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An *in situ* enclosure experiment to test the solar UVB impact on plankton in a high-altitude mountain lake. II. Effects on the microbial food web

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Abstract. We studied the impact of ambient levels of solar ultraviolet B (UVB) radiation on the planktonic microbial food web (viruses, heterotrophic bacteria, heterotrophic nanoflagellates and ciliates) of a high-mountain lake (2417 m above sea level) under *in situ* conditions for 16 days. Enclosures of 1 m^3 receiving either the full sunlight spectrum or sunlight without UVB radiation were suspended at the lake surface. We found that the abundance of heterotrophic nanoflagellates was always lower in the +UVB treatment than in the –UVB one. In addition, bacterial consumption, measured by the disappearance of fluorescently labelled bacteria, was significantly ($P < 0.05$) reduced in the +UVB treatment. The abundance of non-filamentous bacteria (<10 μm long) was also lower in the +UVB treatment, suggesting a direct effect of UVB on their growth. This was supported by the significantly ($P < 0.05$) lower cell-specific activity ([^{3}H]thymidine incorporation) found on the fifth day of the experiment. In contrast, UVB radiation had no effect on filamentous bacteria (>10 μm long), which represented only a small fraction of the total abundance (<4%), but up to ~70% of the total bacterial biovolume. Ciliates, mainly *Urotricha pelagica* and *Urotricha furcata*, were less impacted by UVB radiation, although the net growth rate during the first week of the experiment was lower in the +UVB treatment than in the –UVB one (0.22 and 0.39 day^{-1}, respectively). The abundance of virus-like particles during the first week of the experiment was higher in the –UVB treatment. After reaching the maximum value for the interaction viruses × bacteria, viral abundance decreased dramatically (by ~85%) in both treatments with a decay rate of ~0.017 h^{-1}. This study illustrates the complexity in assessing the impact of UVB radiation when more than one trophic level is considered and indicates the existence of different sensitivity to UVB radiation among components of the microbial food web.

Introduction

The assessment of the potential negative effects of solar ultraviolet B (UVB) radiation (290–320 nm) on aquatic ecosystems has received considerable attention in the last 10 years as a consequence of the degradation of the stratospheric ozone layer observed at different latitudes, but particularly over Antarctica. In addition, this problem has focused the attention of aquatic ecologists on several biological and chemical processes in which solar UV radiation plays a significant role, as well as on the different strategies and mechanisms that organisms have evolved to avoid UV radiation, to protect themselves, and to repair potential damage.

In most studies, the impact of UV radiation has been assessed on single components of the food web, while few investigations have considered the response when two or more trophic levels interact (Bothwell *et al*., 1994; Sommaruga *et al*., 1996; Ochs, 1997). Recently, several studies in freshwater and marine systems included the response of different components of the food web (Bergeron and Vincent, 1997; Cabrera *et al*., 1997; Halac *et al*., 1997; Keller *et al*.,

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1997; Sakka *et al.*, 1997). In those studies, different sizes of containers or enclosures ranging from micro- to mesocosms have been used for that purpose. Although this approach has several advantages, e.g. using the natural solar spectrum and organisms pre-adapted to field conditions, it also presents a series of inconveniences, for instance, the generally long-term exposures, usually days or weeks, needed to observe a potential effect, and the confinement in enclosures that eliminates advection. Confinement in a restricted water column is also critical for species performing vertical migration because their natural avoidance mechanism is eliminated. Nevertheless, this approach represents a useful way to understand the response to UV radiation considering trophic interactions, although it can be considered in most studies as the worst case scenario.

Considering the ecological consequences of enhanced UVB radiation levels also requires the identification of potentially vulnerable aquatic ecosystems. High mountain lakes above the tree line rank among the most exposed aquatic ecosystems because, due to the altitude effect (Blumthaler *et al.*, 1992), they experience higher UVB levels than those situated at sea level. In addition, the lake water is usually highly transparent to UVB radiation due to the low concentration of dissolved organic carbon (DOC) (Morris *et al.*, 1995; Sommaruga and Psenner, 1997). Even more important than the low DOC concentration for the high UV penetration seems to be the source of this dissolved material, which in catchments with poorly developed soils and scarce terrestrial vegetation, like in most alpine lakes, is mainly autochthonous (McKnight *et al.*, 1997). Autochthonous DOC has a lower specific absorbance in the UV range than that terrestrially-derived (McKnight *et al.*, 1994).

In a previous publication (Halac *et al.*, 1997), we have presented the results of an enclosure experiment carried out in a high-mountain lake in order to evaluate the response of phytoplankton growth and species composition when exposed to ambient levels of UVB radiation. The main finding of that study was that no significant differences in phytoplankton species composition and growth were found when UVB radiation was excluded from the sunlight spectrum. Although high fluctuations in the abundance of several species were observed, they occurred in all treatments. In addition, some species were found to be affected by daily fluctuations of solar UVA + photosynthetically active radiation (PAR).

Here we present results from a parallel investigation on the response of the planktonic microbial food web components (viruses, heterotrophic bacteria, heterotrophic nanoflagellates and ciliates) to the exclusion of UVB radiation. This study, the first to our knowledge to report the combined response of these microbial components, indicates the existence of different sensitivity to UVB radiation among them.

Method

The lake and the experimental design

Experiments were carried out between 1 and 16 August 1995 (~2 weeks after ice break-up) at Gossenköllesee, situated at an elevation of 2417 m above sea level in the Central Alps (47°13′N, 11°01′E). Limnological information about the lake

is published elsewhere (Halac *et al*., 1997; Sommaruga and Psenner, 1997). The experimental design was described in detail in Halac *et al*. (1997). Briefly, the experimental system consisted initially of four (two were lost during a storm) enclosures of 1 m^3 each (1 m deep) floating at the surface, with the walls constructed either of acrylic transparent to full sunlight (GS 2458 Röhm®, Germany) or of acrylic that excluded most UV radiation (XT 20070, Röhm®, Germany; 50% transmittance at 385 nm). They will be referred to in the text as +UVB and –UVB treatments, respectively (see below). In order to prevent rain and dust falling into the enclosures, we placed a UV transparent acrylic (GS 2458) roof 20 cm above the upper edge of each enclosure. The roof of the –UVB enclosure was covered with a foil of Mylar® D (50 μm thickness, 50% transmittance at 320 nm; Du Pont de Nemours) to exclude only the UVB. Measurements of UV irradiance inside the enclosures showed that there was a reduction of 92% UVB in the –UVB enclosure, but not in the UVA range (Halac *et al*., 1997; their Table I). The enclosures were arrayed in a line separated from the shore and fixed in a place in the lake where the depth was ~4.5 m. A photograph of the enclosures can be found at the following Internet address: http://pcwww.uibk.ac.at/s06subz2/c71986/enclo.jpg. The enclosures were filled during the night with water from 1 m depth by means of a pump working under low pressure. The final volume of the enclosures was ~900 l. Although the abundance of macrozooplankton was low (B.Tartarotti, personal communication), the water was filtered through a large nylon bag (mesh 100 μm).

Sampling and measurements

The methods used to measure solar UV radiation, phytoplankton abundance, chlorophyll *a* and the chemical analyses (inorganic nutrients, DOC) during the experiment were described in detail in Halac *et al*. (1997). Here we describe the parameters considered in this paper. The enclosures were sampled every second day for chemical analysis, abundance of viruses, bacteria, heterotrophic nanoflagellates and ciliates, and every 4 days for bacterial activity and bacterivory experiments. Samples were collected with a 5 l opaque Schindler–Patalas sampler (40 cm long) from the centre of the enclosure after gently mixing the water with a paddle in order to homogenize the contents of the enclosure.

Particulate organic carbon and nitrogen

For particulate organic carbon (POC) and nitrogen (PON) analysis, between 500 and 800 ml of sample were filtered onto pre-combusted Whatman GF/F filters and dried at 60°C for 12 h. The organic content of the filters was estimated with a Carlo-Erba CHN analyser 1500.

Virus-like particles

Virus-like particles (VLP) were enumerated by transmission electron microscopy according to Bergh *et al*. (1989). Briefly, 10 ml of lake water fixed with

formaldehyde (final concentration 2% v/v) were harvested directly on electron microscope grids (400-mesh Ni grids) supported with carbon-coated Formvar film, using a Beckman L8-60M ultracentrifuge (197 500 *g* for 90 min) with a SW41 swing-out rotor. Afterwards, the supernatant was withdrawn, the grids air dried and the sample stained with 3% uranyl acetate for 20 s. VLP were counted in view fields randomly selected until total numbers exceeded 200 or, in the case of low density, until 200 fields were examined, using a Hitachi-600AB transmission electron microscope at 75 kV and ×80 000 magnification. Taper corrections were implemented in final calculations (Suttle, 1993).

Heterotrophic bacteria and nanoflagellates

Samples fixed with formaldehyde (final concentration 2% v/v) were stained with the fluorochrome 4′,6-diamidino-2-phenylindole (DAPI; final concentration 0.2% w/v) according to Porter and Feig (1980). Between 1000 and 1200 non-filamentous bacteria (<10 μm long) were counted on black membrane filters (Poretics, 0.2 μm pore size) at ×1250 magnification in a Zeiss Axioplan epifluorescence microscope with a BP 365, FT 395 and LP 397 filter set. The distribution of bacteria on the filter was previously checked at lower magnification. A lower number of filamentous bacteria (~200) was counted at ×800 under the same conditions.

The size of the bacteria was determined with an image-analysis system as described in Psenner (1993). Images were recorded with a Hamamatsu high-sensitivity SIT camera mounted on the Zeiss Axioplan microscope. An interference filter (450–490 nm) was used to eliminate the yellow fluorescence of detritus. The image-analysis software LUCIA (Laboratory Imaging, Czech Republic) was used in combination with a real-time image processor (MATROX, MVP-AT, Canada). Digitized images were resolved with 512 × 512 pixels using 8 bits of memory. Between 600 and 1000 non-filamentous cells were analysed, but the number for filamentous bacteria was lower (100–200). Background subtraction was used to minimize variability associated with non-uniformity in illumination and electronic noise. The digital image was filtered using a non-linear filter known as General Filtration (Zamperoni, 1989). Cell volumes were computed with the following formula:

$$V = (w^2 \times \pi/4) \times (l - w) + (\pi \times w^3/6)$$

where V is volume (μm^3), w is width (μm) and l is length (μm). The accuracy of the algorithms used was confirmed with independently calibrated microspheres of known size (Molecular Probes, USA).

Heterotrophic nanoflagellates (HNF) were counted in the same samples for bacteria as described above, but a higher volume (30 ml) was filtered. Samples for counting HNF were treated in the same way as described for bacteria and processed within 2 weeks. At least 100 individuals were counted and checked with a BP 490, FT 510 and LP 520 filter set for the presence of autofluorescence, i.e. the presence of pigments inside plastids.

Ciliates

Samples for ciliates were fixed with acid Lugol and enumerated using the inverted microscope technique (Utermöhl, 1958). Fifty millilitres were settled for 48 h and the entire surface of the settling chamber was examined at ×200 magnification.

Bacterial activity and bacterivory experiments

The method of Fuhrman and Azam (1982) was used to estimate bacterial activity. Methyl [^{3}H]thymidine (83 Ci mmol^{-1}; Amersham, UK) was added at saturating concentrations (10 nM) to three quartz glass tubes containing 30 ml of water from the respective treatments. Experiments were performed following the same conditions of the treatments, i.e. the quartz vessels with samples from the –UVB enclosure were wrapped with Mylar® D, while those with samples from the +UVB enclosure were unwrapped. The tubes were exposed horizontally for 6 h (centred at midday) at 0.5 m depth outside the enclosures. Incubations were finished by the addition of formaldehyde (3% final concentration). Bacteria were concentrated onto cellulose nitrate filters (Sartorius, 0.2 μm pore size). The filters were pre-washed several times with 1 ml of ice-cold 5% (w/v) trichloroacetic acid (TCA), then extracted for 5 min with 5 ml ice-cold TCA, and finally washed again with TCA. The stainless steel filtration tower was removed and the borders of the filter were also washed with TCA. The dry filters were placed into scintillation vials with 5 ml of scintillation cocktail (Ready Safe, Beckman, CA). Disintegrations per minute were determined in a Beckman LS 6000 IC scintillation counter, after complete dissolution of the filters. All samples were corrected for abiotic incorporation by subtracting the radioactivity in one formaldehyde-killed control.

Bacterivory experiments were carried out following the fluorescently labelled bacteria (FLB) disappearance method (Marrasé *et al*., 1992). FLB were prepared from the natural bacterial assemblage of the lake surface according to the procedure described in Sherr *et al*. (1987). Bacteria were concentrated using an Amicon system equipped with a hollow fibre cartridge of 0.1 μm cut-off. Duplicate quartz tubes of 1 l capacity were filled with water from each experimental enclosure and the FLB added at 30–35% of the natural bacterial abundance. As described above, the quartz vessels filled with sample from the –UVB treatment were wrapped with Mylar® D. The tubes were then exposed on a platform made of UV-transparent acrylic (GS 2458) and suspended at 0.5 m depth outside the enclosures for 48 h. The long time exposure was necessary to measure a significant decrease in the number of FLB. The equations developed by Marrasé *et al*. (1992) were used to calculate the bacterivory rate (g) from the decrease in FLB over time and the total number of bacteria consumed (TG, FLB + natural bacteria) per unit volume in 48 h.

Data analysis

As discussed previously (Halac *et al*., 1997), the loss of replicates for each treatment at the start of the experiment during a strong storm seriously restricted the

possibility of checking for significant differences between treatments. Nevertheless, we performed two-way ANOVA, after verifying for normality and variance equality, to compare the effect of the treatments and time of the experiment, on data of bacterial activity and bacterivory, where replicate samples were taken. Post hoc comparisons were made with the Student–Newman–Keuls (SNK) test. Spearman rank correlations were used to investigate the relationship among parameters in each treatment. In order to limit the overall experimentwise error rate, each comparison was tested using a corrected significance level according to the Bonferroni method.

Alternatively, multivariate analysis, i.e. redundancy analysis (RDA; ter Braak, 1988), was used for testing of the effect of UVB (with and without) and the effect of UVA + PAR intensity on changes in the abundance of organisms or concentration of the variables investigated (but see Results). The significance of these variables in explaining abundance variability was tested by means of a Monte Carlo procedure, with 999 permutations (ter Braak, 1995).

Results

Particulate organic carbon and nitrogen

The concentration of POC and PON followed similar patterns in both treatments, increasing rapidly over time, particularly during the first 9 days (Figure 1A and B). This increase in POC was very significantly correlated ($P < 0.01$) with the increment in chlorophyll *a* observed in the enclosures (Tables I and II). The C:N element ratio remained around seven in both treatments all the time (Figure 1C).

Changes over time in the abundance of the microbial components

The abundance of non-filamentous bacteria (<10 μm long) in both treatments increased over time almost in an asymptotic way until day 9, remaining constant afterwards (Figure 2A). While the abundance in both treatments was almost identical at the beginning, after the third day the abundance in the +UVB treatment

Table I. Spearman rank correlations among the abundance of non-filamentous bacteria (BAC), filamentous bacteria (FIL), virus-like particles (VLP), heterotrophic nanoflagellates (HNF), ciliates (CIL) and the concentration of chlorophyll *a* (Chl *a*), dissolved organic carbon (DOC) and particulate organic carbon (POC) in the –UVB treatment

	FIL	VLP	HNF	Chl *a*	DOC	CIL	POC
BAC	–0.524$^{n.s.}$	–0.190$^{n.s.}$	0.643 $^{n.s.}$	0.980**	0.738$^{n.s.}$	1.000**	0.980**
FIL		0.548$^{n.s.}$	0.691$^{n.s.}$	–0.548$^{n.s.}$	–0.738$^{n.s.}$	–0.524$^{n.s.}$	–0.500$^{n.s.}$
VLP			–0.381$^{n.s.}$	–0.167$^{n.s.}$	–0.381$^{n.s.}$	–0.190$^{n.s.}$	–0.238$^{n.s.}$
HNF				0.619$^{n.s.}$	0.809$^{n.s.}$	0.643$^{n.s.}$	0.619$^{n.s.}$
Chl *a*					0.762$^{n.s.}$	0.980**	0.950**
DOC						0.738$^{n.s.}$	0.714$^{n.s.}$
CIL							0.980**

$n = 8$.
*$P \leq 0.05$; **$P < 0.01$; n.s., not significant. The significance level was corrected according to the Bonferroni method.

Table II. Spearman rank correlations among the abundance of non-filamentous bacteria (BAC), filamentous bacteria (FIL), virus-like particles (VLP), heterotrophic nanoflagellates (HNF), ciliates (CIL) and the concentration of chlorophyll *a* (Chl *a*), dissolved organic carbon (DOC) and particulate organic carbon (POC) in the +UVB treatment

	FIL	VLP	HNF	Chl *a*	DOC	CIL	POC
BAC	–0.571$^{n.s.}$	0.214$^{n.s.}$	0.286$^{n.s.}$	0.880**	0.980**	0.857*	0.762$^{n.s.}$
FIL		0.310$^{n.s.}$	0.762$^{n.s.}$	–0.595$^{n.s.}$	–0.595$^{n.s.}$	–0.595$^{n.s.}$	–0.476$^{n.s.}$
VLP			–0.333$^{n.s.}$	–0.040$^{n.s.}$	0.040$^{n.s.}$	–0.090$^{n.s.}$	–0.140$^{n.s.}$
HNF				0.452$^{n.s.}$	0.380$^{n.s.}$	0.548$^{n.s.}$	0.524$^{n.s.}$
Chl *a*					0.900**	0.900**	0.880**
DOC						0.930**	0.857*
CIL							0.980**

$n = 8$.
*$P \leq 0.05$; **$P < 0.01$; n.s., not significant. The significance level was corrected according to the Bonferroni method.

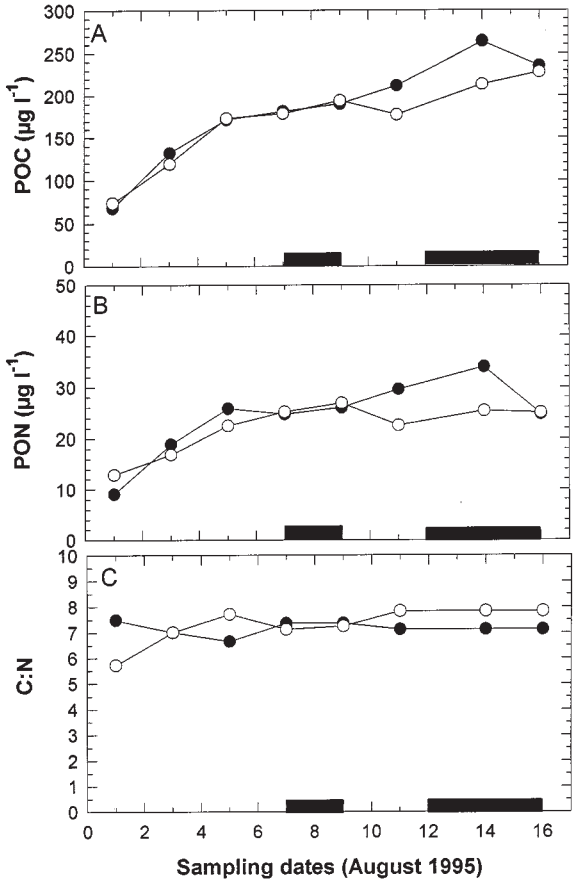

Fig. 1. Time course of particulate organic carbon (**A**), particulate organic nitrogen (**B**) and the carbon:nitrogen element ratio (**C**) in the +UVB (○) and –UVB (●) treatments. Dark bars indicate cloudy days.

was lower than in the –UVB treatment. By the end of the experiment (day 16), the abundance in the –UVB treatment was 27% higher than in the +UVB treatment. In the –UVB treatment, the abundance of bacteria was positively correlated ($P < 0.01$) with the concentration of POC and chlorophyll *a* (Table I), while in the +UVB treatment, bacterial abundance was positively correlated with DOC and chlorophyll *a*. The mean cell volume in the –UVB treatment increased from 0.125 μm^3 on the first day to ~0.17 μm^3 on day 16 (Figure 3A). In the +UVB treatment, the mean cell volume also increased with time, but the maximum (0.20 μm^3) was found on day 5. No significant correlation was found between the mean cell volume and the other tested parameters (data not shown).

The abundance of filamentous bacteria (>10 μm long) was in general 1–2 orders of magnitude lower than that of non-filamentous bacteria (Figure 2B). Their abundance also increased in both enclosures during the first 7 days; however, in contrast to the non-filamentous bacteria, it decreased dramatically afterwards. Changes in the abundance of filamentous bacteria in both treatments were not

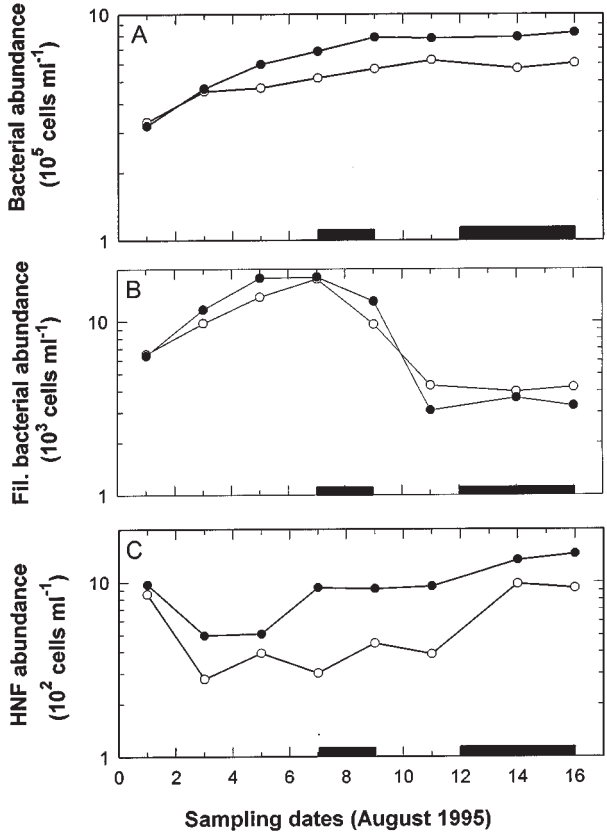

Fig. 2. Semi-log plot of the time course of the abundance of non-filamentous bacteria (**A**), filamentous bacteria (**B**) and heterotrophic nanoflagellates (**C**) in the +UVB (○) and –UVB (●) treatments. Dark bars indicate cloudy days.

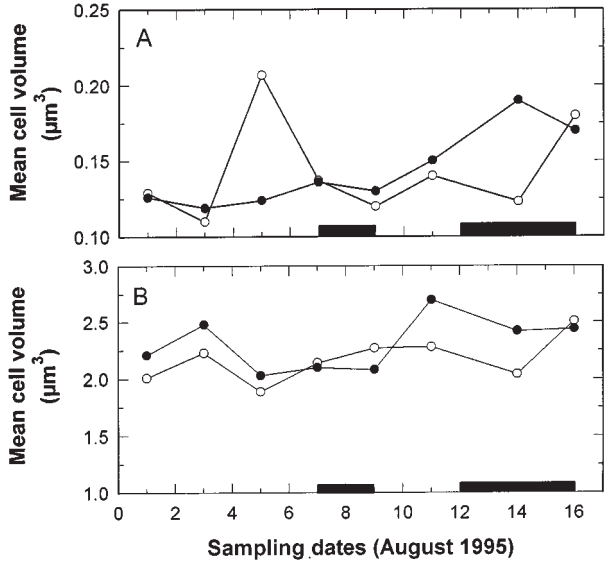

Fig. 3. Time course of the mean cell volume of non-filamentous bacteria (**A**) and filamentous bacteria (**B**) in the +UVB (○) and –UVB (●) treatments. Dark bars indicate cloudy days.

significantly correlated with other variables (Tables I and II). The mean cell volume of filamentous bacteria was relatively constant, ranging in both treatments between ~2 and 2.5 µm^3, and no clear pattern was observed over time (Figure 3B). Non-filamentous bacteria represented up to 70% of the total bacterial biovolume.

The abundance of HNF decreased in both treatments during the first 3 days, although it was more pronounced in the +UVB treatment (Figure 2C). Afterwards, the abundance increased in both treatments, but the abundance in the –UVB treatment always remained higher than that in the +UVB one. Under overcast conditions, i.e. between 7 and 9 and between 12 and 16 August, there was a clear trend of an increase in abundance in the +UVB treatment. There was a higher correlation between the abundance of HNF and bacteria in the –UVB treatment than in the +UVB one; however, both were not significant (Tables I and II).

The abundance of ciliates followed almost a parallel development in both treatments with low values during the first part of the experiment (<1 cell ml^{-1}) and higher ones (between 1 and 3.6 cells ml^{-1}) during the second half (Figure 4). The abundance increased faster in the –UVB treatment than in the +UVB one (net growth rate = 0.22 and 0.39 day^{-1}, respectively) during the first week with sunny conditions. During the prolonged overcast period (12–16 August), a more rapid increase in the abundance was observed in the +UVB treatment than in the –UVB one. The most abundant species in both treatments was *Urotricha pelagica* (>90%), followed by *Urotricha furcata*. The total abundance in both treatments was significantly correlated with the chlorophyll *a* concentration and the abundance of non-filamentous bacteria (Tables I and II).

The abundance of free VLP in the –UVB treatment was generally higher than in the +UVB treatment, except for the sampling dates on 11 and 14 August when the opposite was true (Figure 5). Maximum values were found on day 9 for the –UVB treatment (7.9×10^6 VLP ml^{-1}) and on day 11 for the +UVB treatment (8.7×10^6 VLP ml^{-1}). These days corresponded also with the maximum value for the interaction VLP × bacteria (6.2×10^{12} and 5.4×10^{12}, respectively). Afterwards, the abundance fell dramatically (decay rate of ~0.017 h^{-1} in both treatments) and reached the minimum on day 14 (–UVB: 1.3×10^6 VLP ml^{-1}) or day 16 (+UVB: 1.0×10^6 VLP ml^{-1}). No significant correlation was found in both treatments between VLP and other parameters. The VLP:bacteria ratio changed from ~1.7 to 14 in the +UVB treatment, and from ~1.7 to 13 in the –UVB one. In both cases, the ratio was lowest during the last two sampling dates when the weather was overcast.

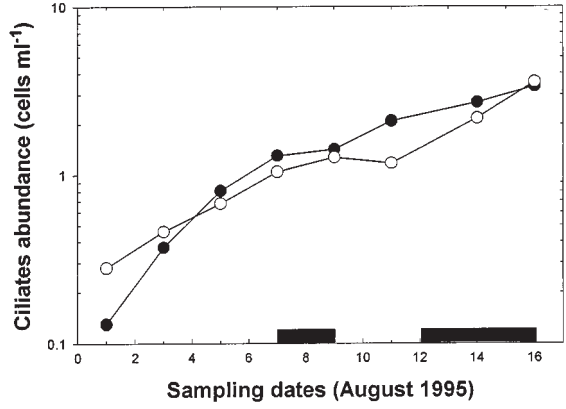

Fig. 4. Semi-log plot of the time course of the abundance of ciliates in the +UVB (○) and –UVB (●) treatments. Dark bars indicate cloudy days.

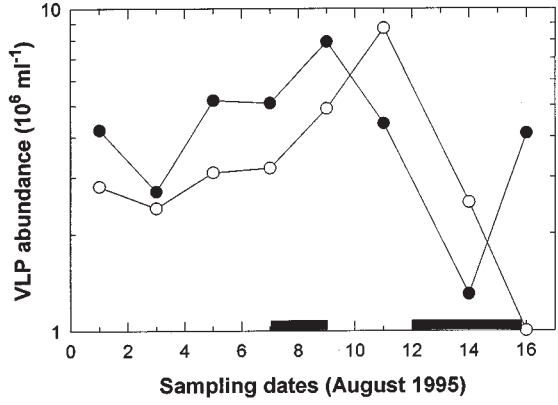

Fig. 5. Semi-log plot of the time course of the abundance of virus-like particles in the +UVB (○) and –UVB (●) treatments. Dark bars indicate cloudy days.

Bacterial activity

[^{3}H]Thymidine (TdR) incorporation rates increased remarkably from 1 to 5 August when bacterial abundance also increased, and decreased afterward in both treatments when bacterial abundance remained nearly constant (Figure 6). Two-way ANOVA for the bacterial activity data from all four experiments showed that there were significant differences between treatments ($P < 0.0001$) and between the experiments ($P < 0.0001$). Post hoc comparisons with the SNK test, however, indicated that there were no significant differences ($P > 0.05$) between the +UVB and –UVB treatments on 1 and 14 August. The interaction term for this ANOVA (treatments × experiments) was also not significant ($P = 0.083$). To compensate for the different bacterial abundance in the treatments, we calculated the cell-specific activity (TdR incorporation rate divided by the number of bacteria at each sampling occasion). The two-way ANOVA and post hoc comparisons with these values indicated significant ($P < 0.05$) differences between the treatments only for 5 August.

Bacterivory

The grazing rates (g) as well as the total number of bacteria grazed (TG) were higher in the –UVB treatment (Table III). The ratio between the total number of bacteria consumed in the –UVB and the +UVB treatments ranged between 1.8 and 3.1. Except for 9 August, when values of TG decreased slightly in both treatments, there was a clear increase between the beginning and the end of the experiment, e.g. from 1.58×10^5 to 4.42×10^5 cells ml^{-1} consumed during 48 h in –UVB and from 0.89 to 1.94 cells ml^{-1} × 10^5 consumed in 48 h in +UVB. A two-way ANOVA for the TG data showed that there were significant differences between treatments ($P < 0.001$) and among the four experiments ($P < 0.001$), as

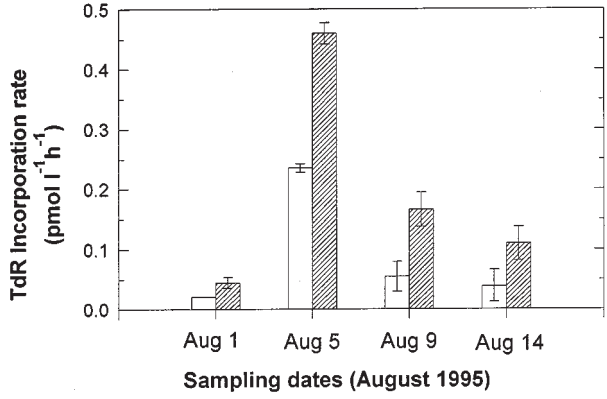

Fig. 6. Thymidine (TdR) incorporation rates on four sampling dates for the +UVB (white bars) and –UVB (black bars) treatments. Error bars represent ± 1 SD of three replicate measurements (quartz tubes).

well as for the interaction term (experiments × treatment). When *TG* was normalized to the abundance of HNF at the beginning of each experiment, the two-way ANOVA and post hoc comparisons showed that except for 2 August there were still significant differences ($P < 0.05$) between the treatments in all experiments. Because the parameter *TG* includes the disappearance of FLB, but also of bacteria, not necessarily all bacteria may have been eliminated by HNF grazing, i.e. lysis by viruses or other losses may have occurred concomitantly. Nevertheless, the two-way ANOVA for *g* normalized to the HNF abundance, which is based only on the disappearance of FLB, indicated a significant difference between treatments in all experiments.

The effect of solar UVB radiation on the microbial components

We assumed that each day's abundance for a given microbial component was a function of the previous day's abundance, their net specific growth, and the variability associated with sampling and measurement. Therefore, if UVB radiation strongly influenced, for example, the growth rate, we expected to find a relationship between the net rate of change of the species [$\ln (N_t/N_o)/t$, where N_o and N_t are the absolute values at two consecutive sampling dates, and t is the time between samplings] and UVB treatment. However, if the effect of radiation was relatively weak, but cumulative, then the rate of change between sampling dates would hardly show any relationship with radiation, because a masking effect could be expected from sampling variability, counting accuracy, and factors influencing the rate of change other than radiation. In that case, it would be more likely to find some relationship between abundance fluctuations and UVB treatment given that we had periods of several days under the same radiation conditions.

Table IV shows the results of the RDA for the abundance and the rate of change of the microbial components using UVB treatment (with or without) as an explanatory variable. The RDA indicated that UVB treatment only explained a significant amount of variance (>20%) for HNF and bacteria when considered as abundance, suggesting a negative and cumulative effect through the experiment. On the other hand, since during the experiment there were strong fluctuations in radiation (UVA + PAR) to which both enclosures were subjected, we also checked their influence in the changes observed. It appeared that UVA + PAR also significantly affected the rates of changes of HNF in a negative way. In this case, there is no sense in checking for cumulative effects since both enclosures experienced the same fluctuations in UVA + PAR.

Discussion

Effects of UVB radiation on bacteria and heterotrophic nanoflagellates

The effects of solar UVB radiation on a certain trophic level may be direct or indirect, i.e. resulting from effects on a higher or lower trophic level, as well as a combination of both. This makes it difficult to identify and separate these effects. The increase in phytoplankton abundance and chlorophyll *a* in both enclosures

Table III. Grazing rates, g (h^{-1}), and total bacteria consumed, TG (10^5 bacteria consumed ml^{-1} in 48 h), in the –UVB and +UVB treatments, calculated from averaged data. Values in parentheses are ± 1 SD of two replicates (quartz tubes). Ratio: TG in the –UVB treatment to TG in the +UVB treatment

Parameter	August 2		August 5		August 9		August 14	
	–UVB	+UVB	–UVB	+UVB	–UVB	+UVB	–UVB	+UVB
g	0.0049 (0.0005)	0.0029 (0.0002)	0.0078 (0.0011)	0.0032 (0.0002)	0.0070 (0.0003)	0.0025 (0.0003)	0.0067 (0.0004)	0.0034 (0.0001)
TG	1.58 (0.0086)	0.89 (0.0423)	3.60 (0.514)	1.24 (0.0930)	3.30 (0.1540)	1.06 (0.1380)	4.42 (0.0200)	1.94 (0.005)
Ratio	1.78		2.90		3.11		2.31	

Table IV. Evaluation of the sensitivity of the different microbial components and parameters to UVB treatment and radiation intensity (UVA + PAR) by means of redundancy analysis (RDA) using either the absolute abundance or their rate of change. Abbreviations of the variables are as in Table II. See the text for more information on the statistical analysis

Parameter	Explained variance (%)		
	UVB		UVA + PAR
	Abundance	Rate of change	Rate of change
BAC	–20	–4	21
Mean cell volume	0	0	1
FIL	1	0	1
FIL mean cell volume	–10	0	0
VLP	–4	–1	1
HNF	–24	–1	–25
CIL	–1	–5	17
Phytoplankton	7	0	6
Chl *a*	2	–4	13
DOC	1	0	–37
POC	–1	0	18
PON	–2	–1	20
Total	11	1	8.9
Significance	n.s.	n.s.	0.27

n.s., not significant.

was not only paralleled by the DOC and particulate phosphorus concentrations already discussed in Halac *et al.* (1997), but also by the POC and PON concentration (Figure 1), as indicated by the high correlation among these parameters (Tables I and II). This increase in abundance is generally observed in enclosure experiments because the walls act as a physical barrier against advection and export of nutrients and sedimentation losses, which stimulate the growth of most microbial components.

The non-filamentous bacteria in both treatments responded positively to the increase in resource conditions, as reflected in the high correlations with chlorophyll *a*, DOC or POC (Tables I and II). However, after the third experimental day, the standing stock of non-filamentous bacteria remained lower in the +UVB

treatment than in the –UVB one. Also the RDA (Table IV) indicated a significant negative effect for the +UVB treatment. The development of HNF was also negatively affected by UVB (Figure 2C). The RDA indicated a negative effect especially noticeable in the absolute values, suggesting a cumulative effect. Negative effects of UVB, but also of UVA radiation, on HNF have been reported for single freshwater and marine species, but have been rarely studied in natural assemblages (Sommaruga *et al*., 1996; Ochs, 1997).

The lower abundance of bacteria found in the +UVB treatment was unexpected because the grazing rates, calculated from the disappearance of FLB, as well as the total number of bacteria consumed (FLB + bacteria) normalized by the abundance of HNF in each experiment, indicated that the bacterivory pressure was suppressed by UVB radiation. One possible explanation for the lower abundance of bacteria is that their growth may also have been negatively affected by UVB radiation. Bacterial activity (TdR incorporation rate) differed significantly between treatments only on 5 and 9 August. Calculation of the cell-specific activity corroborated that even after normalizing by the difference in bacterial abundance between treatments, the negative effect was still significant on 5 August after an extended period of high UV radiation, thus pointing to a negative UVB effect on bacterial growth. As found in this lake and other freshwater and marine ecosystems, short-term exposure of bacteria to sunlight causes a strong inhibition of TdR incorporation rates (Herndl *et al*., 1993; Aas *et al*., 1996; Sommaruga *et al*., 1997). Therefore, one may argue that the effect of UVB discussed above may also include the particular conditions during the short-term exposure. For example, while on 5 August measurements were performed on a clear sunny day, on 9 August the weather was cloudy. However, in this lake, the inhibition of TdR incorporation after short-term exposure (4 h) to sunlight at the surface was found to be caused mainly by UVA and PAR (Sommaruga *et al*., 1997). Moreover, this inhibition has been found to be a transient phenomenon that recovers during the night (R.Sommaruga, unpublished). Therefore, our results stress the importance of considering the potential different response of organisms when exposed to UVB radiation for short- and long-term scales, i.e. longer than their generation times.

In contrast to the non-filamentous bacteria, the filamentous or thread-like forms appeared to be not sensitive to UVB radiation. Thread-like bacteria, although only a small fraction in terms of numbers (<4%), make a significant contribution (up to 70%) to the total bacterial biovolume. Recently, the fraction of thread-like bacteria present in this lake during summer has been assigned to the *Cytophaga–Flavobacterium* cluster (Pernthaler *et al*., 1998).

Effects on ciliates

Urotricha pelagica and *U.furcata* were the dominant ciliates in both treatments. Both species responded positively in both treatments to the increment in phytoplankton and bacteria on which they are known to feed (Sommaruga and Psenner, 1993; Foissner and Pfister, 1997). Effects of UV radiation on ciliate growth have been studied only for a very limited number of species and no information is

available for *Urotricha* sp. One negative effect found under artificial UV exposure in certain species is the so-called retardation effect, i.e. a delay in reproduction rates (Giese *et al*., 1963). Although the total abundance of ciliates at the end of the experiment was the same, the net growth rate in the +UVB treatment during the first week (sunny conditions) was lower than that in the –UVB one, suggesting a retardation effect.

Effects on VLP

The VLP:bacteria ratio inside the enclosures was lower (1.3–14) compared to that found at the lake surface during the same period (4.2–31.1; Pina *et al*., 1998). The abundance of VLP in both treatments was not significantly correlated with changes in the abundance of any of the microbial components considered. However, numbers increased in both treatments during the first part of the experiments following the development of bacteria and phytoplankton, and decreased dramatically during the last part when the weather was cloudy (Figure 5). These changes indicate that viruses were a dynamic component of the plankton. The higher abundance of VLP observed during the first week in the –UVB than in the +UVB treatment may be explained by two non-exclusive processes: a higher viral production or a lower decay rate. However, as the percentage of infected bacteria and the burst size were not estimated we cannot draw a conclusion about their dynamics. Changes in the abundance of free VLP are affected by lytic processes that supply new viruses, and by several loss mechanisms like host infection, sedimentation after adsorption to microaggregates, grazing by protists and digestion by proteolytic enzymes (Suttle and Chen, 1992). On the other hand, infectivity of free viruses seems to be largely affected by solar UVB, but also by UVA radiation (Suttle and Chen, 1992). Nevertheless, this infectivity loss never seems to be 100% (Wilhelm *et al*., 1998). These authors found that despite high DNA viral damage, >50% of the viruses were still infective.

The enclosure approach

The lake considered in this study has a high UV penetration, with ~10% of the UVB (305 nm) intensity at the surface still reaching the bottom (Sommaruga and Psenner, 1997). Consequently, microorganisms at the surface are exposed to a high UVB dose, particularly when trapped in diurnal thermoclines. However, because of partial mixing during the night and cloudy days, the assemblages trapped in consecutive days are not necessarily the same. Therefore, one potential artefact of our experimental approach with enclosures is that organisms were exposed for days in a 1-m-depth water column, while in reality they may be transported up and down by wind-driven vertical mixing. The interaction of UV effects and vertical mixing has rarely been considered and little is known about whether reciprocity, i.e. the effects of UV radiation are independent of the dose rate, applies to heterotrophic organisms or heterotrophic processes. Therefore, it is difficult to evaluate how critical this artefact was for the negative effects of UVB radiation observed in this study. Recently, Neale *et al*. (1998) used a model to

include the interactive effect of vertical mixing with the UV impact on phytoplankton photosynthesis in Antarctic waters. They found that vertical mixing may either enhance or decrease the inhibition on integrated photosynthesis through the water column, depending on the depth of the mixed layer. When the mixing depth was lower than the photic zone, the inhibition was enhanced compared to the static case, while mixing below this zone caused an opposite effect. Their results were also congruent with previous empirical observations made on phytoplankton photosynthesis by Helbling *et al.* (1994). Furthermore, in experiments where vertical mixing was simulated in a relatively turbid shallow lake, the mortality of a copepod was significantly higher in the rotating than in the static treatment (Zagarese *et al.*, 1998). Therefore, mixing may not necessarily reduce the negative effects of UVB radiation under real conditions in Gossenköllesee, although this crucial aspect needs to be investigated further.

Our results show that the impact of ambient levels of solar UVB radiation was different among the components of the microbial food web of this lake and even within one component, e.g. heterotrophic bacteria. Halac *et al.* (1997) showed that no UVB effect on phytoplankton growth and species composition was evident in the same enclosure experiment. In the present study, the bacterial and the HNF assemblages were considered as a 'black box', i.e. no species differentiation was made. However, it may have been possible that UVB-sensitive species were replaced by more resistant ones over time.

Finally, our data suggests that in lakes of low DOC concentration, solar UVB radiation may affect the dynamics of carbon flow in an opposite way to that proposed for lakes rich in humic substances where solar UV radiation was found to have an indirect stimulatory effect on bacteria through DOC photodegradation (Reitner *et al.*, 1995; Wetzel *et al.*, 1995).

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