Limnol. Oceanogr., 49(1), 2004, 125–136

Influence of inlet bacteria on bacterioplankton assemblage composition in lakes of different hydraulic retention time

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Abstract

The influence of inlet bacteria on the assemblage composition of bacterioplankton was investigated in two Swedish forest lakes of different hydraulic retention time. Assemblage composition of the bacteria in lakes and running waters was determined by denaturing gradient gel electrophoresis (DGGE) and sequencing of polymerase chain reaction (PCR)-amplified 16S rDNA. The amount of bacterial cells imported via the inlets in relation to bacterioplankton cells produced in the epilimnion of the lakes was also determined. In the lake with short retention time (theoretical hydraulic retention time of 0.3 yr) the lake bacterioplankton assemblage largely resembled the riverine assemblages, although the extent of similarity varied among inlets, depending on water flow. In the lake with long retention time (theoretical hydraulic retention time of 10 yr) the bacterioplankton assemblage in the lake had low similarity to the inlet assemblages. The degree of similarity between inlets and lakes was well correlated to the amount of imported cells. Thus, our data suggest that import of inlet bacteria could have a large effect on the composition of lake bacterioplankton assemblages and that hydrological factors determined the magnitude of this effect. Since short hydraulic retention times are very common in lakes in the boreal zone, input of allochthonous bacteria can be one major factor influencing bacterioplankton assemblage composition in boreal lakes.

Input of allochthonous matter to lakes is of great importance for ecosystem structure and function as shown in a number of investigations (e.g., Tranvik 1992; del Giorgio and Peters 1994; Hessen and Tranvik 1998; Jansson et al. 2000; Prairie et al. 2002). Most of these studies have focused on the importance of allochthonous dissolved organic carbon. However, it has also been shown that the input of allochthonous bacteria can correspond to up to 70% of the internal production of bacterioplankton in the epilimnion of a lake (Bergström and Jansson 2000). The effect of such allochthonous bacteria on natural lake ecosystems, besides being an additional source of carbon, is to a large extent unknown.

Intuitively, it can be supposed that a large input of allochthonous bacteria may change the composition of bacterioplankton communities in lakes. However, a number of biotic and abiotic factors are known to reduce the survival of allochthonous bacteria in aquatic environments (Barcina et al. 1997). Therefore, the influence of import of inlet bacteria on the composition of lake bacterial assemblages depends also on how well the bacteria establish themselves in the lakes. Experiments have shown a low survival of allochthonous bacteria in freshwater mesocosms (e.g., Höfle 1992; Leff et al. 1998). In reservoirs, the influence by allochthonous bacteria has been found to be local (Simek et al. 2001*a;* Gasol et al. 2002) or low (Dumestre et al. 2002). On the other hand, results from a few natural freshwater systems have indicated that allochthonous bacteria can influence the assemblage composition of the bacterioplankton (Lindström 1998, 2001; Crump et al. 2003; Stepanauskas et al. 2003). In addition, the close relationship often found between bacteria detected in rivers and lakes (Zwart et al. 2002) indicates a frequent exchange of cells between the two habitats. Thus, the input of allochthonous bacterioplankton is a potentially important factor shaping bacterioplankton communities in lakes. Where, when, and to what extent these bacteria influence bacterioplankton assemblage composition is, however, unknown.

Since the introduction of molecular methods in the study of bacterioplankton diversity, a plethora of new taxa has been discovered. Compilation of large amounts of sequence data has shown that the probability is quite high that a sequence recently retrieved from an aquatic environment has close matches to other sequences in public databases (Hagström et al. 2002; Zwart et al. 2002). Thus, a relatively small number of bacterial groups may dominate bacterioplankton communities (Hagström et al. 2002; Zwart et al. 2002). Therefore, instead of putting more effort on the search for new bacterioplankton taxa, the focus can now be directed to

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Acknowledgments

We thank our colleagues, and especially Peter Blomqvist, Stefan Bertilsson, Silke Langenheder, Lars Tranvik, Katarina Vrede, and Tobias Vrede at the Department of Limnology, Uppsala University for theoretical and methodological input. Jan Johansson, Kjell Hellström, and Ulrika Stensdotter at the same department are acknowledged for water chemistry analyses and plankton counts. Further, we thank the microheterotroph group for comments on previous versions of the manuscript. We are also grateful to Mary Ann Moran and two anonymous reviewers for their thorough reviews and suggestions to improve the paper. This work was supported by a grant from the Swedish Environmental Protection Agency/The Swedish research council for environment, agricultural sciences, and spatial planning (21.0/2001-4016) to both authors. Financial support was also given by Oscar and Lili Lamm's, Magn. Bergvall's, and Helge Ax:son Johnson's foundations to E.S.L.

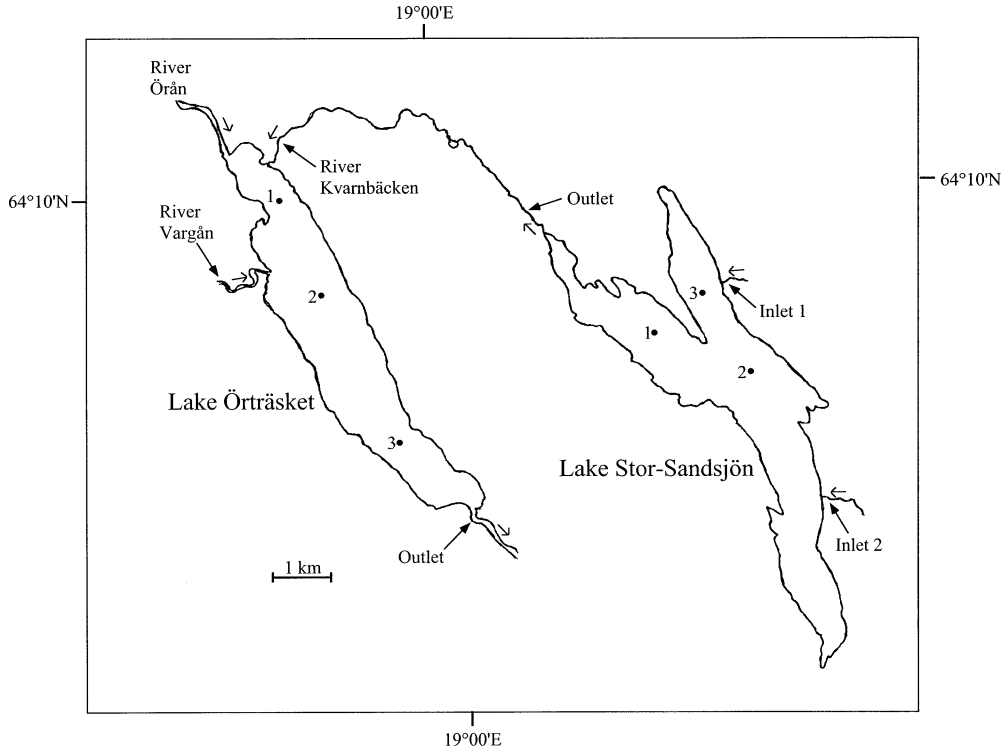

Fig. 1. Map showing the location of the sampling spots of the lakes and their inlets and outlets.

obtaining knowledge about the differences in assemblage composition between different habitats and which environmental factors have effects on such differences. Among the field studies performed in this area, results have indicated that food web interactions, as well as water chemistry, can have effects on bacterioplankton assemblage composition in freshwaters (e.g., Höfle et al. 1999; Methé and Zehr 1999; Lindström 2000; Simek et al. 2001*a*,*b*; Van der Gucht et al. 2001; Lindström and Leskinen 2002; Muylaert et al. 2002; Crump et al. 2003; Stepanauskas et al. 2003).

The aim of this study is to investigate the importance of allochthonous bacteria in determining the assemblage composition of bacterioplankton in lakes. More specifically, we chose to study bacteria in streams and rivers testing the following hypotheses:

1. The similarities in bacterial assemblage composition between lakes and inlets can be very high as a consequence of the import of cells from the inlet that subsequently establish themselves in the lake.

2. The similarity between lake and inlet depends on water discharge and may thus differ between inlets and lakes of different hydraulic retention time.

Two boreal forest lakes that differ in hydraulic retention time were studied. The lakes were sampled on three different occasions during one summer. The assemblage composition of lake bacterioplankton and stream bacteria was determined by denaturing gradient gel electrophoresis (DGGE) and sequencing of 16S rDNA. DGGE is a technique that has been shown to give a good view of the composition of dominating community members (e.g., Torsvik et al. 1998; Casamayor et al. 2002). To compare the amount of imported bacteria to the amount of internally produced cells, bacterial cell budgets were constructed by use of water flow data in combination with microscopic determination of bacterial abundances and measurements of bacterial production in the lakes.

Methods

Study sites and sampling—The two studied lakes, Lake Örträsket and Lake Stor-Sandsjön, are situated in the region of Lappland in northern Sweden, within 10 km of each other (64°10′N, 19°00′E) and are in direct contact via a small stream, River Kvarnbäcken (Fig. 1). The lakes are forest lakes that differ in their theoretical hydraulic retention time (Table 1). The drainage area of Lake Örträsket (2174 km^2) is dominated by coniferous forest (78%) and mires (20%), and only a small percentage consists of arable land. The lake surface area is 7.3 km^2, and the theoretical water retention time is 0.3 years (Table 1). The drainage area of Lake Stor-Sandsjön is considerably smaller in comparison to that of Lake Örträsket, only 25 km^2. Of this area, 9.2 km^2 represent the lake itself (36%). The rest of the drainage area consists of coniferous forest (60%) and mires (3%). The theoretical hydraulic retention time is 10 yr (Table 1).

The lakes and their major inlets and outlets were sampled on three occasions during the summer of 2001 (6–7 June, 3–4 July, 7–8 August). The three sampling dates were selected so that the hydrological regime would differ. Composite samples of the epilimnion (from the surface down to 4–12 m depending on stratification depth) of Lake Örträsket

Table 1. Chemical, physical, and biological character of the epilimnion and the inlets of the two lakes during the period of study.

	Lake Örträsket		Lake Stor-Sandsjön	
	Lake	Inlets	Lake	Inlets
Theoretical water retention time (yr)	0.3	—	10	—
Lake surface area (km^2)	7.3	—	9.2	—
Epilimnion volume (10^7 m^3)	3.4–6.2	—	3.7–5.9	—
Total phosphorus (μg P L^{-1})	14–18	10–29	7–10	14–24
Total nitrogen (μg N L^{-1})	220–310	140–510	140–190	190–460
Water color (Abs 436 m)	0.15–0.22	0.12–0.50	0.02–0.04	0.13–0.41
Total organic carbon (mg L^{-1})	10–12	6–30	5	8–30
pH	5.8–6.8	4.8–6.9	6.7–6.8	5.5–7.0
Potential bacterial grazers (10^6 cells L^{-1})	2.17–4.48	1.30–4.92	1.81–2.88	0.48–1.37
Bacterial abundance (10^9 cells L^{-1})	2.0–3.6	0.7–3.0	0.5–1.6	0.5–0.9
Bacterial production (10^7 cells L^{-1} h^{-1})	3.6–4.1	—	0.1–1.3	—
Bacterial import (10^{16} cells d^{-1})	—	3–530	—	0–0.09

and Lake Stor-Sandsjön were taken at three positions in each lake (Fig. 1). Samples representing hypolimnion of Lake Örträsket were also collected at positions 2 and 3 at depths of 25 m (in July) or 20 m (in August).

Lake Örträsket has two major (River Vargån and River Örån) and several smaller inlets and one outlet (River Öre). The two large inlets, one smaller inlet, and the outlet were sampled. These samples were collected with a Ruttner sampler (2 liters) at a depth of 0.5–1 m. The largest inlet (River Örån) contributed 86% of the water that entered Lake Örträsket. Together, the three sampled inlets contributed 97% of the inflowing water. Thus, the groundwater flow into this lake was less than 3% of the total flow.

Lake Stor-Sandsjön has eight small inlets, draining a total area of 5 km^2. The majority (ca. 75%) of the water that enters Lake Stor-Sandsjön comes from diffuse groundwater input. However, the drainage area of diffuse groundwater input is smaller in Lake Stor-Sandsjön (20 km^2) in comparison with Lake Örträsket (61 km^2). Owing to similar rates of groundwater flow (L km^{-2} s^{-1}), the volume of water entering Lake Stor-Sandsjön as diffuse groundwater only represents one-third of the corresponding amount into Lake Örträsket. In Lake Stor-Sandsjön two inlets and the outlet were sampled (Fig. 1). The two sampled inlets contributed 6% of the water that entered Lake Stor-Sandsjön. The six unsampled inlets contributed another 19% of the total volume of water that entered Lake Stor-Sandsjön.

Three subsamples were taken from the collected samples. One subsample was collected in a sterile bottle and kept at in situ temperature until further processing in the laboratory. This subsample was used for determination of bacterial assemblage composition, abundance, and production (the latter parameter only in the lake samples). The pH and water color were also determined in this subsample after material for bacterial parameters were removed. The second subsample was collected in an acid-washed bottle, kept at in situ temperature, and frozen immediately upon arrival to the laboratory. This sample was later used for analysis of water chemistry. The third subsample was immediately preserved with acidic Lugol's iodine and subsequently used for determination of the abundance of potential bacterivores.

Hydrology—Discharge (m^3 s^{-1}) and the specific runoff (L km^{-2} s^{-1}) were measured daily during the investigation period at the largest inlet to Lake Örträsket (River Örån) by continuous registration of the water level in combination with use of previously established water level/discharge relationships (Jonsson and Jansson 1997). The daily discharge for Lake Stor-Sandsjön (inlets and outlet) and the smaller inlets and outlet of Lake Örträsket was subsequently calculated by using the daily specific runoff measured for River Örån. We tested the accuracy of this assumption by measuring the discharge in the streams at three different occasions during the time period May–August. The difference in discharge between measured values and values calculated from the specific runoff measured for River Örån was always $<5\%$.

Assemblage composition of bacteria—The assemblage composition of bacteria in lakes and streams was determined by denaturing gradient gel electrophoresis (DGGE) of 16S rDNA. Within 2–3 h of sampling, 1.5 ml of the collected samples was pipetted into sterile vials. The cells were collected by centrifugation at 17,000 $\times$ g for 30 min. The su-

pernatant was removed by use of a sterile pipette tip, and the remaining pellet was stored frozen. DNA extraction from the collected cells was done with the DNeasy kit (Qiagen), following the protocol for gram-positive bacteria, which yielded 200 μl of DNA sample. This protocol gives very similar results regarding DGGE patterns as lysis with sodium dodecyl sulfate (SDS) and phenol-chloroform extraction (Lindström and Langenheder unpubl. data)

The 16S rDNA of positions 341–928 (*E. coli* positions) from 20–30 μl of the DNA extracts were amplified in 50 μl polymerase chain reactions (PCRs). The amplification was conducted with 1.25 U AmpliTaq LD (Applied Biosystems) in GeneAmp PCR buffer with 3 mmol L^{-1} $MgCl_2$, 200 μmol L^{-1} of each dNTP, and 25 pmol of each primer. Eubacterial primers GM5F (with GC clamp) and DS907-reverse (Teske et al. 1996) were used. Amplification was done by a touchdown PCR cycle (Lindström 1998).

Forty microliters of the PCR products were analyzed in 40–70% or 37.5–67.5% denaturant gradient gels, which were run for 6 h at 200 V and 60°C. The two different gradients gave essentially the same gel patterns, although the former gradient occasionally led to bad resolution of the bands at the top of the gel. Bands formed in the gels were visualized by staining with SYBRGold (Molecular Probes) and documented under ultraviolet illumination. The contrast of the digital gel images was increased with Photoshop 6.0. These improved gel images were analyzed manually, and matrices over the presence or absence of bands in the different samples were constructed. These band patterns were used as a fingerprint over the numerically dominating members of the bacterioplankton communities (e.g., Torsvik et al. 1998).

Sequence analyses—Bands that were selected for sequencing were excised from the gels by use of a sterile scalpel. The gel piece was placed in 20 μl sterile MilliQ water, and the DNA was eluted by diffusion in 4°C overnight. Five microliters of the eluate were reamplified under the same conditions as above, except for the concentration of $MgCl_2$, which was decreased to 1.5 mmol L^{-1}. The resulting PCR products were run on a DGGE gel under the same conditions as above, and the bands of interest were again excised, eluted, and amplified. These PCR products were subsequently run in a DGGE gel to check for purity and to verify that a band was formed at the expected position in the gel. If the band appeared pure and strong enough, the DNA from the second elution step was again amplified for sequencing. The primers and conditions for this PCR step were as above except that the GC clamp on primer GM5F was excluded. The products from three PCR reactions were pooled and purified by use of the QIAquick PCR purification kit (Qiagen). Sequencing was done commercially by Cybergene AB, Huddinge, Sweden.

To check for chimeras the sequences were analyzed by the check chimera option using the Ribosomal Database Project (RDP; Cole et al. 2003) online services (http://rdp.cme.msu.edu/html/). For further analysis, sequences were collected from GenBank. The three sequences that in a Blast search (http://www.ncbi.nlm.nih.gov/blast/) showed the greatest similarity to our sequences were included. For sequence O4 (*see results section below*), however, no sequences showing a greater similarity than 93% were found, and none of them were freshwater sequences. Therefore, those GenBank sequences were not included. Sequences representing most of the different *β-proteobacteria* and *Cytophaga-Flavobacteria* clusters defined by Zwart et al. (2002) were also included in the analysis. Alignment, distance calculation using Kimura's two-parameter method, and clustering by neighbor joining were performed using the RDP online services (Phylip interface). *Prochlorococcus marinus* was used as the outgroup.

The sequences were submitted to GenBank under accession numbers AY184378–AY184382.

Bacterial production and numbers—Bacterial numbers were determined in lake and stream water by acridine orange staining and epifluorescence microscopy (Hobbie et al. 1977). The samples were preserved with formaldehyde (final concentration 4%) immediately upon arrival in the laboratory and stored at 4°C in darkness for 1–3 months before microscope slides were prepared.

Bacterial production in the lake water was determined by measuring the incorporation of tritiated thymidine into DNA (Bell 1993). Triplicate samples of lake water (10 ml) were incubated with 30 nmol L^{-1} (Lake Stor-Sandsjön) or 50 nmol L^{-1} (Lake Örträsket) [^{3}H] thymidine at in situ temperature for 1 h. The concentration of thymidine used was optimized after saturation experiments. The incubations were started immediately upon arrival to the laboratory, i.e., within 2–3 h of sampling. The growth rate of the bacterial cells (cells L^{-1} h^{-1}) was calculated assuming 2×10^{18} cells per mole incorporated thymidine (Bell 1990).

Construction of bacterioplankton cell budgets—Bacterial cell budgets were constructed in order to compare the amount of bacterial cells flowing into the lakes via their inlets to the amount of bacteria produced within the lakes. Bacterial concentration, multiplied by discharge from the inlets, was used to calculate the magnitude of imported cells. During stratification, inflowing water was assumed to mix only with epilimnetic water, i.e., the hypolimnetic water was regarded as a closed system. This has been found to be the case in previous studies of Lake Örträsket (Bergström and Jansson 2000; Jonsson et al. 2001). Changes in the bacterial concentrations within the lakes were calculated from measured lake bacterial concentrations and calculated lake volumes. Numbers of bacterial cells produced within the lakes were calculated from the lake bacterial production measurements and lake water volumes. The budgets were calculated for the summer stratification period from early June to late August in 2001.

Water chemistry and bacterivores—The total concentration of phosphorus, nitrogen, and organic carbon, as well as pH, was determined by standard methods (Bergström and Jansson 2000). Water color was determined by measuring the absorbance of filtered water (preignited Whatman GF/F-filters) at 436 nm (5 cm cuvette) using a Hitachi U-1100 spectrophotometer.

The abundance of potential bacterial grazers was deter-

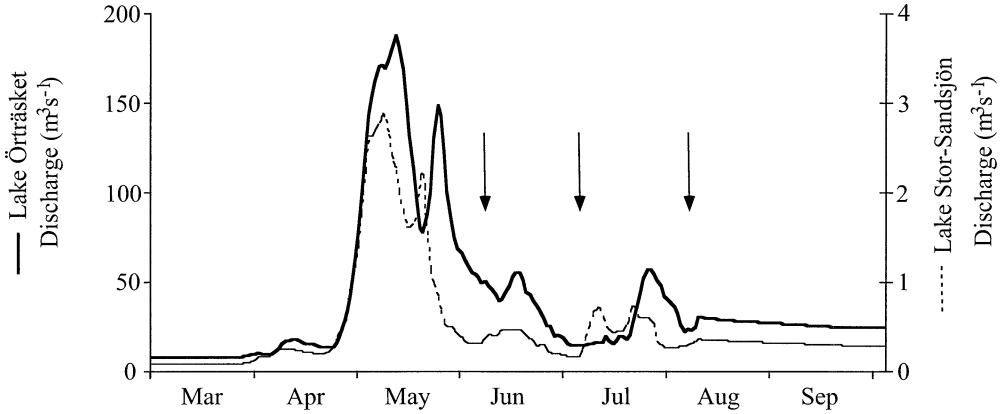

Fig. 2. The daily discharge at the outlet of Lake Örträsket and Lake Stor-Sandsjön in 2001. The arrows show when the lakes were sampled.

mined in an inverted microscope after sedimentation (Utermöhl 1958). Flagellates were determined to genus if possible or otherwise grouped in size classes by order or class. Flagellates belonging to heterotrophic genera were classified as bacterial grazers, as well as flagellates not determined to genus but clearly lacking chlorophyll. Chloroplast-containing flagellates were presumed to be mixotrophic (thus, potential bacterial grazers) except for those belonging to the genera Cryptophyceae and Dinophyceae (Isaksson et al. 1999; Bergström et al. 2000). Ciliates were classified as bacterivores, except for those belonging to the algivorous order Prostomatida.

Statistics—The pairwise similarity between gel patterns was calculated as

$$C_s = 2j/(a + b)$$

where j is the number of bands in common for two samples, a the number of bands in sample A, and b the number of bands in sample B (Lindström 1998). These similarity values were multiplied by 100 to obtain the percentage similarity between samples.

For statistical analyses, the similarity values were converted to dissimilarity values as $1 - C_s$. These values were subsequently analyzed by cluster analysis (unweighted pair-group average, UPGMA) using the program Statistica (StatSoft).

The relationship between the gel patterns and the environmental parameters in the lakes and the inlets was analyzed by pairwise correlation analyses. In this analysis the dissimilarity in gel pattern between a certain inlet and its lake at a certain sampling occasion was analyzed as a function of the absolute values of the difference in water chemistry, temperature, and grazer abundance between the two sampling spots. The gel pattern dissimilarities were also analyzed in relation to the amount of imported cells (cells d^{-1}).

Since normal distribution could not be assumed, the correlation analyses were performed as Spearman rank correlations using the program Statistica (StatSoft).

Results

Character of the lakes and the inlets—Both lakes are slightly acidic and can be designated oligo-mesotrophic based on their content of phosphorus (Table 1). Lake Örträsket is humic as shown by its water color, while Lake Stor-Sandsjön is not humic. The concentration of organic carbon was subsequently higher in Lake Örträsket, as well as the phosphorus and the nitrogen concentration. In addition, the abundance of bacteria and their production as well as the abundance of bacterial grazers was higher in Lake Örträsket.

The inlets to the two lakes had occasionally slightly higher contents of organic and inorganic nutrients and lower pH values compared to the lakes (Table 1). However, there were no great differences in the chemical composition of the water in the inlets and in the lakes. The abundance of the bacterial grazers was also occasionally lower in the inlets to Lake Örträsket and always lower in the inlets to Lake Stor-Sandsjön.

The grazer communities were dominated by flagellates in all samples. The number of ciliate cells was always lower than 1% of the total number of grazer cells.

Hydrology—The hydrology in Lake Örträsket was characterized by a pronounced spring flood in May, which was sustained until the beginning of June. A smaller summer episode occurred in the end of July due to heavy rainfall (Fig. 2). A similar pattern in water flow was observed in Lake Stor-Sandsjön, but the discharge was considerably smaller (<4 m^3 s^{-1}) than the one in Lake Örträsket (Fig. 2). This is a result of the much smaller drainage area for Lake Stor-Sandsjön (25 km^2) in comparison to that of Lake Örträsket (2174 km^2).

Bacterioplankton assemblage composition in Lake Örträsket and its rivers—The samples obtained from the epilimnion of Lake Örträsket and from the rivers were analyzed in three different DGGE gels, depending on sampling date. The assemblage compositions in the three samples from the epilimnion and the outlet were always very similar to each

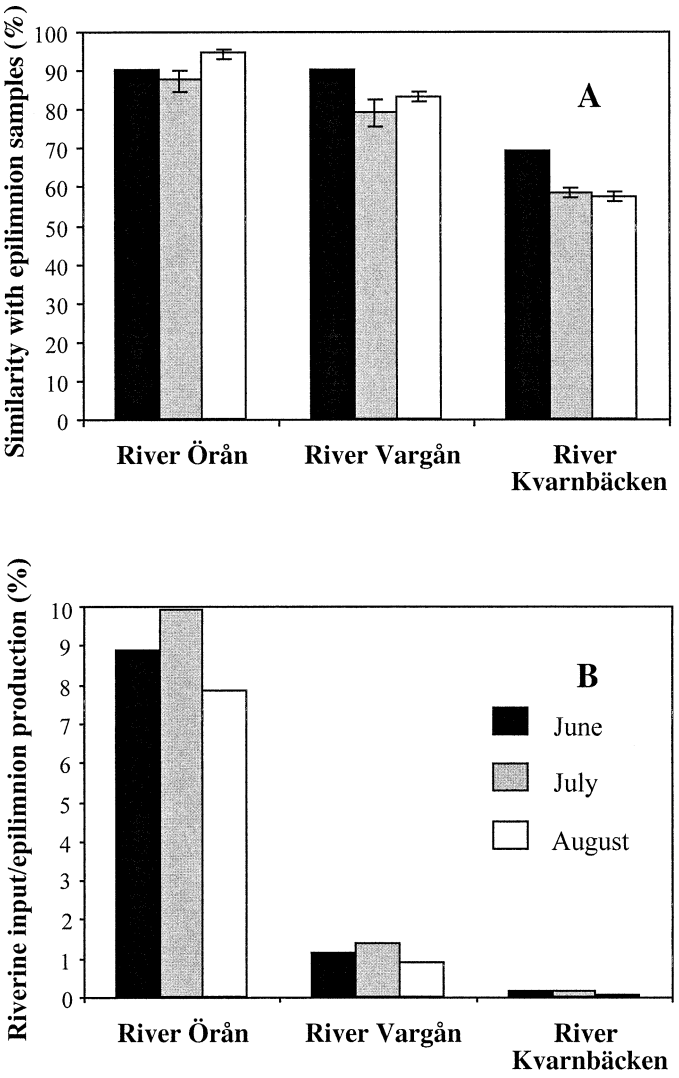

Fig. 3. Comparison between the three inlets and the epilimnion of Lake Örträsket. (A) Similarity in composition of the bacterial communities as determined by DGGE. The results shown are the average value of the comparison between the different inlets and the three lake samples. The error bars show the standard deviation. (B) Input of bacterial cells in comparison to the bacterial production in the epilimnion, as determined by thymidine incorporation.

other, showing similarities of 95–100% (results not shown). River Örån was most similar to the epilimnion, showing 87–94% similarity depending on sampling occasion (Fig. 3A). The similarity of samples from River Vargån and River Kvarnbäcken to the epilimnion samples ranged from 79% to 90% and 57% to 69%, respectively (Fig. 3A).

The amount of bacterial cells introduced via the inlets to the lake also differed between inlets. River Örån contributed an amount of cells corresponding to 8–10% of the cell production in the epilimnion, depending on sampling occasion (Fig. 3B). River Vargån contributed bacterial cells equivalent to 0.9–1.4% of the production in the epilimnion (Fig. 3B). River Kvarnbäcken transported cells equivalent to 0.05–0.16% of the epilimnion production. In total, the different inlets, at the three sampling occasions, transported cells into the epilimnion equivalent to 10.0–11.4% of the production in the epilimnion. Thus, the internal production of cells in the epilimnion was much larger than the amount of imported cells at all sampling occasions, and the largest amount of imported cells came from River Örån.

To simplify comparison of samples from different sampling occasions, all the samples from the largest inlet, River Örån, and one sample per sampling date from the epilimnion and the hypolimnion of the lake were reanalyzed in the same DGGE gel (Fig. 4A). In total, 33 different bands were identified in this gel. There were 18–25 bands detected per sample, the average number of bands was 22.1 per sample. Three strong bands, bands O1–O3 (Fig. 4A), were in common for all samples, although the intensity of band O1 clearly decreased during the summer, indicating a decrease in the relative abundance of that population. Other bands showed different patterns in their appearance, for instance band O4 appeared to be a strict lake population, which was not detected in the inlets.

Cluster analysis of the gel patterns in Fig. 4 showed that there were great similarities (>90%) between the epilimnion and the largest inlet at all sampling dates (Fig. 5A). The assemblage compositions of the hypolimnion samples differed from all other samples, and thus these two samples formed a separate cluster (Fig. 5A). However, the similarities between hypolimnion samples and epilimnion samples were always greater than 80%.

Bands O1–O4 (Fig. 4A) were excised from the gel and sequenced. The analysis showed that the bacteria represented by bands O1 and O2 belong to the group *Cytophaga-Flavobacteria* (Fig. 6). They were relatively closely related to each other (distance 0.025). Sequence O1 was most closely related to sequences Fuku N 24 (distance 0.008) from the humic Lake Fuchskuhle and TAF-B2 (distance 0.017) from River Taff epilithon (O'Sullivan et al. 2002). Sequence O2 was most closely related to sequences clone 09 (distance 0.017) from an experiment in the eutrophic Rimov reservoir (Simek et al. 2001*b*) and SY6-50 from the sediment of Lake Soyang (distance 0.014). SY6-50 was previously defined as a member of a putative freshwater cluster designated Fuku N47, which also includes sequences from humic Lake Fuchskuhle and oligotrophic Crater Lake (Zwart et al. 2002). Thus, both bacteria O1 and O2 are closely related to bacteria from several different types of freshwaters.

Bacteria O3 and O4 were found to be *β-proteobacteria* (Fig. 6), being rather different from each other (distance 0.097). Bacteria O3 was most similar to sequences Fuku S35 (distance 0.000) and Fuku N33 (distance 0.003) from humic Lake Fuchskuhle, and to sequence PRD01a006B from mesotrophic Parker River (distance 0.003). All these three sequences belong to a tight cluster of sequences from freshwaters defined as the *Polynucleobacter necessarius* cluster (Zwart et al. 2002). The sequences in this cluster originate from lakes, rivers, and estuaries, ranging in type from oligotrophic to eutrophic and from clear water to humic (Zwart et al. 2002). Thus, bacterium O3 is also closely related to bacteria from a wide variety of freshwater habitats. Sequence O4 differed from all the freshwater sequences included in the analysis (Fig. 6).

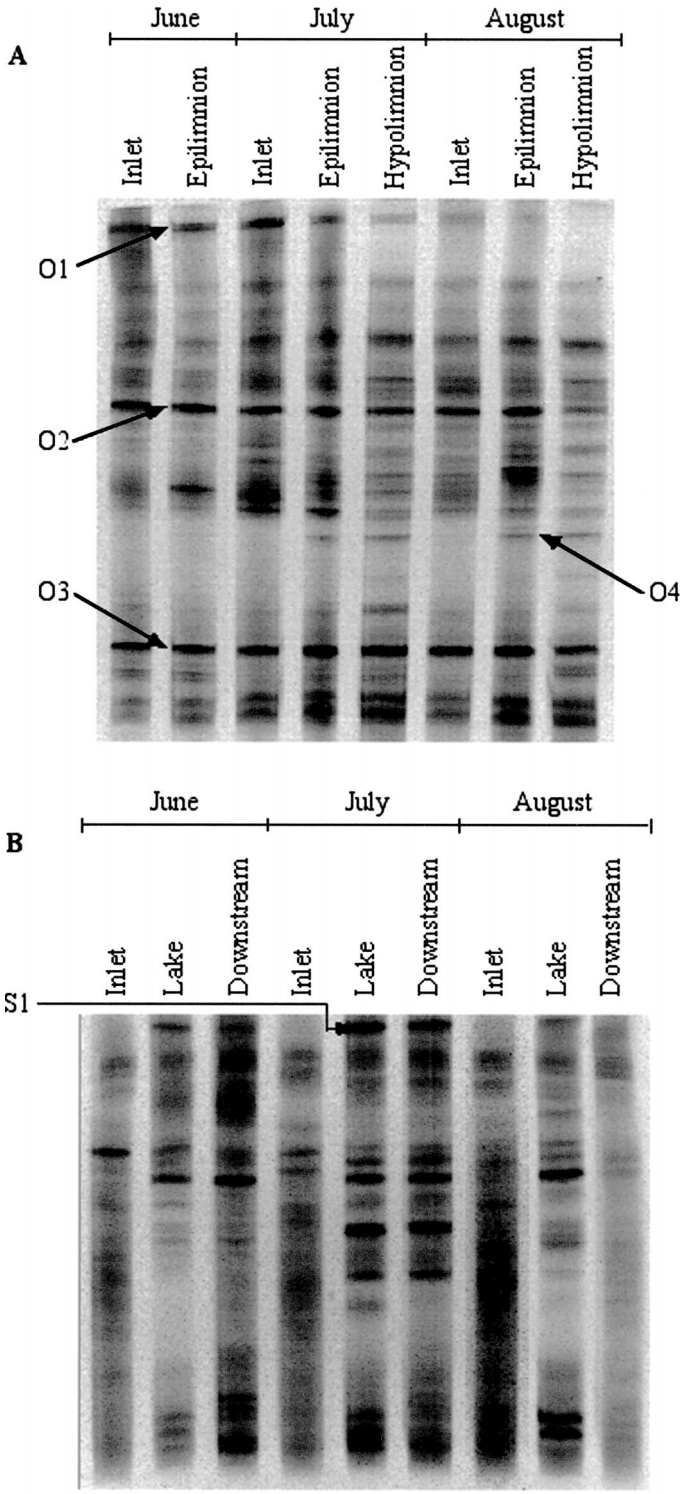

Fig. 4. Negative image over the DGGE patterns. (A) The largest inlet (River Örån) and one epilimnion sample and one hypolimnion sample from Lake Örträsket at the three different sampling occasions. Bands O1–O4 were cut out for sequencing. (B) Lake Stor-Sandsjön at the three different sampling occasions. Samples from the largest inlet, one epilimnion sample, and River Kvarnbäcken, downstream from the lake. Band S1 was cut out for sequencing.

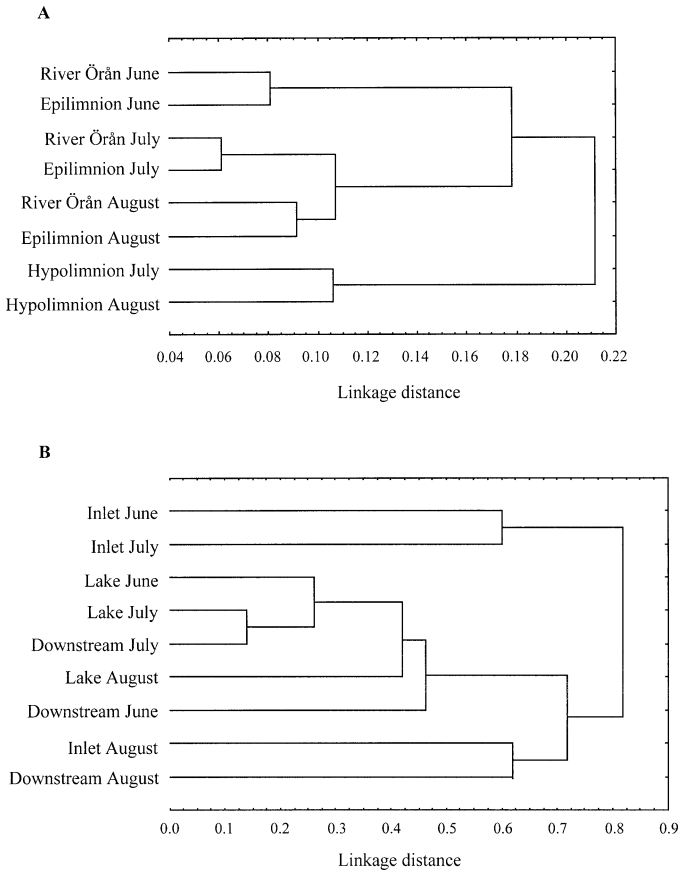

Fig. 5. Result from cluster analysis (UPGMA) of the gel patterns in Fig. 4. (A) Lake Örträsket. (B) Lake Stor-Sandsjön.

Bacterioplankton assemblage composition in the epilimnion of Lake Stor-Sandsjön and its streams—The similarity in bacterioplankton assemblage between the different epilimnion samples and the outlet of Lake Stor-Sandsjön was always 100% at all sampling occasions (results not shown). Samples from inlet 2 (Fig. 1) could only be analyzed at the June sampling occasion, since the stream dried out during the summer. However, at the June sampling occasion it showed great, almost 100%, similarity to inlet 1 (results not shown), and therefore inlet 1 was chosen to represent the inlets in the analysis. To analyze the similarity between the inlet bacterial assemblage and the lake bacterioplankton assemblage during the summer, the sample from inlet 1 and one lake sample per sampling occasion were reanalyzed together on a gel (Fig. 4B). One sample per sampling occasion from River Kvarnbäcken, downstream from the outlet of the lake (Fig. 1), was also included (Fig. 4B). A total of 63 different bands were detected on this gel. There were 12–20 bands per sample, the average being 16.6.

The similarity between the assemblage in the lake and the inlet was much lower in Lake Stor-Sandsjön than in Lake Örträsket and ranged from 6% to 28%. Cluster analysis of the gel patterns in Fig. 4 also showed that the inlet samples from June and July form a separate cluster and that the inlet sample from August forms a separate cluster together with the sample collected downstream of the lake in August (Fig.

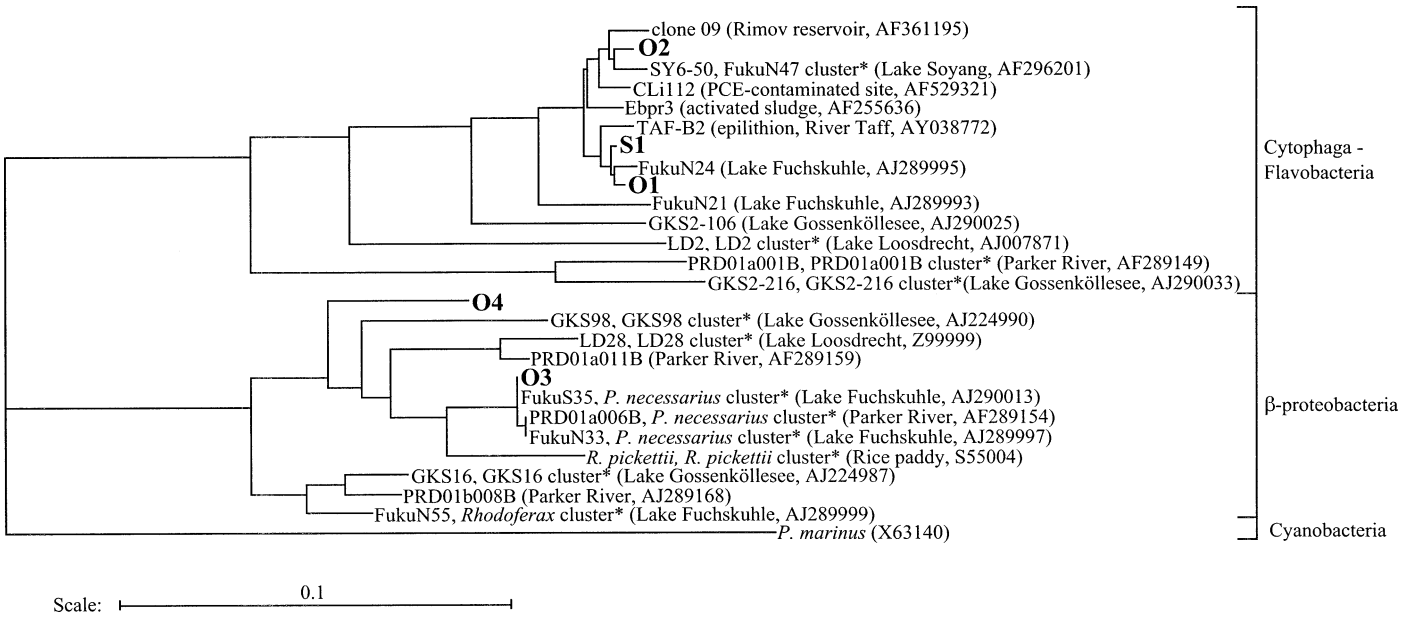

Fig. 6. Results from neighbor joining analysis of DNA sequences retrieved from the DGGE gels (in bold), in relation to sequences from GenBank. O1–O4 were obtained from samples from Lake Örträsket, S1 was obtained from a sample from Lake Stor-Sandsjön (Fig. 4). Representatives of the putative freshwater clusters defined by Zwart et al. (2002) are marked with an asterisk. Study sites and accession numbers of the sequences from GenBank are in brackets.

5B). However, the similarities between the samples in these two clusters were low, below 40%. Most similar to each other were the samples from the lake and downstream of the lake at the July sampling occasion. The gel patterns from these two samples had 86% of their bands in common.

The cell budget of Lake Stor-Sandsjön showed that inlet 1 contributed bacterial cells corresponding to 0.003–0.02% of the cell production in the epilimnion. In total, the different inlets contributed cells corresponding to 0.03–0.4% of the production in the epilimnion. Thus, the input of cells in relation to the internal production of cells was much lower in Lake Stor-Sandsjön than in Lake Örträsket.

One band (S1, Fig. 4B) was excised for sequencing. The band appeared as a relatively strong band in all lake samples except in August, when it was considerably weaker (Fig. 4B). S1 was also detected in samples downstream of the lake, except for in August. It was not detected in the inlet at any of the sampling occasions (Fig. 4B). The band was formed slightly above band O1 from Lake Örträsket in the DGGE gels (result not shown). The sequence analysis showed that the distance between sequences O1 and S1 was only 0.005. Thus, these two bands represent closely related taxa within a cluster of *Cytophaga-Flavobacteria*, most closely related to sequences from humic Lake Fuchskuhle and River Taff epilithon (Fig. 6).

Correlations between gel patterns and environmental parameters—The Spearman Rank correlation analysis showed that dissimilarities in gel patterns between lakes and inlets were best correlated to the amount of imported bacterial cells (cells d^{-1}) from the inlets (Table 2). The cell import was negatively correlated to the gel pattern dissimilarities, i.e., when a large amount of cells were imported, there were small dissimilarities in the bacterial assemblages between

Table 2. Results from Spearman rank correlation (SRC) and Spearman rank partial correlation (SRPC) analyses. The dissimilarities in gel patterns between the inlets and the epilimnion of the lakes, at the different sampling occasions, were analyzed as a function of the absolute values of the difference (Δ) in different parameters between the same samples, as well as to bacterial cell import from the particular inlets. n.s. = not significant, i.e., $p > 0.05$.

Parameter	SRC		SRPC
	p level	ρ	ρ
Import of bacterial cells (cells d^{-1})	<0.000001	−0.957	−0.896
|Δ numbers of potential bacterial grazers|	0.0074	0.727	0.518
|Δ water temperature|	0.0062	0.713	0.081
|Δ TOC|	0.045	0.564	−0.070
|Δ total phosphorus|	0.023	0.623	−0.031
|Δ water color|	0.017	0.646	0.119
|Δ pH|	n.s.	0.597	0.317
|Δ total nitrogen|	n.s.	0.374	−0.069

lake and inlet. The Spearman ρ of this correlation was 0.957 (Table 2).

Another significant (positive) correlation was to potential bacterial grazers (Table 2). This result shows that when there were large dissimilarities in the amount of grazers in inlet and lake, there were also large dissimilarities in the compositions of the bacterial assemblages in the two habitats. However, the ρ value of this correlation (0.727) was lower than the one for bacterial cell import, i.e., the grazer data covaried less with the gel data. Significant positive correlations to temperature and several water chemistry parameters were also found (Table 2).

Many of the parameters analyzed were correlated significantly to each other (results not shown). To avoid certain parameters appearing significantly correlated to our gel data just because they were autocorrelated to something that was truly correlated to our gel data, a Spearman rank partial correlation analysis was performed (Conover 1971). In this analysis the indirect influence on a particular variable from other variables is eliminated. In this second analysis the Spearman ρ was in most cases much lower compared to in the first analysis (Table 2). Bacterial cell import was still best correlated to the dissimilarities in gel patterns, and had a ρ value close to 0.9. Also the amount of bacterial grazers was still relatively well correlated to the gel patterns (ρ = 0.518).

Discussion

In this study we wanted to evaluate the influence of inlet bacteria on bacterioplankton assemblage composition in natural lakes. We hypothesized that this effect can be large in natural lakes and that its magnitude depends on water discharge.

Our results show that in the lake with short hydraulic retention time, Lake Örträsket, there was a larger similarity in bacterioplankton assemblage composition between the inlets and the epilimnion compared to in Lake Stor-Sandsjön, the lake with long retention time (Figs. 4 and 5). We could also see that the inlet to Lake Örträsket, which contributed with the largest amount of riverine cells, was more similar to the epilimnion in terms of assemblage composition of the bacteria compared to the smaller inlets (Fig. 3). The import of cells was in both lakes much lower than the internal cell production in the epilimnion. This is because less than 5% (Lake Örträsket) or 0.05% (Lake Stor-Sandsjön) of the epilimnion water was exchanged on a daily basis at the times of study. The similarities in gel patterns between Lake Örträsket and its largest inlet were, however, very high. Accordingly, the relationship between the amount of imported riverine cells and the similarity between river and lake assemblages does not seem to be a simple function of the extent of ''dilution'' of riverine cells in the assemblage of lake cells.

Our observations may have two explanations:

1. The composition of the bacterioplankton assemblage in the epilimnion of Lake Örträsket was to a large extent determined by the composition of the riverine assemblage, due to the import of bacteria from the inlets. The riverine bacteria subsequently reproduced in the epilimnion, which eventually led to a large similarity between inlet and lake. The lower import of cells to Lake Stor-Sandsjön gave a lower similarity between inlet and epilimnion in this lake.
2. The short hydraulic retention time in Lake Örträsket led to large similarities in chemical, physical, and biological character between the inlets and the epilimnion, causing the same taxa to be selected in the two habitats. The longer hydraulic retention time in Lake Stor-Sandsjön allowed larger differences in character between inlets and lake, and thus different taxa of bacterioplankton were selected in the two habitats.

The statistical evaluation showed significant correlations between the gel patterns and factors that are associated with both explanations. However, the strongest correlation found is a negative relationship between the amount of imported cells and the dissimilarity in gel patterns between inlet and lake (Table 2), i.e., when the cell import was high the dissimilarity between lake and inlet was low. This correlation was still very strong when autocorrelations were accounted for. Thus, the dissimilarities in gel patterns between inlets and lakes were statistically best described as a function of cell import. Unless cell import was strongly autocorrelated to some factor that we did not measure, this suggests that import of bacterial cells from the drainage area influenced bacterioplankton assemblage composition in the lakes (explanation 1).

The factors associated with explanation 2, i.e., the differences in chemical, biological, and physical factors between inlet and epilimnion, were less strongly correlated to the gel patterns, although largely significant. The best correlation was between the numbers of potential bacterioplankton grazers and the gel patterns (Table 2). This factor was still relatively well correlated to the gel patterns when autocorrelations were accounted for (Table 2). Statistical evaluations of DGGE patterns have shown correlations to the grazer abundance in several lake studies (Lindström 2000, 2001; Muylaert et al. 2002). Also, in experimental studies, it has been shown that grazers are capable of shaping bacterioplankton assemblage composition (e.g., Simek et al. 1997; Hahn and Höfle 1998; Gasol et al. 2002). Therefore, differences in grazing pressure may also have had some influence on the differences in bacterioplankton communities between lakes and inlets.

Consequently, the bacterioplankton assemblage composition in the lakes seems to be the result of interplay between import of cells from the inlet, and growth and elimination of bacterial cells in the lake. Since the import of bacterial cells depends on water discharge, the magnitude of the influence of the inlet may be different in lakes of different hydraulic retention time. The influence of the inlet should also depend on how well riverine bacteria establish themselves in the lake. This relationship can be described in a conceptual model (Fig. 7). If survival of riverine bacterial cells in the lake is low or even none, due to, for instance, a higher grazing pressure or an otherwise negative selection against riverine taxa in the lake, the production in the lake is due to bacteria different from those in the inlet. In this

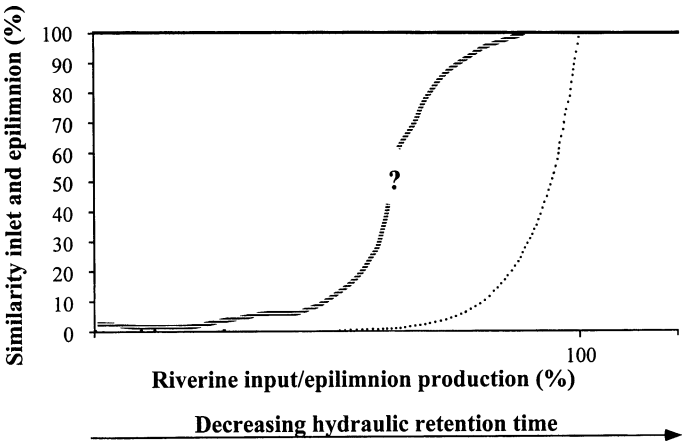

Fig. 7. Conceptual model of the relation between import of bacterial cells and the similarity in bacterial assemblage composition between inlet and epilimnion. Solid straight line: all production of cells in the epilimnion is due to cells identical to the riverine assemblage growing and being eliminated at similar rates. Dotted line: the production in the epilimnion is due to bacteria different from the riverine assemblage. Dashed curved line: hypothetical situation when a significant amount of the production is due to bacteria identical to the riverine assemblage.

case we expect that the similarity between inlet and lake will only be very high when the import of bacterial cells is close to 100% (or higher) of the bacterioplankton production (i.e., dotted line; Fig. 7). On the other hand, if all production of cells in the lake is due to cells identical to the riverine assemblage growing and being eliminated at similar rates, the similarity between lake and river would always be very high no matter how small the import is (i.e., solid straight line; Fig. 7). None of the situations appear to be true for our lakes, where we saw different degrees of similarity depending on import, and where we sometimes saw similarities as high as 90% even though the import was only around 10% of the production. Therefore, we propose that a significant amount (but not all) of the imported riverine bacterial cells survived in the lakes and contributed to the production in the epilimnion. Such a situation leads to large similarities between the inlet and the lake if the hydraulic retention time is short (i.e., hypothetically described as the dashed curved line; Fig. 7). However, in order to more exactly model the relationship between import, growth, and other selective factors in determining the bacterioplankton assemblage composition in lakes, more field and experimental data are needed from a larger set of lakes differing in their hydraulic retention time.

It can be argued that the presented possible relationship between the importance of imported cells and hydrological retention time of lakes cannot be justified based on our data since we only sampled inlets, when in fact the largest source of water flowing into Lake Stor-Sandsjön was groundwater. The number of suspended bacteria in pristine groundwater is variable but generally lower than in surface waters (e.g., Griebler 2001; Griebler et al. 2002). If we assume that the abundance of free bacteria in groundwater flowing into Lake Stor-Sandsjön is similar to that of the inlets of the lake, i.e., 10^9 cells L^{-1} (Table 1), we could calculate a hypothetical inflow of bacterial cells via groundwater. In Lake Stor-Sandsjön, that import would be 1.7–2.7 $\times$ 10^{16} cells per day depending on sampling occasion, which is comparable to the import from River Kvarnbäcken to Lake Örträsket (Table 1). This calculated bacterial import is, however, most probably an overestimate. If we instead assume that the bacterial abundance in the groundwater was only 10^7 cells L^{-1}, which has been found at other pristine groundwater sites (Griebler et al. 2002) and which does not appear to be extremely low (Griebler 2001), the hypothetical import would be 1.7–2.7 $\times$ 10^{14} cells per day. These figures are comparable to the import from the two different sampled inlets to Lake Stor-Sandsjön (Table 1).

In Lake Örträsket, the share of groundwater to the total inflow of water was much lower than in Lake Stor-Sandsjön. If we make the same calculations as above for Lake Örträsket, we find that the groundwater flow could have contributed with 3.4–7.7 $\times$ 10^{16} or 3.4–7.7 $\times$ 10^{14} cells per day, depending on which hypothetical bacterial abundances are used. These figures are similar but slightly higher than the ones of Lake Stor-Sandsjön, which is a result of the larger drainage area for groundwater to Lake Örträsket compared to Lake Stor-Sandsjön (cf. Methods). Altogether, these calculations show that the possible import of groundwater bacteria to the lakes is most uncertain but that it is most likely low. If we consider the highly significant statistical correlation that we found between the amount of imported cells from inlets and the similarity to lake bacterial assemblage composition (cf. Table 2), these data suggest that the influence of groundwater bacteria on bacterial assemblage composition was small in both lakes. Thus, due to low import of cells from both inlets and groundwater, imported cells appear to have been of minor importance shaping the assemblage composition of bacterioplankton in the lake with long hydraulic retention time, in comparison to the lake with short hydraulic retention time where the import of bacterial cells was high.

The hydraulic retention time of lakes is inversely related to water color (Meili 1992; Curtis and Schindler 1997; Dillon and Molot 1997). The water color of Lake Örträsket (Table 1) is only slightly higher than the average of 4114 Swedish lakes in the Nordic Lake Survey conducted in 1995, which had a mean value of light absorbance at 420 nm at 0.133 (http://info1.ma.slu.se/db.html). Thus, it must be concluded that of the two lakes investigated here, Lake Örträsket is the one that, regarding hydraulic retention time, is closest to the majority of the Swedish lakes and therefore most similar to the majority of the lakes in the boreal zone. It is therefore possible that the assemblage composition of bacterioplankton in a large number of boreal lakes is greatly influenced by import of bacteria from the drainage area.

What effect riverine bacteria have on lake ecosystem function must then depend on the taxonomic affiliation of the riverine bacteria and the ecological and metabolic character of those taxa. The four sequences that were extracted from the bands that appeared most representative for Lake Örträsket and its inlets, as well as for Lake Stor-Sandsjön (Fig. 4), were closely related to *Cytophaga-Flavobacteria* (O1, O2, and S1) and *β-proteobacteria* (O3) previously found in various types of freshwaters (Fig. 6). Many *Cytophaga-Flavobacteria* are known to be especially proficient in degrad-

ing biopolymers such as cellulose, chitin, and pectin (Kirchman 2002). A massive input of *Cytophaga-Flavobacteria* into a lake may therefore have consequences for the ability of the bacterioplankton assemblage to degrade high molecular weight organic matter, which in turn implies ecosystem effects. However, a limited number of bands were investigated here. In addition, PCR biases (e.g., Wintzingerode et al. 1997) make it uncertain whether the sequenced populations really were quantitatively important in the lake. Thus, based on this data set it is difficult to draw any certain conclusions about ecosystem effects of the introduction of bacteria from the drainage area.

The sequence labeled O4 did not show a close phylogenetic relationship to other freshwater sequences. This band was selected for sequencing since it did not appear to be introduced from the rivers, thus being a strict lake bacterium in Lake Örträsket (Fig. 4). However, band O4 was always relatively weak, indicating that this bacterium was of minor importance in the lake (Fig. 4). It is possible that this is a lake bacterium that is present in relatively low abundances and, therefore, has escaped detection in other lakes thus far.

Import of inorganic and organic dissolved constituents from the watershed is well known to affect the production of bacterial communities (e.g., Hessen and Tranvik 1998; Jansson et al. 2000). That such factors also may influence the composition of bacterial assemblages is likely (e.g., Methé and Zehr 1999; Simek et al. 2001*a;* Lindström and Leskinen 2002; Muylaert et al. 2002; Crump et al. 2003; Stepanauskas et al. 2003). Here we show that the import of the bacteria themselves also may contribute to the structure of lake bacterioplankton assemblages. We therefore expect that in order to understand and predict the distribution of bacterioplankton taxa among different freshwater habitats, factors such as catchment characteristics and hydrology must be considered.

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Received: 27 November 2002
Accepted: 20 July 2003
Amended: 25 August 2003