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Limnol. Oceanogr., 51(1), 2006, 339–342
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External control of bacterial community structure in lakes

Abstract—We investigated the importance of a regional factor for bacterial communities in lakes. External factors dominated the control of community structures in lakes with retention times up to 200 d, most likely as a result of bacterial import. Because these lakes are numerous in the boreal zone, regional processes can be of great importance for bacterial communities in general. Consequently, we propose that lakes function more like flow through systems, as opposed to the classical “lake as microcosm” concept.

During the last decade, our knowledge about the diversity of bacteria in nature has increased enormously; however, little is understood about which factors are shaping bacterial communities (DeLong and Pace 2001; Zwart et al. 2002). Central to understanding patterns in community structure is knowledge about the relative importance of local versus regional processes (Chase 2003; Cottenie and De Meester 2004). It can be assumed that the degree of isolation, and thereby the rate of exchange of cells and genes between communities, should have consequences for which forces shape local microbial communities (Curtis and Sloan 2004; Papke and Ward 2004). In nature, a gradient in isolation of communities should exist from endosymbionts to those exhibiting a cosmopolitan distribution (Papke and Ward 2004).

Early on, lakes and their ecosystems were assigned as isolated units in the “lake as microcosm” concept (Forbes 1887). This view of the lake was later revised, for instance, because of the discovery of the importance of allochthonous carbon for lake ecosystem function (Hessen and Tranvik 1998; Pace et al. 2004). Thus, lakes today are more often ecologically regarded as a part of a larger unit, i.e., the drainage basin (Soranno et al. 1999). Still, efforts in lake microbial ecology and diversity have largely focused on within-lake selective forces, rather than external influences on community structure and diversity, although it has been shown that lake (Crump et al. 2003; Masin et al. 2003; Lindström and Bergström 2004) and estuarine (Crump et al. 2004) community compositions can be largely influenced by inflowing bacteria. It can be assumed that the degree of isolation a lake bacterioplankton community experiences, and thereby the degree of influence by inflowing bacteria on local community structure, depends on the hydrological retention time of the lakes. Therefore, we used a range of lakes of different hydrological retention times to determine the magnitude of external influence on bacterial community structure in lakes. Our hypothesis was that lakes with short hydrological retention time should have bacterial communities that are more similar to those of the inflowing water than lakes with longer hydrological retention times because of the larger amount of imported bacteria to the former type of lake. Twelve relatively unproductive Swedish lakes with different hydrological retention

times were studied (Table 1). We used denaturing gel electrophoresis (DGGE) of polymerase chain reaction (PCR)–amplified 16S ribosomal deoxyribonucleic acid (rDNA) to characterize bacterial communities in inlets and lakes.

Materials and methods—Study sites and sampling: Twelve lakes were included in the study (Table 1). The lakes differ in theoretical hydrological retention time from 1 d to 10 yr. All the lakes have one inlet or one major inlet.

Four lakes are situated in Lappland, northern Sweden (Lindström and Bergström 2004, 2005), and were sampled in summer 2001 (6–7 June) and 2002 (1–2 June). The eight other lakes are located in Uppland, central Sweden, and were sampled 20–28 May 2003. All lakes were sampled once, in spring, approximately 25–50 d after the start of the latest high-flow event, when flow had subsided.

Temperature, pH, water color, total organic carbon, total nitrogen, total phosphorus, bacterial community composition, bacterial production, bacterial numbers, and abundance of potential bacterivores were determined in lakes and inlets (Lindström and Bergström 2004). In stratified lakes, composite samples of the epilimnion were taken, whereas in unstratified lakes, composite samples of the whole water column were taken.

Assemblage composition of bacteria: The assemblage composition of bacteria in lakes and streams was determined by DGGE of PCR-amplified 16S rDNA (Lindström and Bergström 2004). Eubacterial primers GM5F (with GC-clamp) and DS907-reverse (Teske et al. 1996) were used. The percent similarity between inlet and lakes in the presence and absence of DGGE bands was calculated (Lindström and Bergström 2004) as Sørensen’s index. DGGE data for Lake Örträsketsjön and Lake Stor-Sandsjön were obtained from an earlier study (Lindström and Bergström 2004).

Bacterial cell budgets: Bacterial numbers were determined in lakes and streams by use of epifluorescence microscopy and acridine orange staining (Hobbie et al. 1977).

Bacterial cell division rate in the lake waters was measured by use of the thymidine method (Bell 1993). Tritiated thymidine (30 or 50 nmol L⁻¹, depending on humic content of the lake water) was added to triplicates of 10-ml lake water at the time of arrival at the laboratory. The samples were incubated in darkness at in situ temperature for 60 min. The growth rate (cells L⁻¹ h⁻¹) was calculated by assuming a division rate of 2×10^{18} cells mol⁻¹ of incorporated thymidine (Bell 1990).

Bacterioplankton cell budgets were constructed with the use of data on water flow, epilimnion volumes, bacterioplankton abundance, and growth rate (Lindström and Bergström

Table 1. Description of the 12 lakes included in the study.

Lake	Coordinates	Theoretical hydrological retention time (d)	Lake area (km ²)	DGGE similarity inlet and epilimnion (%)	Cell import rate/ epilimnion cell division rate (%)	Total phosphorus ($\mu\text{g P L}^{-1}$)	Total organic carbon (mg C L^{-1})
Revelstasjön*	59°49'N, 16°57'E	1	0.10	89	81	98	16
Åkerbysjön*	60°22'N, 17°51'E	14	1.11	97	15	13	27
N Björntjärn	64°11'N, 18°47'E	67	0.03	90	12	31	17
Örträsketsjön	64°10'N, 19°00'E	100	7.30	92	9	18	10
Lumpen*	59°58'N, 17°17'E	164	0.25	60	0.4	26	30
N Gäddträsk	64°24'N, 18°49'E	200	1.14	83	0.5	12	12
Bysjön	59°59'N, 18°25'E	276	0.03	36	0.9	5	23
Holmsjön	59°59'N, 18°25'E	349	0.20	43	0.2	24	23
Bredsjön*	59°54'N, 17°10'E	438	1.38	45	0.04	24	19
Hålsjön	59°49'N, 17°15'E	730	0.20	35	0.2	21	13
Skärsjön	59°55'N, 18°01'E	1,460	0.31	30	0.09	9	18
Stor-Sandsjön	64°10'N, 19°00'E	3,650	9.20	17	0.02	10	5

* In unstratified lakes, the whole water column was regarded as the epilimnion.

2004). With this data, the bacterial import could be calculated in relation to the bacterial cell production (%) and the bacterial cell numbers (% d⁻¹) in the epilimnion of the lakes.

Hydrology: To determine the hydrology of the eight lakes in Uppland, water flow data from hydrological stations within the same drainage area as the sampled lakes was obtained from the Swedish Meteorological and Hydrological Institute (SMHI). The specific runoff for the drainage area of the lakes was calculated. On the basis of the size of the drainage area of the lakes and the epilimnion volume (Brunberg and Blomqvist 1998), the water flow and the hydrological retention time of the lake water at the time of sampling were calculated.

Hydrology data for the lakes in Lappland were obtained in a similar manner (Lindström and Bergström 2004, 2005).

Statistics: Three lake categories were defined with the use of similarities in DGGE patterns between inlet and lake. Discriminant analysis was used to determine whether the categories could statistically best be described by bacterial cell import or by similarities in the chemical, physical, and bio-

logical environments in inlet and lake. Discriminant analysis tells whether a particular set of variables is useful in discriminating previously delineated groups (ter Braak and Verdonschot 1995), which in our case are the three lake categories. Because the similarities between inlets and lakes in DGGE patterns showed a nonlinear relationship to several variables, such as cell import rates and water retention time (Fig. 1; Table 1), we considered statistical methods such as correlation analysis less suitable for our data set. The variables included were bacterial import (cells d⁻¹); bacterial import as a fraction of cell production (%) and cell numbers (% d⁻¹) in the epilimnion; and the difference in absolute values between inlet and lake in water chemistry, temperature, and number of bacterial grazers. pH values were converted to [H⁺] before any calculations were made. All data were transformed to z-scores before analysis. Discriminant analysis was run with CANOCO 4.53 software (Biometris-Plant Research International). The environmental factors best describing the lake categories were identified by forward selection. Explanatory variables were added until addition of further variables failed to contribute significant ($p < 0.05$) improvement to the model's explanatory power. This was assessed in permutation tests with 499 unrestricted Monte Carlo permutations.

Modeling of the theoretical hydrological retention time for Swedish lakes: Swedish lakes ($n = 1,640$) were classified into region-specific morphometry classes (Algesten et al. 2004). By use of data on lake surface areas and region-specific runoff obtained from SMHI, the lake volumes and their theoretical hydrological retention times were estimated (Algesten et al. 2004). The lakes included are situated between latitudes 57°5'N and 69°0'N and longitudes 12°E and 24°E (i.e., covering approximately 70% of the land area of Sweden). The lakes were divided into three groups depending on surface area, and for each group, the share of lakes having hydrological retentions times of 1–100, 101–200, and >200 d was calculated. With lake surface area data from SMHI for all Swedish lakes ($n = 92,409$), the number of lakes in each retention time category could be estimated.

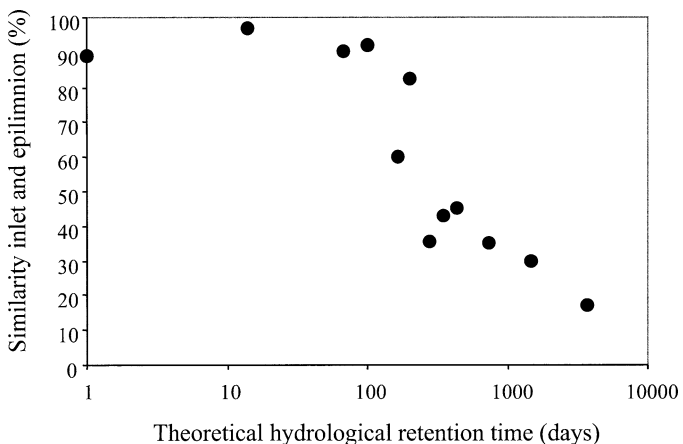


Fig. 1. Relationship between the similarities in DGGE patterns between inlet and epilimnion and the theoretical hydrological retention time of the studied lakes.

Results and discussion—The banding patterns from DGGE and similar methods are commonly used as a proxy for the composition of the numerically dominating populations in communities (Torsvik et al. 1998; Forney et al. 2004). Therefore, it can be assumed that the similarities in DGGE banding patterns between inlet and lakes reflect the influence of the inlets on bacterial communities in the lakes, i.e., the degree of external control of the community compositions. In our study, the degree of similarity in community composition between inlet and lakes showed a nonlinear relationship to the theoretical hydrological retention time of the lakes (Fig. 1). On the basis of the assumptions and our results, the lakes were therefore divided into three categories: (1) external control lakes, which had ~90% similarity in DGGE banding pattern between lake and inlet (i.e., the influence of inlet on the composition of the bacterial communities was very high); (2) intermediate control lakes, which had similarities between inlet and lake lower than for category 1, but still >50% (i.e., the influence of inlet was lower but still the dominating force in shaping the bacterioplankton communities); and (3) low external control lakes, in which the similarities between inlet and lakes were <50% (i.e., the influence of inlet was low). In contrast to the other categories, the communities in category 3 lakes should, to a larger extent, be shaped by internal processes such as food web interactions and competition.

The theoretical hydrological retention times were 1–100 d for external control lakes, >100 and ≤200 d for intermediate lakes, and >200 d for the low external control lakes (Fig. 1). Thus, external control of bacterioplankton community composition appears dominating in lakes with theoretical hydrological retention times up to 200 d.

But how important is external control of lakes in general? According to our estimates, lakes in the boreal zone of Sweden (92,409 lakes) were distributed among the three categories so that 78% have theoretical hydrological retention times of 1–100 d, 10% of 101–200 d, and 12% of >200 d. Thus, if the found relationship between external control and hydrological retention time of the lakes (Fig. 1) is generally valid, the vast majority of the Swedish lakes are external control lakes in which the bacterial composition can be more or less entirely determined by external forces. In addition, short hydrological retention times of lakes are common elsewhere in the boreal zone. For instance in Québec (Canada), the median theoretical hydrological retention time is around 290 days (Y. T. Prairie pers. comm.). Thus, external control of bacterioplankton community compositions should be of considerable importance also in lakes outside Sweden. To what extent our results are applicable to lakes with higher productivity or to lakes outside the boreal zone is an open question. However, a dependence of bacterioplankton community composition on the hydrological retention time was recently demonstrated for estuaries (Crump et al. 2004). These results indicate that bacterial external control can be of considerable importance in widely different aquatic environments.

The nature of the external control was further explored statistically. In theory, two different mechanisms are possible. The first is that cells are transported from the drainage area to the lake and thereby influence the composition of the community by displacement of other cells. The second pos-

sible explanation is that the water flowing from the drainage area influences the character of the lake water, e.g., in the amount and character of the dissolved organic carbon (Sobek et al. 2003), which in turn, being an important substrate for the bacteria, selects a certain composition of the bacterial community. Discriminant analysis was used as a means to investigate which environmental factors statistically best explain the lake categories we defined. We found that among the chemical, physical, and biological variables tested, bacterial cell import rate as a fraction of the cell division rate in the lake (%) was the only factor significantly ($p < 0.05$, 499 unrestricted Monte Carlo permutations) contributing to the model (eigenvalue of the first canonical axis 0.876). These results, therefore, strongly indicate that the external control observed was, to a large extent, caused by bacterial import than by influence of habitat character. However, these results do not rule out the role of selection because growth of bacteria colonizing via the incoming water could be increasingly successful if the habitat in the lake is similar to that in the inflow, which would further augment the external control. That the cells from the inlet establish themselves in the lake and contribute to lake cell production was earlier concluded in a study of one of the external control lakes (Lindström and Bergström 2004). Also, the statistical model, with import rate as the single explanatory variable, statistically explained “only” 43.8% of the variation in DGGE patterns among categories, indicating that more environmental variables than cell import could have contributed to the structures of the bacterial communities in the lakes.

Explaining the exact shape of the curve in Fig. 1 (i.e., the two “break-off points” identified at water retention times of 100 and 200 d) would need additional data. Contributing factors could be pulses of water as well as base flow. In this context, it is interesting to note that in the four external control lakes, the whole epilimnion volume was replaced during the spring flood, whereas in all other lakes only fractions of the epilimnion were replaced (unpubl. data).

Our results demonstrate that the degree of isolation a lake experiences from the surrounding environment, expressed as hydrological retention time, determines the importance of external factors in shaping lake bacterioplankton communities, as similarly proposed previously for microbial communities in general (Curtis and Sloan 2004; Papke and Ward 2004). The most likely mechanism behind the external control is cell transport rate, implying that the local community structure to a large extent is controlled by regional processes, i.e., dispersal rates (Chase 2003). On the basis of these results, we propose that the external control leads to a large number of lakes that function as flow through systems rather than as microcosms (Forbes 1887) with respect to bacterial communities, i.e., that cells and growth media are continuously provided from drainage areas to lakes and thereby influence the composition of the bacterial communities.

Eva S. Lindström¹

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

and

Département des sciences biologiques
Université du Québec à Montréal (UQAM)
Montréal, Québec, Canada

Markus Forslund

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

*Grete Algesten
Ann-Kristin Bergström*

Department of Ecology and Environmental Science
Umeå University
SE-901 87 Umeå, Sweden

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Received: 31 March 2005

Accepted: 12 July 2005

Amended: 8 August 2005

¹ To whom correspondence should be addressed. Present address: Limnology, Department of Ecology and Evolution, Evolutionary Biology Centre, Uppsala University, Norbyv. 20, SE-752 36 Uppsala, Sweden (eva.lindstrom@ebc.uu.se).

Acknowledgments

This paper is dedicated to the memory of Peter Blomqvist (deceased 2004). We thank Jan Johansson and Katarina Vrede for advice concerning water chemistry analyses and bacterioplankton production measurements, respectively. Emmy Blomster did phytoplankton counts. The Swedish meteorological and hydrological institute (SMHI) and the department of Hydrology, Uppsala University, provided us with water flow data. We also thank Yves Prairie and Beatrix Beisner for discussions, and Mats Jansson, Lars Tranvik, Paul del Giorgio, and Silke Langenheder for comments on previous versions of the manuscript.

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 E.S.L. and A.-K.B. Financial support was also given by the Olsson-Borgh and Helge Ax:son Johnson foundations to E.S.L.