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The interaction of phytoplankton and bacteria in a high mountain lake: Importance of the spectral composition of solar radiation

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Abstract

The role of spectral composition of solar radiation has seldom been considered as a critical factor controlling the algae–bacteria relationship. A coupled algae–bacteria relationship mediated by C released from algae was observed during a 2-yr period (1996–1997) in an oligotrophic high mountain lake, except at upper depths. The intensity of photosynthetically active radiation was negatively related to primary production, and the highest percentages of excretion of organic carbon (%EOC) from algae were found at upper depths of the water column. The effect of different spectral regions of solar radiation on the algae–bacteria relationship was assessed by in situ experiments, in which the exposure, tracer uptake by target organisms, and interactions among abiotic and biotic factors were simultaneous. Primary production was ultraviolet radiation (UVR) inhibited by 33–83% depending on depth and date, with ultraviolet-A radiation (UVA) exerting the main effect. EOC and %EOC yielded highest values under UVR exposure. Sunlight affected bacterial production (BP) only at upper depths. UVB inhibited BP by 39–82%, whereas UVA + photosynthetic active radiation (PAR) and PAR enhanced BP three- to fourfold. Full sunlight increased BP 2.5-fold in midsummer but inhibited it (37%) in the late open-water period. The percentage of photosynthetic exudates assimilated by bacteria, and photosynthetic carbon use efficiency by bacteria, showed a similar pattern to that of BP. The experimental results support our hypothesis that increased organic C release from UV-stressed algae stimulates bacterial growth if the bacteria are relatively well adapted to sunlight, determining a coupled algae–bacteria relationship. Thus, sunlight may play a key role as the underlying abiotic factor that regulates algae–bacteria interaction in shallow and clear-water ecosystems.

The degradation of Earth's ozone layer, one of the main causes of global climate change, has allowed an increase in solar ultraviolet-B (UVB) radiation fluxes on the Earth's surface (Crutzen 1992) and aquatic ecosystems (Karentz et al. 1994; Häder 1997). The pelagic planktonic community functions through a web of energy and nutrient exchanges, mediated by a diverse array of producers and consumers that ultimately depend on the energy supplied by sunlight. Thus, alterations in spectral composition of solar radiation can modify the structure (Vinebrooke and Leavitt 1999) and functioning of the pelagic food web. In order to understand the C cycle in aquatic ecosystems, we need to explore the effects of different spectral regions of solar radiation on primary production, C release from phytoplankton, C assimilation by bacteria, and C flux from the microbial loop to higher trophic levels. This knowledge would advance our understanding of the global C cycle and its impact on global warming.

The effect of ultraviolet radiation (UVR) on primary producers in aquatic ecosystems has received considerable attention. UVB radiation (290–320 nm) was reported to inhibit primary production (Vincent and Roy 1993), probably through inhibition of photosystem II (Schofield et al. 1995) and RUBISCO activities (Neale et al. 1993). Other studies showed that ultraviolet-A radiation (UVA, 320–400 nm) is a major inhibitor of primary production (Bühlman et al. 1987; Helbling et al. 2001), regardless of stratospheric ozone concentrations. Some authors demonstrated UVB-induced damage to the DNA (e.g., pyrimidine dimers) of phytoplankton species (Helbling et al. 2001), which could inhibit their growth, while Quesada et al. (1995) showed that UVA can counteract this effect.

In contrast to our knowledge of the effects of UVR on primary producers, there appears to be no published information about the impact of spectral composition of solar radiation on the release of organic C by algae in natural conditions. Nevertheless, it has been reported that phytoplankton release a high proportion of photosynthetic C in highly light-stressed conditions, such as the upper depths of oligotrophic lakes (Maurin et al. 1997), because of an uncoupling between photosynthesis and algal growth (Berman-Frank and Dubinsky 1999).

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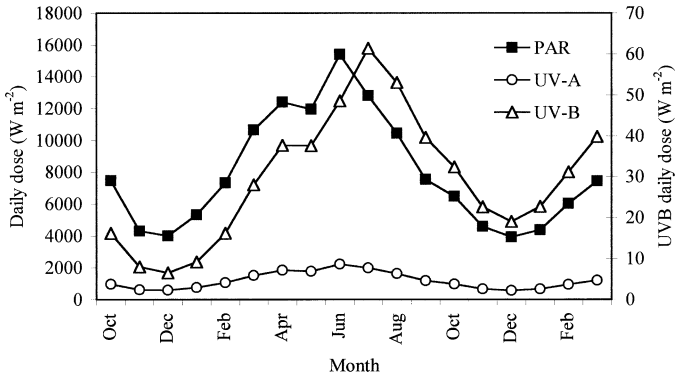

Fig. 1. Daily dose of PAR, UVA, and UVB (W m^{-2}) received from October 1997 to March 1999 at a station near La Caldera Lake at 2,850 m altitude, measured with a Eldonet dosimeter by F. López-Figueroa.

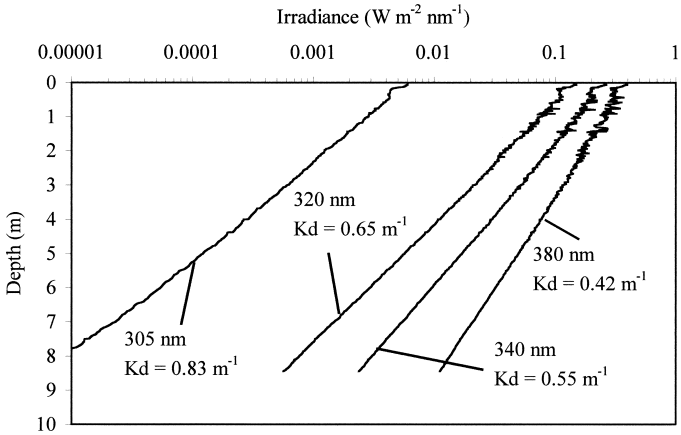

Fig. 2. Semilog plot of UV irradiance at 305, 320, 340, and 380 nm against depth in La Caldera Lake measured on 26 September 1998 with a multichannel radiometer PUV-500B by R. Sommaruga. The diffuse attenuation coefficients (*Kd*) are also shown.

Recent studies reported inhibited bacterial production rates by UVB radiation (Herndl et al. 1993; Aas et al. 1996) and documented the role of UVB radiation in the formation of pyrimidine dimers in bacterioplankton DNA (Jeffrey et al. 1996; Visser et al. 1999). Other authors found similar or greater inhibitory effects of UVA on bacterial activity in comparison with UVB (Aas et al. 1996; Sommaruga et al. 1997; Visser et al. l999). Thus, the negative impact of UVR on bacteria may diminish the bacterial uptake of organic C released by algae. This reduction may constrain immediate C availability at high trophic levels via the microbial loop, causing the potential accumulation in the water column of dissolved organic matter (DOM) that can be photoaltered by exposure to solar radiation (Obernosterer et al. 1999).

On the other hand, UVB-induced damage of the DNA of organisms can be photorepaired by UVA and visible radiation through photolyases (Kim and Sancar 1993) or repaired by light-independent mechanisms such as nucleotide excision and postreplication repair (Sancar and Sancar 1988). Furthermore, UVR may indirectly stimulate bacterial growth by partial photooxidation of DOM to lower molecular weight compounds (Wetzel et al. 1995; Obernosterer et al. 1999). This effect has also been suggested for phytoplankton because more nutrients are made available after the photochemical breakdown of DOM (Wängberg et al. 1999). The scarcity of the research to date precludes reliable predictions of the effects that different spectral regions of solar radiation simultaneously exert on primary and bacterial production and algae–bacteria interactions.

Field studies concerning the relationship between primary and bacterial production reported a tight coupling between these in lakes with low allochthonous inputs and absence of anoxic or hypoxic hypolimnetic depths (*see* discussion in Pace and Cole 1994) or with high inorganic N : P ratio (Le et al. 1994). Although these characteristics are shared by most oligotrophic high mountain lakes above the treeline (Straskrabová et al. 1999*a*), the high fluxes of UVR in the latter ecosystems (Laurion et al. 2000) may also play an important role in this relationship.

Thus, we hypothesized that UVR stress inhibits phytoplanktonic primary production and increases photosynthetic extracellular product release in clear-water oligotrophic ecosystems. As a consequence, short-term bacterial production would augment, establishing a coupled relationship between the two communities. This scenario would only be plausible if the detrimental effects of UVB and UVA on bacteria were counteracted by the beneficial effects of UVA and visible radiation (Kim and Sancar 1993; Kaiser and Herndl 1997; Davidson and van der Heijden 2000), thus allowing the enhancement of bacterial production due to this higher organic C availability.

We tested the above hypothesis as follows: first, the relationship between primary and bacterial production over both seasonal (open-water period) and spatial (vertical profile) scales was analyzed in natural conditions over two years; second, the factors regulating the bacterial and primary productions were established; third, the role of spectral composition of solar radiation in primary production, C release from algae, bacterial production, and their commensalistic interaction was experimentally investigated; and, finally, the experimental results obtained under full sunlight were extrapolated to the natural conditions.

Methods

Study site—La Caldera Lake is a remote oligotrophic high mountain lake situated above the treeline. This ecosystem is located in the Sierra Nevada mountain range (Southern Spain, 36°55′–37°15′N, 2°31′–3°40′W) on siliceous bedrock in a glacial cirque at an altitude of 3,050 m. The lake, which is normally covered by ice from November until mid-July, had a surface area of around 20,000 m^2 and a maximum depth of 14 m (mean depth: 4.3 m) during 1996–1997. The lake water is highly transparent (>10% photosynthetic active radiation [PAR, 400–700 nm] penetrates to maximum depth) and, like other high mountain lakes, receives considerable intensity of UVB (Laurion et al. 2000) (Figs. 1, 2).

There are no visible inlets or outlets, and the drainage basin area : lake surface area ratio was 7.3 in both years (Villar-Argaiz et al. 2001). Inputs of allochthonous organic C are negligible and largely restricted to the ice-melting period.

The dissolved organic carbon (DOC) concentration is nearly 0.5 mg L^{-1}, and most DOC is of autochthonous origin, as indicated by the $F_{450}:F_{500}$ fluorescence ratio of approximately 1.9 (Reche et al. 2001). The balance between new and regenerated production tends to shift toward regeneration as the season progresses. Macrophytes, littoral vegetation, and fish are absent, and the pelagic community is relatively simple (Carrillo et al. 1996*a;* Reche et al. 1996; Medina-Sánchez et al. 1999; Villar-Argaiz et al. 2001, 2002).

Physical, chemical, and biological parameters—Sampling was conducted every 7 to 10 d throughout the open-water period from July to early November in both 1996 and 1997. A total of 64 samples were collected each year. Temperature (*T*) profiles were measured with a YSI meter on each sample date. Chemical and biological samples were gathered with a Van Dorn sampler at the deepest point of the lake from four depths (0.5, 5, 8, and 0.5 m above bottom). Samples (three replicates per depth) of lake water were collected for analysis of total phosphorus (TP) and total nitrogen (TN) and, after filtration through 0.45-μm disposable filters (Sartorius), for analysis of total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP), total dissolved nitrogen (TDN), nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+). We defined dissolved inorganic nitrogen (DIN) as $NO_3^- + NO_2^- + NH_4^+$. Samples for inorganic nutrients were analyzed on the same day as the collection, while samples for TN, TP, TDN, and TDP were persulphate-oxidized and analyzed within 48 h. NO_2^- was analyzed using the sulphanilamide method, NO_3^- by chromatographic analyses, NH_4^+ by the phenol-hypochlorite method, SRP by the acid molybdate technique, TDN and TN by the ultraviolet spectrophotometric screening method, and TDP and TP were analyzed colorimetrically using the acid molybdate technique (American Public Health Association 1992). Spectrophotometric chlorophyll *a* determinations, corrected for phaeopigments, were carried out after filtration at <100 mm Hg of 2–3 liters of lake water (Whatman GF/C) and 24 h cold-pigment extraction in 90% acetone (Jeffrey and Humphrey 1975).

A 50-ml aliquot from each phytoplankton sample was sedimented for 48 h in a Utermöhl chamber of 2.6-cm diameter, and cells were counted in 100 randomly selected fields of view at 2,000× magnification under an inverted microscope. For each sample, at least 600 cells of the more abundant phytoplanktonic species were counted, and 20–30 cells of each species were measured for each date and depth by image analysis (Leica Quantimet 500) in order to estimate the cell volume according to a corresponding geometrical shape. The biovolume density (μm^3 ml^{-1}) for each taxon was determined by multiplying the mean cell volume by the abundance. The cell volume was converted to carbon using the specific conversion factors reported by Rocha and Duncan (1985) and Vadstein et al. (1988).

Bacterial abundance was determined by epifluorescence microscopy from each bacterial sample, preserved with 2% (final concentration, f.c.) 0.2-μm-filtered and neutralized formaldehyde, stained with DAPI, and filtered through 0.2-μm pore-size black Nucleopore filters (Porter and Feig 1980). At least 400 cells were counted for every sample. The bacterial biomass (μg C $cell^{-1}$) was calculated according to the allometric relationship between cell volume and carbon content reported by Norland (1993). The cell volume was calculated by approximating each cell to its geometrical shape from bacterial images obtained by transmission electron microscopy (TEM), after concentrated bacterial subsamples were placed on electron microscopy grids, stained with 1% uranyl acetate, examined, and photographed at 20,000× magnification along with calibration grids.

Measurements of primary and bacterial production within the water column—Primary and bacterial production was determined at different depths throughout the open-water period from July to early November in both 1996 and 1997, yielding a total of 24 measurements (in triplicate). Primary production (PP) was measured with the ^{14}C method proposed by Steeman-Nielsen (1952). Sets of four 70-ml quartz flasks (three clear and one dark), with 0.37 MBq of $NaH^{14}CO_3$ (specific activity: 310.8 MBq $mmol^{-1}$, NEN Dupont) added to each flask, were incubated in situ at 0.5 m under surface, 0.5 m above the bottom of the lake, and an intermediate depth, for 4 h symmetrically distributed around noon. All flask sets were horizontally held during the incubations.

Primary production was measured as total organic carbon (TOC) by acidifying a 4-ml subsample in a 20-ml scintillation vial with 100 μl of 1 N HCl and allowing the vial to stand open in a hood for 24 h (no bubbling), as recommended by Lignell (1992). Particulate primary production was determined by filtering an aliquot of 60 ml through 1.0-μm (particulate organic carbon >1 μm, POC_1) and 0.2-μm (particulate organic carbon 0.2–1 μm, POC_2) pore-size Nuclepore filters of 25-mm diameter (serial filtration). To minimize cell breakage, we applied low pressure (<100 mm of Hg). The filters were placed in scintillation vials and the $DI^{14}C$ was removed by adding 100 μl of 1 N HCl. The filtrate <0.2 μm (dissolved organic carbon, DOC) was also collected and treated as described above for the TOC. We added 16 ml of liquid scintillation cocktail (Beckman Ready Safe) to the vials, and after 12 h the radioactivity was counted in a scintillation counter equipped with autocalibration (Beckman LS 6000 TA). The total CO_2 in the lake water was calculated from the alkalinity and pH measurements (American Public Health Association 1992). In all calculations, dark values were subtracted from corresponding light values.

No autotrophic picoplankton were found in 1-μm-filtered lake-water samples preserved with 1% (f.c.) formaldehyde-cacodylate buffer, after filtering them through black Nuclepore 0.2-μm pore-size filters and examining them by autofluorescence in a Leitz Fluovert FS epifluorescent microscope within 2 weeks after the sampling (Straskrabová et al. 1999*b*). Furthermore, no significant differences (*t*-test for dependent samples, $t = 1.40$, $df = 5$, $p = 0.22$) were found in bacterial counts between whole and 1-μm-filtered water. Both findings imply that the ^{14}C retained on the 0.2-μm pore-size filters from <1-μm filtrates corresponded to the exudates incorporated by heterotrophic bacteria (POC_2). The excretion of organic carbon (EOC) was calculated as the sum of the DOC and POC_2, while the percentage of excretion of organic carbon (%EOC) was estimated as

$$\%EOC = EOC \times PP^{-1} \times 100 \quad (1)$$

The percentage of photosynthetic exudates assimilated by bacteria (%PEA) was calculated as

$$\%PEA = POC_2 \times EOC^{-1} \times 100 \quad (2)$$

Bacterial production (BP) was measured using the radio-labeled thymidine incorporation technique (Fuhrman and Azam 1982, modified following Torreton and Bouvy 1991). A set of ten (6 + 4 blanks) acid-cleaned and sterilized flasks, each flask filled with 25 ml of lake water and with [methyl-^{3}H] thymidine (SA: 2.6–3.2 TBq mmol^{-1}, Amersham Pharmacia) added to reach 10.9 nM (f.c.), was incubated in situ in the dark at each of the above three depths for 1 h. This procedure provided a saturating [^{3}H]TdR concentration and a suitable incubation time (uptake of [^{3}H]TdR was linear over this period) for this system. At the end of the incubation, NaOH was added (0.25 N, f.c.), causing basic hydrolysis of the ^{3}H-labeled RNA and stopping [^{3}H]TdR incorporation. NaOH was added to blanks (0.25 N, f.c.) before the incubations. In the laboratory, the samples were treated with cold trichloroacetic acid (TCA) for 20 min (5%, f.c.), leading to the precipitation of the DNA and proteins. Samples were filtered through 0.2-μm pore-size Nuclepore filters and rinsed twice with cold 5% TCA. Half of the filters (3 + 2 blanks) were then enzymatically digested with DNAse I (Boehringer Mannheim) solution (pH = 7.5) at 37 °C for 2 h under gentle stirring, so that only macromolecules other than DNA remained in the filters. The [^{3}H]TdR incorporated into DNA was calculated as the difference between the two treatments (Torreton and Bouvy 1991). The incorporated [^{3}H]TdR was converted to the number of cells produced by using the conversion factor of 1.07×10^{18} cells mol^{-1} of incorporated [^{3}H]TdR calculated for this system. The amount of bacterial C produced was estimated by using the factor of 2×10^{-14} g C cell^{-1} (Lee and Fuhrman 1987).

The percentage of photosynthetic C use efficiency by bacteria (%CUEb) was calculated as

$$\%CUEb = BP \times EOC^{-1} \times 100 \quad (3)$$

UV measurements—Irradiance-depth profiles were obtained on sunny days in September 1998 (within 3 h of solar noon), for 305, 320, 340, and 380 nm (full bandwidth at half maximum is 8–10 nm) and PAR (400–700 nm) by R. Sommaruga (University of Innsbruck, Institute of Zoology and Limnology, Innsbruck, Austria), using a multichannel radiometer (PUV-500B: Biospherical Instrument). Air UV radiation was measured by F. Figueroa (University of Malaga, Spain) with an Eldonet dosimeter in a nearby station at 2,850-m altitude. Irradiance-depth profiles for PAR (400–700 nm) were established with a spherical radiation sensor LICOR (LI 193SA) for each sampling and experimental date.

Diffuse attenuation coefficients for downward irradiance ($K_{d,\lambda}$) were determined from the slope of the linear regression of the natural logarithm of downwelling irradiance ($E_{d,\lambda}(z)$) versus depth (z). A large sample size (pairs of $E_{d,\lambda}(z)$ and z values, $n > 350$) was used and a good fit ($r^2 > 0.98$) was obtained for all regressions.

Because of the lack of direct measurements of UV radiation in 1997 and the stability of the DOC concentrations (around 0.5 mg L^{-1}) in La Caldera Lake (data from R. Sommaruga and I. Reche, pers. comm.), the percentage of surface UV irradiance reaching the experimental depths was estimated following a modification of the model of Morris et al. (1995) for a DOC concentration <1 mg L^{-1}. This model was the one that best fit the measured UV irradiance profile from among four models tested (results not shown). The equations relating $K_{d,\lambda}$ to DOC concentration were

$$K_{d,305} = 7.62[DOC]^{3.07} \quad (4)$$

$$K_{d,320} = 5.62[DOC]^{2.89} \quad (5)$$

$$K_{d,340} = 4.52[DOC]^{2.96} \quad (6)$$

$$K_{d,380} = 2.68[DOC]^{2.97} \quad (7)$$

where [DOC] is the DOC concentration. The explained variance was high in all equations ($r^2 > 0.89$, $n = 16$).

Experimental design—We conducted the experiments on cloudless days in the middle and at the end of the open-water period of 1997, two distinct periods in the seasonal succession of the pelagic community in La Caldera Lake (Carrillo et al. 1996*a;* Medina-Sánchez et al. 1999; Villar-Argaiz et al. 2001). For each experiment, a composite water sample was constructed from equal volumes of water samples obtained with an acid-cleaned 6-liter horizontal Van Dorn sampler from three depths, spaced evenly within the photic layer affected by >1% UVB (from surface to 5 m), and prescreened through a 40-μm mesh to remove zooplankton. Experimental 70-ml quartz flasks (primary production) and 25-ml quartz flasks (bacterial production) were filled immediately before incubation from the homogenate sample. The respective radio-labeled tracer (*see above*) was added, and the sets of flasks for each light treatment were incubated in situ at 0.5 and 5 m, depths that corresponded to 64% and 1.1% transmission of UVB_{305}, respectively. The light treatments were the following: (1) full sunlight, using quartz flasks; (2) exclusion of UVB (280–320 nm), covering the flasks with Mylar-D foil (Dupont de Nemours); (3) exclusion of UVB and UVA (320–400 nm), covering the flasks with Plexiglas UF3, and (4) darkness, covering the flasks with opaque material. Optical properties of the cutoff filters used in the light treatments were tested prior to the experiments with a double-beam spectrophotometer (Perkin-Elmer Lambda 40). Cutoff filters were replaced for each experiment.

Because water samples were taken from lake water at around noon, the organisms had already been submitted to full sunlight for >5 h. The laboratory procedures to measure primary and bacterial production in the light experiments were the same as those used for the water column (*see above*).

Statistical analysis—Interannual or spatial variation of algae and bacterial activity variables was tested by *t*-test, or by Mann–Whitney *U*-test when conditions of parametric tests were not met. The relationship between bacteria and algae was evaluated through regression analyses of bacterial versus algae abundance, bacterial versus algae biomass, and bacterial production (BP) versus primary production (PP).

To assess the relative influence of potential factors affecting PP (PAR, TP, TN, T) and BP (EOC, TP, TN, T) in each year, stepwise multiple-regression analyses were carried out. Linearity and multiorthogonality between independent variables were verified by previous correlation analysis, and normal distribution of residues was checked by the Shapiro–Wilks *W*-test. Interannual shifts in the slope of the regression lines were tested by parallelism (interaction by covariates) test. The effect that a given spectral region of solar radiation exerted on each response-variable (i.e., PP, BP, etc.) was tested by one-way ANOVA analysis, comparing the light treatments that exclusively differed for this spectral region. Data were checked for normal distribution by the Shapiro–Wilks *W*-test. Homocedasticity was verified with the *F*-ratio variance and Levene's tests, and the data were log transformed when these conditions were not met. Statistics were performed with Statistica 5.1 for Windows (StatSoft, Inc. 1997).

Results

Physical, chemical, and biological parameters—Irradiance-depth profiles for 305, 320, 340, and 380 nm measured in La Caldera Lake in 1998 are shown in Fig. 2. According to Eqs. 4–7 and irradiance-depth profiles for PAR, the 0.5-m depth received approximately 64% of UVB_{305}, 68% of UVB_{320}, 75% of UVA_{340}, 84% of UVA_{380}, and 92% (on average) of PAR, whereas the 5-m depth received approximately 1.1% of UVB_{305}, 2.2% of UVB_{320}, 5.4% of UVA_{340}, 18% of UVA_{380}, and 41% (on average) of PAR. Table 1 displays the mean and intraannual range of some physical, chemical, and biological parameters during early, middle, and late open-water periods in 1996 and 1997 in La Caldera Lake. The lake-water temperature seasonally oscillated within a narrow range during 1996–1997, yielding higher mean values in 1997 than in 1996 (Table 1). The lake exhibited weak thermal stratification in midsummer, with average surface–bottom differences ranging from 1.4°C in 1996 to 2.7°C in 1997. TP values were lower than 10 μg P L^{-1}. Nitrogen was found mainly as inorganic forms during 1996 and from thaw to September in 1997, when DIN represented more than 50% of TN. DIN and NO_3^- slowly decreased over the summer in both years. DIN : TP (by weight) ratio values exceeded 12, which implies a strong likelihood of P limitation for the pelagic community, according to Morris and Lewis (1988).

The autotrophic community was relatively simple and was composed of nanoplankton species, as is characteristic in oligotrophic high mountain lakes (Straskrabová et al. 1999*a*; *see* seasonal shift taxonomic composition in Medina-Sánchez et al. 1999; Villar-Argaiz et al. 2001). Phytoplanktonic standing-stock parameters reached maximum values in the middle of the open-water period in both years. Interestingly, maximum values of algal standing-stock parameters were higher in 1996 than in 1997, alongside a far lower development of zooplankton community (Medina-Sánchez et al. 1999; Villar-Argaiz et al. 2001, 2002).

The heterotrophic bacterial community of La Caldera Lake was composed of free small coccus-like (0.01 ± 0.004 μm^3) and rod-shaped forms (0.05 ± 0.02 μm^3). Filamentous bacteria (0.18 ± 0.07 μm^3) were scarce. Whereas bacterial abundance and biomass showed a significant inverse relationship with algal biomass ($r = -0.85$, $p < 0.001$, $n = 15$) in 1996, abundances of the two communities were positively correlated in 1997 ($r = 0.69$, $p = 0.005$, $n = 15$), reaching their respective maximum density values in late August–early September.

Primary production showed no significant interannual variation ($t = 0.59$, df = 22, $p = 0.56$). Although average values for the entire water column in each year shared a decreasing trend during the open-water period, the most striking results were the accentuated differences in the vertical profile each year, with significantly higher values at deepest versus upper depths (1996, $t = 4.06$, df = 6, $p = 0.0066$; 1997, $t = 5.34$, df = 6, $p = 0.0018$; Fig. 3). This vertical pattern was related to PAR intensity in both years, whereas the vertical distribution of other tested abiotic variables, such as nutrients (TN, TP) or temperature (*T*), did not significantly explain the PP variance (Table 2).

The excretion of organic carbon (EOC) yielded significantly higher values in 1996 than in 1997 ($t = 4.29$, df = 22, $p = 0.0003$). EOC and PP were significantly correlated in both years (1996, $r = 0.98$, $p < 0.001$, $n = 12$; 1997, $r = 0.95$, $p < 0.001$, $n = 12$). The %EOC, like the EOC, yielded significantly higher values in 1996 than in 1997 ($U = 18$, $p = 0.0018$). Interestingly, the %EOC showed higher values at upper versus deeper depths (1996, $t = 4.52$, df = 10, $p = 0.0011$; 1997, $U = 2$, $p = 0.023$).

BP yielded significantly higher values in 1997 than in 1996 ($t = 4.12$, df = 20, $p = 0.0005$), whereas bacterial assimilation of the photosynthetic C released by algae (POC_2) showed the opposite pattern ($t = 3.78$, df = 22, $p = 0.0010$). Nevertheless, when bacterial assimilation was normalized to available exudates, the %PEA values showed no significant differences between the two years ($t = 1.93$, df = 22, $p = 0.0668$; Table 1).

Bacterial production (BP) and PP showed a significant coupled relationship (Fig. 3). BP variance was significantly explained only by the EOC, because neither nutrients (TN, TP) nor temperature were included by stepwise regression analysis (Table 2). Interestingly, the positive BP-PP and BP-EOC relationships were not maintained at upper depths in either year (Figs. 3B,C and 4B,C), and the slopes of BP-PP and BP-EOC regression lines were significantly higher in 1997 than in 1996 (parallelism tests, $F_{1,18}$, $p = 0.0010$ for BP-PP; $F_{1,18}$, $p = 0.0018$ for BP-EOC). Besides, although the %CUEb did not show a clear pattern over the seasonal or spatial scales in either year, the values were significantly higher ($U = 0$, $p < 0.0001$) in 1997 than in 1996 (Table 1).

Experimental effects of spectral composition of solar radiation on primary production and organic C release by algae—Incorporation of ^{14}C by phytoplankton was significantly inhibited by UVR at both depths. Overall, UVR significantly inhibited the primary production (PP) by 81–83% at upper depths ($F_{1,4}$, $p < 0.001$ in each period) and by 33–44% at intermediate depths ($F_{1,4}$, $p < 0.001$ in midsummer; $F_{1,4}$, $p < 0.005$ in late open-water period). PP values at upper depths decreased from 2.36 ± 0.02 μg C L^{-1} h^{-1} under the PAR treatment to 0.45 ± 0.03 μg C L^{-1} h^{-1} under the full

Table 1. Mean and intraannual range of physical, chemical, and biological parameters during early, middle and late open-water periods in 1996 and 1997 in La Caldera Lake. *T*: temperature. TP: total phosphorus. TN: total nitrogen. DIN: dissolved inorganic nitrogen. PA: phytoplankton abundance. PB: phytoplankton biomass. Chl *a*: Chlorophyll *a*. BA: bacterial abundance. BB: bacterial biomass. PP: primary production. POC_1: particulate organic carbon in >1 μm fraction. POC_2: particulate organic carbon in 0.2–1 μm fraction. EOC: excretion of organic carbon. %EOC: EOC normalized to organic C produced. %PEA: percentage of photosynthetic exudates assimilation by bacteria. %CUEb: photosynthetic carbon use efficiency by bacteria. BP: bacterial production.

	1996			1997		
	Early open-water period (Jul)	Middle open-water period (Aug–Sep)	Late open-water period (Sep–Oct)	Early open-water period (Jul)	Middle open-water period (Aug–Sep)	Late open-water period (Sep–Oct)
T	2.6	7.7	5.9	6.3	11.8	8.5
(°C)	(1.2–4.1)	(4.7–9.8)	(5.0–7.0)	(2.6–10.6)	(11.3–12.4)	(7.5–11.1)
TP	4.68	5.36*	3.70	4.72	4.64	3.87
(μg L^{-1})	(4.40–5.23)	(4.73–6.25)	(3.30–3.90)	(3.40–7.33)	(3.25–5.95)	(3.00–4.78)
TN	520	470	402	339	296	293
(μg L^{-1})	(479–555)	(439–525)	(375–436)	(251–477)	(198–464)	(200–393)
DIN	337	258	233	206	141	94.1
(μg L^{-1})	(317–351)	(224–310)	(225–238)	(169–266)	(103–166)	(88.5–104)
DIN : TP	72.3	48.6*	63.3	47.8	31.6	25.1
	(66.3–79.8)	(36.9–65.6)	(57.8–71.0)	(23.0–78.3)	(22.8–43.9)	(18.5–31.0)
PA	3.19	13.2	10.9	6.39	10.3	5.31
(cells ml^{-1}) × 10^3	(0.21–6.14)	(2.70–31.9)	(5.46–23.9)	(3.13–11.7)	(5.80–17.0)	(3.96–6.33)
PB	60.3	35.2	28.0	36.8	39.3	22.1
(μg C L^{-1})	(50.1–78.5)	(12.5–68.1)	(14.4–58.6)	(19.2–49.4)	(29.3–50.0)	(15.0–35.5)
Chl *a*	1.57	1.92	0.89	0.86	0.41	0.22
(μg L^{-1})	(1.05–2.08)	(0.99–2.85)	(0.57–1.48)	(0.51–1.15)	(0.16–0.64)	(0.14–0.39)
BA	2.80	5.33	6.39	1.57	2.50	1.80
(cells ml^{-1}) × 10^5	(1.82–3.85)	(4.09–6.95)	(3.94–7.93)	(1.22–2.15)	(2.10–3.30)	(1.34–2.49)
BB	2.84	5.22	6.31	1.54	2.41	1.73
(μg C L^{-1})	(1.83–4.00)	(4.03–6.74)	(3.92–7.84)	(1.19–2.09)	(2.02–3.20)	(1.28–2.40)
PP	0.96	0.93	0.73	0.97	0.89	0.27
(μg C L^{-1} h^{-1})	(0.46–1.91)	(0.62–1.21)	(0.26–1.22)	(0.64–1.34)	(0.35–1.76)	(0.12–0.42)
POC_1	0.38	0.52	0.25	0.46	0.58	0.11
(μg C L^{-1} h^{-1})	(0.18–0.90)	(0.29–0.72)	(0.08–0.59)	(0.25–0.65)	(0.21–1.10)	(0.05–0.17)
POC_2	0.07	0.06	0.05	0.03	0.03	0.01
(μg C L^{-1} h^{-1})	(0.05–0.10)	(0.03–0.08)	(0.01–0.09)	(0.02–0.03)	(0.02–0.05)	(0.00–0.01)
EOC	0.32	0.28	0.28	0.18	0.17	0.08
(μg C L^{-1} h^{-1})	(0.21–0.48)	(0.23–0.33)	(0.17–0.38)	(0.14–0.23)	(0.08–0.33)	(0.06–0.11)
%EOC	35.4	31.5	45.0	19.9	20.7	36.7
	(25.0–46.1)	(27.5–37.3)	(30.9–64.6)	(16.4–24.9)	(16.2–35.3)	(25.5–48.0)
%PEA	21.3	19.9	16.8	15.1	18.7	10.5
	(18.5–22.1)	(14.3–24.1)	(9.0–23.6)	(12.5–19.0)	(12.8–24.0)	(8.5–12.4)
%CUEb	1.02	0.99	1.01	3.79	4.07	5.94
	(0.92–1.08)	(0.68–1.15)	(0.82–1.09)	(2.85–4.56)	(3.20–5.75)	(2.73–9.14)
BP	3.25	2.91	2.84	7.18	6.40	4.01
(ng C L^{-1} h^{-1})	(1.96–4.58)	(1.57–3.83)	(1.79–3.98)	(4.75–9.94)	(4.11–11.51)	(2.96–5.06)

* A single extreme value of 32.1 for TP associated with dust input was excluded for mean and range calculations.

sunlight treatment in midsummer (Fig. 5A), and from 0.68 ± 0.04 to 0.12 ± 0.01 μg C L^{-1} h^{-1} under the same respective treatments in late open-water period (Fig. 5B). A significant inhibitory effect of UVB on PP was found only in midsummer at upper depths ($F_{1,4}$, $p < 0.001$). The inhibitory effect of UVR on PP was mainly due to UVA radiation at both depths (Fig. 5).

The EOC varied from 0.024 ± 0.003 to 0.184 ± 0.024 μg C L^{-1} h^{-1} (Fig. 6). The UVR produced an increase in EOC values at upper depths ($F_{1,4}$, $p < 0.01$ in each period) and at intermediate depths ($F_{1,4}$, $p < 0.001$ in midsummer; $F_{1,4}$, $p < 0.05$ in the late open-water period, Fig. 6). Finally, EOC values were significantly higher at intermediate than at upper depths, regardless of light treatment and experimental date ($F_{1,10}$, $p = 0.011$).

In order to compare the effect of spectral composition of solar radiation on organic C release between experiments with different phytoplankton assemblages, EOC values were normalized to the respective PP values. Thus, %EOC ranged from 1.3 to 48%, yielding generally higher values in the late open-water period than in midsummer experiments and the highest values at upper depths under UVR exposure (Fig. 6). %EOC values were significantly higher under UVB + UVA + PAR and UVA + PAR treatments than under PAR treatments ($F_{1,4}$, $p < 0.005$ within each depth and period, Fig. 6) and were significantly higher under UVB + UVA +

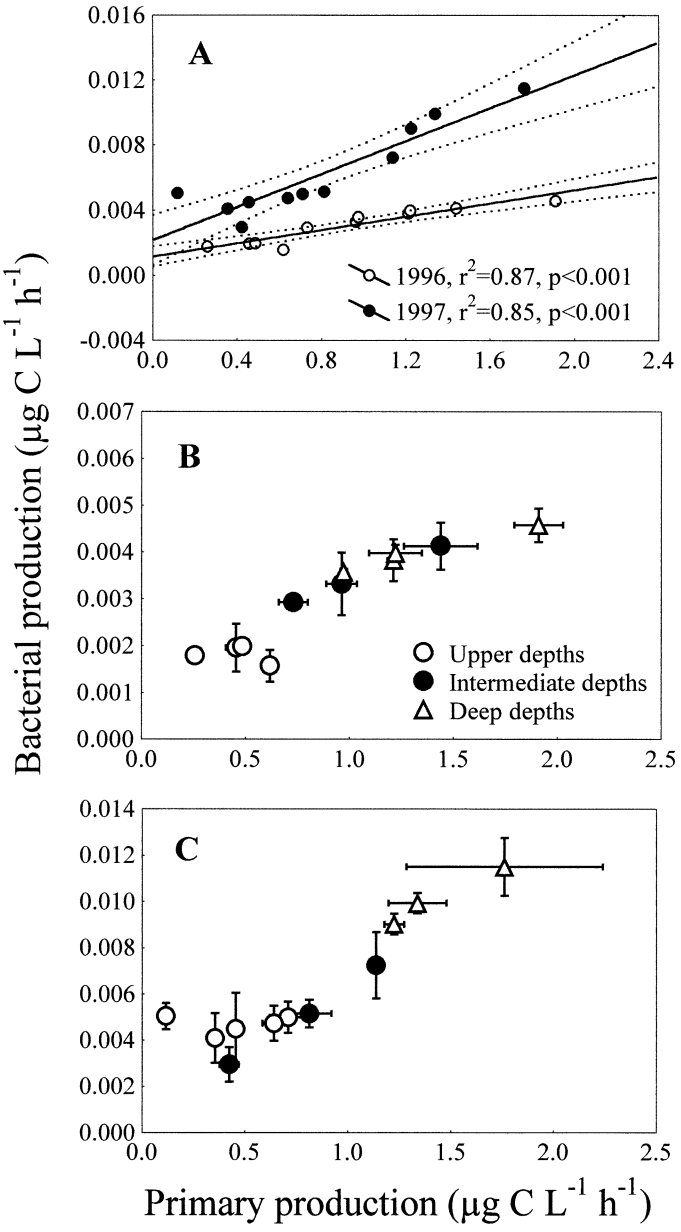

Fig. 3. Annual relationship between bacterial production and primary production for (A) entire water column and discriminating between depths in (B) 1996 and in (C) 1997. Dashed lines indicate 95% confidence intervals around the fitted (regression) line (solid line). Vertical error bars indicate means ± SD of bacterial production, $n = 3$. Horizontal error bars indicate means ± SD of primary production, $n = 3$. Error bars are too small to be visible for some measurements.

PAR treatments than under UVA + PAR treatments only in midsummer, particularly at upper depths ($F_{1,4}$, $p < 0.001$, Fig. 6).

Experimental effects of spectral composition of solar radiation on bacterial [³H]TdR incorporation—Whereas no spectral region of solar radiation exerted any significant effect on experimental [³H]TdR incorporation rates at intermediate depths, a clear pattern was found at upper depths (Fig. 7): [³H]TdR incorporation rates were three- to fourfold higher under UVA + PAR and PAR treatments than under dark treatments and were significantly inhibited by UVB radiation. This inhibitory effect was more pronounced in the late open-water period (82%, $F_{1,4}$, $p = 0.0015$) than in midsummer (39%, $F_{1,4}$, $p = 0.006$). Full sunlight stimulated [³H]TdR incorporation rates in midsummer (twofold increase, $F_{1,4}$, $p < 0.005$) but inhibited them in the late open-water period (37%, $F_{1,4}$, $p = 0.007$).

Effects of spectral composition of solar radiation on bacterial use of C supplied by algae—The bacterial assimilation of the photosynthetic C released by algae (POC_2, Table 3) did not show a clear pattern. However, when normalized to the potentially available substrate, the percentage of photosynthetic exudates assimilated by bacteria (%PEA) showed a similar pattern of variation to that of bacterial [³H]TdR incorporation rates at upper depths, with the highest values found under UVA + PAR and PAR treatments (Table 3). At intermediate depths, the %PEA was significantly higher under the PAR treatments ($F_{1,4}$, $p < 0.001$ in each period) but showed no significant differences between UVB + UVA + PAR and UVA + PAR treatments. Overall, the %PEA values were higher in midsummer than in the late open-water period (Table 3).

The %CUEb yielded significantly higher values in midsummer than in the late open-water period for each light treatment at upper depths ($F_{1,4}$, $p < 0.005$). Furthermore, %CUEb showed significantly higher values at upper than at intermediate depths under every light treatment ($F_{1,4}$, $p < 0.01$). Although no significant differences between light treatments were found at intermediate depths, at upper depths the %CUEb showed a similar pattern of variation to that of bacterial [³H]TdR incorporation rates and %PEA (Table 3).

Discussion

To our best knowledge, the present investigation provides the first in situ evaluation of the role of spectral composition of solar radiation in algal-bacteria interaction. Elucidation of this interaction is crucial to understanding carbon flux from the microbial loop to higher trophic levels, particularly in oligotrophic high mountain lakes, where most organic C is of autochthonous origin (Laurion et al. 2000; Reche et al. 2001). Thus, discernment of the factors regulating PP is key to knowing the functioning of the pelagic food web. In the present study, primary production values were within the characteristic range of oligotrophic high mountain, oligotrophic, and Antarctic ecosystems (Coveney and Wetzel 1995; Arístegui et al. 1996; Straskrabová et al. 1999*a*), and the vertical gradient of PAR irradiance was the main abiotic factor constraining primary production in the water column (Table 2). This finding is consistent with the photoinhibitory effect exerted by high sunlight irradiance on primary producers (Barber and Andersson 1992; Neale et al. 1993; Schofield et al. 1995), which is particularly accentuated in transparent-water systems (Moeller 1994) such as high mountain lakes. In support of our hypothesis, we found that UVR greatly inhibited phytoplanktonic primary production. Over-

Table 2. Results of stepwise regression analysis for primary and bacterial production measured in the lake for each year. R^2 is the multiple coefficient of determination; df_1 and df_2 are the degrees of freedom; F and p-value are the F-test results of the relationship between the dependent variable and the set of independent variables.

Year	Dependent variable	Independent variables included	Multiple R	Multiple R^2	df_1	df_2	F	p-value
1996	PP	PAR	0.78	0.61	1	10	15.6	0.0027
	BP	EOC	0.94	0.88	1	9	66.5	0.0000
1997	PP	PAR	0.83	0.69	1	10	22.8	0.0008
	BP	EOC	0.87	0.76	1	9	28.8	0.0005

all, the effect of UVA was stronger than that of UVB, although both UVB and UVA radiation had a similar negative effect in midsummer at upper depths. Earlier studies showed that UVA radiation is a major cause of depressed photosynthesis rates in phytoplankton and autotrophic picoplankton (Bühlman et al. 1987; Bertoni and Callieri 1999; Helbling et al. 2001). These effects may be due to the greater irradiance of UVA versus UVB reaching the Earth's surface, entering the waters, and causing important biological damage, despite the higher energy per photon of UVB (Karentz et al. 1994).

Although excretion of organic carbon (EOC) was directly related to PP over both vertical and seasonal scales, in line with the findings of other authors (Lignell 1990; Maurin et al. 1997), our experimental results showed that EOC yielded higher values within each discrete depth when algae were exposed to UVR. Overall, UVA radiation was the spectral region most responsible for this increase in EOC. Nevertheless, UVB also caused a significant increase in EOC values at upper depths in midsummer, coinciding with the inhibitory effect of UVB radiation on PP. This result could be due to the greater incident UVB dose in midsummer than in the late open-water period (Fig. 1). When EOC data were normalized to the total organic C production, in order to compare between experiments with different algae assemblages, a sharper pattern of %EOC than of EOC was found (Fig. 6). Besides, %EOC measured seasonally in the water column yielded higher values at upper than at deeper depths in both years. Therefore, %EOC can be considered a physiological stress indicator that may reflect the degree of uncoupling between photosynthesis and growth determined by UVR stress (Berman-Frank and Dubinsky 1999).

Bacterial standing-stock parameters and bacterial production yielded values near the lower limit of the range reported (Reche et al. 1996; Straskrabová et al. 1999*a;* Davidson and van der Heijden 2000). Because our main objective was to evaluate the effect of spectral composition of solar radiation on the algae–bacteria relationship, mimicking the natural environment, we simultaneously exposed bacteria (in whole water) and [^{3}H]TdR to solar radiation in situ in our experimental approach. In this way, solar irradiation, the bacterial [^{3}H]TdR uptake processes, the influence of C release from algae, and other complex interactions among abiotic (e.g., DOM photolysis, free radicals) and biotic (e.g., competence by mineral limiting nutrients) processes were not separated in time. Nonetheless, it is known that [^{3}H]TdR is susceptible to photoalteration when exposed to sunlight (*see* discussion in Sommaruga et al. 1997). However, results from parallel in situ experiments, performed with lake-water samples without algae, showed no significant differences between [^{3}H]TdR incorporation rates obtained under dark versus light conditions when the bacterial elemental composition was P sufficient for growth (Medina-Sánchez et al. in press). Hence, according to the latter findings, potential [^{3}H]TdR photoalteration was negligible after short-term incubations (1 h).

The results obtained using this methodological approach showed that UVB radiation inhibited BP only at upper depths, consistent with the fact that UVB radiation directly damages DNA (Herndl et al. 1993; Aas et al. 1996; Visser et al. 1999). Although the incident UVB irradiance at lake-water surface is lower in October than in August, due to the higher solar angle (Fig. 1), we found a more striking negative effect of UVB on bacterial [^{3}H]TdR incorporation rates in October. As discussed by Laurion et al. (2000), variations in phytoplankton densities in clear waters with low DOC values can also exert an additive effect on UVR attenuation. In fact, although DOC constitutes the primary factor regulating UV attenuation, the values of percentage of attenuation obtained from DOC-based models are rough, first-order estimates that do not take into consideration the absorption or scattering by other substances and particles in the water, which could be responsible for the variability of $K_{d,\lambda}$ that is not explained by DOC (Morris et al. 1995; Williamson et al. 1996). Thus, the decrease in phytoplankton densities (Table 1, Medina-Sánchez et al. 1999) in October versus August could lead to the more pronounced inhibition of BP by UVB.

The significant stimulatory effect of UVA + PAR and PAR on [^{3}H]TdR incorporation rates at upper depths may be explained as a bacterial response to the increase of release of organic carbon, at least under UVA radiation. These results are in agreement with those reported by Aas et al. (1996) in some of their experiments in whole water. Moreover, these authors found that the removal of the particulate fraction >0.8 μm led to loss of the stimulatory effect of PAR, UVA + PAR, and full sunlight. This suggests that interactions between primary producers and bacteria mediated by C released are critical factors influencing the outcome of UV radiation exposure on bacterial activity.

Nevertheless, other underlying processes in the bacterial response to organic C could also be responsible for this pattern. Thus, it is known that DNA damage can be repaired by photolyase enzymes (photorepair mechanisms) that are activated at high wavelengths of UVA radiation (370 to 400

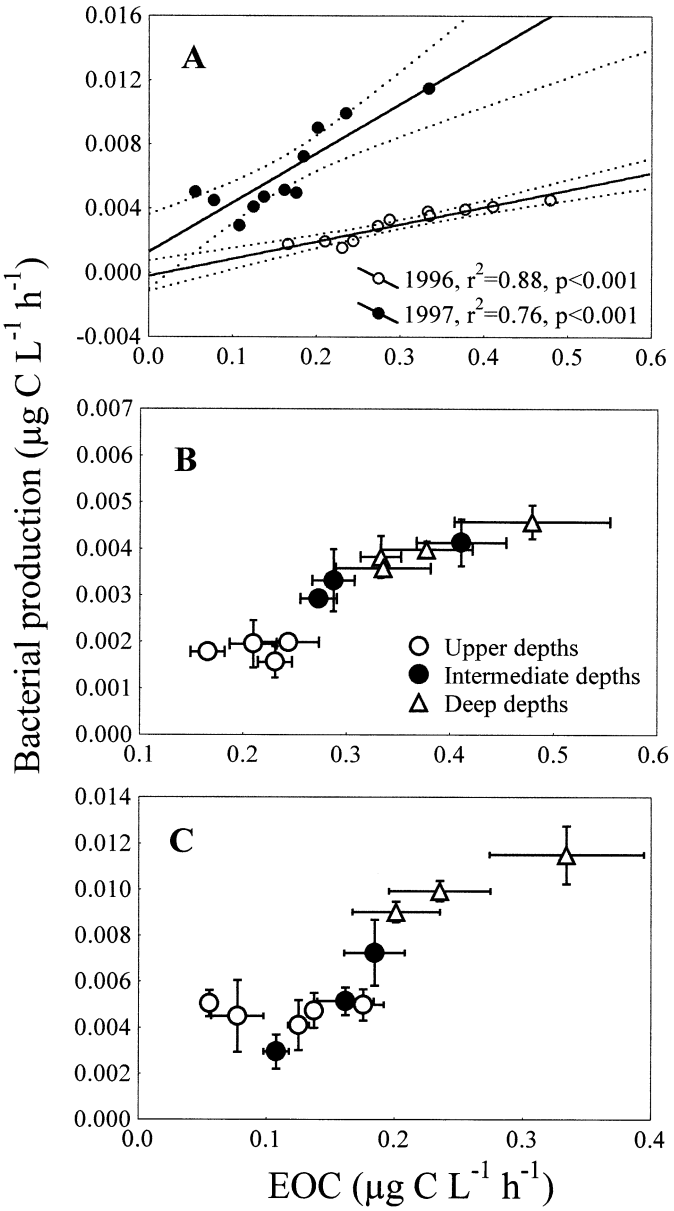

Fig. 4. Annual relationship between bacterial production and excretion of organic carbon (EOC) for (A) entire water column and discriminating between depths in (B) 1996 and in (C) 1997. Dashed lines indicate 95% confidence intervals around the fitted (regression) line (solid line). Vertical error bars indicate means ± SD of bacterial production, $n = 3$. Horizontal error bars indicate means ± SD of EOC, $n = 3$. Error bars are too small to be visible for some measurements.

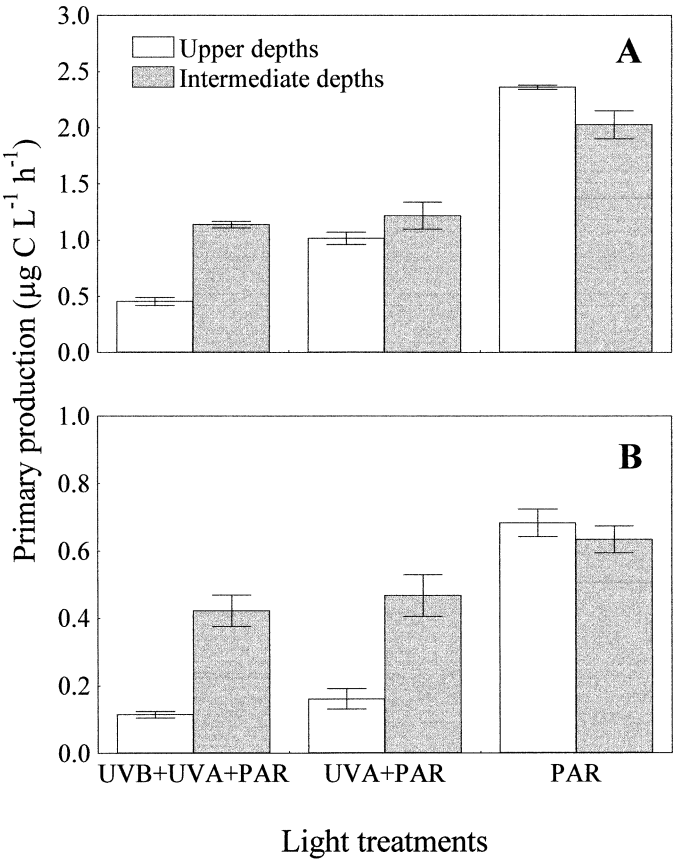

Fig. 5. Primary production measured under light treatments (UVB + UVA + PAR, UVA + PAR, PAR) at upper and intermediate depths in (A) August and (B) October experiments. Bars indicate means ± SD, $n = 3$.

nm) and low wavelengths of PAR (400 to 450 nm) and that reverse the dimerizing effects of UVB (Kim and Sancar 1993). These mechanisms would be relevant in our experimental conditions because bacterioplankton had been naturally exposed to full solar radiation for about 5 h immediately prior to the incubations, so that it is highly probable that bacterial DNA was already partially damaged (Visser et al. 1999). Hence, we interpreted the significant increase of bacterial [^{3}H]TdR incorporation rates under UVA + PAR and PAR treatments as the bacterial response to recovery by photorepair at high intensity UVA and PAR, manifested as enhanced bacterial growth providing that sufficient photosynthetic C is available. In fact, results of parallel experiments carried out in La Caldera Lake showed that, in the absence of algae, this potential recovery did not manifest as enhanced bacterial growth (Medina-Sánchez et al. in press). Moreover, Aas et al. (1996) found a greater inhibitory effect of solar radiation in absence of algae. Our experimental results are consistent with a report by Kaiser and Herndl (1997) of a significant recovery in UVB-inhibited bacterioplankton activity after bacteria were exposed to UVA and PAR. Nevertheless, whereas these authors found this recovery at moderately low intensities of UVA and PAR, we only detected it at high light intensities (i.e., at upper depths). Our results were in line with the finding by Davidson and van der Heijden (2000) that bacteria in whole water were able to repair UV-induced damage and grow while residing in near-surface waters in Antarctic.

In light of the above considerations, we conclude that the net effect of full sunlight (stimulatory or inhibitory) on bacterial activity at upper depths is the result of interplay between the detrimental effects of UVB and the recovery processes promoted by UVA and PAR. Thus, photorepair could completely counteract the negative effect of UVB in mid-

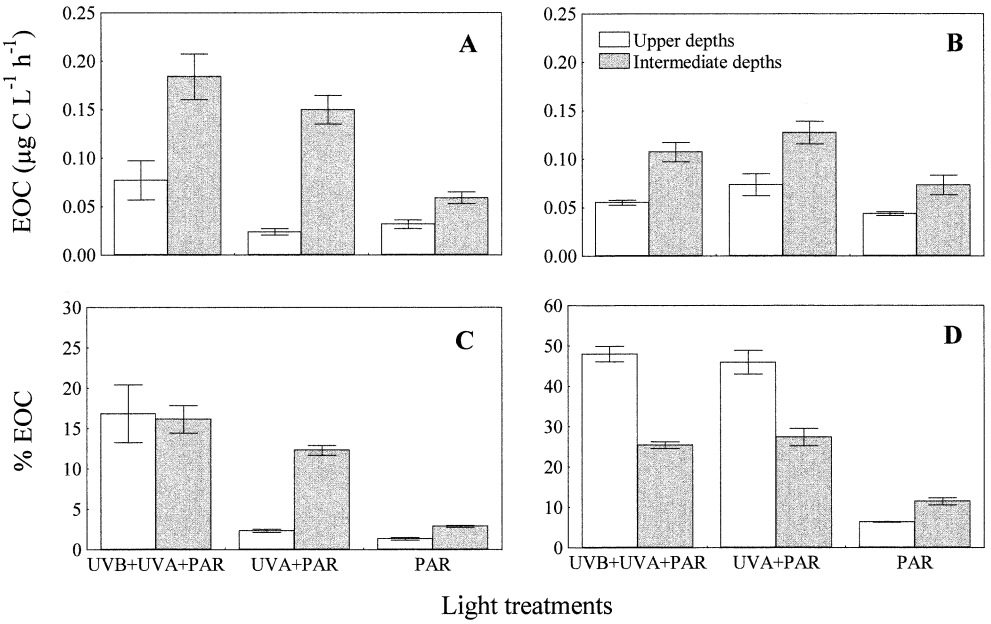

Fig. 6. Excretion of organic carbon (EOC) and %EOC with respect to organic carbon produced measured under light treatments (UVB + UVA + PAR, UVA + PAR, PAR) at upper and intermediate depths in (A, C) August and (B, D) October experiments. Bars indicate means ± SD, $n = 3$.

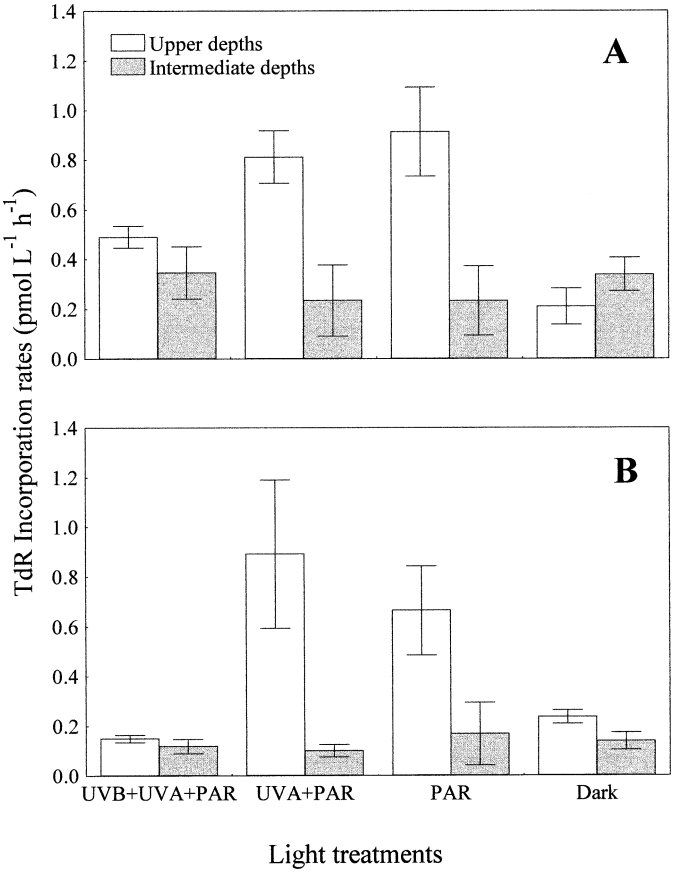

Fig. 7. [^{3}H]TdR incorporation rates by bacteria measured under light treatments (UVB + UVA + PAR, UVA + PAR, PAR, dark) at upper and intermediate depths in (A) August and (B) October experiments. Bars indicate means ± SD, $n = 3$.

summer but not in the late open-water period, probably due to the more severe harmful effect of UVB at the latter time. Photorepair, because of its low-energy cost, would be advantageous for bacteria in environments where sunlight is plentiful and nutrients are in short supply (Weinbauer et al. 1997), such as oligotrophic high mountain lakes. Hence, this ability to recover could be interpreted as an adaptive mechanism to high UVR exposure.

We quantified the proportion of photosynthetic exudates assimilated by bacteria (%PEA) under different light regimes as a further approach to evaluating the effect of spectral composition of solar radiation on bacteria and algae–bacteria interaction. The higher values found under the UVA + PAR and PAR treatments at upper depths or under the PAR treatments at intermediate depths indicate that bacteria can take up fresh C under these light conditions with significantly greater efficiency than under full sunlight (Table 3). This finding agrees with results obtained in laboratory experiments by Pausz and Herndl (1999). Hence, UVB also affected the efficiency of organic C uptake by bacteria, which could be related to UVB-inhibition of bacterial growth (shown in our study), although the bioavailability of C released by phytoplankton could also be diminished by humification processes (Obernosterer et al. 1999; Reche et al. 2001).

Although the total dose of UVR received by bacteria was different in the two experimental approaches, a similar pattern was observed for both %PEA and bacterial [^{3}H]TdR incorporation rates at upper depths. This result would suggest a relationship between the ability of bacteria to take up C and its ability to channel it toward new bacterial biomass. Thus, the percentage of photosynthetic carbon use efficiency by bacteria (%CUEb) may quantify this relationship because bacterial [^{3}H]TdR incorporation rates can be converted to

Table 3. Photosynthetic exudates assimilation by bacteria (POC_2, particulate organic carbon in 0.2–1 μm fraction), photosynthetic exudates assimilation by bacteria normalized to exudates available (%PEA), and photosynthetic carbon use efficiency by bacteria (%CUEb) obtained in the light experiments. Values are means ± SD, $n = 3$.

Month	Depth (m)	Treatment	POC_2 (μg C L^{-1} h^{-1})	PEA (%)	CUEb (%)
Aug	0.5	UVB + UVA + PAR	0.019 ± 0.006	24.0 ± 1.7	14.1 ± 2.9
		UVA + PAR	0.019 ± 0.003	77.8 ± 1.1	73.0 ± 3.2
		PAR	0.021 ± 0.003	65.6 ± 0.5	61.0 ± 4.0
	5	UVB + UVA + PAR	0.034 ± 0.002	18.8 ± 1.3	4.0 ± 0.7
		UVA + PAR	0.030 ± 0.002	20.1 ± 0.6	3.2 ± 1.7
		PAR	0.026 ± 0.002	43.4 ± 1.3	8.2 ± 4.1
Oct	0.5	UVB + UVA + PAR	0.005 ± 0.000	8.5 ± 0.2	5.8 ± 0.3
		UVA + PAR	0.018 ± 0.003	24.2 ± 1.2	25.5 ± 5.5
		PAR	0.011 ± 0.001	25.9 ± 0.2	32.4 ± 7.4
	5	UVB + UVA + PAR	0.014 ± 0.006	12.4 ± 4.3	2.4 ± 0.3
		UVA + PAR	0.017 ± 0.001	13.6 ± 0.2	1.7 ± 0.2
		PAR	0.030 ± 0005	41.2 ± 1.7	4.6 ± 3.0

produced bacterial C by using an appropriate conversion factor, as was calculated for this system. Overall, the highest %CUEb values were found under −UVB treatments at upper depths (Table 3). Hence, %CUEb, %PEA, and bacterial [^{3}H]TdR incorporation rates all shared a similar pattern at upper depths in this study.

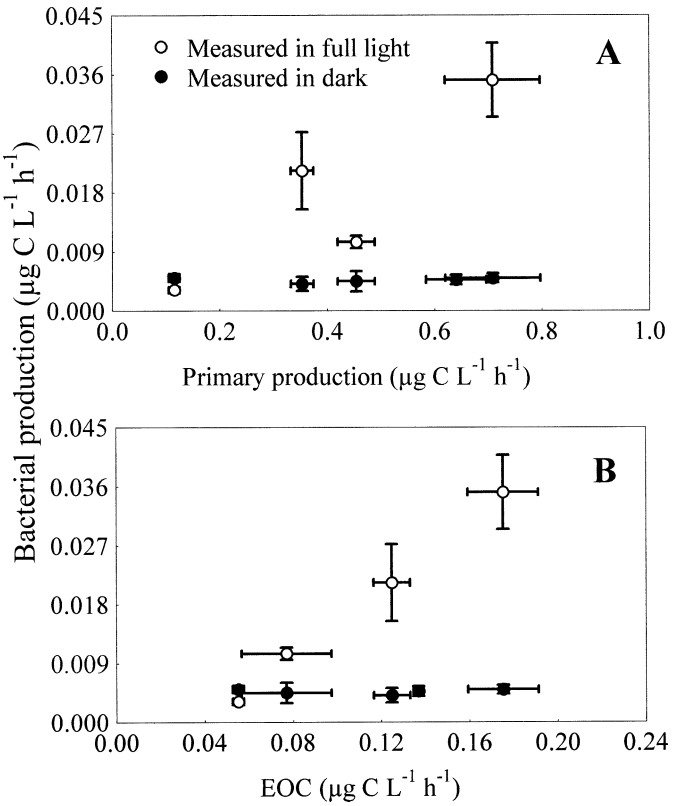

Fig. 8. Relationship between (A) bacterial production versus primary production and (B) bacterial production versus excretion of organic carbon (EOC) measured at upper depths in 1997, with the bacterial production data obtained under full sunlight or dark incubations. Vertical error bars indicate means ± SD of bacterial production, $n = 3$. Horizontal error bars indicate means ± SD of independent variable, $n = 3$. Error bars are too small to be visible for some measurements.

In line with our hypothesis, a coupled relationship between primary and bacterial production was established by direct bacterial dependence on C excreted from algae (EOC) over the seasonal and spatial scales, whereas other potential abiotic factors affecting BP, such as nutrients or temperature, were not able to explain the BP variance (Table 2). Moreover, parallel experiments performed in La Caldera Lake showed a significant reduction (42–95%) in bacterial [^{3}H]TdR uptake when algae were removed (Medina-Sánchez et al. in press). However, the coupled relationship between primary and bacterial production mediated by carbon released from algae was not maintained at upper depths when bacterial production measurements in the dark were considered (Figs. 3, 4). This latter result agrees with previous studies in La Caldera Lake, when the depth of the entire water column ranged between 3 and 5 m (Reche et al. 1996). Nevertheless, when the relationship between BP and PP or between BP and EOC was redepicted, with both variables measured under full sunlight conditions as an extrapolation of our experimental results to natural conditions, a tendency to a coupling between the two variables was also observed at upper depths (Fig. 8). The lack of coupling observed with traditional dark incubations for BP in our study resulted from either an underestimation of actual bacterial production in midsummer or its overestimation in the late open-water period (Visser et al. 1999). Thus, our experimental approach could be of value to establish the role of the spectral composition of solar radiation in coupling the algae–bacteria relationship, particularly in shallow and clear-water ecosystems.

To summarize, our hypothesis was supported by our results. UVR, particularly UVA, promoted a strong inhibition of primary production and enhancement of absolute values of EOC, increasing C availability to bacteria. Because the effect of full sunlight on bacterial activity was restricted to upper depths and was inhibitory only in the late open-water period, we conclude that the bacterial community was well adapted to solar radiation and sustained its ability to uptake

C released by algae (Maurin et al. 1997; Berman-Frank and Dubinsky 1999; Pausz and Herndl 1999, this study). The agreement between the results obtained with our two different methodological approaches ([^{3}H]TdR incorporation and organic ^{14}C uptake by bacteria) strengthens our conclusions and interpretations regarding the pattern of bacteria response to spectral composition of solar radiation and implies that our BP measurements under light conditions were realistic.

Although the bacteria tolerated sunlight-stress and positively responded to the EOC variations, low values of bacterial production, assimilation, and photosynthetic C use efficiency were yielded in this ecosystem, which would lead to the accumulation of organic C in dissolved phase and its subsequent photoalteration (Reche et al. 2001). Other factors than sunlight might explain these low values of bacterial activity. The significantly higher slope of the PB-EOC regression line and the higher %CUEb values in 1997 versus 1996 may be related to the higher water temperature values (Coveney and Wetzel 1995) and the improvement in trophic conditions. The latter is supported by ''normal'' plankton community development in 1997 versus 1996, with a consequent higher P availability from zooplankton recycling (Carrillo et al. 1996*a*,*b;* Villar-Argaiz et al. 2001, 2002). These observations are in line with findings by Shiah et al. (2001) of a higher slope of BP-PP regression lines in mesotrophic compared with oligotrophic conditions.

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