



## Environmental conditions in high mountain lakes containing toxic benthic cyanobacteria

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### Abstract

In glacial lakes on an alpine pasture in Switzerland, benthic cyanobacteria produced microcystin, a cyclic hepatotoxic heptapeptide. The cyanobacteria formed dense mats on sediments and submerged stones. The mats consisted mainly of *Oscillatoria limosa*, *Phormidium konstantinosum* (= *Oscillatoria tenuis*) and *Tychonema granulatum* (= *Oscillatoria granulata*). In order to characterize the ecological conditions of these cyanobacteria, nutrient concentrations were determined, and an automatic data acquisition station was installed in one of the lakes. It continuously recorded air temperature, global irradiance, precipitation, atmospheric pressure, wind speed and direction; as well as temperature, pH, oxygen content and conductivity of the lake water. The nutrient situation in the lakes was mainly influenced by the erosion of the gneissic catchment by glacial meltwater and by precipitation. In the glacial lakes, the concentrations of calcium, iron, magnesium, sodium and sulphate increased throughout the summer season. Conductivity values of 4–110  $\mu\text{S cm}^{-1}$  represent generally low nutrient concentrations. Nevertheless, iron concentrations of up to 20  $\mu\text{M}$  occurred. Biomass, expressed as protein concentration, as well as the microcystin content of the cyanobacterial mats varied within one season and between different years (1994 and 1995). In one cyanobacterial mat community, biomass and microcystin concentrations were highest at the same time, in an other one the microcystin content was maximal three weeks after the highest biomass concentration was reached. Our observations suggest that biomass and toxin production in the mats were strongly influenced by mechanical stress, temporary desiccation and high irradiation.

### Introduction

Cyanobacteria are known to produce a variety of potent toxins which are responsible for animal poisonings and human health problems worldwide. Species of *Microcystis*, *Anabaena* and *Oscillatoria* (*sensu lato*) as well as *Nodularia spumigena* Mertens ex Bornet et Flahault produce hepatotoxic cyclic peptides, the microcystins and nodularins (reviewed by Rinehart et al., 1994). *Anabaena*, *Aphanizomenon* and *Oscillatoria* species synthesize neurotoxins, including anatoxin-a, homoanatoxin-a, anatoxin-a(s) and saxitoxins (Carmichael, 1992). Most of the documented poisoning episodes were associated with blooms of toxic cyanobacteria. It is generally assumed that up

to 75% of all cyanobacterial blooms are acutely toxic (Bell & Codd, 1994). The massive development of planktic cyanobacteria accumulating at the surface of a water body is favoured by certain environmental conditions. These include moderate to high nutrient levels (especially N and P), moderate water temperatures, long retention times of the water and stable stratification (Wicks & Thiel, 1990; Carmichael, 1992). Increasing eutrophication of lakes, either due to natural lake aging or to anthropogenic nutrient enrichment, enhances mass developments of toxic and non-toxic cyanobacteria.

The relations between environmental conditions and toxin production by cyanobacteria are complex,

and laboratory investigations as well as field studies lead to contradictory conclusions. According to Wicks & Thiel (1990), the toxicity of a *Microcystis aeruginosa* (Kützing) Lemmermann bloom in a hypertrophic subtropical lake correlated to solar radiation, temperature, pH and percent oxygen saturation, whereas the toxin content did not or did only weakly correlate to nutrient concentrations. The correlation with biomass production was weak and negative, i.e. optimum conditions for growth did not coincide with those for toxin production. This was also found in experiments with *Microcystis aeruginosa* strains UV-006 and M228 growing at different temperatures and pH values (Van der Westhuizen & Eloff, 1983; Van der Westhuizen & Eloff, 1985; Watanabe & Oishi, 1985). Different light intensities had a marked effect on growth rates of several *M. aeruginosa* strains and correlated positively with toxicity (Van der Westhuizen & Eloff, 1985; Van der Westhuizen et al., 1986; Watanabe & Oishi, 1985; Utkilen & Gjølme, 1992). There was no, or only a weak correlation between toxin production and phosphorus and nitrogen concentrations in the medium (Watanabe & Oishi, 1985; Utkilen & Gjølme, 1992). In a shallow eutrophic lake, the concentration of dissolved inorganic nitrogen influenced the toxicity of a *Microcystis* bloom by affecting the dominance of *M. aeruginosa* over *M. viridis* (A. Braun) Lemmermann (Park et al., 1993). Utkilen & Gjølme (1995) reported a positive correlation of toxicity with iron concentration. *M. aeruginosa* PCC 7806 and *M. aeruginosa* CYA 228/1, however, were found to produce more microcystin-LR in the absence or at low concentrations of iron (Lukač & Aegerter, 1993; Lyck et al., 1996). For several strains of the filamentous *Planktothrix agardhii* (Gomont) Anagnostidis et Komárek (= *Oscillatoria agardhii* Gomont), toxin production correlated positively with temperature and nitrogen concentrations and negatively with light intensities. Different phosphorus concentrations had no influence on toxin production, unless they were very low (Sivonen, 1990). In these experiments, biomass and toxin contents correlated, i.e. optima for growth and for toxin production coincided. This indicates, that for these cyanobacteria, toxin production might not be a stress response.

Toxin production not only occurs in planktic cyanobacteria, but also in benthic *Oscillatoria* (*sensu lato*) species. A strain of *Oscillatoria* sp. from Scotland is known to produce the neurotoxic alkaloid anatoxin-a (Codd et al., 1992). We recently identified a microcystin, a hepatotoxic cyclic heptapeptide, in ben-

thic cyanobacteria from a remote oligotrophic alpine lake. The organisms form mats on lake sediments and submerged rocks. Under certain conditions, they tend to detach and float on the water surface towards the leeside shores of the lake. These toxic cyanobacteria were linked to the death of more than a hundred head of cattle reported during the last two decades from alpine pastures in south-eastern Switzerland (Mez et al., 1996; Mez et al., 1997). In this paper we describe the environmental conditions in high mountain lakes as well as the seasonal variation of toxin and biomass contents of the cyanobacterial mats. Special interest is dedicated to the interrelation between high iron concentrations and microcystin contents in the alpine lakes.

## Material and methods

### Sites of investigation

The area of investigation consists of four alpine lakes situated at an altitude of 2300–2350 m above sea level at Tambo near Splügen in the Hinterrhein valley in south-eastern Switzerland (Figure 1). Lakes A and B are fed by glacial water. They are linked to each other and to lakes C and D by a small brook. Lake D is a small still arm of the brook. Lake E is a small pond situated on a nearby moraine. It is fed only by snow-melt and rainwater and has neither inlet nor outlet (see also Mez et al., 1996). A field laboratory was run from July to September in a nearby mountain cabin at 2000 m a.s.l.

### Species determination

For the taxonomic determination of cyanobacteria we relied on morphological features as described by Geitler (1932) and Anagnostidis & Komárek (1988).

### Chemicals

All chemicals were of standard analytical grade from Merck (Darmstadt, Germany) or Fluka (Buchs, Switzerland). The MCYST-LR (microcystin-LR) standard, glycogen phosphorylase *b* and phosphorylase kinase were obtained from Sigma, [ $\gamma$ -<sup>33</sup>P] ATP from Dupont NEN and ReadSafe scintillation cocktail from Beckman, USA.

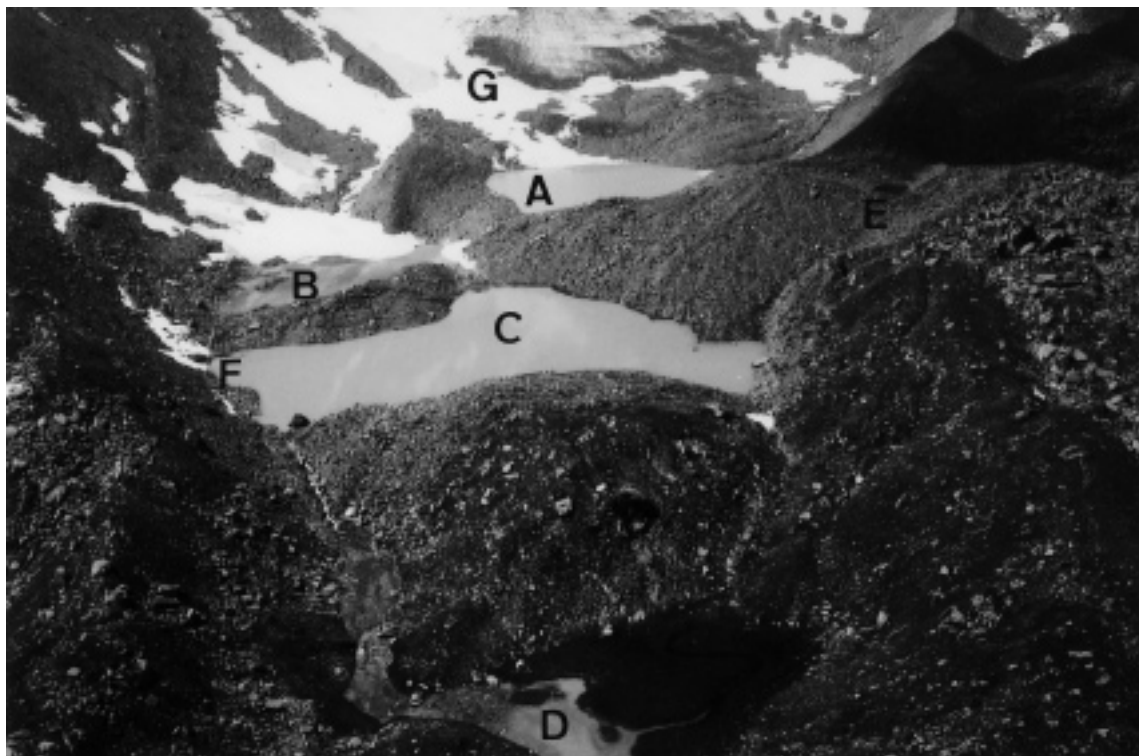


Figure 1. Aerial photograph of the Tambo lakes, near Splügen in the Hinterrhein valley in south-eastern Switzerland. The photograph was taken on July 25 1995 towards the south-west. Letters A-E refer to the lakes described in Table 1, F = location of the meteorological and hydrological data acquisition station, G = Tambo glacier. The diameter of lake C is about 250 m, the altitude difference between lakes A and D is about 50 m.

#### *Protein phosphatase inhibition assay*

The protein phosphatase inhibition assay was performed as described previously (Mez et al., 1996; see also MacKintosh, 1993). Protein phosphatases from crude extracts of oilseed-rape seeds were used with  $^{33}\text{P}$ -glycogen phosphorylase  $\alpha$  as a substrate. Lyophilized cyanobacterial samples were extracted in the reaction buffer by vortexing, freezing in liquid nitrogen, thawing and ultrasonication. Microcystins in filtered water were concentrated on C18 cartridges, then eluted sequentially with 10 ml of 40% aqueous acetonitrile and 10 ml of 100% methanol and analysed for protein phosphatase inhibition. Water-soluble cyanobacterial proteins were quantified in the aqueous extract used for the protein phosphatase inhibition assay according to Mez et al. (1996).

#### *Chemical variables*

All water samples were filtered through Whatman GF/F filters. Samples for HPLC analysis were fixed in 1% formaldehyde (v/v, final concentration) and those for ICP-AES (inductively coupled plasma atomic

emission spectroscopy) in 0.5 M nitric acid (final concentration). The colorimetric analyses were carried out immediately after sampling in the field laboratory. Phosphate concentrations were determined by the phosphomolybdate method (Merck Spectroquant 14848) and ammonia by the indophenol blue method (Berthelot's reaction, Merck Spectroquant 14752). Silicate was quantified as  $\beta$ -silicomolybdate (Merck Spectroquant 14794), total iron by the Ferrospectral method (Merck Spectroquant 14761) and iron (II) by the Ferrozine method (Lovely et al., 1986). Samples for iron (II) determination were taken with a syringe containing HCl (final concentration 0.25 N). Nitrate, sulphate and chloride were separated and quantified by HPLC, using a SUPER-SEP anion column (Metrohm, Switzerland) and a conductivity detector (Wescan 213A). The eluent used at a flow rate of  $1 \text{ ml min}^{-1}$  was ultrapure water containing 0.3 mM phthalic acid and 5% (v/v) acetonitrile (pH 4.2). Cations other than ammonia and iron were determined by ICP-AES (Birch et al., 1996). Alkalinity was determined by Gran-titration (Gran, 1950, 1952).

### *Physical variables*

An automatic data sampling equipment for limnological and meteorological was installed in lake C (Vernez, 1995). The following parameters were recorded at ten-minute intervals: water temperature, pH, conductivity and oxygen content; air temperature (0.05 and 2 m above ground), global irradiance, atmospheric pressure, relative humidity, precipitation, wind speed and wind direction. Oxygen saturation was calculated according to Karagounis (1992). In all other water bodies, temperature and conductivity of the water were measured with a WTW TetraCon 96 conductivity meter. Due to the low conductivity of the water, KCl was added to a final concentration of 55 mM for the manual pH measurement (Palintest pH electrode).

## **Results**

### *Environmental conditions in the Tambo lakes*

The major Tambo lakes are geologically young water bodies created by the melting Tambo glacier during the last few decades. According to old maps and to descriptions by local people, lakes A and B have existed for about forty years, lake C was formed about sixty years ago (Figure 1). Its western basin is 9 m deep, the eastern basin has a depth of about 1 m. All lakes (letters A-E of Figure 1) are embedded in the gneiss rocks of the Tambo nappe (Mayerat, 1989) and their sediments consist almost exclusively of inorganic matter (erosion particles).

On ten days between June 24 and September 23, 1995, temperature, conductivity, pH value and the chemical composition of the surface water of all 5 Tambo lakes were examined. Table 1 lists the average values and the ranges of the physical parameters as well as the nutrients determined. With the detection methods used, the relative standard error was smaller than 5% for concentrations higher than or equal to 20  $\mu\text{M}$  nitrate, 0.6  $\mu\text{M}$  ammonia, 0.2  $\mu\text{M}$  phosphate, 0.2  $\mu\text{M}$  total iron, 20  $\mu\text{M}$  sulphate, 5  $\mu\text{M}$  chloride, 26  $\mu\text{M}$  potassium and 44  $\mu\text{M}$  sodium. For iron (II), silica, calcium and magnesium the relative standard errors were less than 5% throughout the whole range of concentrations determined.

### *Seasonal variations of the environmental conditions*

In lake A, where glacier melt water is running directly into the lake, and lake E, which is fed by rain and

snow-melt water only, the variations of the ion concentrations revealed no seasonal tendencies. In lakes B to D, ammonia, nitrate, phosphate, silica, iron (II), potassium and chloride concentrations were about constant throughout the season. On the other hand, calcium, magnesium, sodium, sulphate and iron(III) contents increased steadily from June to September due to glacial melt water percolating through moraine deposits into lake B (Figure 2). The low ion concentrations on September 13, also detectable in lakes C and D, were due to the diluting effect of a heavy rainfall (100 mm in 20 hours), which was also recorded with high time resolution by the automatic data acquisition station installed in lake C (Figure 3). High solar energy input and daily conductivity changes correlated positively (Figure 4).

In addition to chemical effects due to precipitation and erosion, rain and snowfall events produced mechanical stress for the benthic cyanobacteria in the lakes. Precipitation caused variations in the water surface level of  $\pm 0.2$  m, which led to shoreline fluctuations of  $\pm 1$  m on the flat shores of lake C with resulting irregular dry and wet conditions in the mat habitat. In addition, the benthic cyanobacteria were continuously subject to the forces of waves caused by strong winds. Figure 5 shows the wind characteristics (directions and velocities) throughout the season. Two wind directions clearly prevailed, namely north-northeasterly and south-south-westerly. Precipitations usually were accompanied by northerly winds, whereas southerly winds were dry fall winds. The Tambo lakes are situated north of Piz Tambo, a peak of 3300 m a.s.l.

### *Diurnal dynamics of the environmental conditions*

Long-term variations like the steady conductivity increase throughout the season and patterns of steep diurnal gradients characterize alpine aquatic ecosystems. Table 2 shows minimal and maximal values of the hydrological and meteorological parameters recorded by the automatic data acquisition station. As a representative example, Figure 6 shows the time course of some of these parameters for one week. Daily variations of  $\pm 8$  °C (air temperature),  $\pm 10$  °C (water temperature),  $\pm 1.7$  pH units and  $\pm 0.8$  ppm oxygen (almost 20% of oxygen saturation) were frequently observed. The latter reflected the metabolic activity of a phototrophic biofilm which developed on the surface of the electrodes installed in lake C. Figures 7a and b show the dynamics of photosynthesis

Table 1. Chemical and physical characterization of the Tambo lakes (see also Figure 1) for the summer season 1995 (June 24 to September 23, 1995). All parameters were determined manually around noon. The table shows average values ( $n = 10$ ). Numbers in brackets indicate minimal and maximal values.

Parameter	Lake A	Lake B	Lake C	Lake D	Lake E
Water temperature [°C]	3.7 (0.2–5.8)	4.4 (0.3–13.2)	–	6.1 (0.4–11.2)	10.0 (4.4–20.2)
Conductivity [ $\mu\text{S cm}^{-1}$ ]	28.4 (25.7–35.4)	72.3 (49.4–109.5)	–	52.4 (30.6–96.6)	6.3 (4.3–8.2)
pH <sup>a</sup>	6.9 (6.2–7.5)	7.1 (6.4–7.7)	–	7.1 (6.1–7.7)	7.1 (6.2–7.7)
Nitrate [ $\mu\text{M}$ ]	31.3 (14.7–63.4)	21.2 (4.5–42.4)	39.9 (4.5–136.8)	39.1 (4.5–141)	33.9 (0.0–172)
Ammonia [ $\mu\text{M}$ ]	3.9 (0.4–8.4)	3.3 (0.0–5.5)	3.1 (0.0–6.3)	3.4 (0.3–6.8)	1.7 (0.0–5.7)
Phosphate [ $\mu\text{M}$ ] <sup>a</sup>	0.3 (0.1–0.6)	0.4 (0.2–0.6)	0.2* (0.1–0.5)	0.1* (0.0–0.2)	0.01* (0.0–0.3)
Total iron [ $\mu\text{M}$ ]	1.3 (0.0–3.4)	1.2 (0.1–4.7)	0.9 (0.0–1.5)	0.8 (0.3–1.9)	0.3 (0.1–1.3)
Iron(II) [ $\mu\text{M}$ ]	0.3 (0.01–1.7)	0.3 (0.0–0.8)	0.2 (0.0–0.7)	0.3 (0.0–0.6)	0.2 (0.0–1.4)
Silicate [ $\mu\text{M}$ ] <sup>b</sup>	8.1 (5.6–9.6)	16.1 (14.3–19.7)	12.0 (9.4–13.6)	12.0 (10.7–13.3)	4.4 (3.0–5.2)
Sulphate [ $\mu\text{M}$ ]	19.0* (15.6–21.9)	114.5 (70.7–163.0)	68.2 (31.6–96.1)	65.1 (36.1–89.5)	7.1* (4.6–11.3)
Chloride [ $\mu\text{M}$ ]	11.1 (1.1–51.6)	28.4 (1.1–132.9)	21.3 (0.0–136.1)	27.7 (2.1–122.5)	6.7 (3.2–12.9)
Calcium [ $\mu\text{M}$ ]	149.7 (119.7–168.6)	367.9 (231.7–560.6)	243.9 (133.2–329.4)	244.1 (121.2–355.3)	27.6 (17.7–33.4)
Magnesium [ $\mu\text{M}$ ]	21.8 (14.0–25.5)	65.5 (37.0–97.1)	42.4 (18.9–54.9)	44.2 (21.8–63.1)	3.7 (2.4–4.5)
Sodium [ $\mu\text{M}$ ]	30.1* (7.4–46.5)	41.5* (26.5–82.6)	28.6* (0.0–47.8)	59.5 (7.8–202.6)	22.0* (2.9–37)
Potassium [ $\mu\text{M}$ ]	23.2* (8.2–50.1)	31.7 (0.0–63.9)	21.0* (12.3–40.4)	24.8* (3.8–61.1)	5.2* (0.0–16.1)
Alkalinity [ $\mu\text{eq l}^{-1}$ ] <sup>c</sup>	445	554	310	389	48

– see Table 2 (automatically recorded data)

<sup>a</sup> values from June 24 to September 3 ( $n = 7$ )

<sup>b</sup> three values between July 13 and September 23

<sup>c</sup> determined on a single occasion

\* relative standard error  $\geq 5\%$

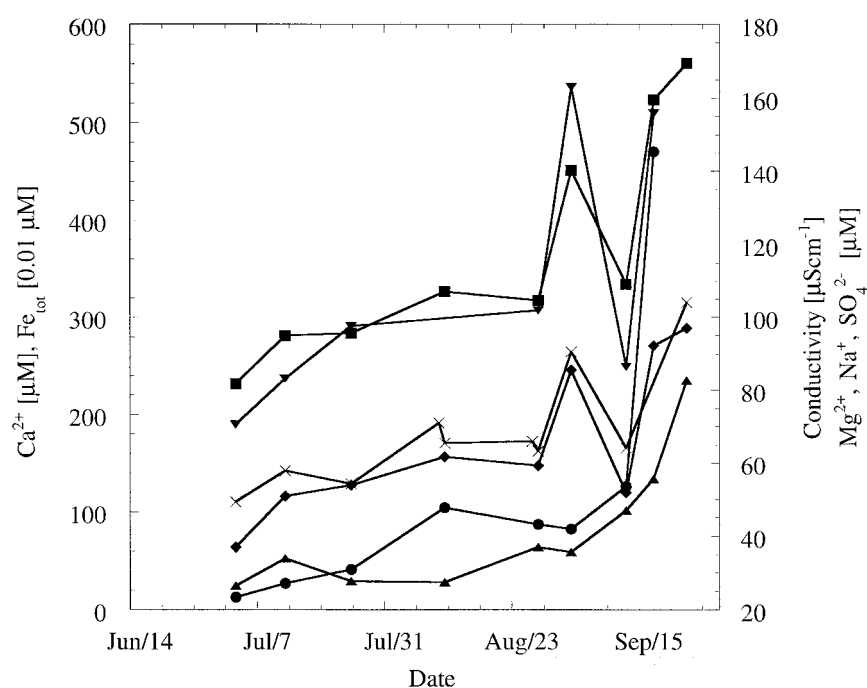


Figure 2. Seasonal dynamics of conductivity (x-x) and major ion concentrations (calcium ■, magnesium ◆, total iron ●, sodium ▲ and sulphate ▼) in lake B.

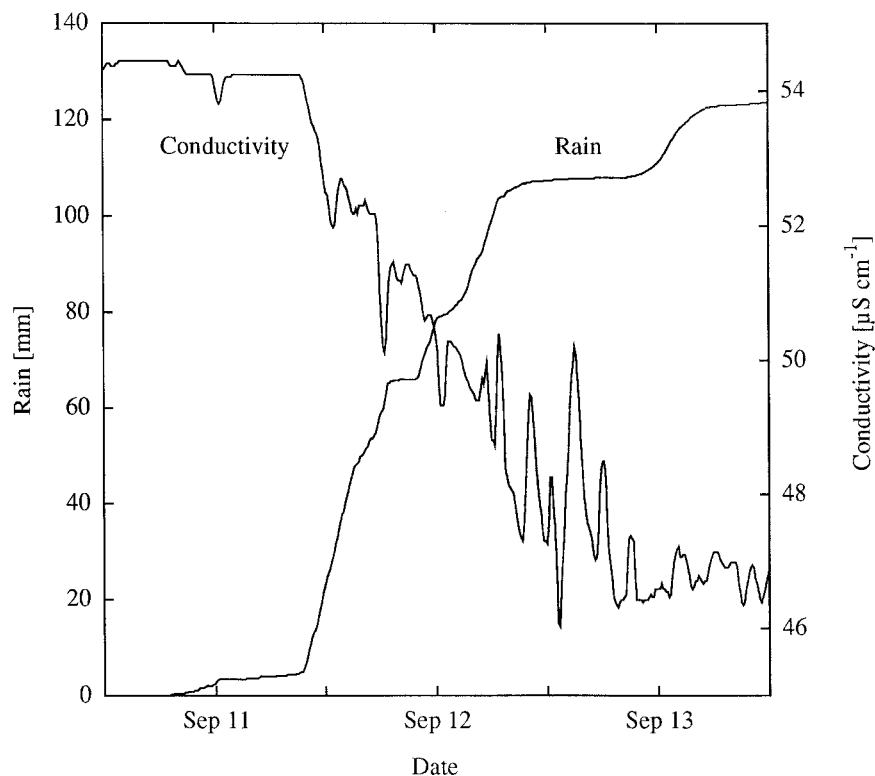


Figure 3. Influence of a heavy rainfall on the conductivity recorded by the automatic data acquisition station installed in lake C.

Table 2. Minimal and maximal values of hydrological and meteorological parameters recorded by the automatic data acquisition station installed in lake C (August 14 to September 24, 1995)

Parameter	Minimum	Maximum
Water temperature [°C] <sup>a</sup>	0.7	13.1
Conductivity [ $\mu\text{S cm}^{-1}$ ]	45.1	55.2
Oxygen [ppm]	6.9	9.3
Oxygen [% saturation]	71.5	103.9
pH	7.3	9.4
Air temperature [°C]		
2 m above ground	-5.3	13.2
0.05 m above ground	-6.5	12.6
Global irradiance [ $\text{W m}^{-2}$ ]	0	977
Precipitations [ $\text{mm 10 min}^{-1}$ ]	0	2.0
Pressure [hPa]	758	773
Wind velocity [ $\text{m s}^{-1}$ ]	0	7.5

<sup>a</sup> values from July 21 to September 24, 1995

(expressed as pH increase and oxygen production) and respiration (pH decrease and  $\text{O}_2$  consumption) for the two days with highest and lowest global irradiance.

Table 3. Concentration of different iron species above and below a cyanobacterial mat growing on the sediment of lake D. The values are averages of 7 determinations throughout a day

Iron species	above the mat ( $\mu\text{M}$ )	below the mat ( $\mu\text{M}$ )
Dissolved		
Total iron	0.9	10.5
Iron(III)	0.7	9.6
Iron(II)	0.2	0.9
Particulate		
Total iron	2.8	10.6
Iron(III)	2.7	7.9
Iron(II)	0.1	2.7

Ion concentrations, determined every two hours on a sunny day in July in lake D, did not reveal any diurnal variations (data not shown). This was also the case for the concentration of different iron species determined in the water 0.5 cm above and below a

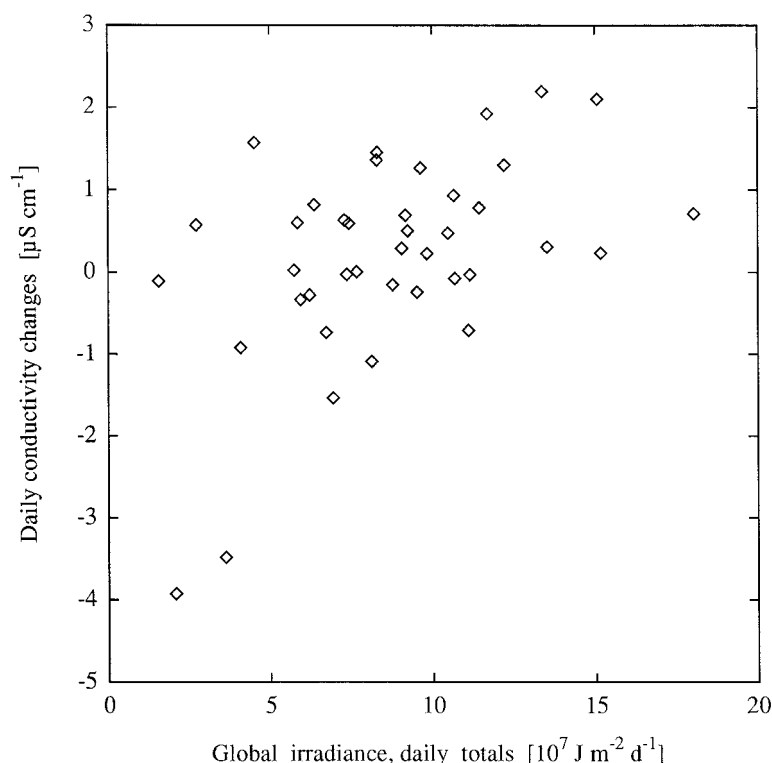


Figure 4. Influence of global irradiance (total energy input between 6:00 am and 7:00 pm) on changes of the average daily conductivity compared to the previous day in lake C (< 0: conductivity decrease; > 0: conductivity increase). Values for the period of August 14 to September 24, 1995 are shown.

cyanobacterial mat growing on the sediment of lake D (Table 3).

#### *Biomass and toxin production by benthic cyanobacteria*

On 6 days between July 7 and October 3, 1994, and on thirteen days between June 15 and October 27, 1995, samples of benthic algae (including cyanobacteria) were taken from the shores of all 5 lakes in order to examine species composition, biomass and hepatotoxin production. The samples consisted of mats growing on sediments and stones or floating on the water surface of the lakes. The mats consisted mainly of cyanobacteria (for a detailed species description see Mez et al., 1997). The composition of the benthic communities did not vary greatly throughout the summer. The most frequent cyanobacteria were *Oscillatoria limosa* Agardh ex Gomont (lakes C and D), *Phormidium konstantinosum* Umezaki et Watanabe (= *Oscillatoria tenuis* Agardh ex Gomont) (lakes A-E), *Limnothrix pseudominima* (Skuja) Umezaki et Watan-

abe (= *Oscillatoria pseudominima* Skuja) (lakes A-D) and *Tychonema granulatum* (Gardner) Anagnostidis et Komárek (= *Oscillatoria granulata* Gardner) in lakes A-D. Filamentous green algae (especially *Zygnema* sp.) were frequently intermingled in the mats (lakes A-D).

In total, 165 samples of benthic cyanobacteria were examined for protein and microcystin content. As described previously (Mez et al., 1997), a microcystin (thereafter called MCYST\*) was identified by HPLC-photodiode array detection in benthic cyanobacteria from lakes C and D (no MCYST\* was detected in lakes A, B and E). We estimated MCYST-LR equivalents from protein phosphatase inhibition, the IC<sub>50</sub> (toxin concentration leading to a 50% inhibition of the enzyme) of the assay corresponded to 0.25 nM for MCYST-LR). For lakes C and D, the development of biomass (expressed as protein content) as well as the seasonal variations of microcystin production in 1994 and 1995 are shown in Figures 8a, b, d and e, respectively. Figures 8c and f show the specific microcystin content, i.e. the concentration of MCYST-

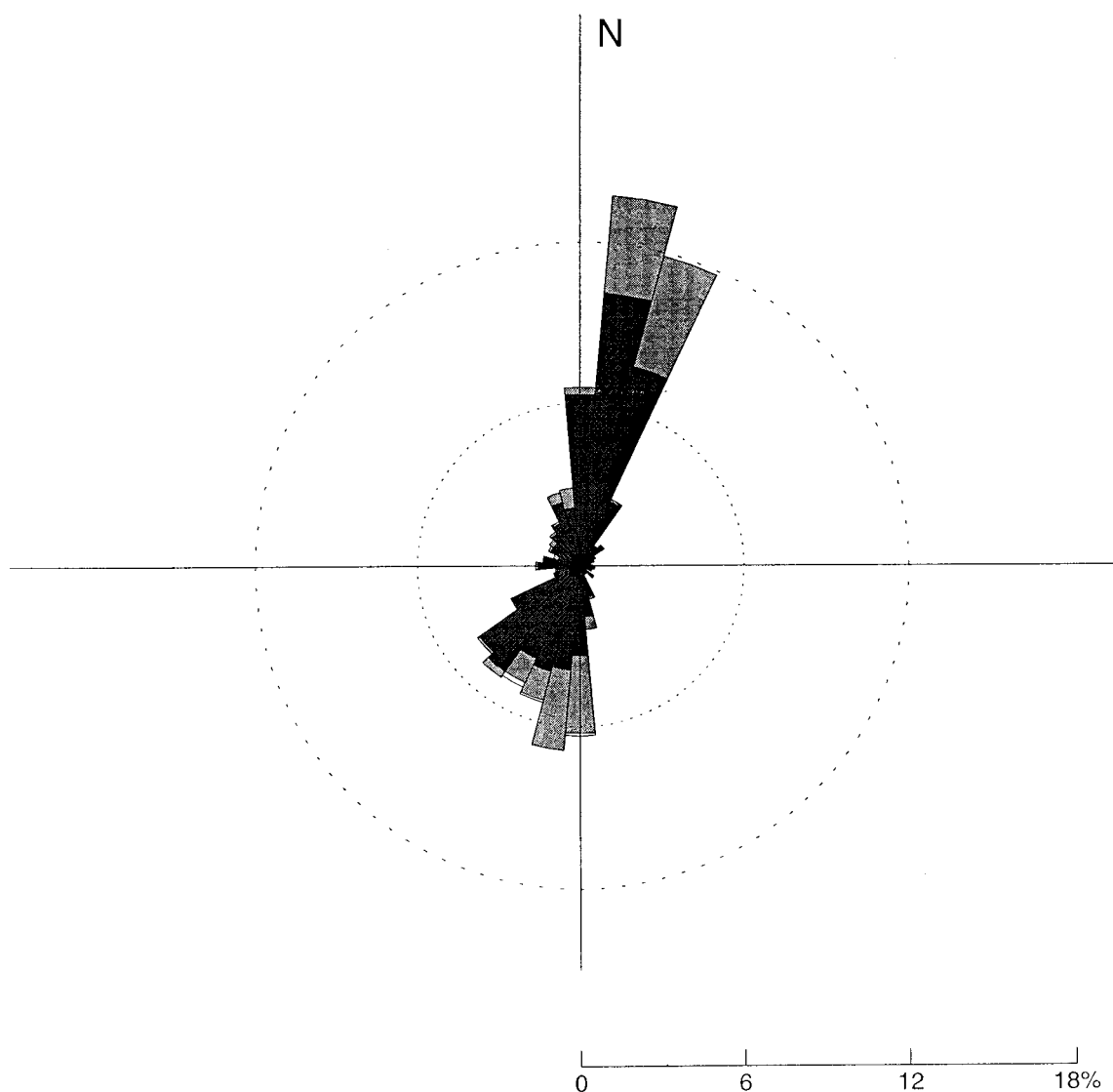


Figure 5. Wind characteristics (velocities and direction) recorded by the automatic data acquisition station. Dark hatching:  $< 2 \text{ m s}^{-1}$ ; grey hatching:  $2\text{--}5 \text{ m s}^{-1}$ ; no hatching:  $> 5 \text{ m s}^{-1}$ . The average wind speed for the period of August 14 to September 24, 1995 was  $1.2 \text{ m s}^{-1}$ . The scale bar represents the percentage of all wind records.

LR equivalents per unit protein for samples from both lakes.

#### *Dissolved MCYST\* in filtered lake water*

In 1995, the microcystin concentration was also examined in filtered water. Again, MCYST\* was only detected in filtered water of lakes C and D. Figure 9 shows the variation of dissolved microcystin estimated by protein phosphatase inhibition for several dates. In order to examine the influence of the high iron con-

centrations in the alpine lakes on the persistence of dissolved MCYST\*, the raw extract of a toxic sample from lake D was diluted in an iron(III) solution to a final concentration of  $10 \mu\text{M FeCl}_3$  (see also Takenaka & Tanaka, 1995). The protein phosphatase-inhibiting activity of the sample was significantly decreased at this iron concentration (Figure 10).



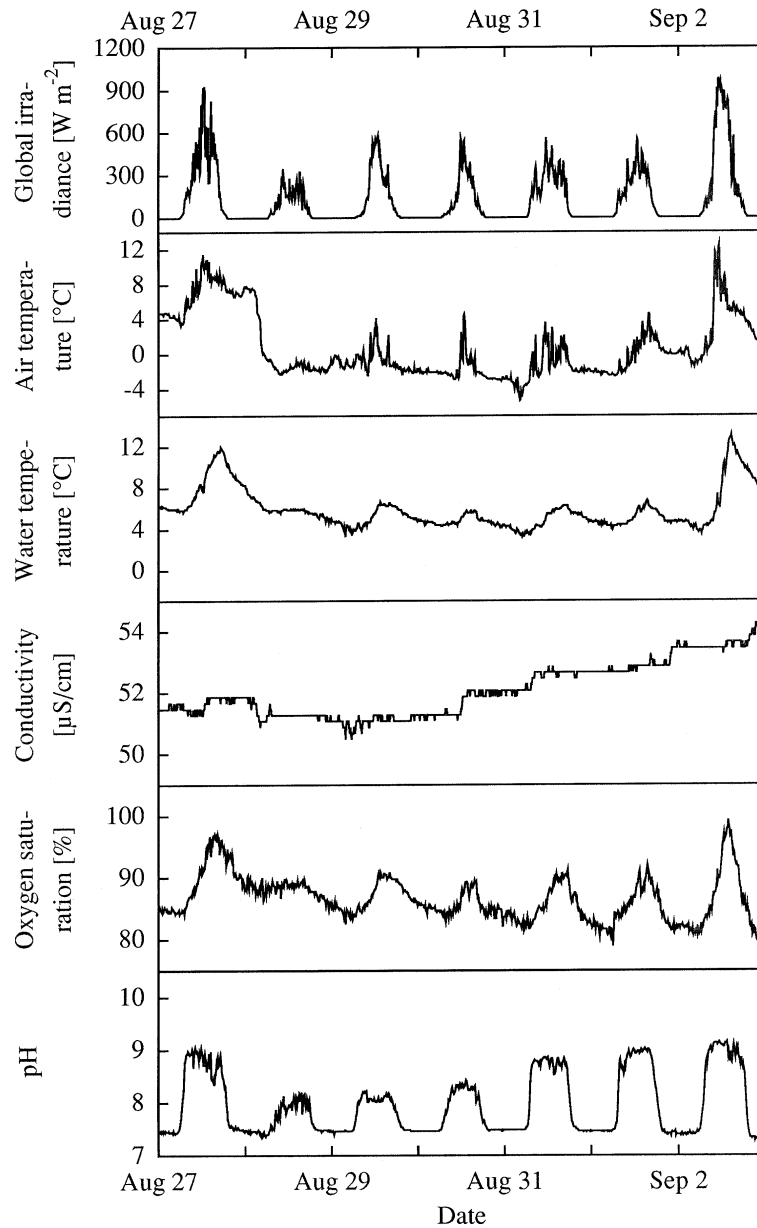


Figure 6. Illustration of the dynamics of several environmental parameters recorded by the automatic data acquisition station in lake C between August 27 and September 2, 1995.

## Discussion

The cyanobacteria identified in this study are pioneer organisms in an environment created and influenced by the glacial meltwater, lithology and erosion in the area, as well as the meteorology of the high alps. Life in this aquatic environment is subject to a short veg-

etation period, low nutrient concentrations, low water temperatures, high turbidity, high energy input (solar irradiance and wind) and diurnal and seasonal changes with high amplitudes (Table 2). The high silica concentration and input of calcium, magnesium, sodium, iron and sulphate into lake B (Figure 2) were due to meltwater percolating through pyrite and feldspar-

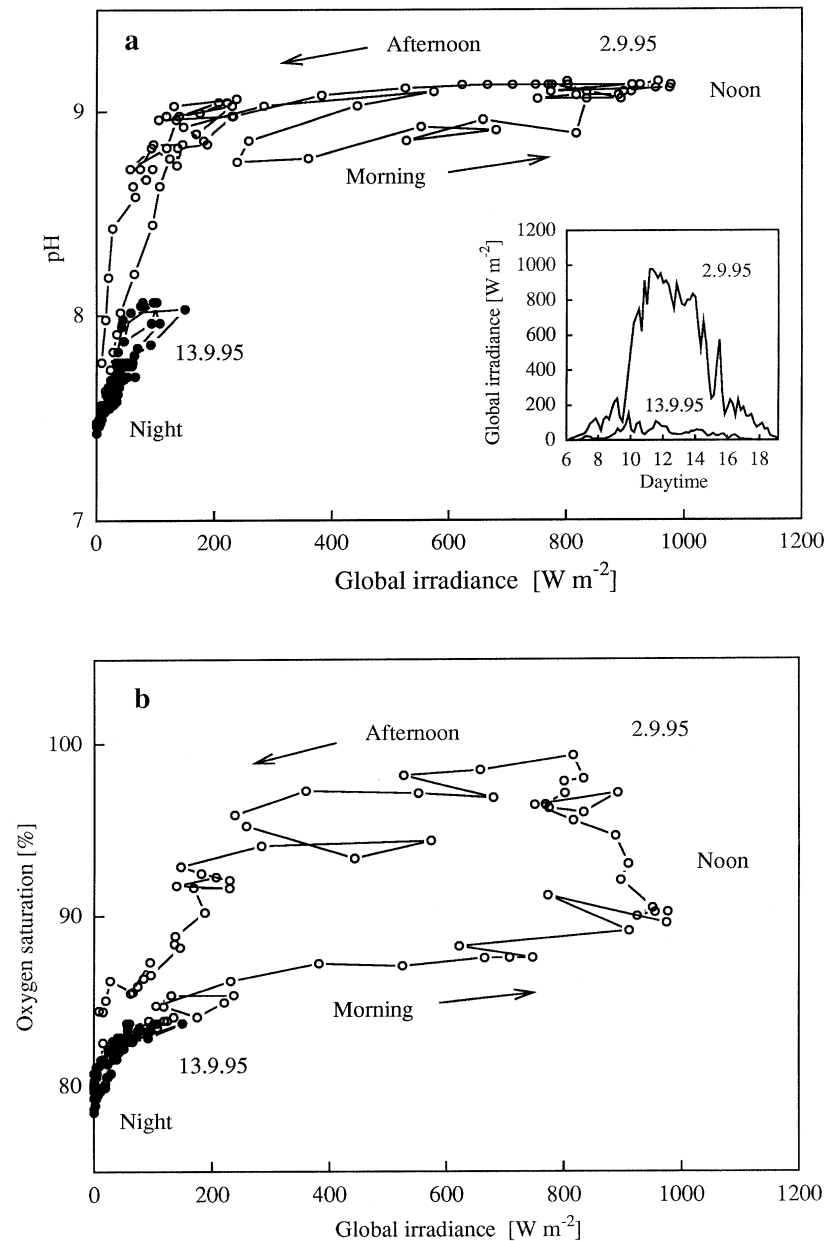


Figure 7. Comparison of the diurnal variations of pH (a) and oxygen saturation (b) as a function of global irradiance on a sunny day ( $\circ$ ), 13.9.95). The insert shows the light conditions for September 2 and 13, 1995, respectively.

containing rocks of glacial moraines. At the mouth of the glacier above lake B, the conductivity of the water was 1.3 fold higher ( $n=3$ ) than the conductivity of the lake water. Warm weather not only enhanced the melting of glacier ice, but also decreased the water volume of the lakes, leading to further concentration of the nutrients. Precipitation, on the other hand, lowered the conductivity due to dilution (Figure 3). Hours

after a rainfall event, dilution was compensated by the inflow of ion-rich water from the catchment. The concentrations of inorganic nitrogen and phosphorus reached mesotrophic levels (Table 1). The N/P ratio was higher than 50 in all lakes and throughout the entire summer season, and its variation did not reveal any seasonal tendency. Phytoplankton development was poor; it did not exceed 8 ppb chlorophyll

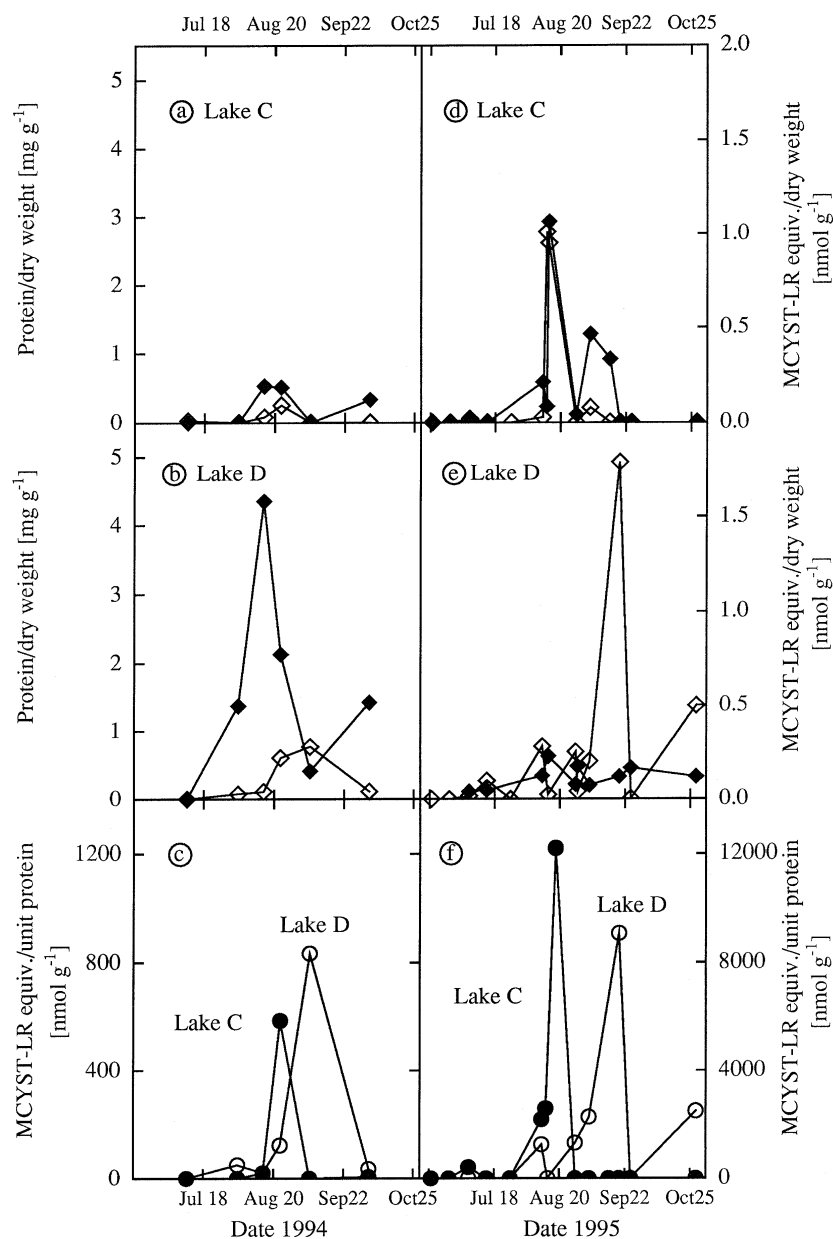


Figure 8. Development of protein (—◆—) and microcystin (—◇—) concentrations in cyanobacterial mats in 1994 (a–c) and 1995 (d–f). 8c and f show the specific toxin contents of the cyanobacterial mats in lakes C (—●—) and D (—○—) for 1994 and 1995, respectively. Please notice that the ordinate scales are different in 8c and 8f. The values are means of 2–4 determinations (1994) and of 2–7 determinations (1995).

*a*, as determined according to Strickland & Parson (1968; data not shown). The metabolic activity of the biofilms on the electrodes installed in lake C were taken to represent the behaviour of phototrophic organisms under the conditions in this lake (Figure 7). The diurnal pH and oxygen fluctuations were neither temporally nor quantitatively consistent. While pH al-

ready reached maximal values at about  $150 \text{ W m}^{-2}$ , significant changes in oxygen values could only be detected at irradiances of more than  $800 \text{ W m}^{-2}$ .

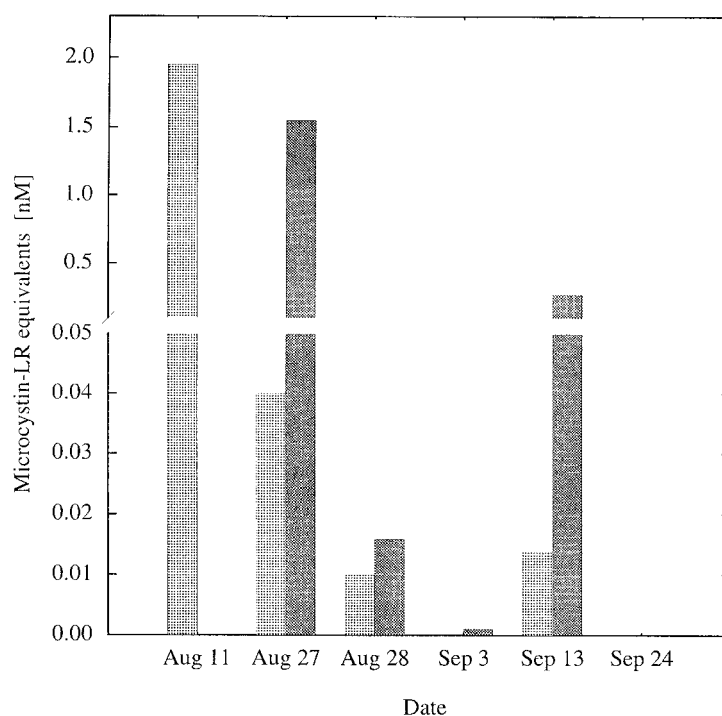


Figure 9. Microcystin-LR equivalents in filtered water of lakes C (grey hatching) and D (dark hatching) on selected days between August 11 and September 24, 1995.

#### *Influence of iron concentrations of MCYST\* production and persistence*

Iron was abundant, especially at the interface between lake sediments and the cyanobacterial mats, where it was sometimes visible as a red precipitate. In lake D, the concentration of ferric iron was 6 times higher, and the concentration of ferrous iron 12 times higher below than above the mats, indicating high ferric iron-reducing activity below the mats (Table 3). The ratio of particulate to dissolved iron (II) was 2.7 below and 0.5 above the mat and for iron(III) 0.8 and 3.9 below and above the mat, respectively. Neither photo-oxidation nor photo-reduction of the different iron species, i.e. no diurnal pattern, was observed above the cyanobacterial mats in the Tambo lakes. As shown in Figure 10, these high iron concentrations decrease the protein phosphatase-inhibitory activity of microcystins (see also Takenaka & Tanaka, 1995). The iron concentrations found below the mats in lake D were in the range of those used in laboratory experiments by Utkilen & Gjølme (1995), who found that iron concentrations up to 10  $\mu\text{M}$  increased the microcystin-RR content in *Microcystis aeruginosa* CYA 228/1. Based on these findings they proposed that micro-

cystins might act as intracellular chelators enhancing iron (II) uptake into the cells. Lukač & Aegerter (1993) observed for *M. aeruginosa* PCC 7806 that iron limitation ( $\leq 2.5 \mu\text{M}$  instead of 28  $\mu\text{M}$  in BG-11 medium) decreased the growth rate but favoured microcystin-LR synthesis. These results, as well as data from other physiological experiments, indicate that microcystin production by cyanobacteria might be a response to certain unfavourable environmental conditions (Van der Westhuizen & Eloff, 1983; Van der Westhuizen & Eloff, 1985; Wicks & Thiel, 1990; Lukač & Aegerter, 1993). The observations made with the benthic cyanobacteria in lake D support this hypothesis. Maximal biomass content was reached prior to maximal hepatotoxin concentration. However, for the cyanobacterial communities in lake C, biomass and toxin content developed simultaneously (Figure 8). A similar situation was also described for *Planktothrix agardhii* (= *Oscillatoria agardhii*) by Sivonen (1990).

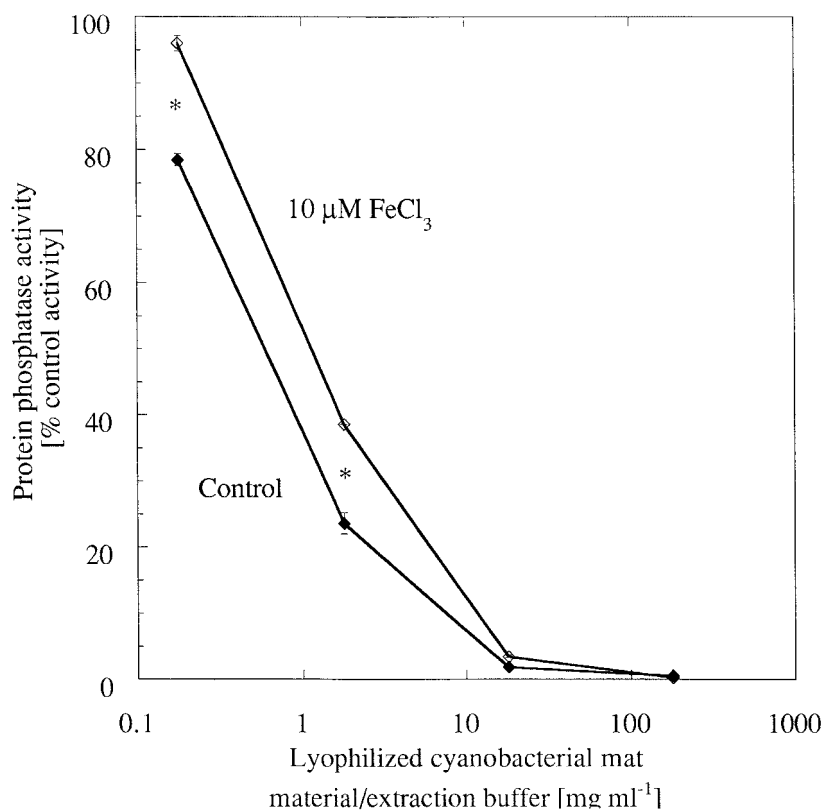


Figure 10. Influence of 10  $\mu\text{M}$  iron(III) on the protein phosphatase-inhibiting activity of a toxic cyanobacterial sample. The asterisks represent significant differences (one-sided student  $t$ -test,  $\alpha = 5\%$ ,  $n = 3$ ).

*Dynamics of biomass and MCYST\* concentrations in the cyanobacterial mats, and of MCYST\* contents in filtered lake water*

Neither biomass production nor hepatotoxin synthesis can easily be correlated to one of the environmental parameters recorded in the Tambo lakes. The low biomass and toxin contents of the mats after the end of August was mainly due to mechanical destruction of the mat communities by repeated heavy rains and intermittent periods of snowfall. In October the benthic cyanobacteria in lake C, consisting almost exclusively of *Oscillatoria limosa* and *Phormidium konstantinosum* at that time, were frozen into the ice cover. The other lakes no longer contained significant cyanobacteria populations at that time.

In August, the concentration of microcystin in filtered water from these high mountain lakes was above the proposed limit for human drinking water (1 nM; Falconer, 1994). It must be assumed that the concentration of free toxin in the Tambo lakes can be significantly higher at a given place and time, taking

into account that the toxins are continuously diluted in the water body and washed out due to the short retention time of the water (especially in lake D) and detoxified by iron(III) (Figure 10). Dissolved microcystins are also degraded microbially (Bourne et al., 1996), or decomposed or transformed to non-toxic isomers by visible and UV light (Tsuji et al., 1994; Beattie & Codd, 1995; Tsuji et al., 1995). At the altitude of the Tambo lakes, light intensities are high, and UV irradiance reaches up to 25 sunburn units per day (Blumthaler et al., 1985).

## Conclusion

Our investigations show that benthic cyanobacteria develop significant amounts of biomass in high mountain glacier lakes. They are obviously well adapted to oligotrophy, to the highly variable environmental conditions, and to the short vegetation period. They produce microcystins in seasonally variable concentrations. No direct correlation could be shown so far

between growth and toxin production by these benthic cyanobacteria and one or several environmental factor(s). Mechanical stress, temporary desiccation and irradiation, determinants which are direct or indirect consequences of the meteorological conditions, as well as the high iron concentration are the most likely factors regulating metabolic processes and thus microcystin production in these mountain habitats. The presence of toxigenic cyanobacteria in low-nutrient mountain lakes enables a new approach towards the understanding of the regulatory role of environmental factors on toxin production.

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