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Investigating Influential Factors on Bacterioplankton Community Composition: Results from a Field Study of Five Mesotrophic Lakes

E.S. Lindström

Department of Limnology, Evolutionary Biology Centre, Uppsala University, Norbyv. 20, SE-752 36 Uppsala, Sweden

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Abstract

In order to investigate which biotic and abiotic factors may have an impact on the community composition of bacterioplankton, five mesotrophic lakes were studied. The composition of the bacterioplankton communities was determined by denaturing gradient gel electrophoresis (DGGE) of 16S rDNA. Multivariate statistical analyses of the gel patterns, in relation to each other and to the chemical, biological, and physical parameters of the lakes, were performed. The analyses showed that the import of allochthonous bacteria and the interaction with other plankton organisms (for example, grazing) in the lakes probably had an impact on the composition of the communities.

Introduction

Bacterioplankton are among the most abundant of planktonic organisms in lakes. They are also known to be responsible for key processes regulating the function and productivity of ecosystems through the "microbial loop" [1] and the "reversed microbial loop" [14]. Thus, it can be concluded that bacterioplankton not only are numerous, but also are important components of lake ecosystems.

Molecular methods for identification of microorganisms that are cultivation-independent have mainly been under development during the past decade (described by, e.g., [10]). The application of such methods on microbial communities in nature has led to the detection of a large number of previously unknown taxa (e.g., [23]). To date, several investigations of bacterioplankton community composition in lakes have been performed. Extensive field studies are still relatively few in number but some information has been gathered. For instance, there are results showing seasonal [9, 12, 16–18, 22, 24] as well as spatial [8, 9, 11, 16, 18–20, 22, 30] variations in lake bacterioplankton communities. The existence of globally distributed taxa [9, 30] has been discovered. Also, several studies show that there might be differences between marine and freshwater communities regarding the proportion of β-proteobacteria to other taxa (e.g., [8, 19]). However, the field of study concerning bacterioplankton community composition in lakes is clearly still at its beginning. For instance, only a few studies have aimed to relate community composition data to environmental

Correspondence to: E.S. Lindström; E-mail: Eva.Lindstrom@ebc.uu.se

Table 1. Biological, chemical, and physical characteristics of the five studied lakes at the dates of study (biomasses of biota are in μg C L^{-1})

	Lake Örträsket	Upper Bear Lake	Lower Bear Lake	Reindeer Lake	Lake Siholma
Manipulation	None	None	P	N	C
Surface area (km^2)[a]	7.3	0.05	0.03	0.01	0.01
Maximum depth (m)[a]	64	8	9.5	9	10
Drainage area (km^2)[a]	2174	3.1	3.5	1.6	0.8
Turnover time (years)[a]	0.3	0.2	0.2	0.1	0.2
Epilimnion depth (m)	6–13	0.5–1	1–2	0.5–1	2–4
Water color (Abs 420 nm)	0.23–0.32	0.53–0.77	0.43–0.67	0.42–0.51	0.09–0.14
Total phosphorus (μg P L^{-1})	18–20	26–29	19–42	12–31	6–15
Total nitrogen (μg N L^{-1})	355–399	494–620	342–574	410–487	172–337
DOC (mg C L^{-1})	10.0–13.5	15.9–24.9	12.8–24.5	13.4–18.3	4.5–5.3
pH	6.5–6.6	4.9–6.3	4.8–5.7	5.0–5.9	5.8–6.0
Temperature (°C)	14.7–16.1	14.3–18.3	14.0–17.9	13.6–18.1	13.6–17.9
Conductivity (mS m^{-1})	31.5–32.8	29.6–37.0	31.4–37.0	27.8–41.7	24–27.1
Bacterioplankton	24–32	17–26	15–30	21–30	6–52
Metazooplankton[b]	1–6	34–84	46–157	29–380	31–43
Ciliates	13–19	3–31	6–23	0–16	3–19
Phytoplankton[c]	16–37	12–58	12–63	14–173	49–146
Picophytoplankton	2–3	0	0	0–1	1–4
Chlorophytes	1–2	1–5	2–3	7–67	42–140
Chrysophytes	6–7	1–7	4–15	7–17	1–5
Cryptophytes	3–20	3–52	1–56	1–94	1–3
Diatoms	4–8	0	0–1	0	0
Dinoflagellates	0–1	0	0	0	0–1

[a] Data from Bergström et al. (unpublished)
[b] Cladocerans, copepodes, and rotifers
[c] Including mixotrophic and heterotrophic taxa of flagellates

data. Results from existing field studies indicate that lake water chemistry [20] as well as the presence of grazers in the lakes [12, 18] can have an impact on the composition of these communities. However, data within this field are still lacking.

The aim of this study was to further investigate which factors affect bacterioplankton community structure. The community composition of the bacterioplankton in five lakes was determined by DGGE of 16S rDNA. This method has previously been used in several studies of bacterioplankton communities in lakes (e.g., [7, 16–18, 22]). It should be recognized that there are several possible sources of errors involved in this technique (see, e.g., [6, 10, 21, 28, 29]). For instance, bands that are formed at the same positions on the gels do not always represent the same taxon. Therefore, differences and similarities in gel patterns may not completely correspond to differences and similarities in nature. However, despite the limitations of the method, it has been concluded that DGGE gives an acceptable view of differences and similarities in the dominating populations of microbial communities [18, 21, 27]. Trends in DGGE patterns have also been confirmed by the simultaneous use of other methods [7, 27, 28], and by experiments including standard DNA [6]. The DGGE profiles obtained in this study were therefore used as means to investigate general patterns in the dominating bacterioplankton populations in the lakes. These patterns were analyzed statistically in relation to each other and to the biological, chemical, and physical parameters of the lakes, to obtain information about relationships between the community composition of the bacterioplankton and their environment.

Materials and Methods

Study Sites and Sampling

Five lakes, four small and one larger, were chosen for the study (Table 1). The lakes are all situated within 13 km of each other in the southern part of the region of Lappland in northern Sweden. Wetlands and coniferous forest dominate the drainage areas of all the lakes. Two of the small lakes, Upper Bear Lake and Lower Bear Lake, are situated within 1 km of each other, Upper Bear Lake being

located within the drainage area of Lower Bear Lake. The lakes have been described previously in more detail (e.g., [3, 5, 15]).

Samples were collected during 25–28 June 1996 (only the four smallest lakes) as well as during 24–27 June and 19–22 August 1997 (all lakes). Composite samples representing the epilimnion of each lake were taken, and chemical and biological analyses (used to determine values of the parameters presented in Table 1) were made as described previously [5, 14, 15].

Three of the small lakes were included in a lake fertilization program, described previously in more detail [5, 15]. The lakes were fertilized every second week during the summer of 1997, starting in mid-June (12–16 June). In one lake, total phosphorus concentration in the epilimnion was increased by 10 μg L^{-1}, while in another, total nitrogen concentration was increased by 100 μg L^{-1}, and in a third the total carbon concentration was increased by 0.5–0.6 mg L^{-1}. For treatment design, see Table 1.

Bacterioplankton Community Composition

Analysis of bacterioplankton community composition was performed as described previously [17]. This procedure involves the collection of bacterioplankton cells from 2–8 L of the composite lake samples on 0.22 μm Durapore filters (Millipore) after prefiltration through an A/E glass fiber filter (nominal pore size 1 μm, Gelman Sciences) within 3–9 h after sampling. DNA extraction and purification was performed, and the 16S rDNA (16S rRNA encoding DNA) of the sampled community was analyzed by denaturing gradient gel electrophoresis (DGGE). Prior to DGGE, polymerase chain reaction (PCR) was performed to amplify a 500 base-pair fragment of the 16S rDNA (907-1406, *E. coli* positions) of the bacterioplankton community. The primers used are "universal," with the following sequences: primer 1: 5′ -CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC GAA ACT YAA AKG AAT TGA CGG-3′ primer 2: 5′-ACG GGC GGT GTG TRC-3′ (GC-clamp underlined; Y = T/C, K = T/G, R = A/G) [17]. When analyzing the samples from the four most humic lakes, it was difficult to obtain enough product for DGGE analysis from the PCR reaction, perhaps because of inhibition by humic substances that were still present in the samples. Therefore, when amplifying DNA from these four lakes, the concentration of $MgCl_2$ in the PCR reaction was increased from 3 mM to 4.5 mM. In some cases the amount of DNA was also increased from 1 to 5 ng (per 50 μl reaction). Between 20 and 30 μL of the PCR products was analyzed on three different gels. Bands formed at the same position in relation to standards (pure cultures of *Bacillus subtilis* and *Serratia marcescens* [17]) were assumed to represent the same taxon of bacterioplankton.

The intensity of the bands was not analysed, since a number of factors, in addition to the abundance of the organism of interest, can influence the intensity of the band formed (see e.g. [10, 29]). Therefore, only the presence or absence of the different bands was considered in the statistical analyses described below, and no quantification of the abundance of different taxa was performed. The gel patterns were assumed to represent the dominating populations of the bacterioplankton communities studied.

Statistical Analyses

Statistical analyses were performed as described in another study of the bacterioplankton community composition in five lakes [18]. To investigate the degree of similarity in bacterioplankton community composition between different samples, semi-strong hybrid nonmetric multidimensional scaling (NMDS) was performed [2]. Relations between community composition and the environmental variables determined (including those in Table 1, with the exception of the data concerning depth, turnover time, and areas of lakes and drainage basins) were investigated using canonical correspondence analysis (CCA) according to ter Braak [26]. Pairwise correlations between the environmental variables were determined using Spearman rank correlations.

Results

Lake Parameters

Based on their conductivity and the total concentrations of phosphorus and nitrogen in the water, all lakes can be considered mesotrophic. The variation in temperature between lakes and sampling occasions were small, since all samples were collected during summer when the air temperature is relatively high. In addition, the lakes did not appear to differ from each other with regard to the biomass of bacterioplankton, ciliates, phytoplankton, or chrysophytes, and they were relatively similar regarding the theoretical turnover time of the water (Table 1).

There was a considerable variation in humic content between the lakes (Table 1). The absorbance of the water at 420 nm ranged between 0.09 and 0.77, which approximately corresponds to a water color of 45 and 385 mg Pt l^{-1}. The three lakes with the highest water color (Upper Bear Lake, Lower Bear Lake, and Reindeer Lake) were considered to be polyhumic, whereas the large Lake Örträsket was classified as humic and the small Lake Siholma as weakly humic (Table 1).

The content of dissolved organic carbon (DOC) in the lakes varied, with the highest concentrations in the polyhumic lakes. The polyhumic lakes also had a shallower epilimnion and smaller biomasses of picophytoplankton and dinoflagellates compared to the other two lakes. In contrast, the biomasses of metazooplankton were higher in the polyhumic lakes. All lakes were slightly acidic, and there was no great variation in pH values between them, although pH values in the polyhumic lakes were slightly lower than those in the other two lakes. Thus, the three polyhumic lakes were similar to each other in terms of their chemical and biological properties, but differed from the other two lakes in these respects (Table 1).

The weakly humic Lake Siholma differed from the other four more humic lakes, in that the biomass of chlorophytes was generally higher in this lake (Table 1). Thus, the humic content of the lakes also appeared to have influenced the biomass of this group of phytoplankton.

Lake Örträsket, which is a relatively large lake, drains a much larger land area compared to the other four lakes, which are very small and have limited drainage areas. Furthermore, Lake Örträsket was the only one of the lakes in which diatoms were found in any significant amounts. Thus, Lake Örträsket was set apart from the other four lakes in several respects (Table 1).

To summarize, the investigated lakes mainly differed in water color, in size, and in the size of their drainage areas.

Bacterioplankton Community Composition

In the 14 samples analyzed, a total of 48 different bands were detected on the DGGE gels. The image of a typical such gel is shown in Fig. 1. There were 4–15 taxa detected per sample, the average being 8.1. One taxon was detected in all of the small lakes on every sampling occasion, but was not found in any of the samples from the large Lake Örträsket. Other taxa showed different distribution patterns, being present on one or several occasions in one or several lakes.

Distances in the NMDS ordination (Fig. 2) reflect the magnitude of dissimilarity in DGGE patterns between samples relatively well, as indicated by a satisfactory goodness of fit (<0.1) of the stress value for the ordination with three dimensions (0.0525). The similarity in bacterioplankton community composition between the polyhumic lakes was no greater than that found between the polyhumic and less humic lakes (Fig. 2). However, the two connected lakes, Lower Bear Lake and Upper Bear Lake, were always very similar to each other, especially in 1997 (Fig. 2).

Samples collected in June 1996 were similar to each other. Furthermore, with the exception of the large Lake Örträsket, all of the samples collected in June 1997 closely resembled each other (Fig. 2). By contrast, of the samples collected in August 1997, three samples (Reindeer Lake, Lake Siholma, and Lake Örträsket) differed from all other samples, and from each other (Fig. 2). Thus, in terms of their bacterioplankton community composition, samples collected on the same occasion appeared to be similar to each other, at least in the four small lakes and at the June sampling occasions. These results indicate that the small lakes had similar patterns of change in their bacterioplankton community composition over time, but also that great changes occurred in several of the lakes during the summer of 1997.

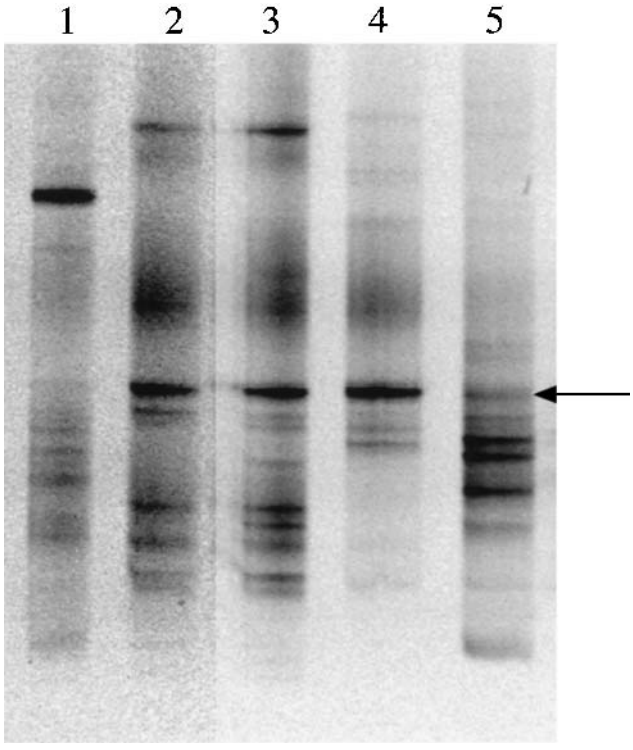

Fig. 1. Negative image of DGGE patterns obtained from analysis of the bacterioplankton community compositions of the studied lakes, in August 1997. Lane 1, Lake Örträsket; 2, Upper Bear Lake; 3, Lower Bear Lake; 4, Reindeer Lake; 5, Lake Siholma. The arrow shows the position of a band that was detected at every sampling occasion in four of the lakes. The image was processed using Adobe Photoshop 3.0 to increase the contrast of the gel images.

Bacterioplankton Community Composition in Relation to Environmental Variables

Three parameters were found to be significantly ($p < 0.05$) related to bacterioplankton community composition: temperature, diatom biomass, and cryptophyte biomass. According to the CCA ordination (Fig. 3), most of the variation in relation to diatoms was due to the samples from the large Lake Örträsket, since diatoms were found almost exclusively in samples from this lake (Table 1). The variation in relation to temperature and chrysophyte biomass was mainly due to the samples collected in August 1997 from the three polyhumic lakes and from Lake Örträsket (Fig. 3). At this time the cryptophytes were especially abundant in these lakes, and the temperatures had reached their maxima.

The results from the CCA also showed that the species–environment correlation was high; i.e., there was a strong relationship between the environmental variables measured and the composition of the bacterioplankton communities

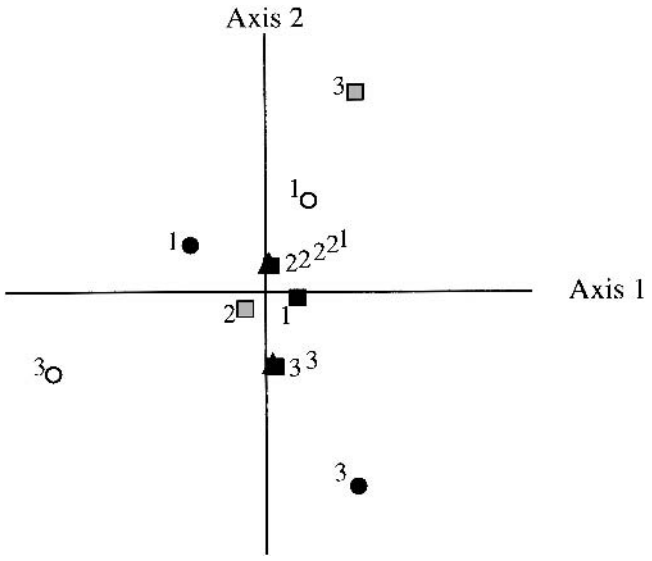

Fig. 2. Results from NMDS analysis of the data concerning the community composition of bacterioplankton in the studied lakes. Open circles, Lake Siholma; gray squares, Lake Örträsket; filled triangles, Upper Bear Lake; filled squares, Lower Bear Lake; filled circles, Reindeer Lake. 1, sample collected in June 1996; 2, June 1997; 3, August 1997.

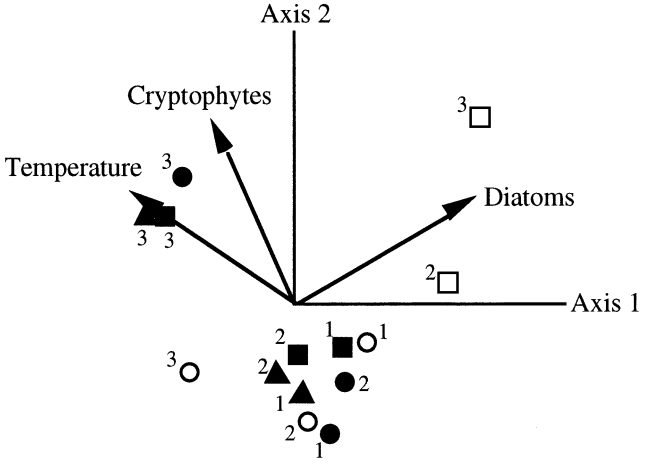

Fig. 3. Results from canonical correspondence analysis (CCA) of the bacterioplankton community composition data. Biplot of the different samples in relation to the strongest environmental variables. Open circles, Lake Siholma; open squares, Lake Örträsket; filled triangles, Upper Bear Lake; filled squares, Lower Bear Lake; filled circles, Reindeer Lake. 1, sample collected in June 1996; 2, June 1997; 3, August 1997.

(Table 2). The eigenvalues were higher for the two first ordination axes compared with the third, indicating that the variation along these two axes was of most importance (Table 2). Axis 1 was mainly composed of temperature and diatom biomass ($r = -0.727$ and $r = 0.792$, respectively), while axis 2 consisted mainly of cryptophytes ($r = 0.848$). Thus, all three of the selected environmental variables were well correlated to bacterioplankton community composition. In total, the three first axes explained 36.6% of the taxonomic variation in the bacterioplankton communities (Table 2).

Table 2. Summary of the results of canonical correspondence analysis of the bacterioplankton community composition data constrained to environmental variables.

Total inertia			4.416
Sum of all constrained eigenvalues			1.615
	Axis 1	Axis 2	Axis 3
Eigenvalues	0.657	0.528	0.430
Species–environment correlation	0.986	0.978	0.987
Cumulative percentage variance			
of species data	14.9	26.8	36.6
of species–environment relation	40.7	73.4	100.0

Correlations between Environmental Variables

Temperature was one of the three factors that according to the CCA showed the highest covariation with the DGGE patterns. This factor was, in turn, significantly ($p = 0.035$; $\rho = -0.586$) and negatively correlated to the biomass of ciliates. The biomass of cryptophytes was also among the strongest factors according to the results of the CCA. Biomass of cryptophytes was, in turn, significantly and positively correlated to the total nitrogen content ($p = 0.048$; $\rho = 0.549$).

The third factor that was significantly related to bacterioplankton community composition in the CCA, was diatom biomass. This factor was not significantly ($p > 0.05$) correlated to any of the environmental parameters determined. However, as mentioned above, these organisms were almost exclusively found in Lake Örträsket, suggesting that the observed relationship between the bacterioplankton community and diatom biomass was largely due to the influence of data from this particular lake.

Notably, bacterioplankton biomass was not significantly ($p > 0.05$) correlated to any of the environmental parameters determined.

Discussion

In general, it can be argued that two different types of processes can affect the composition of the bacterioplankton

communities in lakes. One process is internal to the lake, while the other is external. In the first, the environment influences the organisms' physical and chemical environment, and thereby selects for those taxa that survive and multiply most efficiently. In the external process, a massive input of bacteria occurs from a neighboring ecosystem (allochthonous bacteria), for example, from another lake or from surrounding land, replacing the indigenous community. The results obtained in this study indicate that both types of processes may have been involved in shaping the communities investigated.

All of the lakes studied here have short turnover times (Table 1), and thus should receive a substantial input of inflowing water from the surroundings. An earlier study from Lake Örträsket [3] also showed that the import of allochthonous bacterioplankton cells constituted 20–70% of the bacterioplankton production in the epilimnion in 1997. It is likely that the input of allochthonous bacteria in the other four lakes was of the same magnitude. Some observations made in this study indicate that these allochthonous bacteria also had an impact on bacterioplankton community structure.

First, one of the most striking results obtained from the NMDS-analysis is the consistent similarity between the connected Upper and Lower Bear Lake (Fig. 2), suggesting a constant influence from the former lake on the latter. Secondly, a great change in bacterioplankton community composition occurred in the lakes between the June and the August sampling occasions in 1997 (Fig. 2). These changes coincided with heavy rainfalls in the lake area in July 1997. These rainfalls caused an increased inflow of water into the lakes, which was large enough to replace the entire epilimnion volumes in less than a week [3, 15]. Thus, an increase in the inflow of water from the drainage area appears to have changed the community composition of bacterioplankton in these lakes. Since both fertilized and unfertilized lakes were subject to changes (Fig. 2, Table 1) these changes cannot be attributed to the ongoing fertilization program, especially since these fertilizations did not give rise to a significant ($p > 0.05$) increase in bacterioplankton production or biomass in 1997 compared to 1996 [5, 15].

Selection for certain taxa also appeared to have occurred in the studied lakes, as shown by the strong correlations between internal factors within the lakes and their bacterioplankton community compositions. According to the CCA, in which only within-lake environmental factors were considered, the parameters determined could explain 36.6% of the variation in the bacterioplankton community composition (Table 2). In this analysis, temperature and biomasses of cryptophytes and diatoms, were the variables found to be most strongly related to bacterioplankton community composition.

The variation in temperature was minimal both between and within lakes, ranging between 14 and 18°C (Table 1), thus hardly selecting for different bacterioplankton taxa at the different sampling occasions. An alternative explanation to this statistical relationship is therefore that biomass of ciliates, which was the only factor significantly correlated to temperature, was the factor that had the actual impact on bacterioplankton community structure. In a previous study of five other Swedish lakes [18], grazing by ciliates was suggested to have affected bacterioplankton community structure. Therefore, such an influence should be considered as possible in this set of lakes as well.

Interestingly, in the previous lake study [18], the biomass of cryptophytes was also well correlated to bacterioplankton community composition, thus in agreement with the results obtained here. How cryptophytes should affect the bacterioplankton community structure is difficult to speculate about, since grazing by cryptophytes on bacterioplankton has previously been reported to be minimal in these lakes [4, 13]. However, the results obtained here together with the results from the previous study [18] suggest that other planktonic biota (here ciliates and cryptophytes) should be considered as possible determinative factors in the study of bacterioplankton community compositions.

The statistical relationship between the composition of the bacterioplankton communities and the biomass of diatoms is also difficult to explain. It can be argued that the reason for such a relation is that diatoms provide a substrate for the bacterioplankton, which differs in chemical composition from that provided by other phytoplankton taxa [25]. Such a difference could in turn select for different taxa of bacterioplankton. However, the diatoms, which were almost exclusively found in Lake Örträsket, always had a very low biomass (Table 1). Additionally, the importance of autochthonously produced organic carbon as substrate for bacterioplankton growth in general can be assumed to be low in humic lakes (e.g., [14]). Therefore, it seems more probable that the observed statistical relationship between bacterioplankton community composition and diatoms was due to a general difference in lake characteristics between Lake Örträsket and the other four lakes. Lake Örträsket is much larger than the other four lakes and has a much larger drainage area. Thus, this lake differed from the other four with regard to many lake parameters, which in turn appear to

have influenced the community composition of the bacterioplankton.

One of the differences in the bacterioplankton communities was that the four small lakes showed a consistent presence of a band at one particular position on the gels, whereas no such band was detected in the samples from Lake Örträsket. By sequence analysis it has been confirmed that the populations from which these bands originate belong to *Actinobacteria,* and that they are closely related to an *Actinobacteria* previously [9] detected in Lake Fuchskule in Germany (Lindström and Leskinen, unpublished). Since the bands were always detected, it can be assumed that these particular *Actinobacteria* were of major importance for the composition of the bacterioplankton communities in the four small lakes. In contrast, this taxon was not an important constituent of the community in Lake Örträsket.

To summarize, in this study a number of factors were found to have a potential impact on bacterioplankton community composition. These factors include small-scale within-lake biological factors as well as large-scale factors outside of the lakes. From the results obtained here, it was not possible to determine which factors were most important, but it can be assumed that different types of factors had different impacts at different seasons and in different lakes. The results from the present study together with findings from previous ones, however, suggest that factors such as the input of allochthonous bacteria, the action of grazers [12, 18], and lake chemistry [20] are factors that should be of interest for further study.

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