

Microbial Community Analysis in Oligotrophic, Glacial High Mountain Lakes

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Abstract

In this three years study we report about the temporal responses of remote mountain lake ecosystems to meteorological changes on seasonal and inter-annual time-scales and about the peculiar community structure of the phyto- and bacterioplankton in glacial, oligotrophic mountain lakes.

The study site at the Jöri lakes is located in a remote mountain area of the Eastern Swiss Alps, in the canton of Grisons. The basin comprises 21 lakes and ponds and extends from 2489 m to 3060 m a.s.l. Average water temperature ranges from 4°C to 8°C, and conductivity is very low (15 $\mu\text{S cm}^{-1}$). Total inorganic nitrogen and phosphorus amount to 200 ppb and 10 ppb, respectively. The lakes show great variability in suspended particle concentrations since some get their water directly from the nearby Jöri glacier while others receive it only from snowmelt and precipitation runoff.

Comparison of clear water and turbid lakes showed differences in plankton community structure. For deeper understanding of the interactions between the particles and nutrients, limnocorrals of 13 m³ volume were installed and manipulated by addition of phosphorus, nitrogen and particles. We suggest that particles interact with nitrogen compounds and that such interactions affect the microbial community. In the water column of deep lakes, turbidity always paralleled phosphorus and nitrogen concentrations. They increased towards the bottom.

The period of icebreak has turned out to be a major promoter of community transition and the date of the icebreak influences decisively the seasonal succession of the plankton community. The maximal chlorophyll *a* concentration was often found below the thermocline although only very low amounts of photosynthetic active radiation penetrated to these depths. We suggest that algae live in those deep layers since they are less affected by extreme weather events and profit from increasing nutrient concentrations.

Mixotrophic flagellates were the major grazers on bacteria and clearly outnumbered purely heterotrophic flagellates. Their advantage of being capable of both heterotrophic and autotrophic modes of nutrition seems to be decisive for surviving in these oligotrophic ecosystems. We found a carbon ratio of bacteria, phytoplankton, and autotrophic picoplankton (APP) of 1.5 : 1.1 : 1 which shows a rather high abundance of bacteria and APP compared to larger phytoplankton.

The composition and seasonal variation of the bacterioplankton was studied using temporal temperature gradient gel electrophoresis (TTGE). The separated fragments were sequenced and compared with 16S rDNA sequences available in databases. Some sequences showed a high degree of similarity, 93.8 % to 99.7 %, to other bacteria originating from mountain lakes. Since these species were only found in mountain lakes so far, they seem to be specially adapted to live there. Many of them are not yet identified. Analysis with fluorescent *in situ* hybridization (FISH) indicated the predominance of beta *Proteobacteria*.

This project is part of the European project MOLAR (Measuring and modeling the dynamic response of remote mountain lake ecosystems to environmental change). Our data thus contribute to a comprehensive environmental database on remote mountain lake ecosystems in Europe.

Zusammenfassung

In der vorliegenden Studie wurde während drei Jahren das Plankton (Bakterio- und Phytoplankton) in oligotrophen, glazialen Bergseen untersucht. Dieses Ökosystem zeichnet sich durch lange Kälteperioden, stark variierende Wetterverhältnisse und Nährstoffarmut aus. Untersucht wurden zwei der 21 Jörisseen im Kanton Graubünden (CH). Diese liegen auf einer Höhe von rund 2550 m ü. M. Die mittlere Wassertemperatur lag zwischen 4°C und 8°C, die Leitfähigkeit betrug durchschnittlich 15 $\mu\text{S cm}^{-1}$. Der Gehalt an anorganischem Stickstoff lag im Bereich von 200 ppb, die Phosphorkonzentration bei 10 ppb. Einer der beiden Seen wird mit milchigem Gletscherschmelzwasser gespiesen und enthält deshalb hohe Konzentrationen an suspendierten Erosionspartikeln. Der andere See ist klar und enthält vor allem Regen- und Schneeschmelzwasser.

Um Unterschiede in der Planktonzusammensetzung im trüben und klaren See besser zu verstehen, wurden Limnocorrals mit einem Volumen von 13 m³ installiert und durch Zugabe von Phosphor, Stickstoff und Erosionspartikeln manipuliert. Es stellte sich heraus, dass Interaktionen zwischen Partikeln und Stickstoff stattfinden und dass diese die Zusammensetzung des Planktons beeinflussen. In trüben Seen verlief die Partikelkonzentration parallel zum Phosphor- und Stickstoffgehalt und nahm gegen den Grund hin zu.

Sowohl die Eisschmelze und ihr Zeitpunkt als auch die Wetterbedingungen sind von grosser Bedeutung für die Entwicklung und Sukzession des Planktons. So dominierten bei früher Eisschmelze andere Algenarten als bei später Eisschmelze. Das Chlorophyllmaximum wurde oft in äusserst lichtarmen Tiefen gefunden. Diese Wasserschichten sind weniger von den häufigen extremen Wetterschwankungen betroffen und bieten deshalb stabilere Lebensbedingungen. Ein weiterer Grund könnten die höheren Nährstoffkonzentrationen in diesen Tiefen sein.

Mixotrophe Flagellaten stellten sich als wichtigste Konsumenten der Bakterien heraus; rein heterotrophe Flagellaten wurden fast keine gefunden. Gemessen am Kohlenstoffgehalt spielte das auto- (APP) und heterotrophe (BAC) Picoplankton im Vergleich zu den grösseren Algen (PHY) eine wichtige Rolle ($C_{\text{BAC}} : C_{\text{PHY}} : C_{\text{APP}} = 1.5 : 1.1 : 1$). Sowohl die Mixotrophie als auch das hohe Oberfläche/Volumen-Verhältnis kleiner Organismen scheint in einem nährstoffarmen Lebensraum von Vorteil zu sein.

Die saisonale Dynamik des Bakterioplanktons wurde mit Hilfe der molekularbiologischen Methode ‚Temporal Gradient Gel Electrophoresis‘ (TTGE) untersucht. Fragmente wurden sequenziert und mit 16S rRNA Datenbanken verglichen. Viele zeigten eine hohe Übereinstimmung (bis 99.7%) mit anderen Bakterien aus Hochgebirgsseen. Bei diesen noch nicht beschriebenen Bakterienarten scheint es sich um speziell an solche Lebensbedingungen angepasste Organismen zu handeln. Mit Hilfe der ‚Fluorescent *In Situ* Hybridization‘ (FISH) wurde gezeigt, dass beta-Proteobakterien im Sommer am weitesten verbreitet waren.

Dieses Projekt war Bestandteil des Europäischen Gebirgssee-Forschungsprojektes MOLAR (Measuring and modeling the dynamic response of remote mountain lake ecosystems to environmental change). Die dabei gewonnen Daten dienen als Beitrag zu einer umfassenden Datenbank von Ökosystemen entlegener Bergseen Europas.

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Introduction

Alpine regions are for several reasons of particular interest: some regions are intensively utilized for touristic purposes, and therefore strongly influenced by human civilization. Others represent the least disturbed environments in Europe, and are therefore suitable ecosystems to study atmospheric displacement and deposition of pollutants. Because their catchments have little soil and vegetation, the chemicals are not taken up but are gathered in mountain waters. Moreover, some pollutants (e.g. mercury, volatile organics) accumulate preferentially in cold regions. Mountain lakes are furthermore confronted with acid deposition which affects the lakes harder than in midlands because of their low buffering capacity. In addition, the effects of global warming in Europe are predicted to be highest in arctic and alpine regions. As a result, mountain lakes are not only vulnerable to pollution, they are also excellent sensors of environmental changes. Most of the high mountain lakes have simpler ecological structures than lowland aquatic ecosystems and they are expected to respond more rapidly to environmental changes. They can thus serve as manageable model systems for investigations into the structure and the function of aquatic ecosystems in the alpine environment. Furthermore, their high quality sediment records can be used to infer the speed, direction and biological impact of changing air quality and climate. The European project MOLAR (Mountain Lake Research) has the aim to elucidate some of the questions arising from the peculiar characteristics of mountain ecosystems. The project has four overall objectives, each corresponding to a major strand in the proposal:

1. to measure and model the dynamics of remote mountain lake ecosystems to acid (sulfur and nitrogen) deposition;
2. to quantify and model pollutant (trace metals, trace organics) fluxes and pathways in remote mountain lakes and their uptake by fish;
3. to measure and model the temporal responses of remote mountain lake ecosystems to climate variability on seasonal, inter-annual and decadal time-scales;
4. to continue the development of a high quality environmental database on remote mountain lake ecosystems in Europe and to disseminate results widely to enhance public awareness, environmental education and environmental decision making.

The project is undertaken by a consortium of more than twenty EU- and NON-EU countries (Fig. 1) building a north-south and a east-west transect through Europe. The Jöri site has been accepted to be considered as a contribution to the Climate and Environment

Programme. We are thus integrated into a Europe-wide activity in Alpine Research and contribute to the global network for air quality and climate change research.

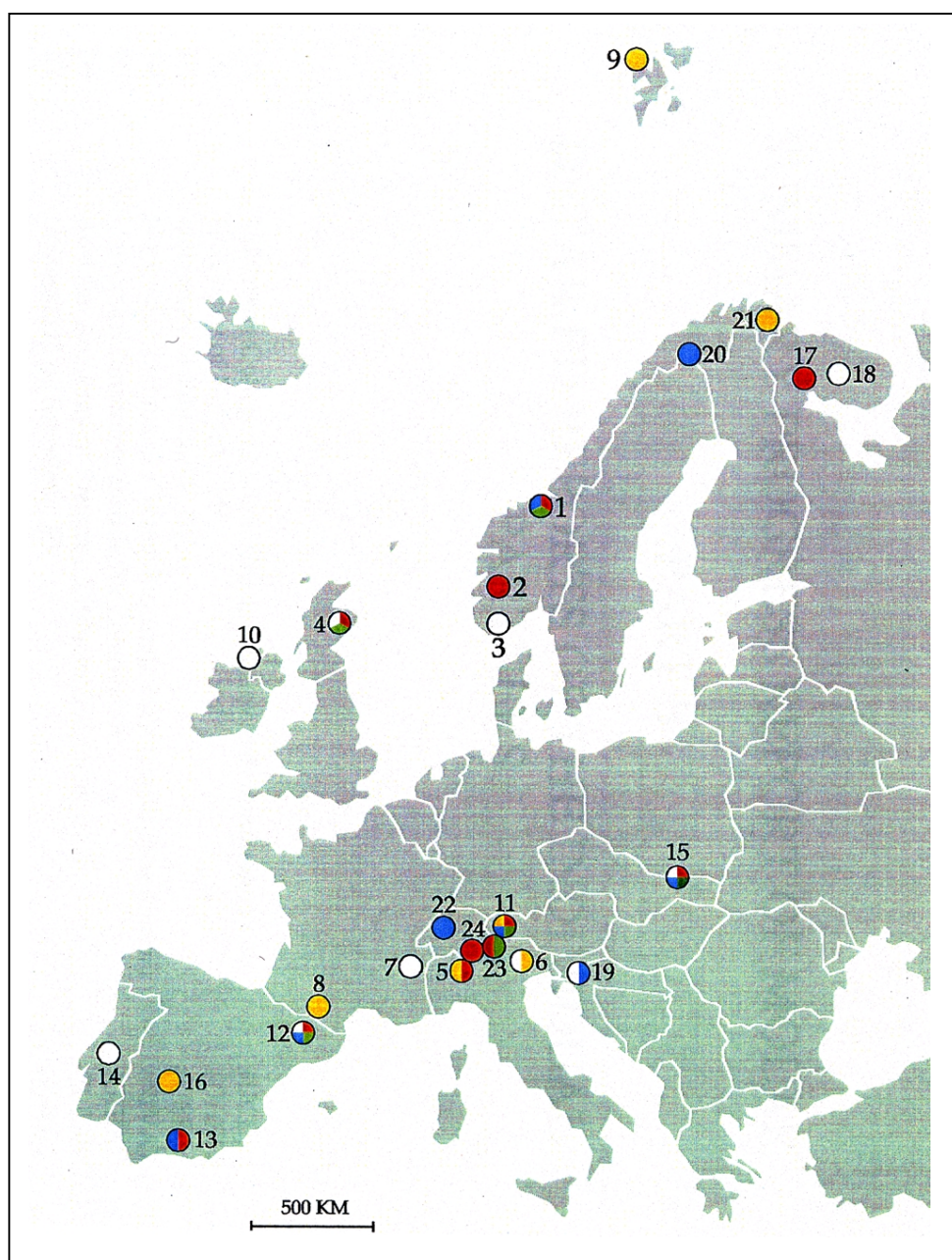


Fig. 1: Sites participating to the European Project MOLAR (Mountain Lake Research): O. Neadalsvatn (1), Stavsvatn (2), Lille Hovvatn (3), Lochnagar (4.1), Sandy Loch (4.2), Loch nan Eun (4.3), Lago Paione Superiore (5.1), Lago Paione Inferiore (5.2), Lago Lungo (6.1), Lago di Latte (6.2), Lac Rond (7.1), Lac Combeynod (7.2), Lac Blanc (7.3), Lac Noir (7.4), Etang d'Aubé (8), Arresjoen (9), Lough Maam (10), Schwarzsee ob Sölden (11.1), Gossenköllesee (11.2), Lago Aguilo (12.1), Lago Redo (12.2), La Caldera (13), Laguna Escura (14), Starolesnianske Pleso (15.1), Terianske Pleso (15.2), Dlugi Staw (15.3), Zieloni Staw (15.4), Laguna Cimera (16), Chuna (17), Chibini (18), Zgornje krisko jezero (19.1), Jezero Ledvicah (19.2), Limgamberggjern (20), Saanajärvi (21), Hagelsee (22), Jörisee (23), Laghetto Inferiore (24).

In addition to model development, much of the field and laboratory work proposed is innovative for studies of such remote sites, especially:

- the focus on the seasonal dynamics of the lake systems;
- the emphasis on nitrogen deposition and its biological impact;
- the study of microbial food webs in relation to acidity;
- the on-site collection and measurement of atmospheric pollutants;
- the use of radio-tracers to validate pollutant transport models;
- the study of trace metal (especially mercury) and trace organic uptake by fish;
- the on-site monitoring of climatic conditions and their relationship to water column behaviour;
- the development of a methodology to infer climate trends from the high resolution analysis of recent sediments.

These objectives were unachievable four years ago because of the almost complete lack of information on remote arctic and alpine lakes. It is now possible to carry out such work because of the knowledge gained about individual sites from the AL:PE project with respect particularly to accessibility, morphometry, chemistry, biology, sediment accumulation rate and pollution status.

The microbiota of alpine ecosystems are constantly challenged by extreme and extremely variable living conditions (Thomas *et al.* 1991, Pernthaler *et al.* 1998). Some of them have to survive for more than half a year in the dark under a thick ice and snow cover (Catalan 1989, Felip *et al.* 1995, Alfreider *et al.* 1996) and afterwards have to grow and propagate within a few weeks. Others are exposed to intense radiation just as the snow has melted and yet others can only scavenge the necessary nutrients for growth from an extremely dilute aqueous medium. During the growing season these ecosystems are heavily influenced by rapid hydrological changes after sudden extreme weather events (Hinder *et al.* 1999).

All organisms that could establish themselves are well adapted to these particular environmental conditions and thus are specialists. It is an open question whether alpine ecosystems are robust due to being constantly challenged by highly variable and extreme conditions or whether they are vulnerable to small environmental changes which they never experienced before. All these environmental factors influence the biodiversity of mountain lake ecosystems.

The Jöri lakes represent a suitable site to investigate the influence of environmental conditions on the diversity of algae and bacteria: There are many lakes with similar bedrocks

in the catchment (Fig. 2), they are exposed to more or less the same weather conditions (except for wind), but they are fed by different water sources and some contain suspended particles which could have various consequences.

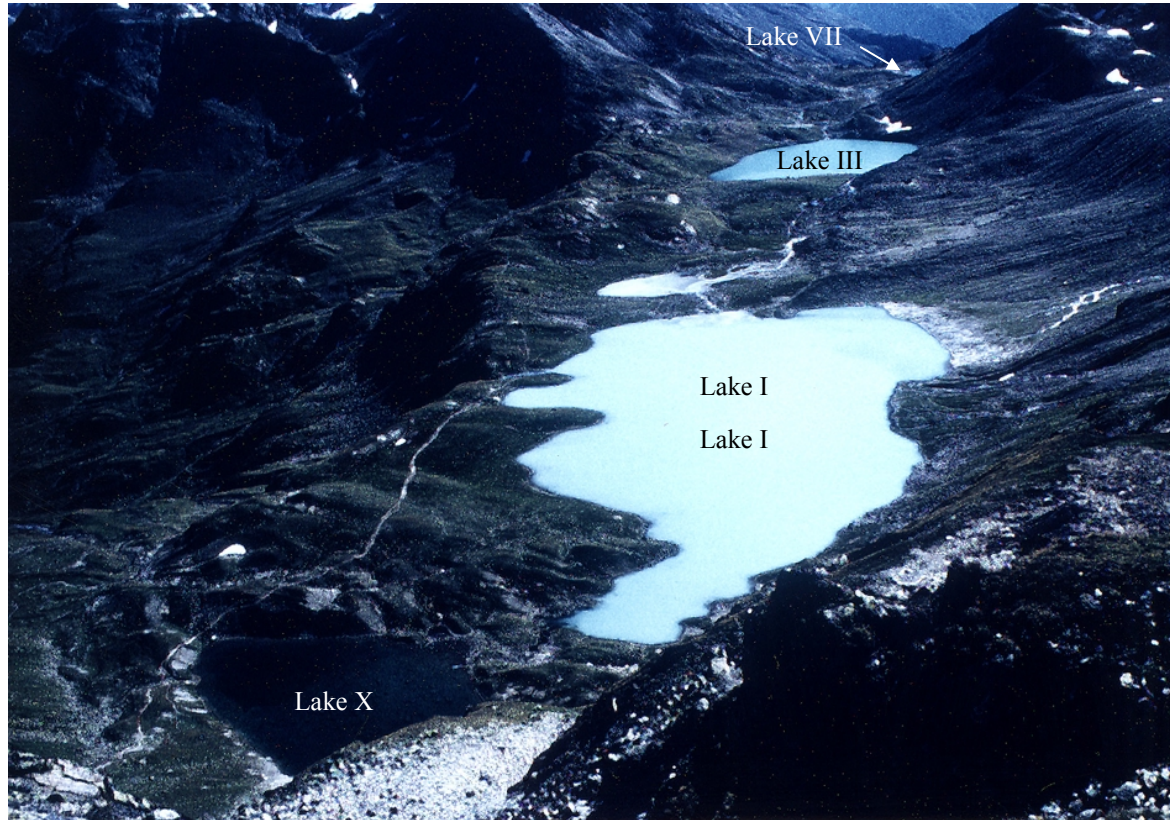


Fig. 2: View from the Jöri-Flüela-Furka Lücke to the Jöri lakes (Eastern Switzerland) towards the south-east. The picture was taken on August 12 1997. The length of Jöri lake I is about 390 m.

Erosion particles may influence the living conditions of microorganisms in many ways: the particles bind or adsorb ions and nutrients, they cause turbidity of the water body thereby influencing the light field of the water column, and some have a certain buffer capacity. They probably very special living conditions for microorganisms which influence the composition of the microbial community, the biodiversity and the seasonal fluctuations of the microbial assemblage. Whether particles act as source or as sink of nutrients, depends on the kind of the nutrient, the type of mineral, their aging, chemical history, loading rates and their duration in suspension (Frölich 1988, Sonzogni *et al.* 1982). The dominant components of the erosion particles in the Jöri lakes determined by XRD-analysis are muscovite, chlorite, plagioclase as well as quartz. Microorganisms mainly interact with small particles. Not only because they have a high surface/volume ratio but also because of their low sedimentation rate and therefore high residence time in the water body. In deeper lakes in the Jöri valley the particles

stay in the water column for six days on average. The high residence times allow the microorganisms to interact with the particles. The cation exchange capacity (CEC) is about 13 meq/100g dry weight which is comparable with the clay mineral caolinite (2-10 meq/100g, Stotzky and Burns 1982) and adsorption experiments with erosion particles from the Jöri lakes' sediment indicate that they are not saturated with the maximal adsorbable amount of cations. The bioavailable, adsorbed ammonium amounts to about 50 $\mu\text{g l}^{-1}$ N per g dry weight. Until today, nothing is known about the role of erosion particles in oligotrophic mountain lakes regarding their ability to concentrate nutrients on their surface.

This study is based on the following questions:

- how do the extreme and extremely variable meteorological conditions affect the development of the phyto- and bacterioplankton?
- how variable/stable are the microbial communities on time scales of days, weeks and seasons and how rapidly do they recover after natural events (e.g. storms) have broken them up?
- what is the influence of suspended erosion particles on the water chemistry of oligotrophic mountain lakes and on the composition of microbial communities? Do they act as source or sink of nutrients?
- what is community structure in an aquatic ecosystem where nutrient concentrations are low and how is the food web organized?

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Seasonal Dynamics and Phytoplankton Diversity in High Mountain Lakes (Jöri Lakes, Swiss Alps)

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ABSTRACT

The development of the phytoplankton in two years with very distinct weather situations was compared. In 1996, the ice on Jöri lake III melted in mid June, summer stratification persisted during two months, and ice began to build up again in mid October. In 1997, the ice melted only at the end of July, which strongly influenced the development of the phytoplankton. Stratification persisted during two months and the lake froze up towards the end of October. The average chlorophyll *a* concentrations were lower in 1996 than in 1997, which reflects the rather high temperatures and stable weather conditions in 1997 after the late melting of the ice. These observations lead us to suggest that the duration of the ice-free season is less decisive for biomass production than the weather conditions during this period. However, the date and duration of melting of the lake ice strongly influence the development of algal species that are typically observed in early season.

KEYWORDS: phytoplankton, community structure, seasonal dynamics, mountain lake, meteorological conditions

1 INTRODUCTION

Organisms of high mountain lake ecosystems are challenged by extreme and extremely variable living conditions, since sudden weather changes are common for high altitudes. The length of the ice-free season as well as the time and duration of ice melting can vary significantly. This affects the relative length of water column stratification, which can last for one to three months between melting and formation of the lake ice cover. The particular conditions which characterize high mountain lakes influence the biomass and community structure of the plankton. For subalpine lakes, Goldman *et al.* (1989) showed that the relative length of water column stratification followed by a sufficiently long mixing period is very important for the primary production. High mountain lakes undergo major physical restructuring during ice melting in spring and cooling in fall. During these periods, the phytoplankton assemblages change dramatically, and the meteorological conditions exert strong regulatory effects on their development and seasonal succession (Harris 1986, Goldman *et al.* 1996, Litaker *et al.* 1993).

At the beginning of this century, Kreis (1921) studied the fauna in 13 of the 21 lakes and ponds in the Jöri catchment, and he described the morphology of the lakes for the first time. About 20 years later, Messikommer (1942) investigated the phytoplankton in the region of Davos, including two of the Jöri lakes.

In this paper, detailed physical, hydrological, chemical and biological features of Jöri lake III and VII are presented. Furthermore, we compare the development of the phytoplankton over two years and correlate them with distinct weather situations. We show, how meteorological factors affect the development of phytoplankton communities in lake III and VII, one containing high amounts of suspended erosion particles (III), the other one with clear water (VII).

Characteristics of the catchment.

The catchment of the Jöri lakes is located in a remote mountain area of the Eastern Swiss Alps, in the canton of Graubünden, at 46°/46'N and 9°/58'E. The entire basin, which comprises 21 lakes and ponds is 3.05 km² in area and extends from 2489 m to 3060 m a.s.l. Accessibility is more difficult in winter, thus requiring special efforts for measurements of variables that can not be obtained by electronic logging. The basin is generally well shielded by high mountain chains against the west and the south side, but it is open to the north, in which direction the water drains from the catchment. There are 16 lakes in the catchment ranging in size from 500 m² to almost 0.1 km² and 5 smaller ponds. The total surface area of

all lakes is slightly over 0.2 km² corresponding to approximately 18 % of the entire catchment area. The Jöri basin was initially formed by the Jöri glacier that is presently retreating, but still covers an area of about 0.3 km². The lakes show great variability in suspended particle concentrations since some get their water directly from the glacier while others receive it only from snowmelt and precipitation runoff. All the lakes lie within the same catchment area which mainly consists of a homogeneous crystalline gneiss formation. The major elements in the rock minerals are Si and Al (Krähenbühl 1984). The alkalinity in the lakes is about 100 µeq l⁻¹ and they are less affected by acidification than the lakes located in the central Alps of the Gotthard region. The surroundings consist mostly of large rocks and some flood plains with erosion mud deposits and only scarce vegetation. Some of the lakes are connected in a cascade-type system which allows to follow chemical and biological changes which occur within a 250 m altitudinal gradient between the highest and the lowest lakes.

2 METHODS

2.1 *Meteorology and Hydrology on-line measurements*

The Jöri weather station is situated on the shore of lake III at an altitude of 2520 m. Meteorological variables are recorded hourly during winter and spring (November to June) and in 10 minutes intervals during summer and autumn (July to October). Variables measured include air temperature, humidity, precipitation, wind speed and direction, global radiation and incoming long wave radiation. In the water, two chains with 11 sensors each measure the temperature profile of lake III every 15 minutes.

2.2 *Chemistry*

Samples for lake water analyses were collected bi-weekly from different depths and filled into polyethylene bottles in a way that no air remained in the bottle. The bottles were kept at 4° C until further analysis. The deposition samples were collected either in a Bergerhoff-type collector for bulk deposition (Minger 1996) or in a polyethylene bottle (wet only deposition). The deposition samples were kept at 4°C until analysis.

The pH was measured with a Methrom pH Meter without stirring but with previous addition of 50 mg suprapur NaCl per 50 ml sample in order to obtain a higher ionic strength. For conductivity measurements a cell with a constant of 1 cm⁻¹ was used and the value was directly normalized to 25° C. The pH and the conductivity of lake water samples were measured *in situ* and double checked in the laboratory. All determinations included a control

of the measured conductivity with the conductivity calculated from equivalent conductances of all species analyzed (Marchetto *et al.*, 1996). The quality criteria are fulfilled if the difference between calculated and measured conductivity is less than 15 %. Alkalinity was determined by Gran titration (Gran 1950, 1952) with a common Methrom pH Meter 632 in presence of suprapur NaCl to obtain an ionic strength of 0.1 M.

Anions (chloride, nitrate and sulfate) were measured with ion chromatography with chemical suppression (DIONEX 2000i, separation column AS4A). Calcium, magnesium, potassium and sodium concentrations were determined by ICP-AES (VARIAN Liberty 150 AX Turbo) and double checked in some samples with ion chromatography. Ammonia was analyzed in the same cation chromatogram. SRP (soluble reactive phosphorus) was determined at the site immediately after sampling employing the method of Murphy and Riley (1962).

2.3 *Phytoplankton*

500 ml samples in PET bottles were taken from three different depths (1 m below surface, thermocline depth and one meter above the bottom) and fixed with Lugol solution. Enumeration of the dominant species of algae was performed using an inverted microscope with phase contrast optics. Biovolume conversion factors were calculated using approximations to geometrical shapes (see MOLAR methods, this volume). The specific carbon content was calculated from $C = 0.1204V^{1.051}$ using the regression after Rocha and Duncan (1985).

2.4 *Chlorophyll a*

Samples were taken with a 3 l plexiglass water sampler from the different depths. 400 ml to 1000 ml water were filtered through glass fiber filter (GF 6, Schleicher and Schuell, Dassel, Germany) within two hours after sampling. Filters were transported frozen to the laboratory for analyses. Chlorophyll *a* concentration was measured after extraction in 90 % acetone using fluorimetric determination after Schanz (1982).

2.5 *Water column profiling*

Water column profiling was done weekly during the ice-free season and at least three times during the ice-covered period. Winter sampling was required to determine the oxygen consumption below the ice cover.

Temperature, pH, oxygen and conductivity were measured with a Hydropolytester (Züllig, Rheineck, Switzerland). Turbidity was determined by light scatter loss in a photometer (PU

8625 UV/VIS Spectrophotometer, Philips, Zürich, Switzerland) in 5 cm glass cuvettes at a wavelength of 660 nm and transformed to NTU (DEV 1996; 38'404-C2).

3 RESULTS

3.1 *Physical characteristics*

3.1.1 *Hydrology*

The lakes in the Jöri catchment area were originally numbered (I to XIII) by Kreis in 1921; we extended the numbering to XXI for those lakes, which have been newly formed during the last 80 years (Fig. 1). The lakes described in this paper are numbers III and VII.

Hydrology lake III.

Lake III has the largest volume of all the Jöri lakes (maximum of 600'000 m³). The surface area is 57'800 m² when the lake is completely filled and up to 5'000 m² less in early winter. The catchment is approximately 1.3 km²; it cannot be precisely delineated, however, due to subsurface inflows from the glacier. The annual average water residence time (R) calculated from yearly average precipitation (P), catchment size (A) and lake volume (V), is about 4 months ($R = V * P^{-1} * A^{-1}$). Lake III receives some water from the glacier, which makes the lake turbid. Early season Secchi depth can be up to 3 m, but it decreases to less than 1 m as more turbid water from the glacier is added. Under conditions of high turbidity, 99 % of the global radiation which penetrates into the water is absorbed in the top 3 m. Due to its turbidity and maximum depth of 22 m the lake can be temperature stratified for up to three months every year.

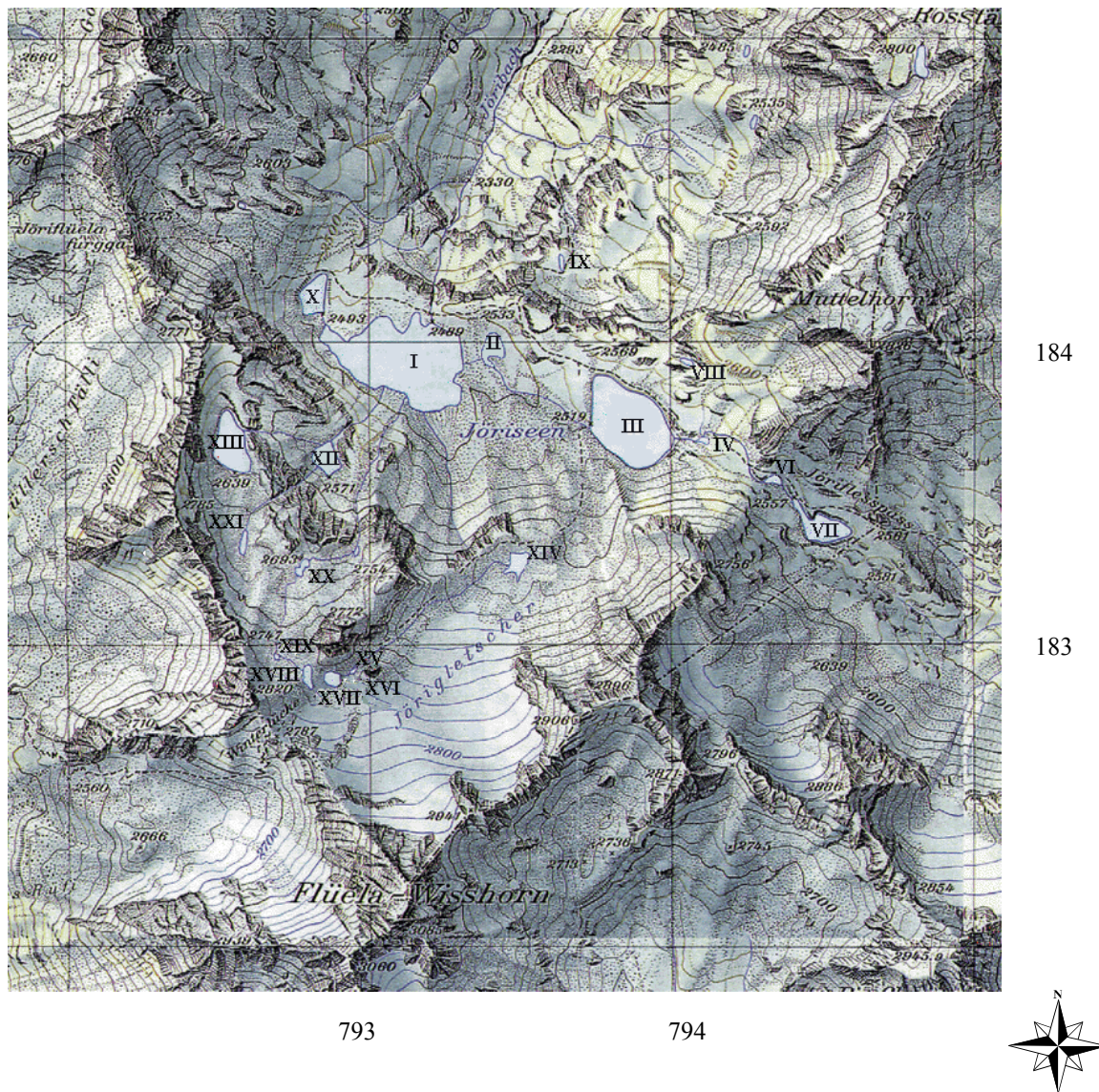


Fig. 1: Map of the study site of the Jöri lakes (9° 57' to 9° 59' N / 46° 46' to 46° 47' E). Numbering of the lakes after Kreis (1921) (I-XIII) and Summa (XIV- XXI). Reproduction with the kind permission of the Federal Office of Topography, Switzerland, on January 7, 1999.

Hydrology lake VII.

Lake VII has an area of 11'600 m² and lies at an altitude of 2557 m a. s. l.. Its maximum depth is 8.5 m, its volume approximately 40'000 m³ and its catchment area 0.16 km². The average water residence time is about 2.5 months. The water in lake VII is clear to the ground with

water inflow from rain and snowmelt only. Secchi depth is always more than 4 m, it can reach more than 8 m early in the season. It is isothermal for most of the season.

3.1.2 Meteorology and climatology

The weather at the Jöri lakes can be characterized by a long cold winter with heavy snowfalls and a short summer with many thunderstorms and a few nice weather spells. Snow may be expected at any day of the year. Maximum air temperatures reach 20° C on rare occasions, daily average temperature is usually below zero from November through April. Yearly average temperature is -2° C as measured at the Jöri weather station. This is slightly less than expected, compared with stations at similar altitudes. When the lakes are frozen, cold air tends to accumulate above the lakes, due to the lack of outflow possibilities. The total yearly precipitation is 1400 mm with approximately 70 % of it falling as snow. Annual average radiation is about 185 W m⁻² (Tab. 1). Winds tend to be strong only on the ridges, but most of the lakes are well shielded by the surrounding mountains. Lake VII on Jörifless Pass usually experiences higher winds than the other lakes which lie deeper in the catchment. Other than exposure to wind, lakes III and VII are similar. Average air temperatures at lake VII are 0.2° C lower than at lake III and due to mountain shading, average global radiation is about 10 W m⁻² smaller.

Tab. 1: Air temperature (°C), precipitation (mm) and radiation (W m⁻²) at Jöri lake III. Long term averages (1981 – 1998) were taken from the SMI (Swiss Meteorological Institute) station at Weissfluhjoch, situated 13 km from Jöri lakes at an altitude of 2690 m. a. s. l.

	<i>1996, Lake III</i>			<i>1997, Lake III</i>			<i>1981-1998, SMI Weissfluhjoch</i>		
	Temp	Prec	Rad	Temp	Prec	Rad	Temp	Prec	Rad
Jun	4.2	125	253	2.7	232	196	2.3	167	239
Jul	4.3	213	234	3.6	265	195	5.8	182	223
Aug	4.3	197	160	6.6	182	186	5.8	171	192
Sep	-1.1	110	166	6.4	45	206	2.9	126	163
Oct	-1.1	122	124	-0.3	83	127	0.2	69	125
Total or average	2.1	767	187	3.8	807	182	3.4	715	188

Snow on the horizontal plains usually disappears between mid June and mid August, depending on the season's maximum snow water equivalent and on early summer weather conditions. The break-up date for the lake ice can be at the end of June at the earliest and the end of August at the latest for lake III. Calculations were made on the basis of a snow height adjusted degree day model (M. Gabathuler, unpublished). The break-up date depends on

temperatures from May up to the disappearance of the ice and on the snow load on the lakes. Deep lakes as well as lakes at higher altitudes tend to break up later. For the build-up of the ice in autumn, altitude is less important than depth, size and accumulated heat. Owing to their greater heat energy content in the autumn, lakes with large volumes tend to freeze later than small and shallow lakes. The freeze-up date of lake III falls usually into the second half of October or the first half of November. Once a lake has reached isothermal conditions, it can freeze when its threshold conditions are fulfilled. These conditions are different for every lake, but always consist of air temperature, wind and average lake water temperature (Gu and Stefan, 1990).

Summer started early in 1996; daily average temperatures regularly came close to 10° C in early June. Due to the small maximum snow water equivalent for this year, snow melted almost a month earlier than usual. The same can be said for the lake ice covers, which disappeared towards the end of June. Between July and October temperatures were regularly below average, especially in September. The few days with nice and warm weather were recorded during the second half of July, allowing lake III to get well stratified. The stratification weakened with a cold front which came up in the middle of August and which completely disappeared during a heavy snowfall at the end of August. August was a wet month with precipitation on 24 days reaching 200 mm in total. Snow constantly covering the area around the lakes started as early as September 13. Although there was no stratification anymore in lake III after September 2, a complete ice cover was not observed until October 17.

June and July of 1997 were not only colder and with less global radiation than the same months in 1996, but there was also a much higher maximal snow water equivalent to begin with, which caused the largest differences in the lake processes. It caused the snow around the lakes to melt more than one month later than in 1996. The same could be observed for the ice cover on the lakes, which did not disappear until the end of July. August was a warm month with precipitation below average. Heavy snowfalls at the end of August caused the stratification in lake III to weaken temporarily. September was extremely dry with only two precipitation events with a total of 45 mm. The average temperature for this month was 7° C higher than in 1996 and 13 cloudless days were recorded. Stratification could be observed until October 2. Snow covered the area around the lakes from October 12 on; the lake froze around the 26 of that month.

3.2 Chemical characteristics

Lake III is fed by a subterranean inlet from the Jöri glacier. In 1996 only surface water had been sampled. Rising concentrations of several ions (K, Mg, NO₃) and therefore increasing conductivity could be observed towards autumn, which was due to the stratification of the lake (Lerman *et al.* 1995). The main ions found in the two lakes were calcium and sulfate which are also the most important components in the deposition (Tab. 2). Lake VII showed similar, mostly smaller, concentrations of ions than lake III for both sampling years. For some components, like K and Mg, it is more pronounced which is probably due to the different water sources of the two investigated lakes. Lake III receives melt water from the glacier, but also rain water and water from upper lakes. In contrast, lake VII receives mainly rain and spring water. The average concentrations for the measured ions are listed in Tab. 2; they did not change substantially from 1996 to 1997. Alkalinity values for both years were around 100 µeq l⁻¹. TP and total inorganic nitrogen (TN, sum of nitrate and ammonium) concentrations after spring mixing amount to about 10 ppb and 200 ppb, respectively. After Wetzel's (1983) trophic state scale, the Jöri lakes are considered as oligotrophic. Increasing TN- and TP-concentrations were measured in the depth profiles towards the sediment (up to 40 ppb TP one meter above the sediment). The same observation was made for turbidity, which could indicate a link between particles and nutrient content.

Tab. 2: Chemical characteristics of Jöri lakes III and VII in 1996 and 1997. x and s are mean and standard deviation, n is the number of samples analyzed.

	n	pH		Cond. _{25°C} [μS cm ⁻¹]	NH ₄ [μg N l ⁻¹]	Ca [mg l ⁻¹]	Mg [mg l ⁻¹]	Na [mg l ⁻¹]	K [mg l ⁻¹]	Alk [μeq l ⁻¹]	SO ₄ [mg l ⁻¹]	NO ₃ [μg N l ⁻¹]	Cl [mg l ⁻¹]	P ¹⁾ [μg P l ⁻¹]											
1996		x	s	x	s	x	s	x	s	x	s	x	s	x	s										
lake III	12	6.75	0.11	19.92	0.99	7.80	3.60	2.28	0.41	0.30	0.06	0.40	0.06	0.36	0.15	103	52	2.99	0.24	194	41	0.17	0.18	36	18
lake VII	8	6.70	0.10	15.74	0.79	n.d.		1.71	0.14	0.20	0.02	0.33	0.03	0.24	0.07	100	46	1.97	0.15	179	52	0.22	0.13	n.d.	
1997																									
lake III	38	7.15	0.54	17.90	3.90	8.80	13.60	3.15	1.32	0.40	0.15	0.52	0.18	0.38	0.14	99	55	2.49	0.77	254	74	0.08	0.11	9	8
lake VII	5	n.d.		n.d.		2.60	1.30	2.27	0.65	0.25	0.07	0.33	0.09	0.24	0.05	n.d.		1.48	0.39	169	14	0.01	0	9	4

1) 1996: soluble reactive phosphorus SRP (after Murphy and Riley, 1962), 1997: Total P (ion chromatography measurement)

Although up to 80% of the chemical species which had accumulated in snowpacks are removed with the first 20% of the melt water, organisms can barely profit from this nutrient peak (Johannessen and Henriksen, 1978). During snow melt, the water in all Jöri lakes is exchanged in less than one month. Since nutrient-rich inflow water has a temperature of around 0° C, it is lighter than lake water and therefore will not be mixed down to great depths but will exit through a surface outflow within a few days.

3.3 Biological characteristics

3.3.1 Phytoplankton

Phytoplankton composition in Jöri lakes III and VII.

In both lakes the Chlorophyta is the most abundant algal division. Almost all species can be found in both lakes (Tab. 3).

Tab. 3: Dominant species of algae in Jöri lakes III and VII.

Chlorophyta	<i>Eutetramorus fottii</i>
	<i>Dictyosphaerium subsolitarium</i>
	<i>Monoraphidium subclavatum</i>
	<i>Chlamydomonas braunii</i>
Cryptophyta	<i>Rhodomonas lacustris</i>
Chrysophyta	<i>Dinobryon cylindricum</i> var. <i>alpinum</i>
	<i>Kephyrion doliolum</i>
	<i>Chromulina</i> sp.
	<i>Chrysococcus furcatus</i>
Dinophyta	<i>Gymnodinium lantzschii</i>
	<i>Amphidinium elenkinii</i>

Therefore, phytoplankton biodiversity in terms of the number of species is about the same in lake III and VII. The dominance and the abundance of the species differ considerably, however (Fig. 2). In lake III several species develop with almost the same dominance, but still *Eutetramorus fottii* is the most abundant species. As *Eutetramorus fottii* is a rather large algae, it consequently represents an important fraction of the total algal carbon content of lake III (Fig. 2). In lake VII only few species dominate the community: *Dictyosphaerium subsolitarium* in early season, as well as *Gymnodinium lantzschii* (1996) and *Monoraphidium subclavatum* (1997) towards the end of the season. In lake VII cell numbers are higher than in lake III. This is due to the dominant but small species *Dictyosphaerium subsolitarium*. Taking biovolume and specific carbon content into consideration, biomasses in lake III are distinctly higher.

Seasonal dynamics.

Both lakes show strongly developed seasonal dynamics. Comparing the two years 1996 and 1997 it seems that the date of the ice melting is very important for the development of some species. In 1996 when the ice melted early (late June) *Rhodomonas lacustris* developed rapidly and dominated in the first weeks of the ice-free period in lake III (Fig. 3). After one month, the Chlorophyta (*Dictyosphaerium subsolitarium* and *Eutetramorus fottii*) appeared in higher cell numbers and started to dominate the community (Fig. 3). On a C-content basis, *Eutetramorus fottii* was the most dominant taxon. Towards the end of the ice-free season, *Kephyrion doliolum*, and *Monoraphidium subclavatum* also developed well.

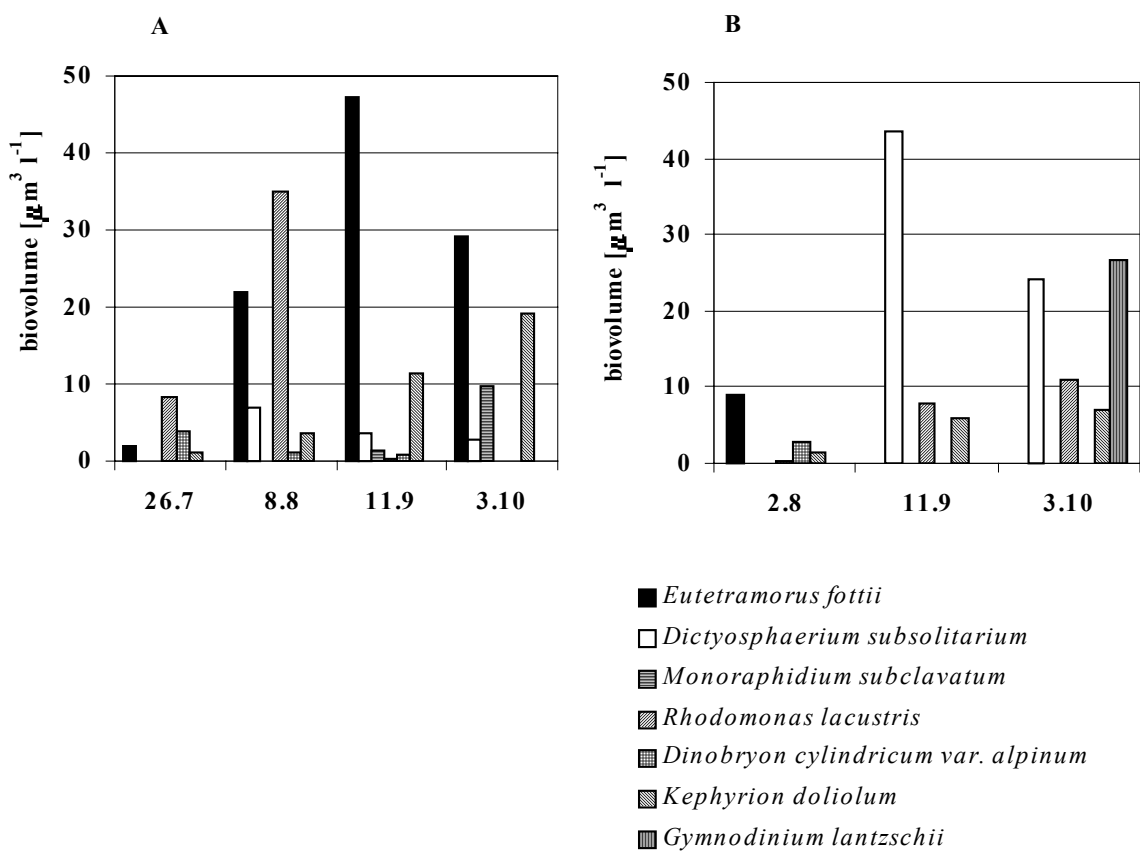


Fig. 2: Seasonal dynamics of dominant species of phytoplankton in 1996 in Jöri lake III (A) and in Jöri lake VII (B).

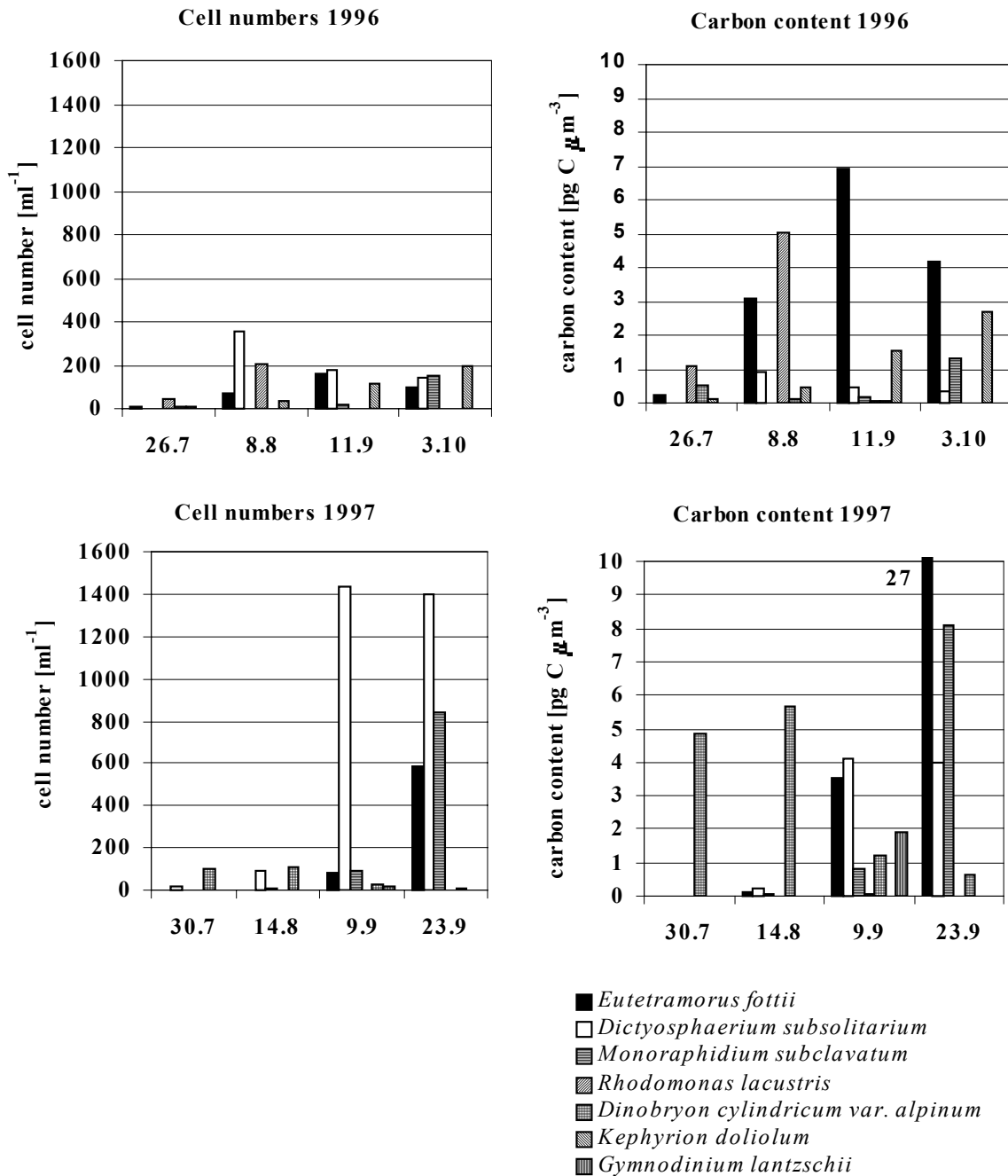


Fig. 3: Seasonal dynamics of dominant algal species on a cell number- and a carbon content-basis in Jöri lake III in 1996 and 1997.

In 1997, when the ice melted very late (end of July), there was a great number of cysts, mainly of Chrysophyta, which sometimes persisted during almost the entire season. After the late melting of the lake ice, it took longer for biomass production to take off than the year before. An exception was *Dinobryon cylindricum var. alpinum*, which developed even better than the year before (Fig. 3). On a C-content basis, it was by far the most dominant taxon in July and August. In contrast to 1996, *Rhodomonas lacustris* was very scarce. Chlorophyta

development was one month late compared to 1996, but towards the end of the season, *Eutetramorus fottii*, *Dictyosphaerium subsolitarium* and *Monoraphidium subclavatum* propagated even better. In summary, the proportional composition of the algal divisions differed significantly in the two years: Cryptophyta, which were typical for the early season in 1996, did not develop well during the entire season of 1997. In the second part of the ice-free season, Chlorophyta were even more dominant in 1997 than in 1996 (Fig. 4). For lake VII similar observations have been done (data not shown).

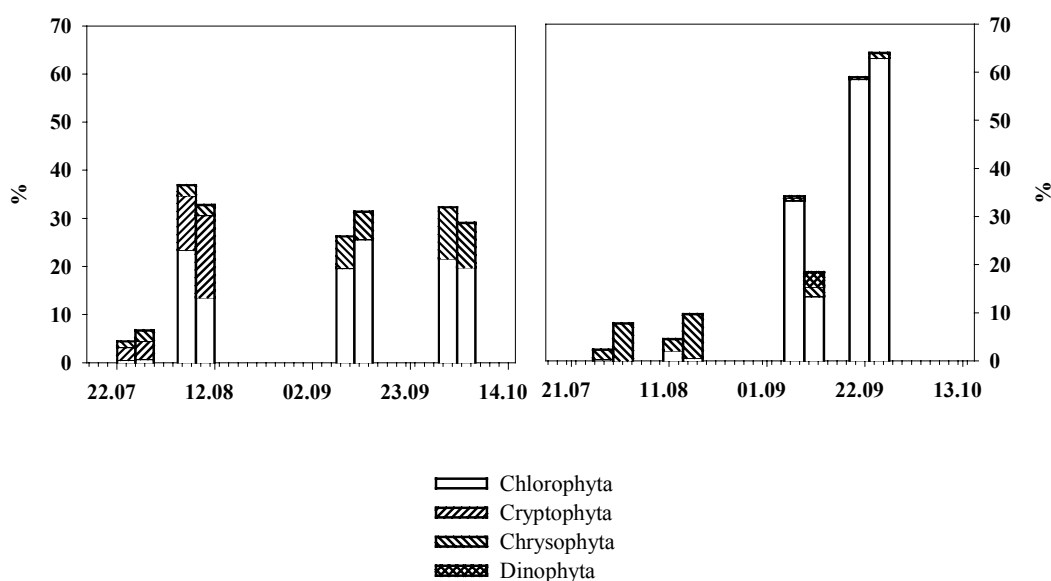


Fig. 4: Occurrence of algal divisions in Jöri lake III in 1996 and 1997. 1: cell numbers [counts ml⁻¹]. 2: Carbon content [pg μm⁻³], both scaled to percentage. 100% is total annual cell counts, and carbon content, respectively.

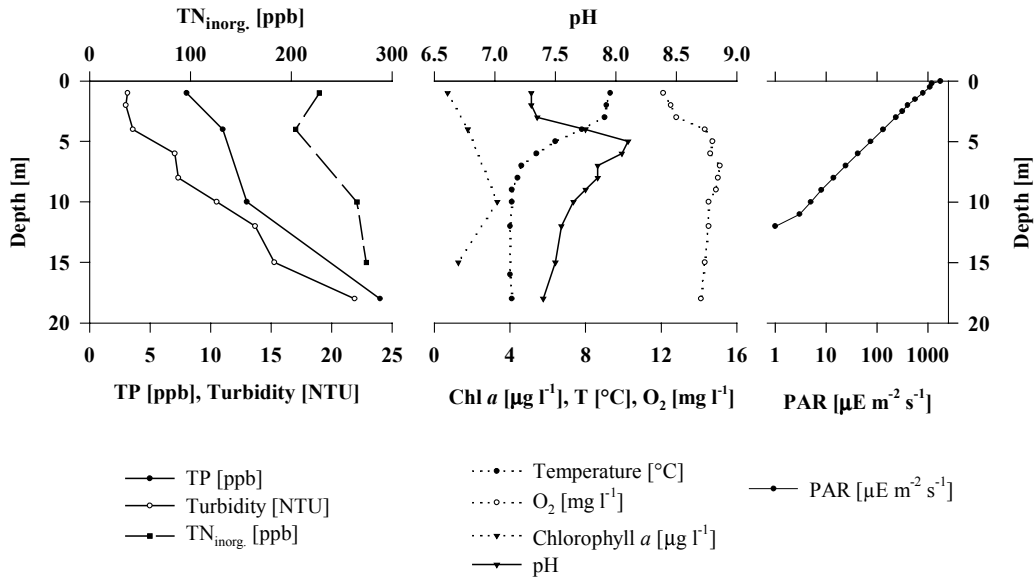


Fig. 5: Physical, chemical and biological parameters in the water column of Jöri lake III on September 9, 1997. TP: total phosphorus, $TN_{inorg.}$: total inorganic nitrogen .

3.3.2 Chlorophyll *a*

Chlorophyll *a* concentrations in lake III range from 0.4 to 5.0 $\mu\text{g l}^{-1}$. In 1996 average concentrations were slightly lower than in 1997 (Tab. 4). The maximal concentration in 1996 (2.4 $\mu\text{g l}^{-1}$) was only about half the maximal concentration of 1997 (5.0 $\mu\text{g l}^{-1}$). Stratification persisted in both years for two months. In 1996 the highest chlorophyll *a* concentrations were found just below the thermocline (6 m depth) when the lake was stratified, although maximal chlorophyll concentrations were found in October when the lake was no longer stratified after a cold weather period in early September (Tab. 4). This supports the findings of Goldman *et al.* (1989) in subalpine lakes, that biomass production is highest after a relative long water column stratification followed by a sufficiently long mixing period. This allows to recirculate nutrients from the hypolimnion. In 1997, the highest values were found at the end of September a few meters below the thermocline in 10 m depth (Tab. 4).

Tab. 4: Chlorophyll *a* [$\mu\text{g l}^{-1}$] in Jöri lake III in 1996 (above) and 1997 (below).

<i>depth</i>	26.7.	8.8.	21.8.	22.8.	23.8.	10.9.	11.9.	12.9.	3.10.	<i>mean</i>	<i>std. dev.</i>
<i>1 m</i>	0.4	0.4	0.5	0.6	0.6	1.1	1.2	1.5	2.3	1.0	0.7
<i>thermocline (4-6 m)</i>	0.8	1.6	1.7	2.0	1.9	1.4	1.6	1.7	2.1	1.6	0.4
<i>10 m</i>	2.2	1.1	0.9	0.7	0.8	1.2	1.6	1.6	-	1.3	0.5
<i>1 m above bottom</i>	1.1	0.4	-	-	-	-	-	-	2.4	1.3	1.4
<i>depth</i>	30.7.	14.8.	19.8.	26.8.	2.9.	9.9.	17.9.	23.9.	7.10.	<i>mean</i>	<i>std. dev.</i>
<i>1 m</i>	1.0	1.1	0.7	0.6	0.8	0.7	0.7	0.9	1.1	0.8	0.2
<i>thermocline (4-5 m)</i>	0.8	1.0	0.7	0.9	0.6	1.8	1.2	1.6	1.2	1.1	0.4
<i>10 m</i>	1.0	1.2	3.6	3.2	3.4	3.3	5.0	4.7	2.5	3.1	1.4
<i>1 m above bottom</i>	-	1.0	0.9	1.2	0.9	1.3	1.2	0.9	0.9	1.0	0.2

This correlates also with higher algal biomasses in this depth, although this correlation was not always reliable. Only very low amounts of photosynthetic active radiation (PAR) penetrates to this depth (maximal $5 \mu\text{E m}^{-2} \text{s}^{-1}$) and the temperature never exceeds 4°C (Fig. 5). An advantage for algae to live at these depths are the slightly increased ion and nutrient concentrations (Fig. 5). In a different study, we have shown that the higher nutrient concentrations were always linked to increased turbidity due to suspended particles (data not shown). Algae could thus profit by living in a less oligotrophic environment. Moreover, algae living in the upper hypolimnion are less affected by sudden weather changes which happen usually at least once during the ice-free season.

3.3.3 Effects of weather events on algae

The effects of weather events on algae were recorded at the end of August 1997. During this period, lake profiles were taken once before and twice after a strong cold front. The lake was well stratified. The strong cold front hit the lakes at 15 UTC (Universal Time Coordinated) on August 28, reducing air temperatures from 8°C to -2°C within a few hours. Heavy snowfall built up a snow cover of about 30 cm. Chlorophyll *a* concentration and pH were measured as indicators for the presence of algae and their photosynthetic activity, respectively. On August 26, a maximum pH value of 7.84 was found at 4 m depth (Fig. 6). Surface water temperature decreased from 12°C to 7°C and stratification became less distinct. During this period of cold weather the thickness of the epilimnion increased. As the surface temperature decreased, the epilimnion was mixed and consequently approached the water temperature of the surface. Due to these lower temperatures, some of the original thermocline became isothermal, making the epilimnion thicker and the thermocline steeper. This process resulted in a one meter

thicker epilimnion on September 9, which is also reflected in the vertical pH-profile (Fig. 6). Low temperatures were recorded up to the morning of August 31. Stratification intensified quickly again due to the large energy input during the first days of September. On September 2, two days after the cold front had passed by, a maximum pH value of 7.60 was found three to four meters deeper (Fig. 6). If photosynthesis was the main factor influencing the pH, the proton consumption in seven to eight meters depth indicates restarting photosynthesis and suggests that primary production broke down during the storm. One week later, we again recorded a strongly increased proton production with maximal values at 5 m depth. A few days of sunshine doubled or even tripled the chlorophyll *a* concentration (Tab. 4). In 1 m depth, chlorophyll *a* concentrations were always low, probably due to rough living conditions. Chlorophyll *a* concentrations in 10 m depth were not affected by the cold front indicating stable environmental conditions in this layer.

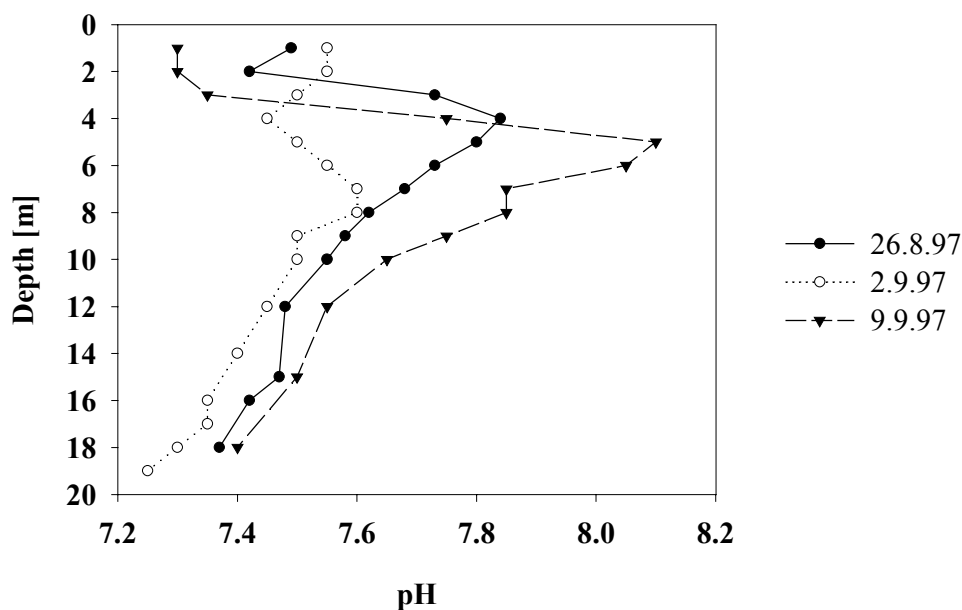


Fig. 6: Vertical profile of the pH in Jöri lake III on three dates in 1997.

4 DISCUSSION

During the two years of studying the environmental conditions in the Jöri lakes, high intra- and interannual variability was registered. The timing of the melting of the lake ice seems to strongly influence the starting conditions for the development of the phytoplankton. The community structure during the ice-free seasons differed distinctly. While Cryptophyta were frequent during the early season of 1996, they occurred only in low numbers in 1997 and were replaced by Chrysophyta. A large number of cysts, mostly of Chrysophyta, were observed in 1997 when the ice cover on the lake persisted until the end of August. Only little is known about the influence of the timing of lake ice melting on phytoplankton diversity. Scavia *et al.* (1986) hypothesized that abnormal phytoplankton biomass in 1977 in Lake Michigan could have been attributed to prolonged spring ice cover. Goldman *et al.* (1996) showed that horizontal differences in nutrients, phytoplankton and productivity in Lake Baikal - apart from other reasons - probably reflected the variation in the timing of lake ice melting in different parts of the lake. The authors concluded that during the period of pre-stratification, phytoplankton growth in Lake Baikal is regulated by physical factors. However, we found only small diversity of phytoplankton after the late ice-break in 1997. Harris (1986) assumed that small diversity might be expected to occur in the seasonal sequence if species grow slowly in comparison to the rate of environmental change; for example when the lake undergoes major physical restructuring in spring and fall. In both years at Jöri, the dominant fraction of total biomass in the second part of the ice-free season was contributed by Chlorophyta. In 1997, when the weather was brighter and stable for almost two months, biomass production was higher than in 1996. This corroborates with the findings of Goldman *et al.* (1989) for subalpine lakes, that biomass production is highest if a relatively long water column stratification is followed by a sufficiently long mixing period.

Litaker *et al.* (1993) found that periodic ecological processes operating on time scales equivalent to phytoplankton cell division rates are important in controlling chlorophyll *a* biomass changes. Such an event was studied at the end of August 1997 when a cold front reached the lakes and surface water temperature was reduced from 12° C to 7° C in only a few hours. It took a few days to restore the chlorophyll *a* biomass in the epilimnion. Photosynthetic activity two days after the cold front had passed by, was still low. After one week sunny weather, it was completely restored or even higher than before the cold front had passed. The chlorophyll *a* biomass below the thermocline was not affected by this weather breakdown. In fact, chlorophyll *a* biomass was highest below the thermocline in 1997. We suggest, that algae live at these depths since the environmental conditions are more stable and

the nutrients more abundant. In the frame of this study, migration of flagellated species has not been investigated. Higher nutrient concentrations might be linked to higher concentrations of suspended particles. Many studies demonstrated interactions between particulate matter and ions or nutrients (e.g. Cuker *et al.* 1990, Fukushima *et al.* 1991, Cuker 1993, Mez *et al.* 1998) and concluded that particulates can have strong effects on the nutrient situation in the lake and the phytoplankton community. Suspended particulate matter entail also a different underwater light quality. Several authors have shown, that this is a controlling factor for phytoplankton growth as well (Glover *et al.* 1987, Talling 1971). Hence, the presence of suspended particles may also be a determinant for the phytoplankton community structure in the two Jöri lakes III and VII.

5 CONCLUSIONS

The seasonal succession and community structure of the phytoplankton was distinctly different in the two years during which they were studied. We suggest that the reasons for the variabilities are the different meteorological conditions. The timing of the lake ice melting might be most relevant to the development of some taxa: *Rhodomonas lacustris* was abundant in 1996, when the ice melted early (late June). In 1997, when the lake ice melted more than one month later, we found a great number of Chrysophyta cysts; the dominant fraction of total phytoplankton biomass was contributed by *Dinobryon cylindricum* var. *alpinum*. The phytoplankton community during mid- and end-season was dominated by Chlorophyta. Highest biomass production was observed at the end of the season 1997 after two months of stable stratification and following mixing of the water column. In 1997 the highest chlorophyll *a* concentrations were found several meters below the thermocline. This might be due to higher nutrient concentrations and more stable environmental conditions at this depth. The lakes containing and those lacking suspended particles showed similar phytoplankton diversity but different dominance of the algal species. According to the findings of Cuker *et al.* (1990), this could be due to the presence of the particles but also due to different light quality caused by the particles (Glover *et al.* 1987, Talling 1971).

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Bacterial Diversity in High Mountain Lakes (Jöri Lakes, Switzerland) as Revealed by Temporal Temperature Gradient Gel Electrophoresis (TTGE)

ABSTRACT

We studied the seasonal dynamics of microorganisms during the ice-free period of a clear water and a turbid high mountain lake using temporal temperature gradient gel electrophoresis (TTGE). Eight of the TTGE separated fragments were sequenced and compared with 16S rDNA sequences available in databases. Some sequences showed 93.8 to 99.7 % similarity to other sequences of unidentified bacteria originating from mountain lakes. Analysis with fluorescent *in situ* hybridization (FISH) indicated the predominance of beta *Proteobacteria*. The influence of suspended erosion particles on the nutrient availability for the microbiota in glacial high mountain lakes is studied for the first time. Microbial communities were shifted *in situ* by adding P, N and / or surface reactive erosion particles to limnocorrals of 13 m³ volume. The results suggest that erosion particles interact with nitrogen compounds and that the presence of suspended particles affects the microbial community.

KEY WORDS. temporal temperature gradient gel electrophoresis (TTGE), fluorescent *in situ* hybridization (FISH), 16S rDNA, bacterial diversity, high mountain lake, erosion particles.

1 INTRODUCTION

Microorganisms living in mountain lakes are often challenged by low nutrient concentrations, rapid hydrological changes and a short ice-free period, during which they grow and propagate most actively. Lakes fed by glacial melt water contain high amounts of erosion particles which are supposed to regulate the availability of nutrients and radiation thereby changing the living conditions for the microbiota. The effects of suspended particles on the phytoplankton are multifaceted: lower chlorophyll *a* concentrations in turbid waters are attributed to the

higher light attenuation (Cuker *et al.* 1990, Dokulil 1994, Lind *et al.* 1997), and to the adsorption capacity of the particles for nutrients like phosphate and ammonia (Henley 1998). Interactions between particles and bacteria are reported for estuaries, e.g. Crump *et al.* (1998) showed that 90% of the bacterial carbon production was contributed by particle-associated bacteria. Bidle and Fletscher (1995) compared the free-living and particle-associated bacterial community in the Chesapeake Bay and showed that the two communities differed in composition. Jannasch *et al.* (1972) made observations which are relevant for oligotrophic waters. In laboratory experiments these authors showed a negative correlation between the nutrient concentration in the medium and the bacteria living particle-associated. They suggested that particle-associated life styles become more favorable for the bacteria at lower nutrient concentrations. Due to their adsorption capacities for ions suspended particles could provide a profitable microhabitat for bacteria in oligotrophic lakes.

The Jöri lakes consist of 21 small lakes and ponds situated at elevations between 2489 and 2730 m a. s. l. in the Vereina region of the eastern Swiss Alps. Crystalline rocks dominate in the catchment (Krähenbühl 1984). New lakes in this region get formed as the Jöri glacier retreated. Some lakes are fed by turbid glacial inlets, others contain clear water. They offer a suitable test site to study the influence of suspended particles on the microbiota.

For microbial community analysis molecular techniques were used, not only because they offer the possibility to include uncultured species (Ward *et al.* 1990, Giovannoni *et al.* 1990, Amman *et al.* 1995) but because the information obtained allows one to reconstruct phylogenetic relationships. The application of molecular-phylogenetic methods to study natural microbial ecosystems has resulted in the discovery of many unexpected evolutionary lineages. Members of some of these lineages are only distantly related to known organisms but they are sufficiently abundant to have an impact on the chemistry of the habitat (Pace 1997).

In this study, we try to answer the following questions: how is the seasonal and inter-annual variability of the microbial community in high mountain lake's ecosystem where meteorological conditions are extremely variable? Which bacterial species live there and how is the community influenced by the presence of suspended erosion particles?

To study the microbial composition of the lake, we choose the temporal temperature gradient (TTGE) approach, which gives similar information as the DGGE technique described by Muyzer *et al.* (1993). In their work, they showed that it is possible to identify populations which represent only 1% of the total community. TTGE is a very simple approach since it allows to directly determinate the genetic diversity of complex microbial communities

without cloning. It provides an immediate pattern of the constituent population DNA in a qualitative and semiquantitative way.

2 MATERIALS AND METHODS

Site description. The Jöri lakes are numbered after Kreis (1921) who investigated the lakes at the beginning of this century. In our work, we focused on lake III and VII. Lake VII (2557 m a.s.l.) lies about 50 m higher than lake III and water of lake VII is fed into lake III. Today, the glacier ends several hundred meters behind lake III but turbid glacial water percolates through the front moraine and enters the lake at subsurface springs. Lake III has a maximal depth of 22 m. Lake VII (maximal depth 8 m) is fed by rain and snowmelt water and is clear.

Sample collection. Water from 1 m depth was collected with a 3 L plexiglass sampler (home-made construction by J. Fott, Charles University, Prag) and filled into 100 ml sterile polyethylene bottles. 50 ml of water were filtered through 0.22 µm pore-size filters (Durapore, Millipore, Bedford, USA) using a syringe filtration device. The filters were placed in sterile 2 ml centrifuge tubes and covered with 1.5 ml lysis buffer (50 mM Tris, pH 8.0, 20 mM EDTA, 50 mM sucrose). After processing, the tubes were immediately frozen on dry ice until returning to the laboratory and then stored at -20°C until extraction.

For fluorescence *in situ* hybridization (FISH), 20 to 50 ml of water were filtered with polycarbonate membrane filters (pore size, 0.2 µm; diameter 47 mm, Millipore, Ireland) and fixed as described by Glöckner *et al.* (1996). Filters were stored in small petri dishes at -20°C until further processing.

Nucleic acid extraction. For total nucleic acid extraction, microorganisms were washed off the thawed filter by rinsing the membrane with lysis buffer. The cells were centrifuged and the pellet was resuspended in 0.5 ml lysis buffer. 10 mg ml⁻¹ of lysozyme were added, and the suspension was incubated at room temperature for 10 min. After adding 1% (vol/vol) sodium dodecyl sulfate (SDS) and 100 µg ml⁻¹ proteinase K, the mixture was incubated at 37°C for 30 min and at 55°C for 10 min. DNA was obtained from the lysates by using standard phenol-chloroform extraction and ethanol precipitation procedures (Sambrook *et al.* 1989). RNA was removed by incubating the aqueous solution with 5 U of DNase-free RNase for 15 min at 37°C. The total amount of nucleic acids extracted from 50 ml water samples was approximately 0.5 - 1.0 µg DNA.

Amplification of 16S rRNA genes. 16S rDNA was amplified from genomic DNA by PCR with the oligonucleotide primers S-D-Bact-0008-a-S-20 (5'-AGA GTT TGA TCC TGG CTC

AG-3') and S-*-Univ-0536-A-18 (5'-GWA TTA CCG CGG CKG CTG-3'). The combination of these primers amplifies a 520 bp fragment of the 16S rDNA suitable for TTGE analysis. The GC-clamp at the 5'-end of the forward primer (Muyzer *et al.* 1993) prevents strand dissociation at high temperature during separation in the gel. Amplification was carried out in 25 µl reactions with a Techne thermocycler (Witec AG, Luzern, Switzerland). Each reaction tube contained Expand High Fidelity reaction buffer (Roche Diagnostics, Rotkreuz, Switzerland), 1.5 mM MgCl₂, 200 µM deoxynucleotide triphosphate, 0.7 U of HiFi-DNA polymerase (Roche Diagnostics, Rotkreuz, Switzerland), 5 pmol of each forward and reverse primer, and 10 ng template DNA. Touchdown PCR was carried out with the Techne thermocycler with the following conditions: First, the samples were heated to 94°C for 3 min to denature template DNA. Subsequently, 20 cycles were performed beginning with an annealing temperature of 65°C for 30 sec and lowering it 0.5°C every cycle, followed by 15 cycles with annealing at 55°C. Primer extension was carried out at 72°C for 90 sec. For environmental samples the hot start technique was used, adding the enzymes after the initial denaturing step at 75°C. Negative controls showed no amplification in all experiments. Products were run on an agarose gel (1% agarose, 1× TBE [90 mM Tris-borate, pH 8.3, and 8 mM EDTA]) and viewed with ethidium bromide (0.5 µg ml⁻¹).

TTGE analysis. TTGE-analysis was performed with a DCode system (Bio-Rad Laboratories, Glattbrugg, Switzerland). 2.5 µl of the PCR samples were applied directly onto 6% (w/v) polyacrylamide gels (acrylamide:*N,N'*-methylene bisacrylamide, 37.5:1 [w/w]; 7 M urea; 1× TAE). The temperature ranges were between 54 and 64°C, temperature ramp rates between 0.6 and 3°C h⁻¹, electrophoresis time was between 3 and 15 h according to the temperature range and ramp rate, and voltage was between 90 and 150 V. After completion of electrophoresis, the gels were stained in ethidium bromide and photographed under UV transillumination.

Sequencing of TTGE bands. For sequence determination of TTGE bands, small pieces were excised from the acrylamide gel and placed into sterile vials. After 20 µl sterilized water were added, the samples were subjected to passive diffusion (-20°C overnight, 12 h at 4°C). 10 µl of the supernatant were then used as template for a reamplification PCR. PCR amplification products were bidirectionally sequenced with an automated DNA sequencer (Applied Biosystems, model 310), using the Taq DyeDeoxy terminator sequencing kit as described by the manufacturer with 500 ng of template and the primers S-D-Bact-0008-a-S-20 and S-*-Univ-0536-a-A-18. Excising was checked by rerunning an aliquot of this PCR product on the

TTGE. PCR amplification products were bidirectionally sequenced with an automated DNA sequencer (Applied Biosystems, model 310), using the Taq DyeDeoxy terminator sequencing kit as described by the manufacturer with 30-90 ng of template and the primers S-D-Bact-0008-a-S-20 and S-*-Univ-0536-a-A-18.

Analysis of sequencing data. The FASTA search option for the EMBL database was used to search for closest phylogenetic neighbors. Sequences were submitted to the CHECK_CHIMERA program of the ribosomal database project (RDP) to detect possible chimeric artifacts (Kopczynski *et al.* 1994, Larsen *et al.* 1993). Further analysis was performed by using the program ARB (Strunk *et al.* 1999). The next relatives were inserted along with our sequences into the ARB environment. The sequences were initially aligned by using the ARB automatic aligner and then verified and corrected manually. PCR primer regions were excluded from phylogenetic analyses. To avoid possible treeing artifacts caused by multiple mutational changes and/or regions that could not be unambiguously aligned, 50% conservation filters were generated by using the appropriate tool of the ARB package. Only those positions which contained identical residues in at least 50% of all sequences of interest were included in the analysis. Filters were adjusted to the length of each sequence. The sequences were added to the consensus tree provided in the ARB database by the maximum-parsimony approach. The overall phylogenetic affinity was evaluated using a consensus filter for all bacteria. This resulted in the use of 368 to 555 aligned sequence positions for our own sequences and 1152 to 1724 aligned positions for the next relatives. For accurate phylogenetic reconstruction, group specific filters were used on subdivision, order or family level for each sequence using 309 to 515 aligned sequence positions for our own sequences and 1152 to 1537 aligned positions for the next relatives.

FISH with group specific probes. One of the filters of the sample collection (see above) was thawed, the cells were washed off and used for FISH (Glöckner *et al.* 1996). Cells were concentrated on 47 mm diameter polycarbonate filters (pore size 0.2 µm, type GTTP; Millipore, Volketswil, Switzerland). Filter sections were hybridized with 150 ng of CY3-labeled probe. Cells were viewed with an Olympus BX50 epifluorescence microscope (Olympus Optical, Volketswil, Switzerland). The following group specific probes (Manz *et al.* 1992) were used: EUB338 (domain *Bacteria*), ALF1b (alpha *Proteobacteria*), BET42a (beta *Proteobacteria*) and GAM42a (gamma *Proteobacteria*). In addition, a probe specific for *Archaea* was used.

Cultivation of pelagic microorganisms of Jöri lake III. 100 ml surface water of Jöri lake III were collected in sterilized polypropylene bottles and transported at 4°C to Zurich. Aliquots of 0.1 ml were plated on agar surfaces with 10-fold diluted Luria-Bertani (LB) medium. Colonies were distinguished after colors and forms and inoculated to a liquid LB medium. After letting them grow for several days, the pureness of the culture was checked by plating an aliquot on agar surfaces with LB medium.

Experiments with limnocorrals. For the construction of the limnocorrals, the design of Cuker *et al.* (1990) was modified. We used open cylinders made of polyethylene film (volume 13 m³, diameter 1.9 m, depth 4.5 m). The bottoms were held open by steel rings. Hoops of 2.5 cm in diameter PVC pipe maintained the shape of the cylinder tops. Polyurethane foam (20 x 2 cm) was inserted into sleeves at the top of the columns to provide flotation and a barrier to waves. In contrast to the design of Cuker *et al.* (1990), who pressed the bottoms into the sediment, we kept the enclosures floating in open water. The bottoms were closed with a polyethylene ring. The limnocorrals were installed in the clear lake VII in 1997 and 1998 and manipulated with NH₄NO₃ (800ppb), KH₂PO₄ (50ppb), and sterilized erosion particles from lake XIV (200g) and all the combinations of it. One limnocorral was untreated for control. Weekly controls of the P, N, and particle concentrations were performed and adjusted if necessary. The weekly to bi-weekly samplings included chlorophyll *a*, bacterial abundance and samples for TTGE.

Nucleotide sequence accession numbers. Nucleotide sequences have been deposited in the GenBank database under the accession numbers AF187307 - AF187316.

3 RESULTS

3.1 *Climatology in 1997 and 1998*

Meteorological aspects of the Jöri region have been discussed in Hinder *et al.* (1999). Here, we will mention ecological pertinent observations for the two sampling years 1997 and 1998:

June and July of 1997 were not only colder and had less radiation than the same months in 1998 (Tab. 1), but there was also a much higher maximal snow water equivalent to begin with. The lake ice melted at the beginning of August - more than one month later than in 1998 - which caused large differences in the lake processes. June and July 1998 were exceptionally warm, the lake was ice-free by the middle of July and stratification established rapidly. In both years, August was warmer than the long-term average. While the start of the ice-free

season was cold and cloudy in 1997, the end was warmer and sunnier than the year after. The lakes froze towards the end of October in both years.

Tab. 1: Air temperature (Temp, °C), precipitation (Prec, mm) and radiation (Rad, W m⁻²) at Jöri lake III. Long term averages (1981-1998) were taken from the SMI (Swiss Meteorological Institute) station at Weissfluhjoch, situated 13 km from the Jöri lakes at an altitude of 2690 m a. s. l.

	1997			1998			1981-1998		
	Temp	Prec	Rad	Temp	Prec	Rad	Temp	Prec	Rad
June	2.7	232	196	4.1	203	256	2.3	167	239
July	3.6	265	195	6.0	194	204	5.8	182	223
August	6.6	182	186	7.0	118	202	5.8	171	192
September	6.4	45	206	1.9	171	140	2.9	126	163
October	-0.3	83	127	-1.7	164	106	0.2	69	125
Average	3.8 ± 2.7		182 ± 32	3.5 ± 3.5		182 ± 59	3.4 ± 2.4		188 ± 46
Total		807			850			715	

In early summer 1998, lake III was fed by large volumes of turbid glacial melt water, which caused a high turbidity (Tab. 2). Also the total phosphorus (TP) and total inorganic nitrogen (TN) concentrations were significantly higher in 1998 than in 1997. These values always paralleled the turbidity which indicates that the particles carried phosphate and nitrogen. Chlorophyll *a* concentrations were higher in 1998 than in 1997, probably due to the favorable growing conditions at the beginning of the ice-free period in that year (Tab. 2).

Tab. 2: Physical and chemical characteristics in Jöri lakes III and VII in 1997 and 1998 (n = number of samples, Temp. = temperature, Cond. = conductivity, TN = total inorganic nitrogen, TP = total phosphorus, Chl *a* = chlorophyll *a*).

	<i>n</i>	Temp. [°C]	pH	Cond. 25°C [μS cm ⁻¹]	Turbidity [NTU]	TN [ppb N]	TP [ppb P]	Chl <i>a</i> [μg l ⁻¹]
<i>lake III</i>								
1997	9	8.6 ± 7.3	7.2 ± 0.5	17.9 ± 3.9	4.7 ± 0.9	204 ± 61	9 ± 8	0.8 ± 0.2
1998	7	8.0 ± 4.1	7.0 ± 0.4	19.7 ± 4.9	26 ± 25	362 ± 59	20 ± 7	1.3 ± 0.8
<i>lake VII</i>								
1997	5	8.4 ± 6.7	6.6 ± 0.3	11.8 ± 3.7	1.4 ± 1.1	186 ± 15	9 ± 4	0.9 ± 0.3
1998	5	9.2 ± 4.4	7.3 ± 0.3	11.2 ± 2.3	2.6 ± 1.1	288 ± 26	15 ± 7	2.1 ± 1.3

3.2 Community analysis by TTGE

Lake III. Although the weather situation was distinctly different in 1997 and 1998, the pattern on the TTGE gel was the same in both years. The abundance of the fragments was different, however (Fig.1). In 1998, bands 12, 9, and 11b were weak or did only occur during a short period.

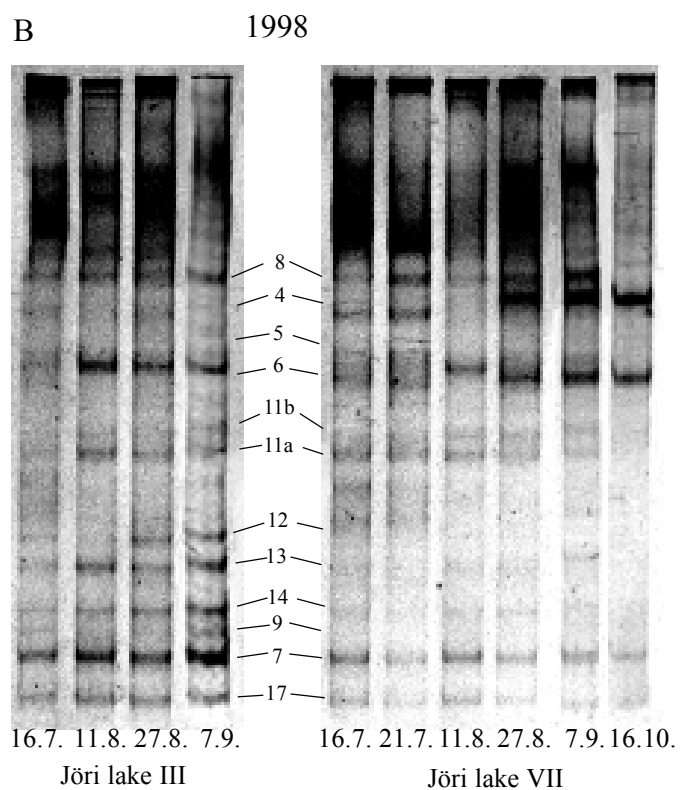
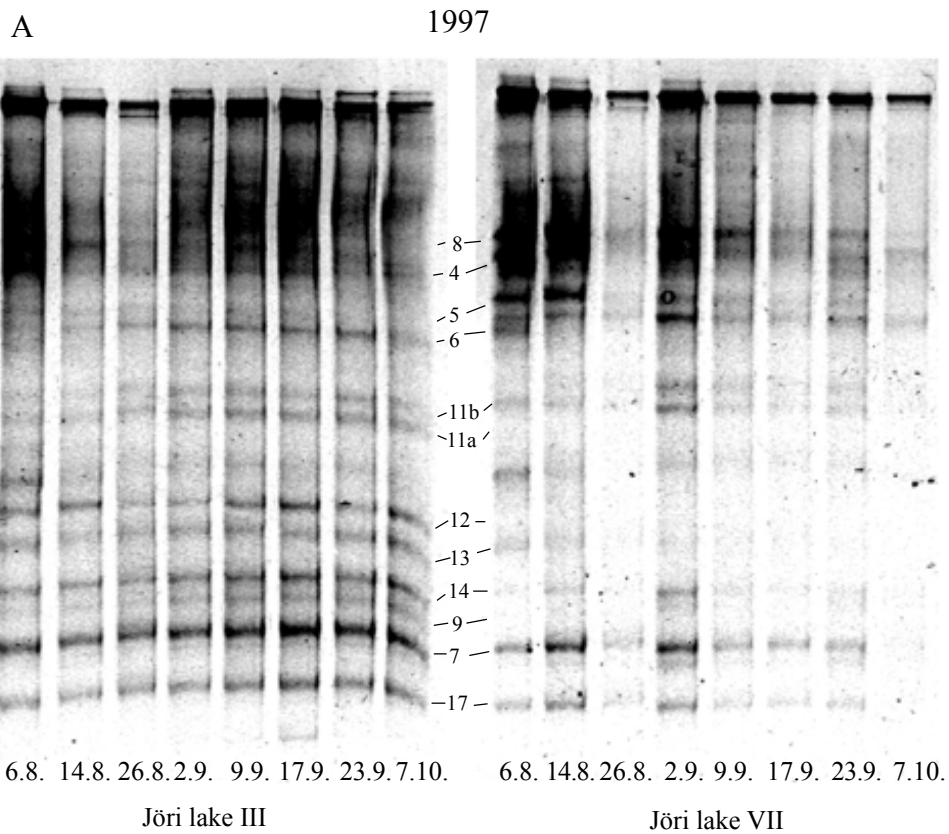


Fig. 1: TTGE patterns of surface water samples of Jöri lake III and VII in 1997 (A) and 1998 (B). Samples were collected between August and the beginning of October in 1997 and between July and October in 1998.

The most fundamental change in the community structure occurred during and shortly after the period of icebreak at the beginning of August in 1997 and in the middle of July in 1998. While the bands 12 and 14 were detected immediately after the icebreak in 1997, they were only found towards the end of summer in 1998. Band 7 was present in both years during the entire ice-free season. The highest number of bands was always found in September. Most of the bands persisted until the time when the ice cover was formed again.

Lake VII. As in lake III, the occurrence of the DNA fragments detected by TTGE did not differ significantly in 1997 and 1998 (Fig. 1). As in lake III, the most evident changes were observed after the period of ice-break. In contrast to lake III, the seasonal succession and the dominance of the fragments were similar in both years.

Comparison of lake III and VII. Some fragments obtained from lake III were not found in the clear water lake VII: Band 12 is missing, bands 4, 5 and 6 are more pronounced whereas band 14 is less dominant. The seasonal succession shows a different trend as well: towards the end of the ice-free period, almost all bands disappear except bands 4 and 6. The highest number of bands in lake VII was slightly less than in lake III.

Phylogenetic affinity. Up to sixteen different bands could be distinguished on TTGE gels of water samples of the lakes III and VII (Fig. 1). The bands were excised and the DNA was sequenced. Most of the sequences have high similarities with those of so far uncultured bacteria (Fig. 2), others have a low similarity with known sequences. Eight different bands of the TGGE gel and two isolates of Jöri lake III were sequenced and their sequences were added to a phylogenetic tree constructed using the program ARB (Fig. 2). Sequences were named using the prefix J for Jöri lake followed by a roman numeral identifying the lake of origin and the band number from the TTGE gel pattern and the sampling year; e.g. band 4 becomes JIII-04-98 (Tab. 3).

Tab. 3: Sampling sites and dates of the sequenced fragments, their nomenclature and accession numbers.

<i>Accession number</i>	<i>Sequence number</i>	<i>Sampling site</i>	<i>Date of sampling</i>	<i>Phenotypic characteristics</i>
AF187307	JIII-04-98	Lake III	27.8.98	uncultured
AF187308	JIII-05-98	Lake III	27.8.98	uncultured
AF187313	JVII-06-98	Lake VII	7.9.98	uncultured
AF187309	JIII-07-98	Lake III	27.8.98	uncultured
AF187314	JVII-08-98	Lake VII	27.8.98	uncultured
AF187310	JIII-12-97	Lake III	23.9.97	uncultured
AF187311	JIII-15-98	Lake III	20.10.98	culture 'yellow'
AF187312	JIII-17-98	Lake III	27.8.98	uncultured

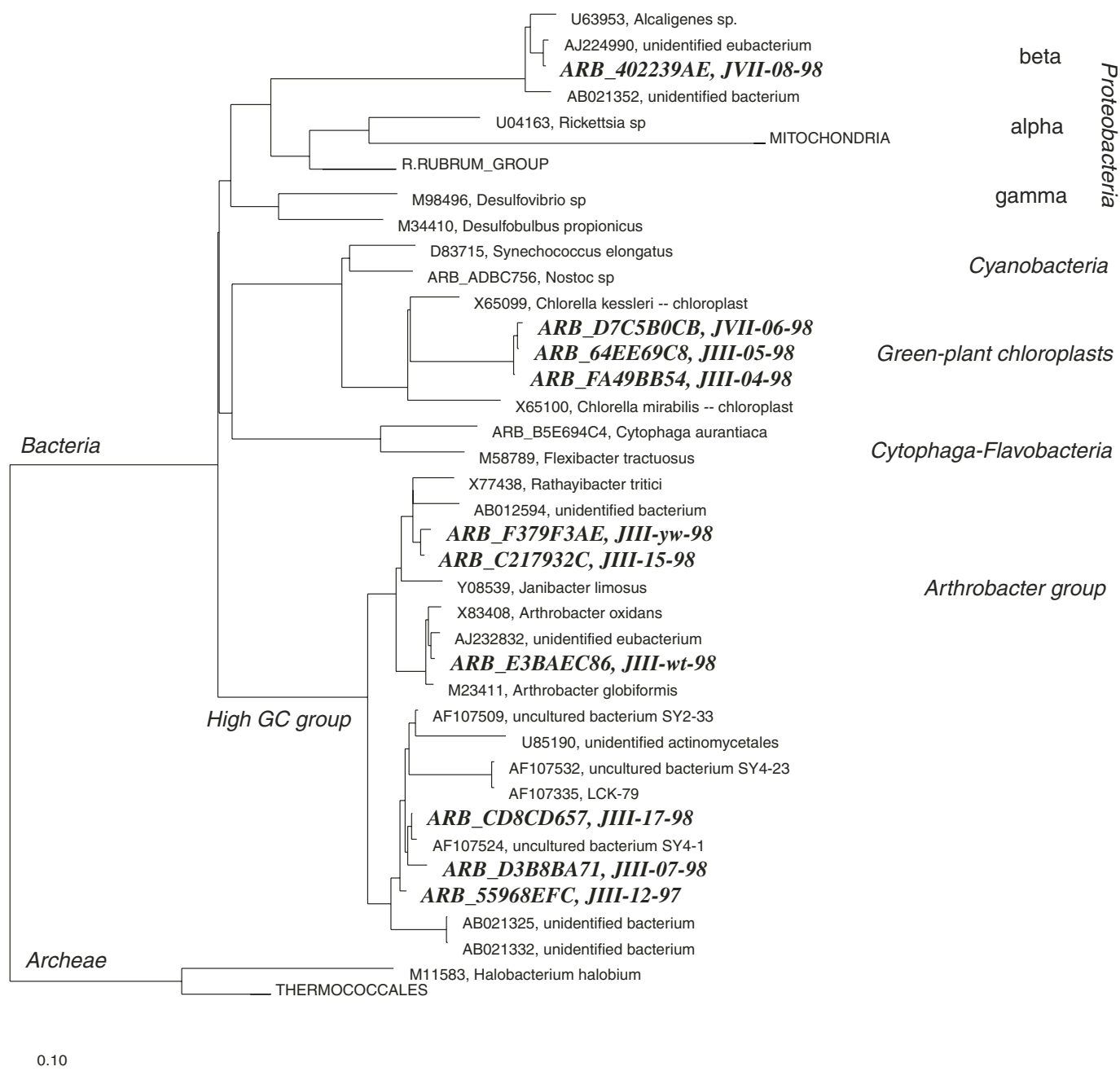


Fig. 2: Phylogenetic affinity of sequenced fragments excised from TTGE gels of Jöri lakes III and VII.

Sequences JIII-04-98, JIII-05-98 and JVII-06-98 showed only low similarities to known sequences. They matched most closely in the group of green plant chloroplasts (87% similarity) and with the group of cyanobacteria (85% similarity). Interestingly, sequences JIII-07-98, JIII-12-97 and JIII-17-98 branched into a new cluster within the group of *Actinomycetales*. The closest relatives are the uncultured bacteria SY1-4 found in Lake Soyang, Korea (Han *et al.* 1999). In this group we also find several other unidentified bacteria, which originate from mountain lakes: an other unidentified *Actinomycetales* (accession number U85190), which was found in a lake in the Adirondack Mountains of New York State (Hiorns *et al.* 1997) and LCK-79 (accession number Af107335), which was found in Lake Cadagno, Switzerland.

Two isolates from lake III (cultures ‘yellow’ and ‘white’) belong to the *Arthrobacter* group. Sequence JIII-15-98 and the yellow culture are identical. The white culture is closely related to *Arthrobacter globiformis*, which is normally associated with terrestrial habitats. Bahr *et al.* (1996) have found this species in an oligotrophic arctic lake and suggested a particle-associated origin for these cell types. This corroborates with our findings that bacteria in the particle-rich Jöri lake could live particle-associated profiting from higher nutrient concentrations on particle surfaces. The nearest relative of our isolate JIII-15-98 (98% similarity) was an unidentified soil bacterium (accession number AJ232832) found by Marilley and Aragno (1998).

Sequence JVII-08-98 was positioned in the *Bordetella* group and is probably identical with the clone GKS98 (accession number AJ224990) found by Pernthaler *et al.* (1998) in an Austrian high mountain lake (Gössenköllesee). Clone GKS98 is a rod-shaped bacterium, 2 to 4.5 µm long. It was most abundant during the period of ice formation and then declined below the limit of detection during early spring.

Bacterial community as revealed by FISH. To check the abundance of the bacterial groups found by sequencing in the natural habitat, FISH was applied with a sample of lake III taken on 7.9.98. 57% of all DAPI-stained objects could be visualized with bacterial probe EUB338. As in other lakes (Bahr *et al.* 1996, Hiorns *et al.* 1997, Pernthaler *et al.* 1998) organisms belonging to the beta *Proteobacteria* predominated (26% of all DAPI-stained cells). Alpha *Proteobacteria* represented the second abundant group with 10% of all DAPI-stained cells, whereas gamma *Proteobacteria* (4%) and *Archaea* (2%) were scarce. These findings correspond to the observations of Pernthaler *et al.* (1998) who showed that pelagic archaea were abundant only during autumn and hardly present thereafter.

3.3 Interactions of suspended inorganic particles and nutrients and their effect on the microbiota

We employed limnocorrals in lake VII to study the effect of suspended particles on the nutrient availability for the microbiota. Although limnocorrals do not reflect the natural habitat and many environmental determinants like light, internal seiches, and wind are different from the open water, they allow one to study phenomena that cannot be imitated in the laboratory or be observed under steady state conditions in natural environment. An undisturbed limnocorral served as control for all experiments.

Experiments of Summa (1998) showed that the suspended particles from the Jöri lakes carry substantial amounts of ammonia. The binding sites are not saturated and still able to adsorb more ions. Erosion particles have a high affinity to adsorb ammonia (Scheffer and Schachtschabel 1979). It remains to be shown whether the particle-associated part of ammonia is bioavailable or not. Lab experiments of Theis (1999) showed that adding particles to a dilute media significantly enhanced the growth of *Rhodotorula glutinis* (an isolate of the Jöri lakes) and decelerated the die-off rate. Suspended particles in Jöri lake III may thus positively influence the living conditions for certain microorganisms.

The band pattern on the TTGE gel did not change in corrals to which particles were added (Fig. 3). Feeding with KH_2PO_4 and NH_4NO_3 resulted in a visible change on the TTGE pattern, however: band 8 vanishes in nutrient amended corrals. This corroborates with the sequence analysis indicating that band 8 represents a typical mountain lake bacterium which was only found in oligotrophic mountain lakes so far. While band 8 vanishes, band 4 - which is probably a sequence of a chloroplast - becomes stronger. Band 7 is fading in the nutrient-enriched corrals whereas band 14 becomes stronger. The sequences of these two bands are closely related to each other. Furthermore, it seems that the addition of particles together with N weakens the effect of adding N only. This is in agreement with the findings that ammonia is bound tightly to particles (Summa 1998).

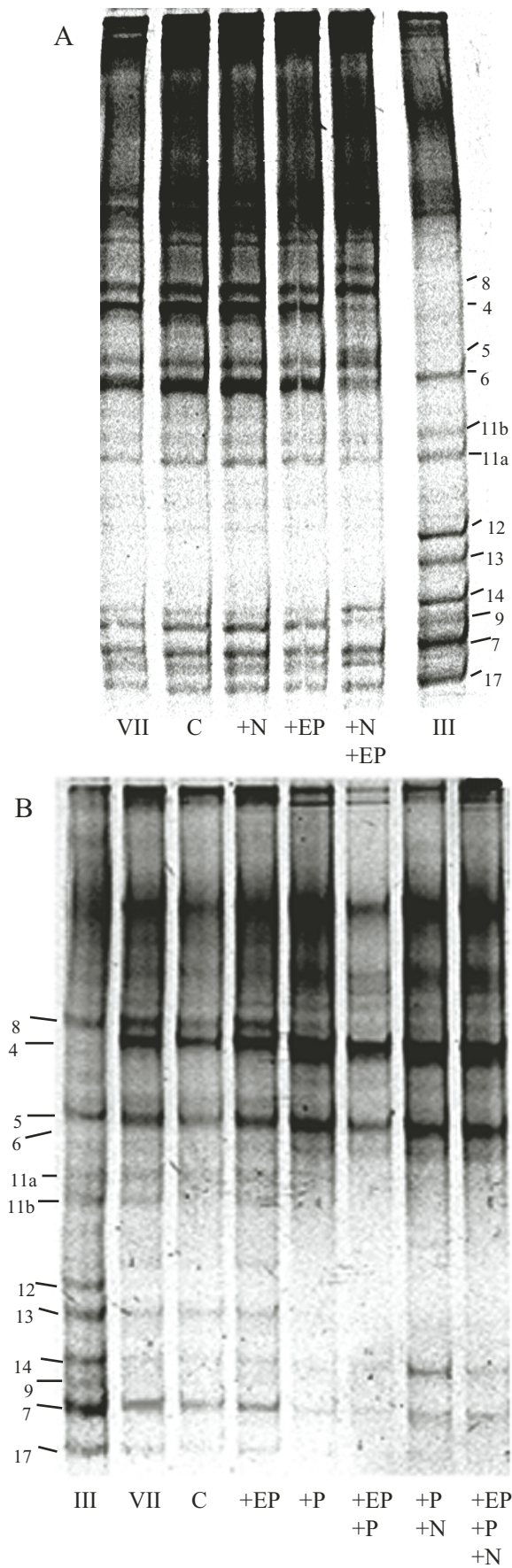


Fig. 3: TTGE pattern of the experiments with the limnocoralls in 1997 (A) and 1998 (B). III: Jöri lake III, VII: Jöri lake VII, C: control, EP: addition of erosion particles, P: addition of K_2HPO_4 , N: addition of NH_4NO_3 .

The chlorophyll *a* concentration reflects a clear response to the changes in the trophic level (Tab. 4). In the control corral, chlorophyll *a* concentrations were more or less the same as in the open lake. The addition of particles had no effect on the chlorophyll *a* concentration. Feeding KH_2PO_4 almost doubled the chlorophyll *a* concentration which suggests that the growth of the phytoplankton is P-limited. Adding particles to P-amended corrals had no effect which indicates that the interaction between particles and KH_2PO_4 is weak. Adding KH_2PO_4 and NH_4NO_3 led to a threefold increase in chlorophyll *a* concentration as compared to the concentration in the control corral while adding only NH_4NO_3 had no effect (data not shown). P was apparently the first limiting factor for the growth of the phytoplankton and N became the second one, once P-limitation was overcome. Interestingly, the chlorophyll *a* concentration was diminishing again when particles were added to the P- and N-amended corral, suggesting that the particles are able to bind N. This coincides with the findings of Summa (1998) that erosion particles of the Jöri lakes have a considerably capacity to bind ammonium.

Tab. 4: *In situ* experiments in lake VII in 1998 with limnocorrals: effect of adding erosion particles (EP), KH_2PO_4 (P), and NH_4NO_3 (N) on the chlorophyll *a* concentration.

<i>Manipulation</i>	<i>Chlorophyll a [$\mu\text{g l}^{-1}$]</i>		
	<i>September 7</i>	<i>September 29</i>	<i>October 17</i>
Lake VII outside corrals	3.5	2.6	1.9
Control	3.3	3.2	2.2
+EP	3.7	3.5	1.8
+P	6.3	5.7	3.1
+EP, +P	6.4	6.2	3.9
+P, +N	13.2	10.2	3.9
+EP, +P, +N	9.8	5.4	2.4

4 DISCUSSION

Although hardly all species are detected by TTGE, the method is a powerful tool to follow seasonal successions of the species present and to display community changes in natural waters. In both lakes, III and VII, we observed a distinct succession of species in 1997 and 1998 – two years with very different weather situations. There are only few data available about seasonal changes in microbial populations in mountain lakes (Pernthaler *et al.* 1998, Alfreider *et al.* 1997). In the Jöri lakes, the most pronounced changes occurred after the icebreak and some species were dominant only during this period. The icebreak period is a major promoter of community transition (Pernthaler *et al.* 1998) mostly due to (i) the allochthonous nutrient input from the winter ice cover and the melt water from the surroundings, (ii) the subsequent thermal mixing of the water body followed by stratification and (iii) the increasing light intensity. The importance of the date of icebreak for the phytoplankton has been reported by Hinder *et al.* (1999). The same is also true for bacterial populations as the heterotrophic processes are coupled to the primary production. The causes of the population dynamics observed remain unknown, however.

The diversity and composition of the bacterial communities as revealed by TTGE were not basically different in the lakes III and VII, although lake VII showed fewer bands, and the dominance of the bands differed from lake III. This is not only due to the presence of inorganic particles in lake III, also depth, exposure to wind, mixing of the water body etc. might have large influences. In 1998, when lake III was very turbid and higher TP and TN concentrations were measured, slightly fewer species were detected than the year before. This coincidences with the results of the limnocorral experiments where the bacterial diversity decreased when particles and the nutrients N and P were added. The differences observed on the TTGE pattern were smaller in the clear water lake VII. The influence of suspended particles in a lake are multifaceted. They not only affect the physical characteristics, e.g., the light quality and penetration, but also chemical determinants. Due to the mostly negative charges, they interact with the cations present in the lake water. This is supported by the observation that TN and TP always parallel the turbidity in lake III. Summa (1998) showed in laboratory experiments that particles from the Jöri lakes carry substantial amounts of ammonia. Henley (1998) reported interactions between particles with ammonia and phosphate as well. In 1998, when solar insolation was high and much more turbid melt water from the glacier percolated into the lake, the total concentrations of N and P were elevated as well.

Interactions between particulate matter and nitrogen and phosphorus is a well known phenomenon (e.g. Fukushima *et al.* 1991) but has never been reported for glacial mountain lakes. The turbidity of the Jöri lakes is very different from one year to the next due to different weather situations. This is one explanation for the conspicuously high variations of nutrient concentrations in different years and it represents one factor for the seasonal differences in the community structure of the plankton.

In lake III, the group of beta *Proteobacteria* was quantitatively most abundant as it is also reported for other lakes (Bahr *et al.* 1996, Hiorns *et al.* 1997, Pernthaler *et al.* 1998). None of the sequences of the excised fragments can be correlated to a known culturable organism but several are identical or closely related to sequences of other unidentified bacteria listed in the EMBL databank. Interestingly, some of these were collected in mountain lakes as well, another one was found in an arctic lake which is a similar habitat to mountain lakes. These findings suggest that there are bacteria which well adapted to live in these cold habitats where nutrient concentrations are low, rapid hydrological changes are common and the ice-free periods are very short.

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Microbial Food Web in an Oligotrophic High Mountain Lake (Jöri Lake III, Switzerland)

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ABSTRACT

Jöri lake III (2512 m a.s.l., $z_m = 22$ m, $A = 57.81 * 10^3$ m², $V = 601.1 * 10^3$ m³) is situated in the Vereina region in the eastern part of the Swiss Alps. We studied microbial grazing on bacteria and bacterial productivity during the ice-free period. The lake normally gets thermally stratified for two months between July and September. In 1996, chlorophyll *a* concentrations varied from 0.5 to 2.0 µg l⁻¹ with maximum values just below the thermocline (6 m depth), in 1997, they were between 0.6 and 5.0 µg l⁻¹ with maximum values at 10 m depth – several meters below the thermocline. Bacterial densities varied between 0.7 and $1.7 * 10^6$ ml⁻¹ with maxima in the thermocline, one to two meters above the chlorophyll maximum. The areal bacterial biomass (volume beneath 1 m² to a depth of 8 m) was 10 µg C l⁻¹ which remained more or less constant for the periods investigated. In 1997, bacterial growth rate and production rates were determined using [³H]-thymidine incorporation. The rates were as low as 0.002 to 0.006 h⁻¹ and 0.01 to 0.03 µg C l⁻¹ h⁻¹, respectively. We found a carbon ratio of bacteria, phytoplankton, and autotrophic picoplankton (APP) of 1.5 : 1.1 : 1 which shows a rather high abundance of bacteria and autotrophic picoplankton (APP) compared to larger phytoplankton. Bacterial growth followed a temperature dependence similar to the one observed for bacteria from Lake Zürich, a prealpine and mesotrophic lake which was studied for comparison. Microbial food web in Jöri lake III was not top down controlled during the periods of our study and mixotrophic algae like *Dinobryon cylindricum* var. *alpinum* and autotrophic nanoflagellates (ANF) were the dominant bacterial grazers observed.

KEY WORDS: alpine lake, microbial food web, bacterial growth rates, autotrophic picoplankton, mixotrophic algae.

1 INTRODUCTION

Productivity and nutrient cycling in high mountain ecosystems are governed by the extremely variable meteorological conditions. One of the characteristics of high mountain lake ecosystems is the long period of ice cover which may persist from October to July. High mountain lakes normally have low concentrations of nutrients as long as the drainage area is not fertilized and no wastewater is released to the catchment (Niederhauser 1993, Mez *et al.* 1998). In some lakes the nutrient budget is further affected by high turbidity due to suspended erosion particles from nearby glaciers. These inorganic particles can adsorb nutrients and affect the nutrient balance of the entire lake. Moreover, organic and inorganic matter transported over long distances as dust via the atmosphere from highly fertilized agricultural land can carry substantial amounts of nutrients with it (Schanz 1984). Suspended particles not only diminish light penetration, they also change the light quality thereby affecting growth and composition of the phototrophic plankton (Wyman and Fay, 1986, Glover *et al.* 1987, Talling 1971).

Primary production is low in oligotrophic high mountain ecosystems (Tilzer 1972) and low productivity of bacterial biomass is expected as well since the heterotrophic bacterial production depends on the release of oxidizable organic carbon by photosynthetic organisms (Vrede 1996; Laybourn-Parry and Walton 1998). This was found by Tilzer (1972) in the Vorderer Finstertaler Lake (Austria), and it was reported for ultra-oligotrophic Antarctic lakes (Laybourn-Parry *et al.* 1995). There is a contradiction in reports which state that the significance of bacteria increases at low primary productivity and that bacterial production can temporarily exceed primary production (Scavia and Laird 1987, Lavandier 1990, Coveney and Wetzel 1995). This implies that the ratio of phytoplankton and bacterial biomass depends on other sources of organic carbon than exudates of phytoplankton. In oligotrophic lakes, bottom-up forces are strong compared to top-down grazing control. Algal taxa capable of mixotrophic modes of nutrition become more important and replace heterotrophic nanoflagellates (HNF) and ciliates in the foodweb (Jones 1994). Similarly, the importance of autotrophic picoplankton (APP) is expected to increase in nutrient-limited habitats, since small organisms have a higher surface to volume ratio than larger phytoplankton species. One might expect 40 – 60 % of the total phytoplankton carbon being contributed by APP in oligotrophic ecosystems (Stockner 1991).

The scientific interest in the limnology of the Jöri lakes dates back to the beginning of the century. Kreis (1921) studied the fauna in 13 Jöri lakes and ponds and described the morphology of the lakes for the first time. When Messikommer investigated the phytoplankton in lakes of the Davos region in 1942, he included two of the Jöri lakes (Messikommer 1942). Renewed interest in studying the Jöri lakes emerged in connection with investigations on climate change impacts on the hydrological cycle in mountain regions. Comprehensive physical, hydrological, chemical and biological studies have been conducted in the Jöri catchment since 1996 (Hinder *et al.* 1999). In the frame of the European Project MOLAR (Mountain Lake Research, Patrick *et al.* 1998) the dynamics of the bacterioplankton was investigated in Jöri lake III. We studied growth and loss rates of heterotrophic bacteria in order to understand the significance of the microbial loop in oligotrophic lakes as outlined by Azam *et al.* (1983) for sea ecosystems. Lake Zürich was investigated to check the methodology of the determination of the bacterial production.

2 STUDY SITE

Jöri lake III is one of 21 small lakes and ponds situated at elevations between 2489 and 2730 m a. s. l. in the Vereina region in eastern Switzerland. Crystalline rocks dominate in the drainage area. All lakes get formed as the Jöri glacier retreats. Today, the glacier ends several hundred meters behind lake III but turbid glacial erosion water percolates through the front moraine and enters the lake at subsurface springs. Lake III has a maximal depth of 22 m. The water level is highest during the ice and snow melt period and falls continuously by up to 3 m during fall and early winter. The maximal surface area and volume are 57810 m² and 601100 m³, respectively. Surface temperatures may reach 15° C in August when a stable thermocline gets established. The high concentration of suspended erosion particles creates turbidity which causes strong light attenuation: In August 1996 K_d was 0.5 m⁻¹, in July and August 1997 it was 0.6 m⁻¹. Alkalinity and pH were 65 to 80 µeq l⁻¹ and 6.4 to 6.9, respectively, during the sampling periods. Conductivity (20° C) was 14 to 20 µS cm⁻¹, total phosphorous < 0.7 µM, and total organic carbon 0.7 mg C l⁻¹. The algal division Chlorophyta (e.g. *Eutetramorus fottii*, *Dictyosphaerium subsolitarium*, *Monoraphidium subclavatum*) represents the major fraction of the total phytoplankton biomass in Jöri lake III (Hinder *et al.* 1999). There are also representatives of the divisions Chrysophyta (e.g. *Dinobryon cylindricum* var. *alpinum*, *Kephyrion doliolum*), and Cryptophyta (e.g. *Rhodomonas lacustris*). It is striking but ecologically meaningful that some of

them are flagellates and potentially mixotrophic: e.g. *Dinobryon cylindricum* var. *alpinum* and *Kephyrion doliolum*. The lake is regularly stocked with fish (*Salvelinus namaycush*, *Salmo trutta* var. *fario*). The zooplankton community is dominated by rotatoria (*Polyarthra* sp. and *Keratella* sp.). Copepoda (*Arctodiaptomus alpinus* and *Cyclops abyssorum* var. *tatricus*) are also abundant, while *Chydorus sphaericus* is very scarce (pers. communication J. Fott and J. Cejkova).

3 MATERIAL AND METHODS

3.1 Sampling

Samples in Jöri lake III were taken at the location of maximum depth with a 3 liter home-made plexiglass sampler (Charles University, Prag) from July to October 1996 and in July and August 1997. Alkalinity, pH, and total phosphorus were determined as described by Niederhauser (1993). Chlorophyll *a* (chl *a*) concentration was measured using the fluorimetric determination after Schanz (1982). Bacterial numbers in Jöri lake III were determined in layers from the surface to 8 m depth (63% of the total water volume). Due to the large number of erosion particles it was difficult to recognize the bacterial cells in deep layers.

3.2 Bacterial and autotrophic picoplankton (APP) densities and biomass

Bacteria samples were fixed with glutaraldehyde (2% final concentration, v/v) immediately after sampling and stored in 50 ml polyethylene bottles at 4° C in the dark. Bacteria were stained with DAPI (final concentration 1 - 1.5 mg l⁻¹; Porter & Feig 1980), filtered through membrane filters (25 mm diameter, 0.2 µm pore size) and counted using an epifluorescence microscope (Leitz Dialux 20, objective 100x). For the samples collected in 1996 Anodisc filters (Whatman, U.K., Nr. 6809 6022) and for the 1997-samples Black Nuclepore filters (Costar, U.S.A., Nr. 8242) were used. Anodisc filters were shown to give up to 30% higher cell counts (Jones *et al.* 1989). At least 400 cells were counted in eight or more randomly chosen grids. Bacterial volume was estimated by semi-automatic image analysis (Psenner 1993). An allometric model was used (Loferer-Kroessbacher *et al.* 1998) for the conversion of measured biovolumes (in µm³ l⁻¹) to biomass (in mg C l⁻¹). For studies on short time dynamics of the bacterial population, we sampled at six depths every three hours over several days during the period from July to September 1996. APP-counting was performed by flow cytometry with samples collected in August and October

1996. For this purpose samples were first filtered through a 50 μm -net, 1.5 ml of the filtered sample were then fixed in a 2 ml cryotube with 150 μl of a paraformaldehyde/glutaraldehyde solution (Marie *et al.* 1996) and stored at -80°C until analyzed. The analysis was carried out at the Biological Station in Roscoff (F). The APP biovolume was calculated from the counted cell numbers using an estimated cell diameter of 2 μm (average value for eukaryotic picoplankton, Reynolds 1984). The biovolume was transformed to carbon using the conversion factor of 200 $\text{fg C } \mu\text{m}^{-3}$ (Weisse 1993).

3.3 *Phytoplankton biomass*

For Jöri lake III, phytoplankton biomass was calculated from the micro- and nanophytoplankton biovolumes (Hinder *et al.* 1999).

3.4 *Bacterial activity and bacterial production rate*

The [^3H]thymidine method (rate of [^3H]thymidine incorporation, $\text{pmol l}^{-1} \text{h}^{-1}$) described in detail by Straskrbova *et al.* (1999) was used. To convert the [^3H]thymidine-incorporation rate ($\text{pmol l}^{-1} \text{h}^{-1}$) into cell production rate ($\text{cells l}^{-1} \text{h}^{-1}$), a factor of $2 * 10^6$ ($\text{cells pmol}^{-1} [\text{H}]^3\text{thymidine}$) was used for Jöri lake III samples (Bell 1990). Cell production rate was converted to bacterial production rate (in $\mu\text{g C l}^{-1} \text{h}^{-1}$) using the allometric model factors ($\mu\text{g C cell}^{-1}$) determined for Jöri lake III after Loferer-Kroessbacher *et al.* (1997).

3.5 *Specific bacterial growth and loss rate*

The specific bacterial growth rate μ (h^{-1}) was calculated based on the number of cells produced ΔN (cells l^{-1}) during the incubation time t , and the cell concentration at the beginning N_0 (cells l^{-1}) as $\mu = t^{-1} * \ln(1 + (\Delta N * N_0^{-1}))$.

Alternatively, specific bacterial growth was determined by the dilution culture method after Painchaud *et al.* (1996). Two cultures from each water sample are needed: 750 ml of undiluted sample, and 750 ml of a mixture (1:4) of undiluted sample and 0.2- μm -filtered water were incubated for 66 h at the sampling depth. Each experiment was sampled five times. From the net growth rates, μ_{net} , of each sample one calculates gross growth and loss rates, μ_{gross} and v , respectively. At the same time, a second experiment was carried out replacing the untreated water by 3- μm -filtered water in order to measure loss rates caused by grazers $< 3 \mu\text{m}$ and autolysis.

4 RESULTS

4.1 Bacterial morphology, biomass and abundance

In 1996 Jöri lake III began to stratify in July. At the beginning and at the end of August the depth profiles of temperature and chl *a* concentration were not subjected to daily changes (Fig. 1). During this period, bacterial numbers fluctuated between 0.7 and $1.7 \cdot 10^6$ cells ml^{-1} . A pronounced maximum was found between 4 and 6 m in the thermocline.

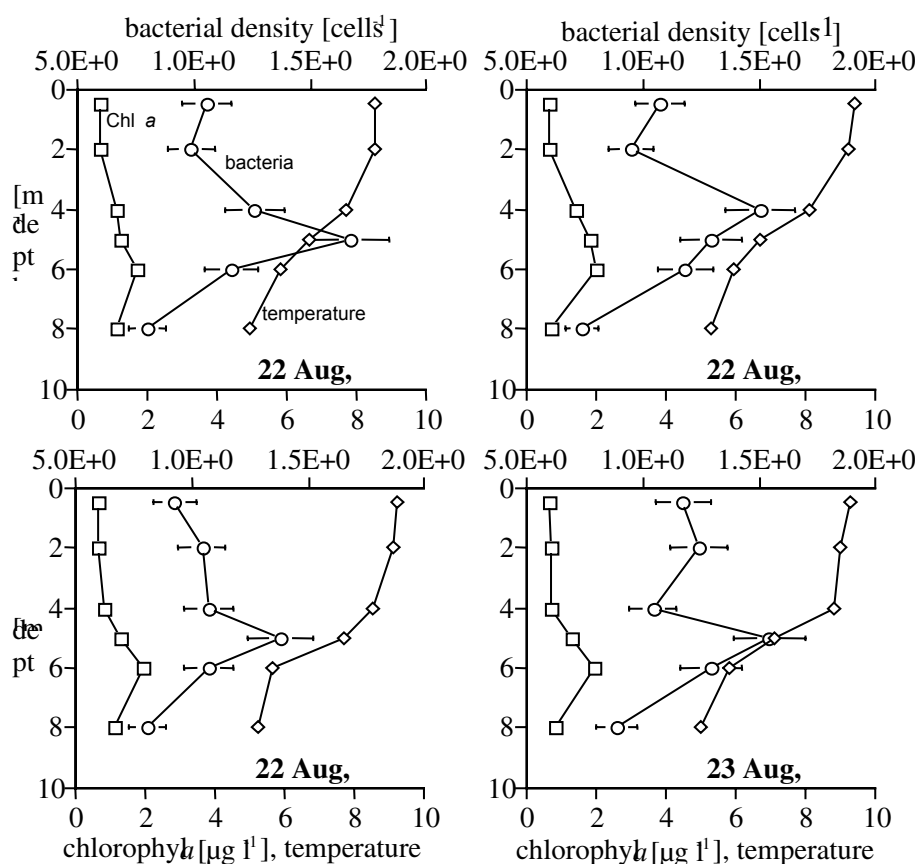


Fig. 3: Profiles of chlorophyll *a*- concentration, temperature and bacterial density during a daily cycle on August 22 and 23, 1996 in Jöri lake III. Bars are 95% confidence intervals of the means of bacterial density.

On August 22 this maximum was displaced from 5 m at 7 a.m. to 4 m at 1 p.m., and back to 5 m by 7 p.m. Temperature changed up to 1°C in the depth interval from 4 to 6 m which points to activities of internal seiches. Chl *a* concentrations varied between 0.5 and $2.0 \mu\text{g l}^{-1}$. Its maximum was at the bottom of the thermocline in 6 m depth, usually 1 m below the maximum of the

bacteria. By the beginning of September the thermocline and stratification of bacteria and chl *a* had vanished.

The differences of the areal bacterial concentration (integral over the depth interval from 0 to 8 m, in cells m⁻²) determined in three hour-intervals on ten days between July and September 1996 fluctuated between -0.06 and +0.04 h⁻¹, but the so calculated net growth rates were insignificant at the 5%-confidence level. For two dates, August 9 (between 7 a.m. and 10 a.m.) and September 10 (between 1 p.m. and 4 p.m.) statistically significant net growth rates could be determined as $\mu_{\text{net}} = -0.06 \pm 0.03 \text{ h}^{-1}$ and $\mu_{\text{net}} = -0.06 \pm 0.02 \text{ h}^{-1}$, respectively.

The bacterial biomass at depths between 0 and 8 m was $12 \pm 2.3 \text{ } \mu\text{g C l}^{-1}$ ($n = 4$) during the ice free period of 1996. Bacteria were rod-shaped and rather small ($0.028\text{-}0.034 \text{ } \mu\text{m}^3 \text{ cell}^{-1}$) (Tab. 1). Their mean cellular C-content was $10.9 \text{ fg C cell}^{-1}$.

Tab. 1: Average length, width, volume and C-content of bacteria from Jöri lake III in 1997. *n* is the number of measured cells, \bar{x} is the mean and \bar{x}_s the standard error.

sample			length [μm]		width [μm]		volume [$\mu\text{m}^3 \text{ cell}^{-1}$]		C-content [fg C cell ⁻¹]	
date	depth	<i>n</i>	\bar{x}	\bar{x}_s	\bar{x}	\bar{x}_s	\bar{x}	\bar{x}_s	\bar{x}	\bar{x}_s
29 July	2m	447	0.470	0.009	0.305	0.003	0.028	0.001	10.2	0.3
31 July	1m	557	0.618	0.017	0.292	0.003	0.034	0.001	12.1	0.3
	5m	495	0.537	0.014	0.281	0.003	0.028	0.001	10.0	0.3
7 Aug	2m	662	0.544	0.022	0.304	0.003	0.033	0.001	11.5	0.3

4.2 Bacterial activity, growth and loss rates

Due to the late ice melt, Jöri lake III was only weakly stratified on the sampling days in July and August 1997 (Fig. 2). Bacterial densities, specific growth rates, and production rates did not differ significantly at the depths considered. Bacterial numbers were between 6.1 and $8.1 \cdot 10^5$ cells ml⁻¹. The specific growth rates varied between 0.002 to 0.006 h^{-1} and only very low amounts of [³H]thymidine were assimilated. Two out of ten samples showed uptake rates which differed

significantly from zero (t-test, $P < 0.02$), the ones determined for July 29 at 6 m and the ones from August 7 at 1 m depth. For both cases a bacterial growth rate of 0.003 h^{-1} was calculated resulting in production rates between 0.01 and $0.03 \text{ } \mu\text{g C l}^{-1} \text{ h}^{-1}$. The bacterial growth and loss rates determined by the dilution culture method were equal in the non filtered samples and in a fraction after filtering through $3 \text{ } \mu\text{m}$ pore-size filters. The values were $0.008 \pm 0.002 \text{ h}^{-1}$ which is the same order of magnitude as the values found with $[^3\text{H}]$ thymidine incorporation.

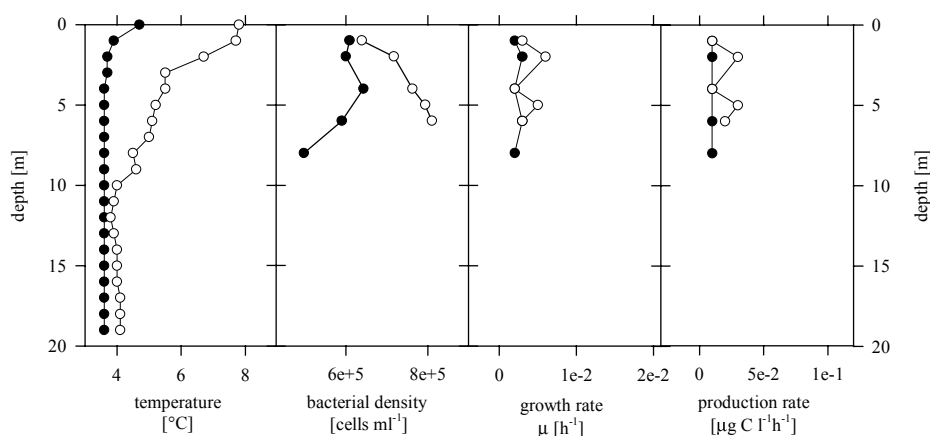


Fig. 2. Profiles of temperature (A), bacterial density (B), specific growth rate μ (C), and bacterial production rate (D) for Jöri lake III in 1997 (closed symbols 29 July; open symbols 7 August).

4.3 Phytoplankton biomass and abundance

The phytoplankton biomass (without autotrophic picoplankton) at depths from 4 to 5 m during the ice free period in 1996 amounted to $9.1 \pm 0.5 \text{ } \mu\text{g C l}^{-1}$ ($n = 3$). The flow cytometric analyses of the APP showed red ($> 650 \text{ nm}$) but almost no orange ($585 \pm 21 \text{ nm}$) cell fluorescence. This suggests that the Jöri lake III APP consisted mostly of eukaryotic algae while cyanobacteria

represented a minor portion of the phytoplankton community. The cell density of APP was $1.0 \cdot 10^4$ cells ml⁻¹ in the average with a maximum at the same depth as the chl *a* maximum. In August 1996, the calculated average APP biomass for the depth 4 to 5 m was 8 µg C l⁻¹.

5 DISCUSSION

Although the bacterial growth rates determined by the [³H]thymidine method show considerable uncertainty due to the low incorporation rates, they indicate the order of magnitude. They were all in the same range which was also confirmed by the dilution culture results. To check the reliability of the results, we did the same experiments in the prealpine, mesotrophic Lake Zürich (unpublished results). As expected, we found higher specific growth rates (0.008 to 0.044 h⁻¹) with lower standard errors (9%, n = 3). The reliable results obtained with the [³H]thymidine method for Lake Zürich give evidence that the high uncertainty of the Jöri lake III results was not caused by methodological errors but rather by sensitivity of the [³H]thymidine method when applied to the low bacterial activity in Jöri lake III.

The carbon ratio of bacteria, phytoplankton, and autotrophic picoplankton (APP) was 1.5 : 1.1 : 1, a rather high abundance of bacteria and APP compared to larger phytoplankton. This result corroborates observations by Simon *et al.* (1992) for different lakes and it allows to conclude that bacterial biomass and phytoplankton biomass are in the same order of magnitude when phytoplankton biomass is low. The biomass of larger phytoplankton and the APP biomass were approximately the same in Jöri lake III. This confirms that APP have a selective advantage in oligotrophic environments which is due to their high surface to volume ratio (Stockner 1991). Nevertheless, APP and in particular picocyanobacteria are generally negligible in mountain lakes due to photoinhibition. Jöri lake III is an exception as the suspended erosion particles diminish the light penetration significantly and change the underwater light climate.

In Jöri lake III, APP might contribute an important part of extracellular carbon, from which bacteria can profit. On the other hand, the rather high TOC content suggests that allochthonous input of organic carbon might be a major C-source for heterotrophic growth. Since TOC is 0.7 mg l⁻¹ and the sum of bacterial, APP and phytoplankton biomass is about 0.03 mg C l⁻¹, one might assume an allochthonous input of slowly degradable carbon from the watershed which could promote bacterial growth independently of extracellular carbon release by algae.

Bacterial production and loss rates were in balance during the sampling periods, since the loss rates did not differ significantly from the production rates. In Jöri lake III the main grazers were smaller than 3µm which was in contrast to Lake Zürich. Similar results were reported for other eutrophic and mesotrophic systems (Simek *et al.* 1997, Jürgens and Güde 1991). Possible bacteria grazers are *Daphnia* species, protozoa (heterotrophic nanoflagellates HNF, ciliates), and mixotrophic algae like *Dinobryon* species or autotrophic nanoflagellates (ANF, Pace *et al.* 1990). *Daphnia* species were never observed in Jöri lake III and HNF and ciliates are very scarce (<28 cells ml⁻¹ and 0.04 cells ml⁻¹, respectively; K. Simek and M. Macek, personal communication). Probably the most important bacterial grazers in Jöri lake III are mixotrophic algae. Both, *Dinobryon* species and ANF were frequent in 1997 in Jöri lake III with concentrations up to 50 cells ml⁻¹ and 182 cells ml⁻¹, respectively (K. Simek, personal communication, B. Hinder, unpublished). This suggestion is supported by the observation that the maximum bacterial numbers were always found just above the chl *a* maximum within the thermocline. Since bacterial production is enhanced by the release of organic carbon from phytoplankton, bacteria are expected to be abundant where phytoplankton densities are high. Algae are not expected to release more organic carbon in upper layers as a consequence of photoinhibition, as the photosynthetic active radiation (PAR) at four meters depths amounts to 250 µE m⁻² s⁻¹ maximally. Bacterial numbers were low at the site of the chl *a*-maximum where mixotrophic algae, the most important bacterial grazers, are most numerous. Since water densities are lower than biomass densities and since water density differences are small at low temperatures there are no physical reasons to justify why bacteria and phytoplankton prefer to accumulate in the thermocline of Jöri lake III. UV-radiation is also of little importance for the vertical distribution of phyto- and bacterioplankton at the depths where they are observed since the lake's turbidity prevents UV-radiation to reach deeper than 1 m below the surface. Mixotrophic algae seem to be the major players in the microbial loop of this ecosystem.

Temperature is about 2° C higher at depths where bacteria are most abundant compared to the depth of the chl *a*-maximum (Fig. 2). The two pairs of data sets from Jöri lake III (Fig. 3), obtained by the [³H]thymidine method, fit well into the Lake Zürich data, suggesting that the same temperature control might apply to bacterial growth in Jöri lake III. It could not be shown, however, that slightly enhanced bacterial activity would lead to higher biomass production as

observed in Lake Zürich where bacterial growth was clearly temperature dependent up to 22° C with a Q_{10} of 3.2 (Fig. 3).

The measured bacterial production rates of 0.01 to 0.03 $\mu\text{g C l}^{-1} \text{ h}^{-1}$ are in the same range as those found in a Spanish high mountain lake (Reche *et al.* 1996), where the primary production was 10 times the bacterial production (0.2 to 1.5 $\mu\text{g C l}^{-1} \text{ h}^{-1}$). For Jöri lake III primary production differs from the one observed in the Spanish mountain lake since alkalinity, conductivity and phosphate concentrations are lower.

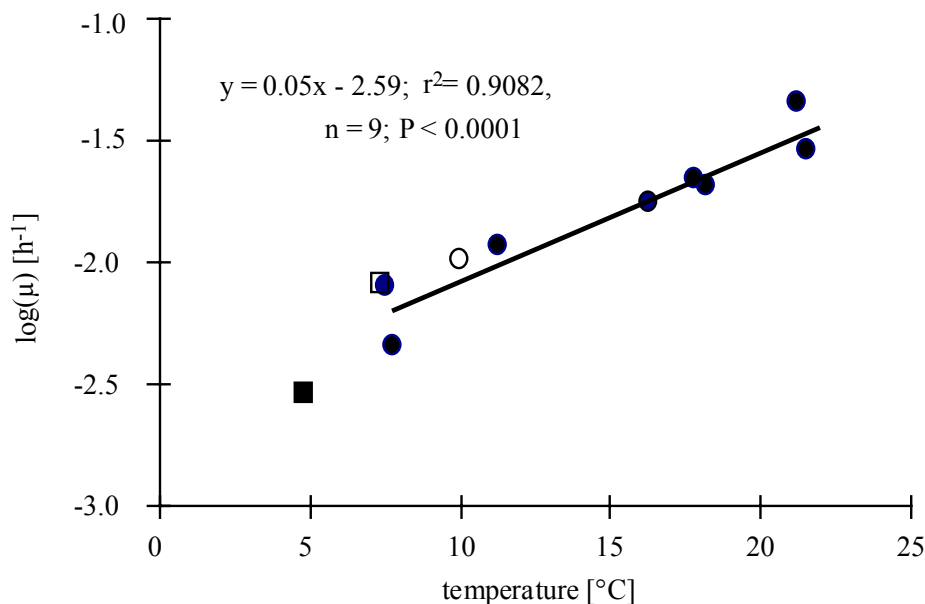


Fig. 3: Correlation between temperature and specific bacterial growth rates for Lake Zürich with two data points from Jöri lake III for comparison. Circles represent Lake Zürich data, squares the Jöri lake III data; open symbols are results obtained by the dilution culture method, closed symbols are results obtained by the [^3H]thymidine method (for Jöri lake III the averaged value of the statistically significant experiments are represented).

6 CONCLUSIONS

The microbial loop in Jöri lake III is not mainly top down controlled since bacterial grazing is very low. The carbon ratio of bacteria, phytoplankton, and autotrophic picoplankton (APP) was

1.5 : 1.1 : 1 which shows a rather high abundance of bacteria and APP compared to larger phytoplankton. It emphasizes the importance of bacteria and APP in this oligotrophic, turbid lake. APP biomass was similar to the biomass of the larger phytoplankton. The high proportion of APP in the total phytoplankton biomass reflects the selective advantage of small organisms with a high surface to volume ratio in oligotrophic environments. Although, APP are generally negligible in mountain lakes due of photoinhibition, in the turbid Jöri lake III they obviously find good conditions to propagate.

Bacterial growth could be enhanced by allochthonous organic carbon originating from the watershed, a source which would be available more constantly than extracellular organic carbon released by algae.

The taxa capable of both heterotrophic and autotrophic modes of nutrition become more important; in Jöri lake III it is interesting to note that purely heterotrophic nanoflagellates and ciliates are virtually absent. Our observations suggest that mixotrophic algae of the genus *Dinobryon* and other mixotrophic nanoflagellates are the major bacterial grazers. Their mode of nutrition must provide advantages for survival and competition in oligotrophic high mountain lakes.

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Conclusions

Microorganisms living in high mountain lake ecosystems are confronted with adverse living conditions which are primarily controlled by extremely variable meteorological determinants. Most characteristic are long winters with a heavy snow cover and short summers with intensive radiation, irregular rain and snowfalls, many thunderstorms and sometimes nice weather spells. The period of the icebreak has turned out to be a major promoter of community transition and the date of the icebreak influences decisively the seasonal succession of the plankton community. Snow may be expected at any day of the year. A snowfall event at the end of the summer was studied in detail. It revealed that the phytoplankton population living in the epilimnion broke down and was able to re-establish itself within one week. The maximal chlorophyll *a* concentration was found below the thermocline although only very low amounts of photosynthetic active radiation penetrated to these depths. We suggest that algae live in those deep layers since they are less affected by extreme weather events and offer more stable living conditions. In addition, algae living there profit from higher nutrient concentrations which increased towards the bottom, parallel to higher amounts of suspended particles.

The carbon ratio of bacteria, phytoplankton, and autotrophic picoplankton of 1.5:1.1:1 suggests a rather high abundance of bacteria and autotrophic picoplankton compared to larger algae. This could be a consequence of the higher surface to volume ratio of smaller organisms which gives them an advantage in nutrient-poor lakes. We observed two populations of autotrophic picoplankton but were not able to determine which species they included. This would afford detailed analysis with a well adjusted flow cytometer.

The major grazers of bacteria are mixotrophic flagellates which dominated over purely heterotrophic flagellates. The advantage of being able to switch between heterotrophic and autotrophic modes of nutrition seems to be decisive for surviving in these oligotrophic ecosystems. The main controlling mechanism of the microbial food web is not top down but nutrient limited. The bacterial biomass and bacterial production are more important in oligotrophic lakes than in eutrophic ones which makes the microbial loop more relevant in nutrient-poor habitats. In order to estimate the processes quantitatively, it would be necessary to measure at the same time bacterial and primary production. It would be interesting to know if there are differences in the microbial loop of clear water and turbid lakes which are both present in the Jöri catchment.

In some of the Jöri lakes suspended erosion particles regulate the nutrient availability for the microbiota. The particles mainly interact with nutrient cations like ammonia. Experiments with limnocorrals revealed that while more NH_4NO_3 and KH_2PO_4 enhances chlorophyll *a* concentration, the presence of erosion particles together with N and P diminishes it. These findings suggest that erosion particles can act as sinks for some nutrients which are then less accessible to the phytoplankton. It is an open question whether bacteria or phytoplankton are able to interact more actively with nutrient-loaded erosion particles.

The composition of the bacterial community was studied by TTGE and subsequent sequencing of excised fragments. Many sequences showed highest similarity with sequences of unidentified bacteria, some of them originating from other mountain lakes as well. One of them branched closest to the sequence of a bacterium found in an arctic lake which is similar to the high mountain lakes investigated in this study. Since only a fraction of all species are recorded by TTGE, it would be interesting to compare the results of community analyses obtained with different methods, like amplified ribosomal DNA restriction analysis (ARDRA) or cloning. To know more about the abundance of the species in the natural habitat, other molecular techniques, like fluorescent *in situ* hybridization with specific probes should be applied. Analysis with TTGE showed no significant differences in bacterial community in particle-enriched limnocorrals as compared to fertilized ones.

High mountain lakes are interesting for studies on ecosystem functioning under adverse and variable conditions. They offer opportunities to study nutrient cycling and energy flow under naturally extreme conditions and basic ecological principles emerge on how microorganisms manage to exist in these inhospitable habitats.

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