supor-200; Gelman). Filters were placed in cryovials, frozen immediately in liquid nitrogen, and stored at –80°C until DNA extraction via the FastPrep DNA purification kit (BIO101).

Single samples were collected on each sampling date in 2000 and January 2001, whereas replicate samples were collected at each sampling date during the rest of 2001. The replicate samples from 2001 were 94.74% ± 1.47% (mean ± SE; $n = 45$) similar to each other (as assessed by Sorenson's similarity; see below), and thus the authors feel that the lack of replication in the 2000 data does not preclude its use in this study.

Table 1. Selected chemical, morphometric, and biological parameters for the three study lakes for the year 2000^a

	Lake Mendota (eutrophic)	Crystal Lake (oligotrophic)	Crystal bog lake (humic)
Surface area (ha)	3937.7	36.7	0.5
Maximum depth (m)	25.3	20.4	2.5
Mean depth (m)	12.8	10.4	1.7
Landscape position	Low	High	High
Total phosphorus ($\mu\text{g L}^{-1}$)	39.5–221	1.5–8.5	11.5–19.67
Total nitrogen ($\mu\text{g L}^{-1}$)	895–3157	102–187.5	394–678
DOC (mg L^{-1})	5.28–6.67	1.84–2.23	7.72–9.74
Chlorophyll a ($\mu\text{g L}^{-1}$)	0.7–23.2	0.27–5.4	3.3–54.1
Conductivity (μS)	N/M	14	11
ANC ($\mu\text{eq L}^{-1}$)	N/M	16	10
pH	8.48–8.74	6.48–6.74	5.19–6.17

N/M, not measured

^a Ranges are for across the entire year. All values are derived from NTL-LTER database ([35, 57]; http://limnosun.limnology.wisc.edu/lter_lake.html)

Data on the limnological conditions of the lakes, including water temperature and the depths of various strata, were obtained from the NTL-LTER database [35, 57]. These datasets, as well as details on the methods used to collect and calculate the associated parameters, can be found and queried on the World Wide Web at <http://lterquery.limnology.wisc.edu/>.

Community Composition of Bacterioplankton

Bacterioplankton diversity and community composition were assessed by automated ribosomal intergenic spacer analysis (ARISA), following the method of Fisher and Triplett [21] with the following modifications. PCR reactions contained 1× PCR buffer (consisting of 50 mM Tris [pH 8.0], 250 μg of bovine serum albumin per mL, and 3.0 mM MgCl_2 [Idaho Tech, Salt Lake City]), 250 μM (final concentration) of each dNTP, 10 pmol of each primer, 1.25 U of *Taq* polymerase (Promega), and 1 μL of lake-extracted DNA in a final volume of 25 μL . The primers used were 1406f (universal, 16S rRNA gene; 5'-TGACAC ACCGCCGT-3') and 23Sr (bacteria-specific, 23S rRNA gene; 5'-GGGTTBCCCCATTCRG-3'; [39]). The 1406f primer was labeled at the 5' end with the phosphoramidite dye 6-FAM. The PCR was carried out in an Eppendorf MasterCycler Gradient (Eppendorf) with an initial denaturation at 94°C for 2 min, followed by 30 cycles of 94°C for 35 s, 55°C for 45 s, and 72°C for 2 min, with a final extension carried out at 72°C for 2 min.

Denaturing capillary electrophoresis was carried out for each PCR reaction using an ABI 310 Genetic Analyzer (PE Biosystems). Electrophoresis conditions were 60°C and 15 kV with a run time of 50 min using the POP-4 polymer. A custom 200- to 2000-bp Rhodamine X-labeled size standard (Bioventures) was used as the internal size standard for each sample. ARISA fragment sizes were determined using the local Southern size calling method in GeneScan 3.1.2 [2]. To include the maximum number of peaks while excluding background fluorescence, a fluorescence cutoff of 100 fluorescence units for peak height was used. Because GeneScan estimates the lengths of fragments to the nearest 0.01 bp, the analyzed community profiles were then aligned using the help of Genotyper 2.1 [1]. This ensured that ARISA fragments of

the same length were consistently identified with each other despite minor variations in size calling that occurred from run to run. Sample community fingerprints were then generated from the aligned profiles, with each different-sized ARISA fragment being scored as “present” (1) or “absent” (0) from a sample. The presence/absence of ARISA fragments, rather than their relative abundances (i.e., as reflected in the fluorescence), was used because PCR has been shown to skew the ratio of its products away from that of the original ratio of templates [51, 59, 60].

Data Analysis

The datasets derived for the present study were multivariate, with many more variables (ARISA fragments, in this case) than samples. On any given sample date, there were many more ARISA fragments scored as absent than as present. These features violated several of the assumptions of traditional parametric statistics. In particular, the data were not amenable to transformations to normality. Nonparametric approaches are more appropriate for datasets with distinctly nonnormal distributions and with a low sample-to-variable ratio [9, 18]. Therefore, all data analyses utilized in the present study were nonparametric. Because nonparametric tests have less power than parametric statistics, reliance upon them may have hindered the ability to detect significant differences in some cases, and thus the results presented here may be viewed as conservative. Mardia and others [46] and McCune and Grace [47] offer a thorough discussion of this.

Pattern Discovery. The similarity of ARISA profiles from pairs of sample dates was determined using the Sorenson's Index of Similarity [45]. For the 2000 and 2001 datasets, and for each lake separately, a similarity matrix was prepared in which the Sorenson's Index was calculated for all possible pairs of sample dates. These similarity matrices were used to create nonmetric multidimensional scaling (MDS) plots for each of the datasets. MDS is an ordination technique that performs well regardless of the linearity (or lack thereof) in the relationships between variables, and it has been shown to faithfully represent the

relationships inherent in multivariate datasets [47]. MDS attempts to preserve the ranked order of the similarity of any two sample communities as an inverse function of the distance between the points representing those communities on the plot [37]. That is, the two communities with the highest similarity in the dataset are represented on the plot with the two points that are plotted closest together, and the two communities with the lowest similarity are represented on the plot with the two points located furthest apart. The degree to which the plot matches the similarity matrix can be judged by examining the stress, defined here as Kruskal's stress formula 1 [38], with values less than 0.1 representing good ordinations with little risk of misinterpretation of pattern [9]. The prepared MDS plots were used to visualize the relationship between the bacterial communities, as determined by their ARISA profiles, over the range of the sampling period. MDS was performed here using 20 random starting configurations of sample points, and the final configuration was assumed to be the optimal one if no other configurations had a lower stress. In all cases, two-dimensional solutions are presented here.

Serial Correlation. This test was carried out to determine whether the observed patterns were the same from lake to lake and from year to year. Each lake dataset for each year was recorded so that sample dates corresponded to the number of weeks since the observed ice-out date on that lake, and new similarity indices were generated. The resultant similarity matrices for each lake/year pair were compared using the Spearman rank correlation coefficient (ρ). High ρ -values would suggest that patterns in different lakes or in the same lake in different years were very similar. Null distributions for correlation coefficients were generated by randomly reshuffling the elements of the similarity matrices. Comparison of the ρ -values to these null distributions allowed for the determination of the likelihood that ρ -values of the observed magnitude would occur if communities were randomly distributed in time or space.

Analysis of Similarity (ANOSIM). Analysis of similarity, as described by Clarke and Green [9, 11], was used to test hypotheses regarding the seasonal differences in lake bacterial communities. This method is an example of a multi-response permutation procedure [47]. Sample ARISA profiles were grouped according to the temperature and stratification state of the lake on the dates on which they were recovered, and ANOSIM was used to test the null hypothesis that the within-group similarity was equal to the between-group similarity. ANOSIM generates a test statistic, R , and the magnitude of R is indicative of the degree of separation between groups, with a score of 1 indicating complete separation and 0 indicating no separation [9, 11]. Monte-Carlo randomization of the group labels was used to generate null distributions in order to test the hypothesis that within-group similarities are higher than would be expected if sample ARISA profiles were grouped at random.

Because replicate samples in 2001 had a high degree of similarity, the inclusion of both replicates in ANOSIM analyses

would have biased the results, as these would more properly be considered "pseudoreplicates" [27] for ANOSIM. Therefore, replicate samples collected on the same date were used to generate "pooled" ARISA profiles. Only ARISA fragments present in both replicate samples were considered to be present in the pooled profile. The pooled ARISA profiles were used for the generation of all 2001 similarity matrices and for all analyses conducted on 2001 data.

Identification of Important Pattern-Forming Elements. It is possible to recreate the pattern inherent in a similarity matrix with only a subset of the original data [12]; this subset is composed of the most important pattern-forming elements of the original dataset. The method of Clarke and Warwick [12] was used to determine which ARISA fragments constituted the most important pattern-forming elements from each lake/year combination. In brief, this method used an algorithm to assemble new sample-by-ARISA fragment matrices whose calculated similarity indices best matched the original similarity matrix, as determined by Spearman rank correlation coefficient. ARISA fragments were randomly added and removed from a subset of fragments until the newly assembled similarity matrix was 95% correlated with the original similarity matrix, or until the improved fit was less than 0.01%. This process was performed 10 times, beginning with random subsets of 50% of the original data. The best subset was considered to be the one with the fewest ARISA fragments that still produced a correlation of >90% with the original similarity matrix.

To generate lists of the most important pattern-forming ARISA fragments, this algorithm was applied to each lake/year dataset. The ARISA fragments identified as the best pattern-forming elements were then deleted from the dataset, and the procedure was repeated with the new, modified dataset. This process continued until the modified dataset failed to produce a subset of ARISA fragments that was >90% correlated to the original similarity matrix. The previously deleted ARISA fragments, which had been identified as important pattern-forming elements, were then assembled into a new dataset. This dataset was presumed to contain the ARISA fragments whose patterns of presences and absences contained the most information about the overall pattern.

All data analyses were performed with the PRIMER 5 for Windows software suite [10]. The relevant subroutines were MDS, ANOSIM, RELATE, and BVSTEP.

Results

Comparisons among Lakes and Years

The richness of ARISA fragments differed substantially across the three lakes (Fig. 1). In general, the profiles from the humic lake (Crystal Bog) were the poorest in terms of total number of ARISA fragments. Richness in all three

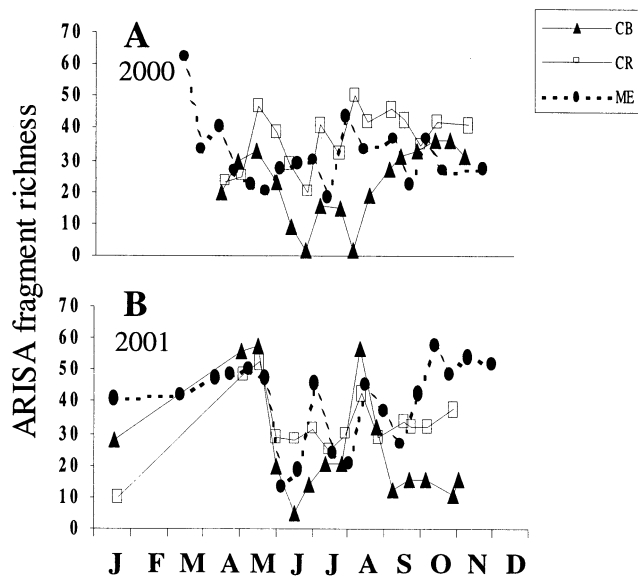


Fig. 1. Time series of ARISA fragment richness for the study lakes. Filled triangles are Crystal Bog Lake (CB; humic), open squares are Crystal Lake (CR; oligotrophic), and filled circles are Lake Mendota (ME; eutrophic). (A) Series for the year 2000. (B) Series for the year 2001.

lakes tended to be higher in the spring and fall months, with periodic declines to extremely low richness observed in the summer. These declines were most notable in the humic lake and the eutrophic lake (Lake Mendota). Richness in the oligotrophic lake (Crystal Lake) was comparatively stable in both years (Fig. 1).

The distribution of ARISA fragments in the lakes is summarized in Table 2. Roughly 40% of all ARISA fragments identified in this study were unique to one of the three study lakes. Only 2% of all ARISA fragments were found in all three lakes and in both years. These fragments, identified here by their estimated size in base pairs, were 555, 579, 593, 601, 619, 655, 741, 759, 864, 929, and 950.

The ARISA profiles from the three lakes were readily distinguished from each other (Fig. 2). In general the variation in ARISA profiles was higher between samples from different lakes than between samples obtained from the same lake at different times, and ANOSIM indicated that samples from each lake were more similar to each other than to samples from other lakes (Table 3). Samples from the humic lake showed the greatest variability in ARISA profiles. There was somewhat greater within-lake variability seen in profiles from 2001 in all three lakes (Fig. 2).

Seasonal Patterns for Bacterial Communities. The time-series of change in ARISA profiles from these lakes showed a general pattern of seasonal change (Figs. 3,4,5). Community composition appeared to be stable in the spring and the fall, and most of the variability occurred in the summer, with dramatic changes in composition identified by large, cross-plot jumps in Figs. 3,4,5. Spring and fall samples tended to plot close together, but distinct spring and fall clusters were often identifiable (Figs. 3,4,5).

Table 2. Distribution of ARISA fragments in study lakes

	Total fragments	Fragments unique to this lake	Fragments in common in these lakes
Year 2000			
Crystal	117	49	—
Mendota	137	60	—
Crystal Bog and Crystal	—	—	35
Crystal Bog and Mendota	—	—	45
Crystal and Mendota	—	—	53
All three lakes	237	—	20
Year 2001			
Crystal Bog	161	33	—
Crystal	137	20	—
Mendota	195	51	—
Crystal Bog and Crystal	—	—	77
Crystal Bog and Mendota	—	—	103
Crystal and Mendota	—	—	92
All three lakes	273	—	52
Crystal Bog 2000 and 2001	—	—	59
Crystal 2000 and 2001	—	—	64
Mendota 2000 and 2001	—	—	98
All lakes, both years	—	—	11

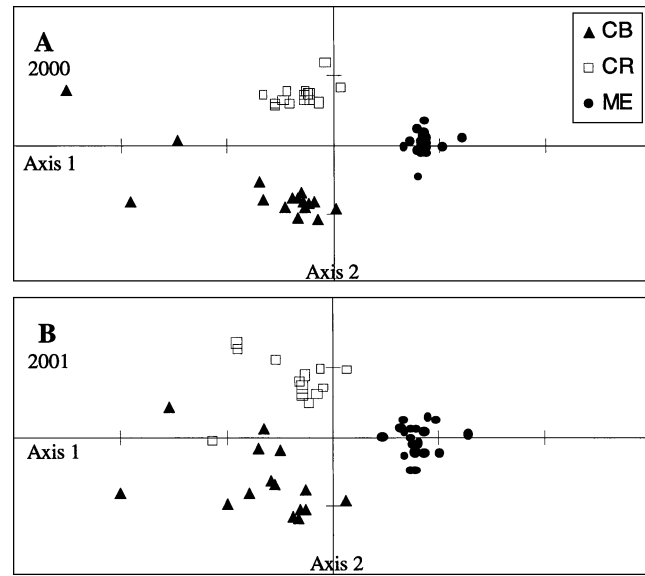


Fig. 2. MDS plots for all ARISA profiles collected from the study lakes. Because plots were generated from separate ordinations, absolute distances on different plots cannot be considered equal. Filled triangles are Crystal Bog Lake (CB; humic), open squares are Crystal Lake (CR; oligotrophic), and filled circles are Lake Mendota (ME; eutrophic). (A) Profiles for the year 2000. Stress = 0.13. (B) Profiles for the year 2001. Stress = 0.21.

Comparison of Figs. 3,4,5 with Fig. 2 showed that many of the dramatic summer shifts in community composition were associated with dates with lower ARISA fragment richness. The sole exception to this was the oligotrophic lake in 2001, for which there was no marked drop in richness (Fig. 2). Samples from low diversity dates did not necessarily plot close together (e.g., points f and i in Fig. 3A).

Based on water temperature data and on the patterns seen in 2000 (Figs. 3A, 4A, and 5A), seasonal phases were defined for these lakes. For the humic lake (Fig. 3A), it was determined that the variable “summer” phase included sampling dates with surface water temperatures greater

than or equal to 20°C. “Spring” and “fall” phases were defined to be when the water was cooler than 20°C. For the oligotrophic lake (Fig. 4A), four phases were defined. These phases were “spring” (water column mixed), “early summer” (water column stratified but average epilimnetic temperature less than 20°C), “late summer” (epilimnetic temperature greater than or equal to 20°C), and “fall” (beginning with the deepening of the epilimnion and the point at which the average epilimnetic temperature consistently decreased; included fall mixis). For the eutrophic lake (Fig. 5A) there were also four phases: “spring” (mixed water column), “early summer” (stratified water column but average epilimnetic temperature less than 22°C), “late summer” (lasted until fall mixis), and “fall” (mixed water column). These criteria were used to place samples from 2001 into the same categories. In addition, as some 2001 samples were collected while the lakes were ice covered, an “ice” phase was added to each lake for 2001.

ANOSIM determined that these criteria successfully grouped the 2001 samples into separate clusters for the oligotrophic and eutrophic lakes, but not so for the humic lake (Table 4). Separation was best for the oligotrophic lake (global $R = 0.525$) but was significant for the eutrophic lake as well. Individual contrasts carried out for the seasonal phases revealed that many of the phases were well separated from the phases immediately preceding and following (Table 4), and in most cases there was good, but not necessarily statistically significant, separation between temporally distant phases, such as spring and fall. The low number of replicate samples in some phases, particularly the ice phases, made statistical inference difficult because of low power (Table 4).

Serial Correlation. The correlations of the time series for lakes are shown in Table 5. The 2000 time series from the oligotrophic lake was significantly, though poorly,

Table 3. ANOSIM (analysis of similarity) statistics for tests involving comparisons of all three lakes

Comparison	Sample statistic R	Monte-Carlo permutations with scores $\geq R$	P -value
Year 2000 global test	0.795	0 out of 999	0.001
Crystal Bog vs Crystal	0.718	0 out of 999	0.001
Crystal Bog vs Mendota	0.743	0 out of 999	0.001
Crystal vs Mendota	0.961	0 out of 999	0.001
Year 2001 global test	0.782	0 out of 999	0.001
Crystal Bog vs Crystal	0.604	0 out of 999	0.001
Crystal Bog vs Mendota	0.783	0 out of 999	0.001
Crystal vs Mendota	0.922	0 out of 999	0.001

Crystal Bog is humic, Crystal is oligotrophic, and Mendota is eutrophic

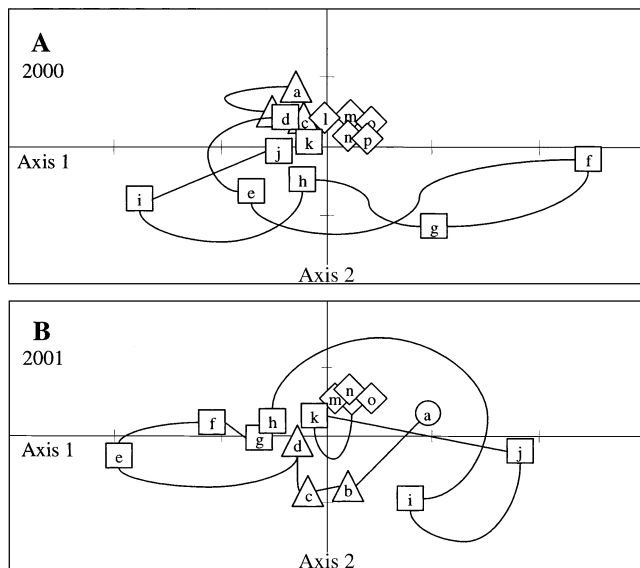


Fig. 3. MDS plots for ARISA profiles collected from humic Crystal Bog Lake. Because plots were generated from separate ordinations, absolute distances on different plots cannot be considered equal. Consecutive sampling dates have been connected to illustrate the time series. Circles represent samples collected underneath the ice, upward-pointing triangles represent spring samples, squares represent summer samples, and diamonds represent fall samples. (A) Profiles for the year 2000. Stress = 0.09. Dates are as follows: a = 17 Apr, b (hidden under point d) = 1 May, c = 17 May, d = 3 Jun, e = 15 Jun, f = 28 Jun, g = 10 Jul, h = 28 Jul, i = 8 Aug, j = 22 Aug, k = 9 Sep, l = 18 Sep, m = 2 Oct, n = 18 Oct, o = 31 Oct, p = 13 Nov. (B) Profiles for the year 2001. Stress = 0.10. Dates are as follows: a = 16 Jan, b = 2 May, c = 15 May, d = 30 May, e = 14 Jun, f = 26 Jun, g = 11 Jul, h = 24 Jul, i = 8 Aug, j = 22 Aug, k = 5 Sep, l (hidden under point n) = 19 Sep, m = 2 Oct, n = 24 Oct, o = 30 Oct.

correlated with the time series from the same lake in 2001, indicating a degree of regularity in the annual pattern of change that occurs in this lake following ice-out. In 2001, the serial patterns observed in the humic lake and in the oligotrophic were significantly correlated. The magnitude of the correlation coefficient (Table 5) suggested that a fair degree of synchrony was present in the serial patterns of change observed in these northern Wisconsin lakes.

Important Pattern-Forming ARISA Fragments

Certain ARISA fragments were identified as the most important pattern-forming elements for the three lakes in 2000 and 2001 (Figs. 6,7,8). A total of 221 distinct ARISA fragments were significant in determining the community

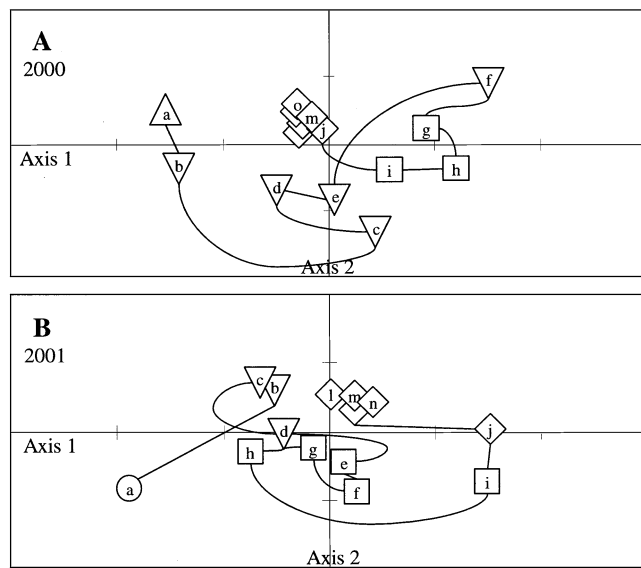


Fig. 4. MDS plots for ARISA profiles collected from oligotrophic Crystal Lake. Because plots were generated from separate ordinations, absolute distances on different plots cannot be considered equal. Consecutive sampling dates have been connected to illustrate the time series. Circles represent samples collected underneath the ice, upward-pointing triangles represent spring samples, downward-pointing triangles represent early summer samples, squares represent late summer samples, and diamonds represent fall samples. (A) Profiles for the year 2000. Stress = 0.12. Dates are as follows: a = 16 Apr, b = 3 May, c = 18 May, d = 3 Jun, e = 14 Jun, f = 29 Jun, g = 11 Jul, h = 27 Jul, i = 10 Aug, j = 21 Aug, k (hidden under point l) = 10 Sep, l (hidden under point m) = 21 Sep, m = 5 Oct, n (hidden under point o) = 19 Oct, o = 15 Nov. (B) Profiles for the year 2001. Stress = 0.10. Dates are as follows: a = 19 Jan, b = 3 May, c = 10 May, d = 31 May, e = 14 Jun, f = 29 Jun, g = 13 Jul, h = 27 Jul, i = 10 Aug, j = 24 Aug, k (hidden under point m) = 13 Sep, l = 20 Sep, m = 4 Oct, n = 25 Oct.

patterns in these lakes, and this represented less than half of the total number of ARISA fragments identified in this study. Thus, roughly half of the ARISA fragments from this study were involved in structuring the communities in question. Of these, 55 were found in more than one lake. In 2000, these fragments, identified here by their estimated size in base pairs, were 493, 535, 544, 555, 556, 576, 579, 583, 591, 593, 610, 619, 626, 650, 653, 659, 670, 680, 715, 735, 746, 780, 864, 884, and 920. In 2001, these fragments were 478, 487, 516, 521, 523, 528, 544, 552, 555, 562, 571, 593, 601, 610, 612, 614, 615, 619, 621, 629, 632, 647, 648, 690, 702, 722, 737, 747, 749, 762, 807, 814, 907, 929, and 934. The majority of these fragments did not show the same seasonal patterns in all of the lakes in which they occurred. For example, fragment 619, which was found in

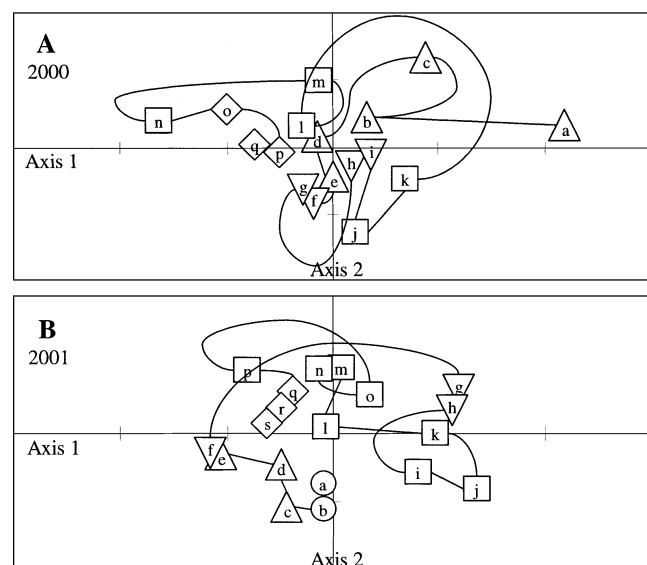


Fig. 5. MDS plots for ARISA profiles collected from eutrophic Lake Mendota. Because plots were generated from separate ordinations, absolute distances on different plots cannot be considered equal. Consecutive sampling dates have been connected to illustrate the time series. Circles represent samples collected underneath the ice, upward-pointing triangles represent spring samples, downward-pointing triangles represent early summer samples, squares represent late summer samples, and diamonds represent fall samples. (A) Profiles for the year 2000. Stress = 0.13. Dates are as follows: a = 15 Mar, b = 30 Mar, c = 14 Apr, d = 27 Apr, e = 11 May, f = 25 May, g = 6 Jun, h = 20 Jun, i = 5 Jul, j = 17 Jul, k = 1 Aug, l = 17 Aug, m = 12 Sep, n = 26 Sep, o = 10 Oct, p = 24 Oct, q = 28 Nov, (B) Profiles for the year 2001. Stress = 0.20. Dates are as follows: a = 17 Jan, b = 13 Mar, c = 10 Apr, d = 23 Apr, e = 7 May, f = 21 May, g = 4 Jun, h = 18 Jun, i = 2 Jul, j = 16 Jul, k = 30 Jul, l = 13 Aug, m = 28 Aug, n = 10 sep, o = 26 Sep, p = 9 Oct, q = 22 Oct, r = 6 Nov, s = 26 Nov.

the spring and the fall of 2000 in the humic lake, was found primarily in the summer in the oligotrophic lake and in the fall of the eutrophic lake that same year (Figs. 6A, 7A, 8A), and it was found in the summer and fall in the oligotrophic lake in 2001 (Fig. 7B). Only 17 fragments appeared to have the same seasonal distributions in all of the lakes they occurred in.

Within each lake, less than 30% of the ARISA fragments identified as important pattern-forming elements were identified as such in both 2000 and 2001 (Figs. 6,7,8). Thus, the patterns observed in MDS plots were not caused by the same set of elements from year to year. The seasonal distribution of these important elements was apparent (Figs. 6,7,8); however, a majority of ARISA fragments seen in both years in these lakes did not show the same seasonal patterns of distribution from year to

Table 4. ANOSIM (analysis of similarity) statistics for tests involving comparisons of seasonal categories in the lakes for the year 2001 data

Comparison	Sample statistic R	Monte-Carlo permutations with scores $\geq R$	P-value
Crystal Bog			
Global test	0.164	158 out of 999	0.159
ice vs spring	1.000	1 out of 4	0.250
ice vs summer	0.109	3 out of 8	0.375
ice vs fall	1.000	1 out of 5	0.200
spring vs summer	-0.059	66 out of 120	0.550
spring vs fall*	0.889	1 out of 35	0.029
summer vs fall	0.103	83 out of 330	0.252
Crystal Lake			
Global test***	0.525	0 out of 999	0.001
ice vs early summer	1.000	1 out of 4	0.250
ice vs late summer	0.880	1 out of 6	0.167
ice vs fall	1.000	1 out of 6	0.167
early vs late summer	0.303	6 out of 56	0.107
early summer vs fall*	0.554	1 out of 56	0.018
late summer vs fall*	0.432	2 out of 126	0.016
Lake Mendota			
Global test*	0.343	12 out of 999	0.013
ice vs spring	0.333	2 out of 10	0.200
ice vs early summer	0.625	2 out of 10	0.200
ice vs late summer*	0.494	1 out of 45	0.022
ice vs fall	1.000	1 out of 10	0.100
spring vs early summer	0.630	1 out of 10	0.100
spring vs late summer**	0.421	1 out of 165	0.006
spring vs fall	0.704	1 out of 10	0.100
early vs late summer*	0.375	1 out of 165	0.012
early summer vs fall	0.704	1 out of 10	0.100
late summer vs fall	-0.050	86 out of 165	0.521

Crystal Bog is humic, Crystal Lake is oligotrophic, and Lake Mendota is eutrophic

* Indicates significance at the $\alpha = 0.050$ level

** Indicates significance at the $\alpha = 0.010$ level

*** Indicates significance at the $\alpha = 0.001$ level

year. Notable exceptions to this included fragments 544, 555, 593, 601, 680, and 864, which had year-round distributions (Figs. 6,7,8), and others that seemed restricted to the spring (747 in Fig. 7), summer (650 in Fig. 7 and 648 in Fig. 8), fall (735 in Fig. 8), or cold water phases (762 in Fig. 6; 890 in Fig. 7; 402 and 759 in Fig. 8).

Discussion

Despite the temporal variation in ARISA profiles observed in this study, it is clear that among-lake differences accounted for the greatest share of variation in bacterial communities (Fig. 2). This is in contrast to the observations of Lindström [41]. However, the lakes studied by Lindström [41] were all relatively eutrophic compared to

Table 5. Correlations of the serial patterns of ARISA profile changes, with sample dates coded as “weeks past ice-off,” for the study lakes in 2000 and 2001

Comparison	Spearman rank correlation ρ	Monte-Carlo permutations with scores $\geq \rho$	P-value
CB 2000 and CR 2000	0.225	128 out of 999	0.129
CD 2000 and ME 2000	-0.042	532 out of 999	0.533
CR 2000 and ME 2000	0.140	214 out of 999	0.215
CB 2001 and CR 2001***	0.625	0 out of 999	0.001
CB 2001 and ME 2001	0.083	283 out of 999	0.284
CR 2001 and ME 2001	0.234	59 out of 999	0.060
CB 2000 and CB 2001	-0.010	500 out of 999	0.501
CR 2000 and CR 2001*	0.325	31 out of 999	0.032
ME 2000 and ME 2001	0.219	61 out of 999	0.062

CB is Crystal Bog (humic), CR is Crystal Lake (oligotrophic), and ME is Lake Mendota (eutrophic)

* Indicates significance at the $\alpha = 0.050$ level

*** Indicates significance at the $\alpha = 0.001$ level

the humic and oligotrophic lakes in this study (c.f. Table 1 of [41] and Table 1 of this work).

Despite the among-lake differences observed in this study, variation in the bacterial communities as assessed by ARISA showed some general patterns. In all three lakes in both years, the cold-water communities in the spring and the fall appeared to be much more stable than the stratified summer periods, where the vast majority of the yearly variation in ARISA fragments was seen (Figs. 3,4,5). In addition, the ARISA profiles in the summer showed

much larger changes over shorter spans of time, suggesting that the pacing of community change can be quite different, depending on the season under consideration.

Each of these lakes, except for the oligotrophic lake in 2001, experienced a mid- or late-summer drop in ARISA fragment richness (Fig. 1), which suggested periods of extremely low bacterial diversity. In the humic lake, DAPI-stained counts of bacteria were also extremely low on these dates (data not shown), suggesting that this decline in bacterial diversity was also reflected in the population sizes

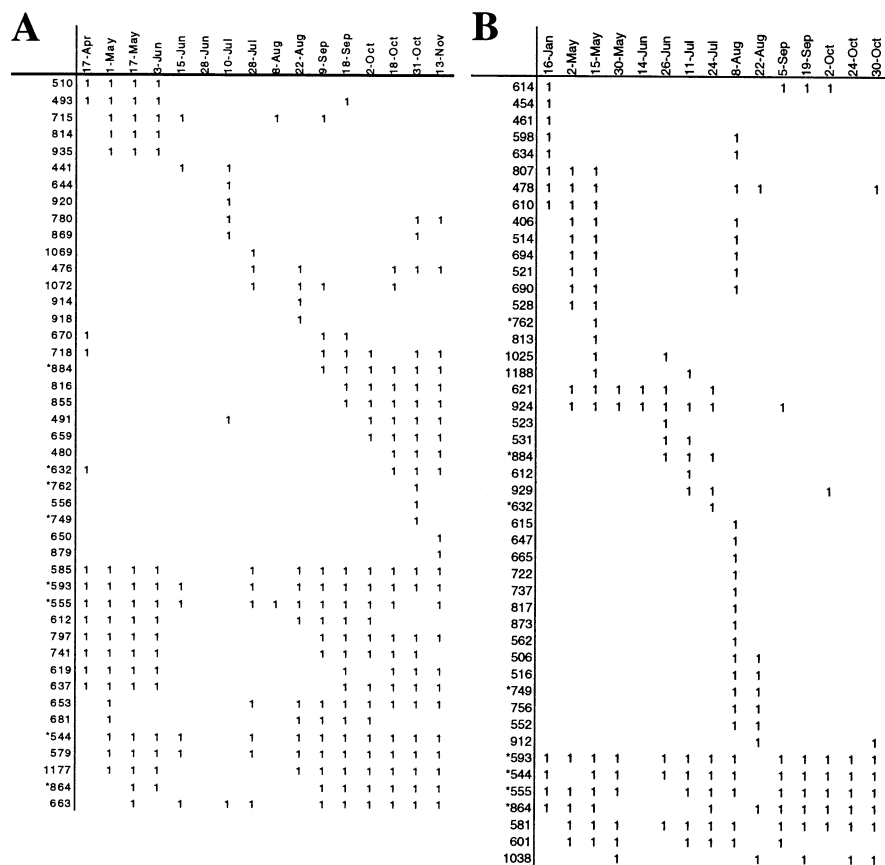


Fig. 6. Best pattern-forming ARISA fragments found in humic Crystal Bog Lake. Rows represent ARISA fragments, identified as the estimated fragment size in base pairs, and columns represent sample dates. “1” indicates the presence of the ARISA fragment in question on the given date. Fragments labeled with an asterisk (*) were identified as important pattern-forming elements in both 2000 and 2001. (A) Data for the year 2000. (B) Data for the year 2001.

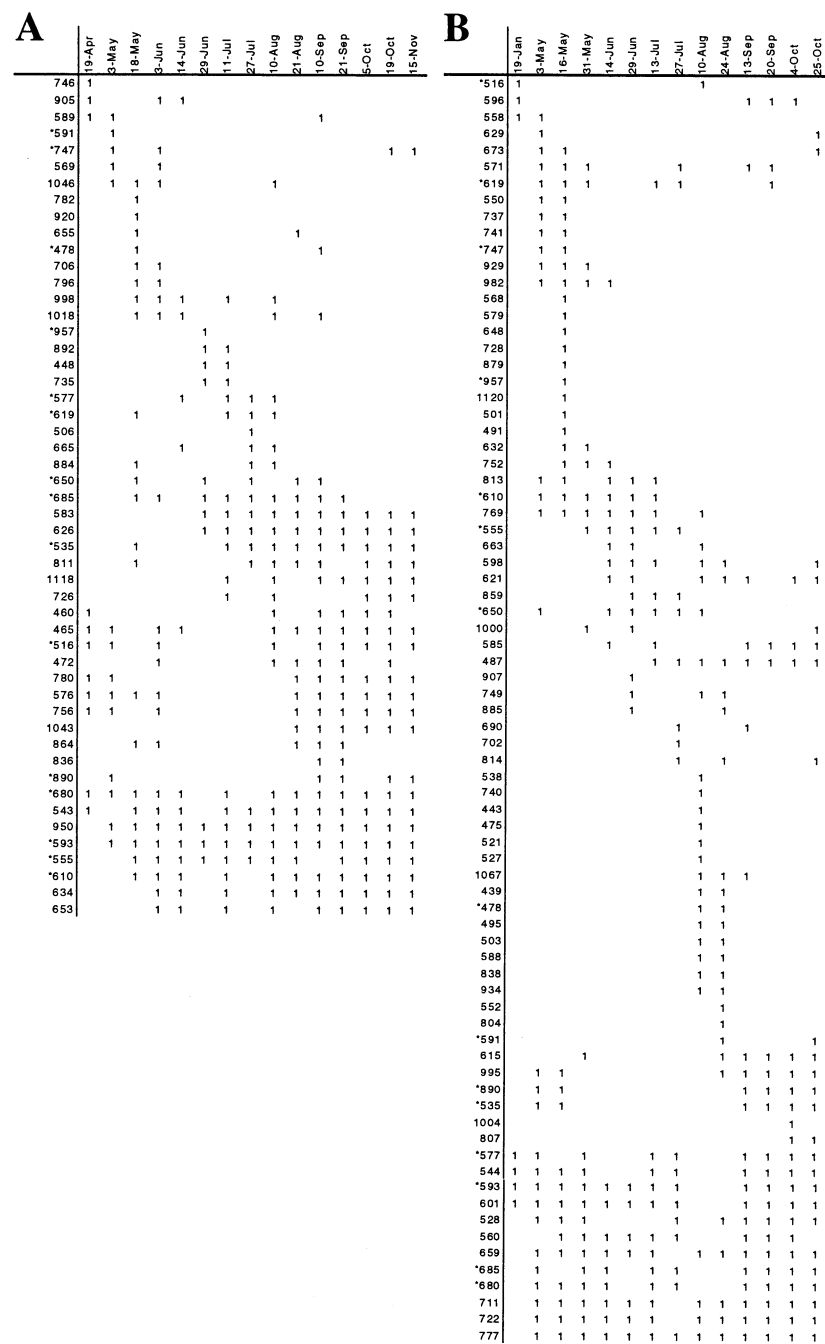


Fig. 7. Best pattern-forming ARISA fragments found in oligotrophic Crystal Lake. Rows represent ARISA fragments, identified as the estimated fragment size in base pairs, and columns represent sample dates. "1" indicates the presence of the ARISA fragment in question on the given date. Fragments labeled with an asterisk (*) were identified as important pattern-forming elements in both 2000 and 2001. (A) Data for the year 2000. (B) Data for the year 2001.

of bacteria present. In a study of the eutrophic reservoir Lake Plußsee, Höfle and others [26] also noted dramatic decreases in bacterial diversity occurring during the spring clear-water phase, midsummer, and late summer. The spring and late-summer diversity drops in Lake Plußsee had clear correlates with macroplankton dynamics [26, 56], with the former being caused by zooplankton grazing and the latter associated with the dominance of a single dinoflagellate species in the phytoplankton communities. In the present study, the oligotrophic lake followed this same pattern closely. There were three notable declines in diversity

occurring on 25 May, 17 Jul, and 26 Sep of 2000 and on 4 Jun, 30 Jul, and 10 Sep of 2001 (Fig. 1). The earliest declines occurred during the clear water phase of this lake in both years (e.g., chlorophyll *a* on 25 May 2000 is at 0.7 µg/L, down from a value of 10.3 µg/L the previous month; please see <http://lterquery.limnology.wisc.edu/>). This suggests that grazing by zooplankton was a likely cause. This was also a probable cause of the 29 Jun drop witnessed in the oligotrophic lake, as this event also occurred in a period of depressed chlorophyll *a* levels (<http://lterquery.limnology.wisc.edu/>). The July and September drops in the eutrophic

The use of basic water temperature and stratification state criteria to predict the occurrence of closely related assemblages of bacteria was moderately successful in this study. The most notable failure of this model was seen in the humic lake (Table 4), which was also the most variable lake in terms of its ARISA profiles (Fig. 2). The lack of fit to the predicted groups of samples was primarily due to the extreme variability witnessed in the summer, as the spring and fall samples were readily distinguished from one another (Fig. 3B; Table 4). Thus, water temperature alone does not appear to be sufficient to predict the pattern of change in bacterial community composition as described by ARISA, and this is particularly true when water temperatures are relatively high. The summer period in lakes should be characterized by intense biological interactions that change along with fluctuating populations, leading to rapid changes in bacterial community composition [29]. In addition, the humic lake in this study was a small, shallow, darkly stained bog lake, and these features may make it susceptible to short-term weather-induced changes that the larger lakes may be buffered against.

The seasonal model was more successful in the other two lakes, suggesting that these larger lakes undergo more predictable seasonal changes from year to year. This was particularly true of the oligotrophic lake, where criteria established using 2000 data generated seasonal groupings that again showed a high degree of separation in 2001 (Fig. 4; Table 4). The pattern of change in this lake was the most stable from year to year, and the serial progressions of change in both years were significantly correlated with each other (Table 5). It is likely that the interaction of water temperature, lake stratification, and the nutrient-poor condition is a powerful force structuring the behavior of bacterial communities in this lake. Although the serial correlation in the eutrophic lake in 2000 was not related to the serial correlation in 2001 (Table 5), the criteria for the year 2000 were still useful at predicting the times when ARISA profiles from this lake would be the most similar (Fig. 5; Table 4). The fit of this seasonal model would have been greatly improved with slight modifications to the cutoff criteria used to define the groups. For example, from Fig. 5B, the fit would have improved significantly by moving the point for 21 May (point f) into the spring group instead of the early summer group, and inclusion of the point for 9 Oct (point p) in the fall group would also have improved the fit. Such slight modifications would also have improved the model for the oligotrophic lake. It is not surprising that changes in the

composition of biological communities would lag behind changes in the environment [3], and incorporating these time lags would improve these seasonal models considerably. Thus, the seasonal changes associated with changing water temperature and stratification dynamics provide a good first-order predictor of the degree of autocorrelation and community relatedness inherent in a temporal series of samples.

Of all ARISA fragments found in each lake in a given year, 30–50% were unique to that particular lake, and this suggests the existence of assemblages of organisms that are indigenous to particular lakes or to lakes of similar trophic status. Several multilake studies have shown a great deal of overlap in the composition of bacterial communities among different lakes [34, 41]. However, these studies used DGGE to characterize bacterial communities. Since this technique focuses on the 16S-rDNA, which is more conserved than the intergenic transcribed spacer [7, 21], it may have lacked the taxonomic resolution to distinguish between populations of closely related organisms. There was a small set of ARISA fragments that appeared to be ubiquitous in these lakes. It is likely that these fragments were derived from organisms that have a widespread distribution and are common to freshwater systems, and the existence of such organisms has been the focus of previous work [23, 42, 66].

Only 30–50% of the ARISA fragments found in each lake in 2001 had previously been seen in the same lake in 2000. This indicates that the overall composition of the communities in these lakes was different from year to year. The important pattern-forming elements in these lakes also tended to be different from year to year (Figs. 6,7,8). In addition, many of the recurrent fragments did not show the same seasonal patterns of occurrence in both years. Given the highly individualistic nature of the behavior of ARISA fragments, it is somewhat surprising that these lakes appear to manifest regular seasonally associated patterns. That such patterns exist implies that the influence of seasonal changes in the environment imposes strong constraints upon the behavior of these systems as a whole, despite the fact that those same constraints may not apply to the individual parts. Alternatively, there may be a large number of functionally redundant phylotypes present in these lakes, and only a small subset of each functional group may be present in a lake in any given year. This observation is very similar to a phenomenon that has been noted for phytoplankton, which show much clearer patterns of occurrence when organized into polyphyletic

functional groups than when examined on a per-species basis [36, 52, 53]. This strongly suggests that a functional classification of bacterioplankton would be helpful in the development of hypotheses regarding patterns of community composition.

In conclusion, it appears that seasonally induced changes act as important structuring forces for lake bacterial communities. Knowledge of the water temperature and stratification dynamics in lakes can provide a good first approximation about the consecution of bacterial communities in some lakes, but the impact of specific biological and environmental factors needs to be more thoroughly investigated. This is particularly true of lake communities in the summer, when the most dramatic changes appear to take place. More detailed knowledge of the factors creating these patterns, the identity of the organisms involved, and the regularity of species or functional group replacement is needed before such patterns can legitimately be characterized as ecological succession.

Acknowledgements

We thank B. Benson, S. Lake, J. and L. Graham, S. Carpenter, D. Armstrong, and the NTL-LTER technicians for their valuable assistance with this manuscript. This work was funded by NSF grant DEB 9977903, awarded to the Center for Limnology at University of Wisconsin-Madison.

References

1. PE Applied Biosystems (1996) ABI Prism Genotyper
2. PE Applied Biosystems (2000) ABI Prism GeneScan Analysis
3. Allen TFH, Bartell SM, Koonce JF (1977) Multiple stable configurations in ordination of phytoplankton community change rates. *Ecology* 58:1076–1084
4. Amann R, Fuchs BM, Behrens S (2001) The identification of microorganisms by fluorescence *in situ* hybridisation. *Curr Opin Biotechnol* 12:231–236
5. Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiol Rev* 59:143–169
6. Biddanda B, Ogdahl M, Cotner J (2001) Dominance of bacterial metabolism in oligotrophic relative to eutrophic waters. *Limnol Oceanogr* 46:730–739
7. Borneman J, Triplett EW (1997) Molecular microbial diversity in soils from Eastern Amazonia: evidence for unusual microorganisms and microbial population shifts associated with deforestation. *Appl Environ Microbiol* 63:2647–2653
8. Caron DA (1994) Inorganic nutrients, bacteria, and the microbial loop. *Microb Ecol* 28:295–298
9. Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. *Aust J Ecol* 18:117–143
10. Clarke KR, Gorley RN (2001) PRIMER 5 for Windows PRIMER-E Ltd., Plymouth, UK
11. Clarke KR, Green RH (1988) Statistical design and analysis for a “biological effects” study. *Mar Ecol Prog Ser* 46:213–226
12. Clarke KR, Warwick RM (1998) Quantifying structural redundancy in ecological communities. *Oecologia* 113:278–289
13. Cole JJ, Findlay S, Pace ML (1988) Bacterial production in fresh and saltwater: a cross-system overview. *Mar Ecol Prog Ser* 43:1–10
14. Cole JJ, Likens GL, Gene EE, Hobbie JE (1984) Decomposition of planktonic algae in an oligotrophic lake. *Oikos* 42:257–266
15. Danovaro R, Marralle D, Della Croce N, Dell’Anno A, Fabiano M (1998) Heterotrophic nanoflagellates, bacteria, and labile organic compounds in continental shelf and deep-sea sediments of the Eastern Mediterranean. *Microb Ecol* 35:244–255
16. Ducklow HW, Carlson CA (1992) Oceanic bacterial production. In: Marshall KC (Eds.) *Advances in Microbial Ecology* Plenum Press, New York, pp 113–181
17. Felip M, Pace ML, Cole JJ (1996) Regulation of planktonic bacterial growth rates: the effects of temperature and resources. *Microb Ecol* 31:15–28
18. Field JG, Clarke KR, Warwick RM (1982) A practical strategy for analysing multispecies distribution patterns. *Mar Ecol Prog Ser* 8:37–52
19. Fisher MM, Klug JL, Lauster G, Newton M, Triplett EW (2000) Effects of resources and trophic interactions on freshwater bacterioplankton diversity. *Microb Ecol* 40:125–138
20. Fisher MM, Klug JL, Lauster G, Newton M, Triplett EW (2001) Effects of resources and trophic interactions on freshwater bacterioplankton. *Microb Ecol* 41:82–82
21. Fisher MM, Triplett EW (1999) Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. *Appl Environ Microbiol* 65:4630–4636
22. Glöckner FO, Fuchs BM, Amann R (1999) Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence *in situ* hybridization. *Appl Environ Microbiol* 65:3721–3726
23. Glöckner FO, Zaichikov E, Belkova N, Denissova L, Pernthaler J, Pernthaler A, Amann R (2000) Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of actinobacteria. *Appl Environ Microbiol* 66:5053–+
24. Hahn MW, Höfle MG (2001) Grazing of protozoa and its effect on populations of aquatic bacteria. *FEMS Microbiol Ecol* 35:113–121

25. Hansson LA, Rudstam LG, Johnson TB, Soranno P, Allen Y (1994) Patterns in algal recruitment from sediment to water in a dimictic, eutrophic lake. *Can J Fish Aqua Sci* 51:2825–2833
26. Höfle MG, Haas H, Dominik K (1999) Seasonal dynamics of bacterioplankton community structure in a eutrophic lake as determined by 5S rRNA analysis. *Appl Environ Microbiol* 65:3164–3174
27. Hurlbert SH (1984) Pseudoreplication and the design of ecological field experiments. *Ecol Monogr* 54:187–211
28. šimek K, Kojecá P, Nedoma J, Hartman P, Vrba J, Dolan JR (1999) Shifts in bacterial community composition associated with different microzooplankton size fractions in a eutrophic reservoir. *Limnol Oceanogr* 44:1634–1644
29. Jaspers E, Nauhaus K, Cypionka H, Overmann J (2001) Multitude and temporal variability of ecological niches as indicated by the diversity of cultivated bacterioplankton. *FEMS Microbiol Ecol* 36:153–164
30. Jeppesen E, Erlandsen M, Sondergaard M (1997) Can simple empirical equations describe the seasonal dynamics of bacterioplankton in lakes: An eight-year study in shallow hypertrophic and biologically highly dynamic Lake Søbygard, Denmark. *Microb Ecol* 34:11–26
31. Jürgens K, Pernthaler J, Schalla S, Amann R (1999) Morphological and compositional changes in a planktonic bacterial community in response to enhanced protozoan grazing. *Appl Environ Microbiol* 65:1241–1250
32. Kirchman DL (1994) The uptake of inorganic nutrients by heterotrophic bacteria. *Microb Ecol* 28:255–271
33. Kitts CL (2001) Terminal restriction fragment patterns: a tool for comparing microbial communities and assessing community dynamics. *Curr Issues Intest Microbiol* 2:17–25
34. Konopka A, Bercot T, Nakatsu C (1999) Bacterioplankton community diversity in a series of thermally stratified lakes. *Microb Ecol* 38:126–135
35. Physical limnology of the North Temperate Lakes Primary Study lakes, North Temperate Lakes Long Term Ecological Research Program, NSF (<http://www.limnology.wisc.edu>), Kratz TK, Center for Limnology, University of Wisconsin–Madison
36. Kruk C, Mazzeo N, Lacerot G, Reynolds CS (2002) Classification schemes for phytoplankton: a local validation of a functional approach to the analysis of species temporal replacement. *J Plankton Res* 24:901–912
37. Kruskal JB (1964) Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. *Psychometrika* 29:1–27
38. Kruskal JB, Wish M (1978) *Multidimensional Scaling* Sage Publications, Beverly Hills, CA
39. Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (Eds.) *Nucleic Acid Techniques in Bacterial Systematics* Wiley & Sons, Chichester, UK
40. Langenheder S, Jürgens K (2001) Regulation of bacterial biomass and community structure by metazoan and protozoan predation. *Limnol Oceanogr* 46:121–134
41. Lindström ES (2000) Bacterioplankton community composition in five lakes differing in trophic status and humic content. *Microb Ecol* 40:104–113
42. Lindström ES, Leskinen E (2002) Do neighboring lakes share common taxa of bacterioplankton? Comparison of the 16S rDNA fingerprints and sequences from three geographic regions. *Microb Ecol* 44:1–9
43. Liu WT, Marsh TL, Cheng H, Forney LJ (1997) Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Appl Environ Microbiol* 63:4516–4522
44. Magnuson JJ, Kratz TK, Allen TF, Armstrong DE, Benson BJ, Bowser CJ, Bolgrien DW, Carpenter SR, Frost TF, Gower ST, Lillesand TM, Pike JA, Turner MG (1997) Regionalization of long-term ecological research (LTER) on north temperate lakes. *Verh Internat Verein Limnol* 26:522–528
45. Magurran AE (1988) *Ecological Diversity and Its Measurement* Princeton University Press, Princeton, NJ
46. Mardia KV, Kent JT, Biddy JM (1979) *Multivariate Analysis* Academic Press, London
47. McCune B, Grace JB (2002) *Analysis of Ecological Communities*. MjM Software Design, Gleneden Beach, Oregon
48. Methé BA, Hiorns WD, Zehr JP (1998) Contrasts between marine and freshwater bacterial community composition: Analyses of communities in Lake George and six other Adirondack lakes. *Limnol Oceanogr* 43:368–374
49. Methé BA, Zehr JP (1999) Diversity of bacterial communities in Adirondack lakes: do species assemblages reflect lake water chemistry? *Hydrobiologia* 401:77–96
50. Osborn AM, Moore ERB, Timmis KN (2000) An evaluation of terminal-restriction fragment length polymorphism (T-RFLP) analysis for the study of microbial community structure and dynamics. *Environ Microbiol* 2:39–50
51. Polz MF, Cavanaugh CM (1998) Bias in template-to-product ratios in multitemplate PCR. *Appl Environ Microbiol* 64:3724–3730
52. Reynolds CS (2000) Phytoplankton designer—or how to predict compositional responses to trophic-state change. *Hydrobiologia* 424:123–132
53. Reynolds CS, Huszar V, Kruk C, Naselli-Flores L, Melo S (2002) Towards a functional classification of the freshwater phytoplankton. *J Plankton Res* 24:417–428
54. SimeNgando T (1997) Viruses in aquatic ecosystems. A review. *Anee Biol* 36:181–210
55. Simon M, Byong C, Azam F (1992) Significance of bacterial biomass in lakes and the ocean: comparison to phytoplankton biomass and biogeochemical implications. *Mar Ecol Prog Ser* 86:103–110
56. Sommer U (1993) Phytoplankton competition in Plußsee: a field test of the resource-ratio hypothesis. *Limnol Oceanogr* 38:838–845
57. Chemical Limnology of North Temperate Lakes Primary Study lakes, North Temperate Lakes Long Term Ecological Research Program, NSF (<http://www.limnology.wisc.edu>),

- Stanley EH, Center for Limnology, University of Wisconsin-Madison
58. Stepanauskas R, Edling H, Tranvik LJ (1999) Differential dissolved organic nitrogen availability and bacterial aminopeptidase activity in limnic and marine waters. *Microb Ecol* 38:264–272
 59. Suzuki M, Rappe MS, Giovannoni SJ (1998) Kinetic bias in estimates of coastal picoplankton community structure obtained by measurements of small-subunit rRNA gene PCR amplicon length heterogeneity. *Appl Environ Microbiol* 64:4522–4529
 60. Suzuki MT, Giovannoni SJ (1996) Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. *Appl Environ Microbiol* 62:625–630
 61. Tuomi P, Torsvik T, Heldal M, Bratbak G (1997) Bacterial population dynamics in a meromictic lake. *Appl Environ Microbiol* 63:2181–2188
 62. Vila X, Abella CA, Figueras JB, Hurley JP (1998) Vertical models of phototrophic bacterial distribution in the metalimnetic microbial communities of several freshwater North-American kettle lakes. *FEMS Microbiol Ecol* 25:287–299
 63. Vila X, Cristina XP, Abella CA, Hurley JP (1999) Effects of gilvin on the composition and dynamics of metalimnetic communities of phototrophic bacteria in freshwater North-American lakes. *J Appl Microbiol* 85:138S–150S
 64. Weiss P, Schweitzer B, Amann R, Simon M (1996) Identification in situ and dynamics of bacteria on limnetic organic aggregates (lake snow). *Appl Environ Microbiol* 62:1998–2005
 65. White PA, Kalf J, Rasmussen JB, Gasol JM (1991) The effect of temperature and algal biomass on bacterial production and specific growth rate in freshwater and marine habitats. *Microb Ecol* 21:99–115
 66. Zwart G, Hiorns WD, Methé BA, Van Agterveld MP, Huismans R, Nold SC, Zehr JP, Laanbroek HJ (1998) Nearly identical 16S rRNA sequences recovered from lakes in North America and Europe indicate the existence of clades of globally distributed freshwater bacteria. *Syst Appl Microbiol* 21:546–556