

DNA damage and photosynthetic inhibition induced by solar ultraviolet radiation in tropical phytoplankton (Lake Titicaca, Bolivia)

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(Received 6 September 2000; accepted 15 December 2000)

Experiments were conducted during October 1998 in Lake Titicaca, Bolivia (16° S, 68° W, 3810 m a.s.l.), to determine the effects of solar ultraviolet radiation (UVR) on phytoplankton photosynthetic rates and DNA damage. Water samples were taken daily and incubated *in situ* or in simulated *in situ* conditions using sharp cut-off filters to eliminate various portions of the UVR spectrum. The total inhibition of photosynthesis due to UVR in surface waters was 85%; the greatest part of this inhibition (65%) was due to UVAR (315–400 nm), the rest (20%) being due to UVBR (280–315 nm). The inhibition of photosynthesis decreased with depth so that there were no significant differences among treatments at 1–3 optical depths (K_{PAR}). The loss of carbon assimilation in the integrated production over the euphotic zone (4–6 optical depths) was 17.4%, with 14% due to UVAR and an additional 3.4% due to UVBR. Lake Titicaca phytoplankton had a threshold for inhibition of photosynthesis at about 0.3 W m⁻² for UVBR and 5 W m⁻² for UVAR, below which no inhibition was detected. Above this threshold, photosynthetic inhibition increased steadily, with UVAR having the greatest effect. Analysis of biological weighting functions (BWFs) indicated that phytoplankton from Lake Titicaca was less sensitive to UVR than phytoplankton from other regions. DNA damage (evaluated through the formation of cyclobutane pyrimidine dimers, CPDs) was observed for a simulated worst-case situation (i.e. samples incubated in full sunlight) and significant CPD accumulation was found as a result of UVBR exposure, but not with UVAR. However, absolute levels of damage were relatively low when compared with results obtained at other locations, also suggesting the low sensitivity of Lake Titicaca phytoplankton to UVR. It seems that UVBR stress in these organisms acts via at least two cellular targets: the photosynthetic apparatus and nuclear DNA. Our results suggest that an eventual enhancement of UVBR, due to ozone depletion, would have little impact on the phytoplankton of Lake Titicaca.

Key words: cyclobutane pyrimidine dimers, DNA damage, Lake Titicaca, photosynthesis, phytoplankton, ultraviolet radiation

Introduction

Tropical lakes are normally exposed to high fluxes of solar radiation throughout the year due to a combination of geographic and atmospheric factors (Madronich, 1993). In particular, high-altitude tropical lakes receive higher irradiance because, in addition to the latitudinal factor, there is an increase of 10–20% in solar ultraviolet radiation (UVR, 280–400 nm) for every 1000 m of increase in elevation (Blumthaler & Rehwald, 1992; Andrade *et al.*,

1998). Optical and physical characteristics of the water body (amount of particulate and dissolved organic matter, mixing processes, etc.) also affect the amount and spectral properties of solar radiation received by aquatic organisms (Helbling *et al.*, 1994; Scully & Lean, 1994).

Lake Titicaca is a relatively clear lake located on the border of Bolivia and Peru (16° S, 68° W, 3810 m a.s.l.), and its organisms are exposed to high levels of natural solar radiation (Villafañe *et al.*, 1999). In spite of its very interesting characteristics, few photobiological studies have been carried out in this tropical, high-altitude lake (Vincent *et al.*, 1984; Richerson *et al.*, 1986; Neale & Richerson, 1987; Villafañe *et al.*, 1999). Indeed, most of the photo-

biological research on the impact of UVR has focused on polar areas, especially Antarctica (e.g., Helbling *et al.*, 1992; Smith *et al.*, 1992; Weiler & Penhale, 1994; Neale *et al.*, 1998), due to the fact that this region is influenced by annual stratospheric ozone depletion events which allow increased amounts of solar UVBR (280–315 nm) to reach the surface (Kerr & McElroy, 1993; Madronich *et al.*, 1995).

High levels of visible radiation (PAR, 400–700 nm) and UVR are considered to be a stressor for many physiological mechanisms in diverse organisms (Häder, 1993; Holm-Hansen *et al.*, 1993a; Caldwell *et al.*, 1995). Many studies have focused on a variety of effects of solar radiation on phytoplankton, as these autotrophic cells constitute the base of the food web in aquatic ecosystems, and any effect on them would have the potential to affect (directly or indirectly) higher trophic levels. One of the effects of solar radiation (in particular UVR) that is most studied in phytoplankton is the inhibition of photosynthesis (Cullen *et al.*, 1992; Helbling *et al.*, 1992; Smith *et al.*, 1992; Behrenfeld *et al.*, 1993; Holm-Hansen *et al.*, 1993b, among others). Many studies have demonstrated that solar UVAR (315–400 nm) is responsible for most of the photosynthetic inhibition (Bühlman *et al.*, 1987; Kim & Watanabe, 1993; Villafañe *et al.*, 1995, 1999). Another negative effect caused by solar UVR (particularly UVBR) on living organisms is the induction of DNA damage (Mitchell & Karentz, 1993; Buma *et al.*, 1996a, 1997; Jeffrey *et al.*, 1996a, b) that occurs, among other mechanisms, through the formation of dimeric products between adjacent pyrimidines (i.e. cyclobutane pyrimidine dimers, CPDs: Karentz *et al.*, 1991; Mitchell & Karentz, 1993; Buma *et al.*, 2000). Numerous species have mechanisms to mitigate the deleterious effects of solar radiation. These include the synthesis of UVR-protective compounds such as mycosporine-like amino acids (MAAs: Dunlap & Shick, 1998) and DNA repair (Sancar & Sancar, 1988). Such repair mechanisms include photoreactivation (mediated by PAR and UVAR), nucleotide excision repair ('dark' repair) and recombination repair (Mitchell & Karentz, 1993).

Here we present results of experiments carried out during the spring of 1998 with phytoplankton communities of Lake Titicaca (Bolivia) that were designed to determine the impact of UVR on photosynthetic rates and DNA damage under extreme conditions of natural solar radiation. In the following paragraphs we discuss the importance of photosynthetic inhibition in Lake Titicaca, the impact of solar UVBR on DNA, and the potential acclimation of phytoplankton to cope with the extreme fluxes of solar radiation received in this environment.

Materials and methods

This study was carried out in the austral spring during October 1998. Water samples were taken daily from the Bolivian sector of Lake Titicaca, in the Chua Basin – Lago Menor (Fig. 1), where the depth of the water column was 30 m.

Experimentation

Different experiments were done in order to determine UVR-mediated inhibition of photosynthetic rates and DNA damage in natural phytoplankton populations:

In situ incubations. *In situ* experiments (total of three) were done with samples incubated at six depths in the water column. Surface water samples were collected with an acid-cleaned (1 N HCl) polycarbonate carboy, dispensed in 50 ml quartz tubes and inoculated with labelled radiocarbon to determine photosynthetic rates (see below). At each depth three radiation treatments were used (duplicate samples for each treatment): (1) samples receiving full radiation (UVR, 280–400 nm, and PAR, 400–700 nm)–uncovered quartz tubes; (2) samples receiving UVAR (315–400 nm) and PAR–quartz tubes covered with Mylar-D film (50% transmission at 323 nm); and (3) samples receiving only PAR–quartz tubes covered with Plexiglas UF-3 (50% transmission at 400 nm). The tubes were put in black anodized aluminium frames and incubated for 6 h (centred around local noon) at 0, 3, 6, 9, 12 and 15 m depth (corresponding to 0, 0.66, 1.32, 1.98, 2.64 and 3.3 optical depths; $K_{PAR} = 0.22 \text{ m}^{-1}$).

Simulated in situ experiments. These experiments were done to determine UVR effects on phytoplanktonic communities under conditions of maximum radiation (i.e. the worst-case scenario). Vessels or tubes containing the samples were placed in a water bath with running surface lake water for temperature control (13 °C). Three different sets of experiments were done using this set-up:

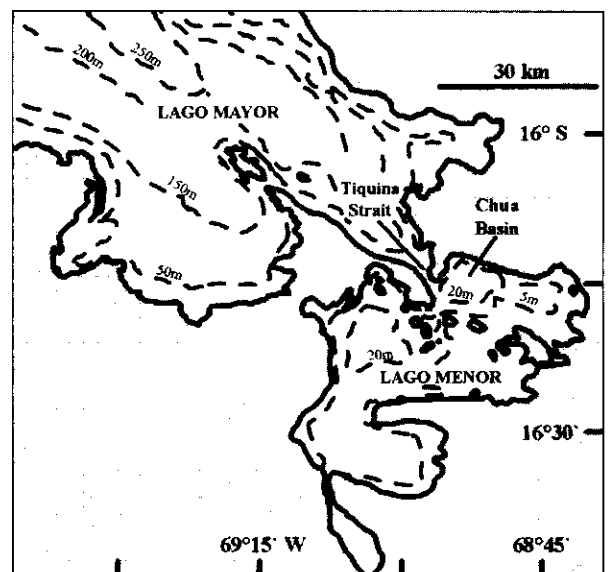


Fig. 1. Map of Lake Titicaca showing the location of the Chua Basin, the area where this study was done.

(i) To determine the presence of a threshold of solar radiation for photosynthetic inhibition, water samples were dispensed in 50 ml quartz tubes, inoculated with labelled radiocarbon (see below) and exposed to several irradiance treatments. This was done by the use of a combination of filters/materials and layers of neutral density screens (the latter allowing 100%, 50%, 25%, 12%, 6% and 3% of incident solar radiation to pass). Each irradiance treatment (total of six) had samples receiving UVR+PAR (two unwrapped quartz tubes), samples receiving UVAR+PAR (two quartz tubes covered with Mylar-D film) and samples receiving only PAR (two quartz tubes covered with Plexiglas UF-3). The incubations (total of three) lasted 6 h and were centred on local noon.

(ii) To assess the wavelength dependence of photosynthetic inhibition (i.e. biological weighting functions, BWFs), phytoplankton samples were placed in 50 ml quartz tubes and inoculated with labelled radiocarbon (see below). The tubes (four for each treatment) were placed in a black aluminium frame with the following six radiation treatments: uncovered quartz tubes (receiving both UVR and PAR), and quartz tubes covered with various sharp cut-off filters (Schott) cutting incident solar radiation at 295, 305, 320, 360 and 400 nm. The incubations were centred on local noon and lasted 6 h, with two tubes from each treatment removed after 3 h of exposure, the rest being removed at the end of the incubation period. The BWF-PI model (Cullen *et al.*, 1992; Neale & Kieber, 2000) was used to calculate a BWF for Lake Titicaca phytoplankton as our data indicated that inhibition was a function of the irradiance (see Results, Figs 5 and 6). The photosynthetic inhibition for each wavelength interval (Schott filters) over the incubation period was expressed as a function of the average irradiance; the spectral dependence of the BWF in the broadband intervals was extracted using the method of Rundel (1983). An exponential decay function (base 10) was used to fit the data for each experiment, and the exponent of the function was expressed as a third-degree polynomial function and fitted by iteration to achieve the best fit (the smallest R^2 obtained was 0.95). Five different and independent experiments were done to determine BWFs, and from these data a mean BWF was obtained for Lake Titicaca phytoplankton.

(iii) To study the formation of CPDs (i.e. DNA damage) and the potential for DNA repair, natural phytoplankton assemblages from surface water were placed in two 25 l, acid-cleaned (1 N HCl), plastic (50 × 35 × 15 cm), open incubators for 24 h. One container was exposed to full solar radiation (UVR+PAR, uncovered), whereas the other was exposed to UVAR+PAR (covered with Mylar-D film). A subsample (2–3 l) was removed from each incubator every 2 h for DNA damage analyses (see below); a total of six subsamples were taken from each container during daylight and one additional subsample was taken at the end of the experiment, early in the morning of the following day. At the same time, photosynthetic rates were determined using 50 ml quartz tubes exposed to three radiation treatments (UVR+PAR, UVAR+PAR and PAR only). All tubes were exposed to solar radiation from the beginning of the experiment and duplicate samples from each treatment were removed together with the DNA samples. This experiment was performed twice during the field season in Lake Titicaca.

The water samples used in all incubations were pre-filtered through a 250 μm mesh size to remove zooplankton. For every experiment we checked the chlorophyll *a* (chl *a*) content in both unfiltered and pre-filtered samples: the loss due to the filtration process was less than 5%.

Analyses and measurements

The analytical procedure for each determination/measurement was as follows:

Photosynthetic rates. Samples contained in the 50 ml quartz tubes were inoculated with 0.1 ml (5 μCi , 0.185 MBq) of labelled sodium bicarbonate (ICN Radiochemicals) (Steeman Nielsen, 1952). After incubation, the samples were filtered onto a Whatman GF/F glass fibre filter (25 mm). Then the filter was placed in 7 ml scintillation vials, exposed to HCl fumes overnight, dried, and counted using standard liquid scintillation techniques (Holm-Hansen & Helbling, 1995). Photosynthetic parameters of the *P* vs *I* curve were determined according to Platt & Jassby (1976).

DNA damage. Samples were filtered onto a Whatman GF/F glass fibre filter (47 mm) and immediately frozen in liquid air (-120°C) until analysis, which was carried out at the University of Groningen. DNA was extracted using a modified method from Doyle & Doyle (1991). Filters were incubated at 60°C for 30 min with 750 μl preheated CTAB isolation buffer (2% (w/v) CTAB (Sigma), 1.4 M NaCl, 0.2% (v/v) β -mercaptoethanol, 20 mM EDTA, 100 mM Tris-HCl pH 8.0). An aliquot of 750 μl of CIA (chloroform/isoamylalcohol, 24:1) was added to extract the DNA from cell debris and proteins. After centrifugation (Eppendorf 5417C, 14000 rpm (20000 g), 10 min), 0.5 ml of cold isopropanol was added to the upper (water) phase to precipitate the DNA (1 h, 4°C). Following another centrifugation (14000 rpm, 30 min, at 4°C) the supernatant was removed and the pellet washed with 1 ml of 80% ice-cold ethanol (15 min, -20°C , followed by centrifugation, 30 min, 4°C). Finally the DNA pellet was dried under vacuum and re-suspended in TE buffer (1 mM Tris-HCl pH 8.0, 0.1 mM EDTA). To remove the RNA, the DNA was incubated for 1 h with 75 $\mu\text{g ml}^{-1}$ RNase (Boehringer Mannheim) at room temperature. The amount of DNA was determined fluorometrically using Picogreen dsDNA quantitation reagent (dilution 1:400, Molecular Probes) on a 1420 Victor multilabel counter (Wallac, excitation 485 nm, emission 535 nm).

The amount of CPDs was determined using the method of Boelen *et al.* (1999) employing a primary antibody directed mainly to thymine dimers. In brief, 100 ng of heat-denatured DNA samples was blotted onto nitrocellulose membranes (Schleicher and Schuell, Protran 0.1 μm). The membranes were baked at 80°C to immobilize the DNA. After a 30 min blocking step, followed by three washing steps (PBS-T), the membranes were incubated with the primary antibody H3 overnight at 4°C . After repeated washing, incubation with the secondary antibody (HRP rabbit anti-mouse, Dako P0260) was done for 2 h at room temperature. The detection of CPDs was done using ECL detection reagents (RPN2106 Amersham) in combination with photosensitive films

(Kodak X-AR-5). Finally, the films were scanned and the quantification of dimers was done using Image Quant software (version 4.2, Molecular Dynamics). Each blot contained two dilution series of standard DNA with known amounts of CPDs (Boelen *et al.*, 1999), allowing for the calculation of absolute amounts of CPDs.

Chlorophyll *a* (chl *a*). Chl *a* concentration was measured by filtering 2 l of water sample onto a Whatman GF/F glass fibre filter (47 mm) and extracting the photosynthetic pigments in 90% acetone. The optical density of the acetone extract was measured in an UV-VIS Hitachi U-2000 spectrophotometer using a 5 cm quartz cuvette, and the chl *a* concentration determined using the formula of Strickland & Parsons (1972).

Floristic analysis. Water samples were taken daily during the 10 days of experimentation and fixed with buffered formalin (final concentration in the sample = 0.4% of formaldehyde). The quantitative analysis of phytoplankton cells was carried out using an inverted microscope (Utermöhl, 1958). The samples (50 ml) were settled for 48 h, and then counted with $\times 300$ magnification for microplanktonic cells ($> 20 \mu\text{m}$) and with $\times 600$ for nanoplanktonic cells ($< 20 \mu\text{m}$). A drop of Rose Bengal was added to the sample in the settling chamber to distinguish better between cells which were living or dead at the time of collection (Villafañe & Reid, 1995).

Radiation measurements. Incident solar UVBR was continuously monitored using an ultraviolet pyranometer (Yankee Environmental System, YES Model UVB-1, 280–320 nm) that was installed in a shade-free area close to the experimentation site. Data were obtained at a frequency of 1 min and recorded on a 486 computer. The YES sensor was calibrated against a Brewer #110 spectrophotometer that is permanently installed at Laboratorio de Física de la Atmósfera (UMSA) in La Paz (16.5°S, 68.1°W, 3420 m a.s.l.). Data from the Brewer instrument were also used to obtain detailed information on the UVBR spectral energy distribution. In addition, and by applying relevant algorithms to the data (i.e. STAR; Ruggaber *et al.*, 1994) we estimated UVAR, PAR and total ozone column concentrations. The attenuation coefficient for PAR in the water column was determined from the data obtained with a Topcon photocell illuminometer.

Results

Natural phytoplankton assemblages in October 1998 were composed mainly of the following species of colonial cyanobacteria: *Gomphosphaeria pusilla* (Van Goor) Komarek, *Microcystis aeruginosa* Kützing and *Anabaena* sp. The most important species of Chlorophyta present in Lake Titicaca samples were *Sphaerocystis Schroeteri* Chodat, *Oocystis crassa* Wittrock and *Chlorhormidium subtile* (Kützing) Fott, whereas diatoms (Bacillariophyceae) were represented mostly by the colonial genera *Fragilaria* and *Tabellaria* (Table 1). Phytoplankton were distributed rather homogeneously in the water column and no significant variations in

chl *a* concentration (mean of $2.1 \mu\text{g chl } a \text{ l}^{-1}$, SD = 0.12) with depth were observed (data not shown), suggesting a well mixed condition, at least in the upper 16 m of the water column. Temperature data (Fig. 2) also showed little variations from surface (12.8°C) down to 18 m (12.7°C), indicative of a well-mixed epilimnion.

Ozone concentrations showed a small variation during the study period with a mean value of 259 D.U. (SD = 3.3; Fig. 3). Radiation conditions during the month of October 1998 (Fig. 3) showed a day-to-day variability that was related to cloud cover. Daily doses of UV-B varied between 24 and 48 KJ m^{-2} (Fig. 3A), whereas UV-A ranged from 800 to 1150 KJ m^{-2} , and PAR from 8 to almost 16 MJ m^{-2} (Fig. 3B).

Studies carried out under *in situ* conditions allowed us to obtain a baseline for UVR effects on phytoplankton photosynthesis in the water column. Similar results were obtained in all *in situ* experiments and a typical profile conducted during the study period is shown in Fig. 4. Carbon fixation in surface waters (Fig. 4A) was about $28 \mu\text{g C l}^{-1}$ (samples receiving only PAR), with photosynthetic rates increasing slightly with depth so that at 2 optical depths (9 m depth) the amount of carbon fixed was $33 \mu\text{g C l}^{-1}$. This increase in carbon fixation with depth indicates a slight photoinhibition due to high levels of PAR. However, a sharp decrease in carbon fixation was noticed in both PAR+UVR and PAR+UVAR treatments, as compared with the treatment that received only PAR. In surface waters, 65% of inhibition was due to UVAR, while UVBR accounted for an additional 20% (Fig. 4B, C). Photosynthetic inhibition, however, decreased with depth, with UVAR causing

Table 1. Mean concentration (cells ml^{-1}) and standard deviation (in brackets) of the most abundant phytoplankton species during the studies at Lake Titicaca

Class	Species	Cells ml^{-1}
Chlorophyta	<i>Chlorhormidium subtile</i>	39 (10)
	<i>Closterium acutum</i>	23 (6)
	<i>Oocystis crassa</i>	29 (9)
	<i>Pediastrum boryanum</i>	5 (1)
	<i>Scenedesmus quadricaudata</i>	8 (1)
	<i>Sphaerocystis Schroeteri</i>	90 (12)
	<i>Staurastrum glaucile</i>	6 (2)
	<i>Zygnema</i> sp.	17 (5)
	Cyanophyta	<i>Anabaena</i> sp.1
<i>Anabaena</i> sp.2		46 (11)
<i>Chroococcus minutus</i>		51 (4)
<i>Gleocapsa</i> sp.		21 (8)
<i>Gomphosphaeria pusilla</i>		205 (28)
<i>Microcystis aeruginosa</i>		129 (30)
Bacillariophyceae	<i>Fragilaria</i> sp.	80 (14)
	<i>Tabellaria</i> sp.	48 (7)

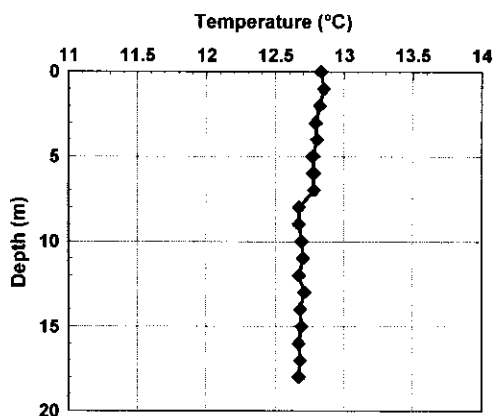


Fig. 2. Representative temperature profile of the upper water column at Chua Basin.

more inhibition than UVBR at all depths (Fig. 4B, C). No significant inhibition was observed below 1.2 optical depths (i.e. at 6 m depth). The integrated daily production in the euphotic zone (i.e. 4.6 optical depths) was approximately 0.62 g C m^{-2} (only PAR) and the integrated losses due to UVBR and UVAR were 3.4% and 14%, respectively.

The exposure-response curve showed a maximum photosynthetic assimilation number, P_{\max} , of $2.6 \text{ [mg C (mg chl a)}^{-1} \text{ h}^{-1}]$ for the PAR treatment (Fig. 5A). There was a slight photoinhibition due to PAR; however, in the treatments that received UVAR and UVR in addition to PAR, photoinhibition was much more pronounced (lower P_{\max} values) and increased significantly with increasing irradiance. The experiments carried out with increasing irradiance levels showed the presence of a threshold for photosynthesis inhibition due to UVR (Fig. 5B). This threshold was at relatively low dose rates (0.3 W m^{-2} and 5 W m^{-2} for UVBR and UVAR, respectively) in comparison to the irradiances normally received at Lake Titicaca. As also seen in the exposure-response curve, photosynthetic inhibition increased steadily with increasing UVAR, reaching maximum values of about 70%, while the inhibition caused by UVBR increased with irradiance up to 1 W m^{-2} and then remained rather constant (Fig. 5B).

The effect of solar ultraviolet radiation on phytoplankton varied through the day as a function of the irradiance received by the cells (Fig. 6). Fig. 6A represents a typical UVBR curve during our day-long experiments, with a maximum irradiance of 5.8 W m^{-2} at local noon (note that the data correspond to the YES radiometer that has a broad UVB band response of 280–320 nm). Photosynthetic inhibition was significant even after the first 2 h of incubation (Fig. 6B). As with the *in situ* incubations, UVAR accounted for most of the inhibition, while UVBR was shown to be less important in terms of photosynthetic inhibition. In

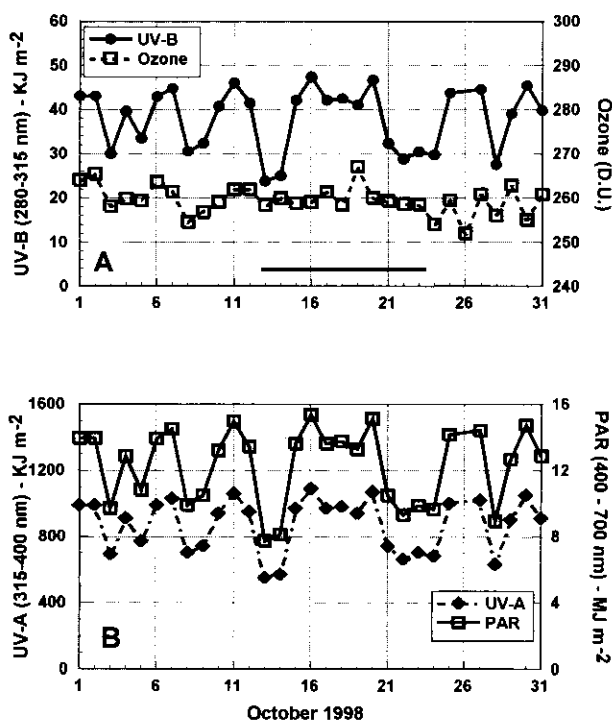


Fig. 3. Radiation conditions and ozone concentration during October 1998 at Lake Titicaca. (A) Daily doses of UV-B (280–315 nm) in KJ m^{-2} and total ozone column concentrations (Dobson Units). (B) Daily doses of UV-A (315–400 nm) in KJ m^{-2} and PAR (400–700 nm) in MJ m^{-2} . The line in (A) indicates the time when the experiments were conducted.

contrast, UVBR induced DNA damage, judging from the clear accumulation of CPDs over the daily period (Fig. 6C). No CPD formation was observed in the UVAR+PAR treatment, as CPD levels remained close to initial values over the whole daily period (approx. $20 \text{ CPDs (10}^6 \text{ nucleotides)}^{-1}$). In the samples exposed to full-spectrum radiation (UVR+PAR), CPDs increased significantly after 4 h of exposure to solar radiation, with a maximal damage accumulation rate immediately after the time when incident UVBR was highest (Fig. 6A, C). No significant decrease in CPDs was observed overnight, indicating that little or no repair occurred in the dark (data not shown).

Discussion

Solar ultraviolet radiation can affect phytoplankton in different ways (Döhler, 1990; Goes *et al.*, 1994; Holm-Hansen *et al.*, 1993a), but two of the most studied forms of UVR stress are photosynthetic inhibition and DNA damage (Karentz *et al.*, 1991; Helbling *et al.*, 1992; Smith *et al.*, 1992; Buma *et al.*, 1996a, 1997). Many studies (e.g. Helbling *et al.*, 1992; Vernet *et al.*, 1994) determined the impact of UVR on photosynthesis under worst-case conditions (i.e. surface irradiance). However, the inhibition of photosynthesis decreases with depth even

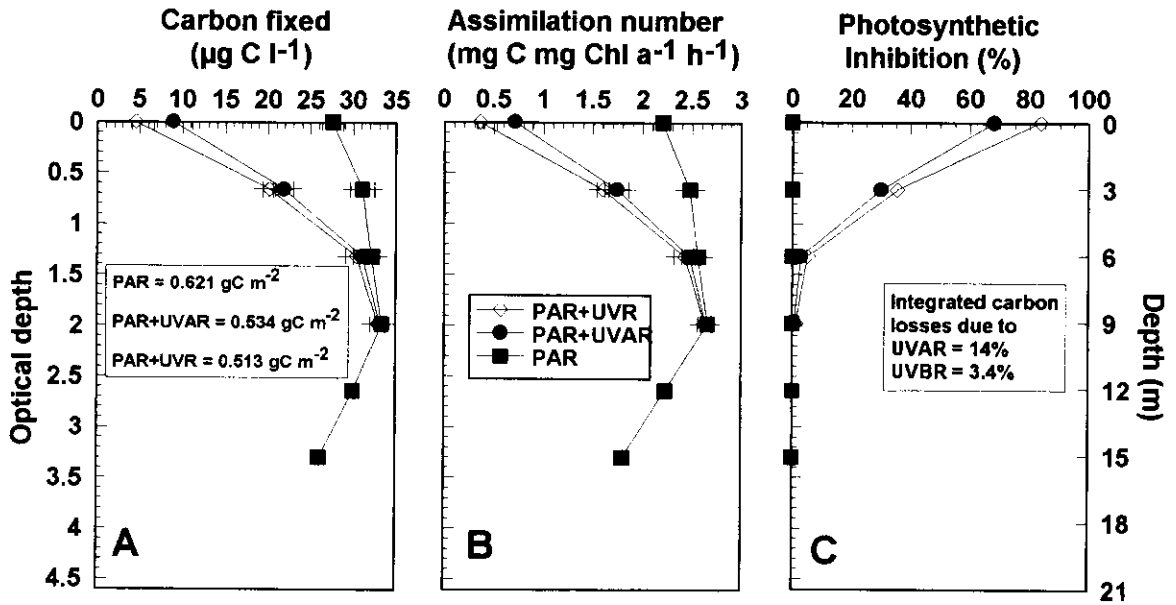


Fig. 4. Results of *in situ* incubations conducted with natural phytoplankton populations. (A) Carbon fixed during the 6 h incubation period in $\mu\text{g C l}^{-1}$ as a function of the optical depths. Inset shows integrated production in the euphotic zone (i.e. 4.6 optical depths) for the three treatments implemented. (B) Photosynthetic assimilation numbers (in $\text{mg C mg chl a}^{-1} \text{h}^{-1}$) as a function of the optical depth. (C) Photosynthetic inhibition (%) in the water column by UVR. The inhibition was calculated as $[(P_{\text{PAR}} - P_{\text{UV}}) / P_{\text{PAR}} \times 100]$, where P_{PAR} indicates the carbon fixed in the PAR treatment and P_{UV} the carbon fixed in either the PAR + UVAR or PAR + UVR treatments. Inset shows carbon losses in the euphotic zone due to UVAR and UVBR. The + symbols indicate 1 standard deviation ($n = 2$ for each treatment).

in a relatively clear lake such as Lake Titicaca (Fig. 4). From an ecological point of view, it is thus important to determine the impact of UVR on the integrated production of the water column, as this would take into account the contribution of various processes. Some of these processes have been addressed in the literature and they include the differential attenuation of solar radiation in the water column (Smith & Baker, 1979; Holm-Hansen *et al.*, 1993a) – that in freshwater environments is greatly influenced by dissolved organic carbon (Morris *et al.*, 1995) – and distribution of phytoplankton as affected by mixing processes (Helbling *et al.*, 1994; Neale *et al.*, 1998). The inhibition of photosynthesis in the water column of Lake Titicaca reached down to 1.2 optical depths, about 6 m depth (Fig. 4), with most of the inhibition due to UVAR. The daily integrated water column production (Fig. 4) was fairly high (0.62 g C m^{-2} , PAR only) but much less than in other environments where phytoplankton blooms normally occur (for instance in the Bransfield Strait, Antarctica), where integrated production values of up to 3 g C m^{-2} were found (Holm-Hansen & Mitchell, 1991). The integrated carbon losses at Lake Titicaca (e.g. 14% and 3.4% due to UVAR and UVBR, respectively; Fig. 4C) were comparable, however, to those found for Antarctic phytoplankton (Holm-Hansen *et al.*, 1993b). This latter study reported an integrated loss of carbon of 4.9% due to UVBR in the euphotic zone under normal ozone conditions, and calculated an additional carbon loss of 3.8% when ozone

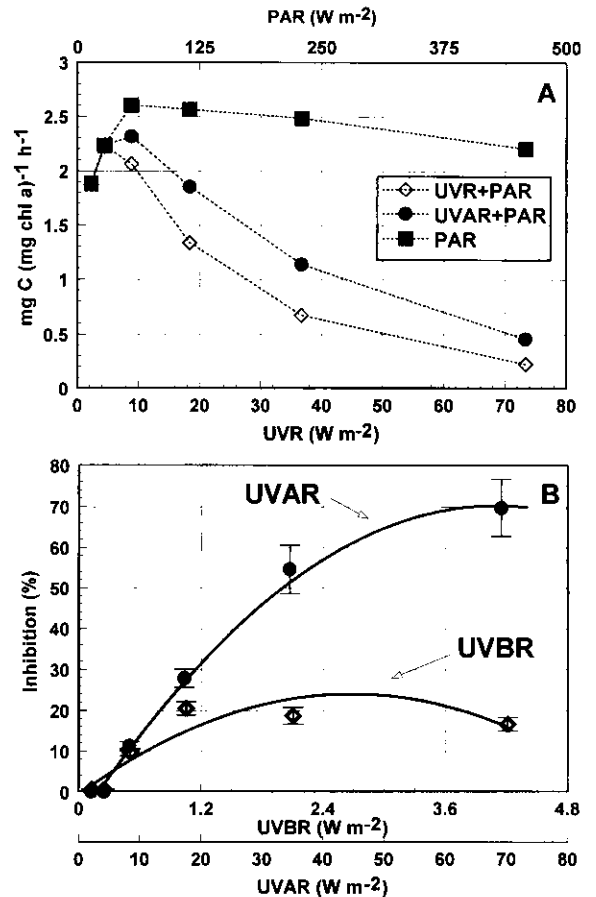


Fig. 5. Response of natural phytoplankton populations to increasing irradiance using temperature-controlled experiments. (A) Photosynthesis versus irradiance (P vs I) curves. (B) Photosynthetic inhibition (%) as a function of increasing UVR and UVBR. The vertical lines indicate 1 standard deviation ($n = 2$ for each treatment).

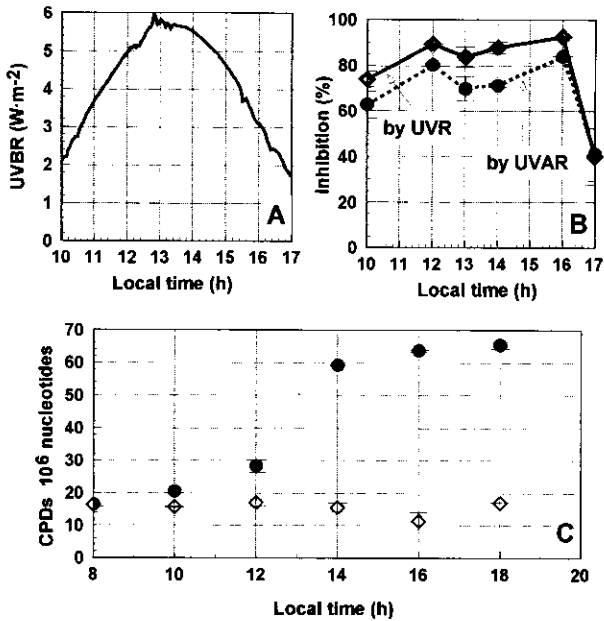


Fig. 6. Phytoplankton photosynthetic inhibition and DNA damage as a function of time of day and solar radiation. (A) UVBR (280–320 nm; in $W m^{-2}$) as a function of time; data obtained with the YES radiometer. (B) Variations in photosynthetic inhibition (%) due to UVAR and UVBR as a function of time of day. (C) Number of CPDs formed as a function of time of day and type of radiation received by the cells; the filled circles indicate samples exposed to UVR + PAR, and the open diamonds indicate samples exposed to UVAR + PAR. The vertical lines indicate 1 standard deviation ($n = 3$ or 4 measurements for each treatment) to indicate methodological errors.

column concentration was reduced to 150 Dobson Units (DU). During our studies, ozone column concentrations were 'normal' for this time of the year and ranged from 250 to 270 DU (Fig. 3).

Tropical marine phytoplankton have been hypothesized to be better acclimated to solar UVR than species from high latitude, due to their evolutionary history of exposure to higher radiation levels (Helbling *et al.*, 1992). A recent paper (Villafañe *et al.*, 1999) has shown that phytoplankton at Lake Titicaca also seems to be more acclimated to solar UVR as a result of the high radiation fluxes received at this high-altitude, low-latitude environment. The lower sensitivity of Lake Titicaca phytoplankton to solar UVR was observed when calculating a BWF (Fig. 7) and comparing it with other functions (Neale *et al.*, 1994; Helbling & Villafañe, 2001). The BWF obtained by us at Lake Titicaca (Fig. 7) showed a steeper decline at wavelengths shorter than 315 nm, than the BWFs for natural phytoplankton assemblages from Antarctica (Neale *et al.*, 1994), but with a significantly higher biological weight at wavelengths lower than 300 nm. The BWF for Lake Titicaca phytoplankton had, however, a significantly lower biological weight than the Arctic BWF at all wavelengths; but only in the UVAR region when compared with the Antarctic BWF.

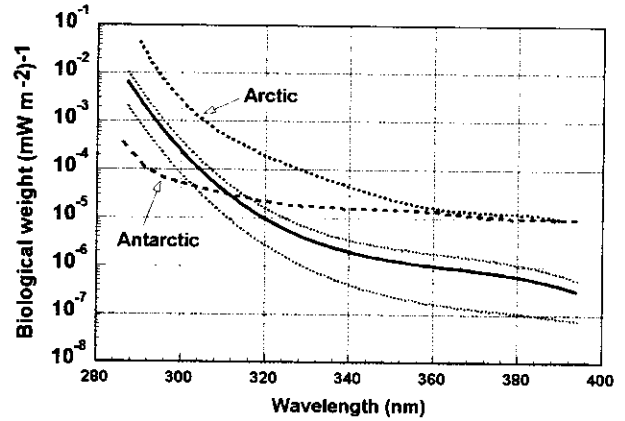


Fig. 7. Mean biological weighting function $[(mW m^{-2})^{-1}]$ for Lake Titicaca phytoplankton. The thin dotted lines indicate the 95% confidence limit. The BWFs for natural populations of Antarctic (from Neale *et al.*, 1994) and Arctic phytoplankton (Helbling & Villafañe, 2001) are shown for comparison.

This suggests a lower sensitivity of phytoplankton from Lake Titicaca to UVR as compared with these other locations. However, it is interesting to note that in this study phytoplankton had a lower radiation threshold for inhibition ($0.3 W m^{-2}$ for UVBR; Fig. 5) as compared with the value of about $1 W m^{-2}$ for Antarctic phytoplankton (Helbling *et al.*, 1992), although higher than the one reported for Arctic phytoplankton (less than $0.1 W m^{-2}$; Helbling *et al.*, 1996b), and in other studies (Behrenfeld *et al.*, 1993) that showed no threshold at all for phytoplankton of the Pacific Ocean.

The acclimation status of phytoplankton, as reflected by parameters such as the threshold value for UV damage, depends upon the previous exposure to UVR and high PAR, as well as other factors such as nutrient supply (Halac *et al.*, 1997). Therefore, it is expected that the depth of the epilimnion and the rate of mixing (Helbling *et al.*, 1994; Neale *et al.*, 1998) would have a strong influence on this threshold value. With a shallow epilimnion, phytoplankton would be distributed near the surface and would receive higher irradiances, as compared with a deeper epilimnion where the cells would be distributed deeper in the water column. The depth of the epilimnion would in turn affect the acclimation processes of phytoplankton (Helbling *et al.*, 1996a) as the mean irradiance received by the cells would vary with this depth. Near-surface diurnal thermoclines (Vincent *et al.*, 1984) may retain phytoplankton and expose the cells to high irradiances. Morales (1996) showed, however, that temperature profiles at Chua Basin from 1992 to 1994 were relatively homogeneous and the thermocline was found consistently at about 20 m depth. This observation was also supported by temperature–depth profiles conducted during our studies (Fig. 2) and thus the phytoplankton assemblages

used in our experiments were from a relatively deep (almost all the euphotic zone was mixed; see Fig. 4), but 'normal' epilimnion.

With regard to the DNA damage induced by solar UVR, and despite the fact that CPD formation was significant as a result of UVBR exposure (Fig. 6C), vulnerability for DNA damage induction in the Lake Titicaca communities was low compared with that of tropical marine picophytoplankton communities. As has been shown recently (Jeffrey *et al.*, 1996a, b; Boelen *et al.*, 1999) several hundreds of CPDs are formed within hours in picoplankton assemblages from Caribbean waters (30° N) when incubated in bags under full solar radiation. This is much higher than the levels found at Lake Titicaca, even though incident UVBR levels were higher here. In the present study CPD accumulation in the phytoplankton may also have been overestimated, due to the fact that (heterotrophic) bacteria, which are more vulnerable to CPD induction than eukaryotes (Buma *et al.*, 2001), were also retained by the filters. Differences may not be explained by repair capacity between communities: obviously dark repair is not occurring at sufficient rates in phytoplankton of Lake Titicaca to adequately remove damage overnight. Also, even if photorepair is active, it is not efficient enough to suppress further CPD accumulation during the course of the day (Fig. 6C). In the water column, however, initial CPDs levels were low, suggesting that the differential attenuation of solar radiation with depth together with mixing processes were favourable for removing CPDs more efficiently than in the worst situation that we imposed on the samples (i.e. full solar radiation). Again, possibly the mean cell size also determines the extent to which damage is induced, as suggested by Karentz *et al.* (1991). The size range of marine tropical phytoplankton is typically between 0.2 and 2 μm , whereas at Lake Titicaca cells are in the nanoplankton size range (2–20 μm). Also, natural Antarctic picoplankton assemblages displayed significantly higher CPD levels than larger, diatom-containing size fractions, as a result of exposure to solar UVBR (Buma *et al.*, 2000b).

This study has demonstrated that UVR stress may be brought about by multiple target effects, at least at incident irradiance levels for UVR, as was also shown for mid-latitude marine communities (Helbling *et al.*, 2001). Both UVAR and UVBR inhibit photosynthesis, with UVAR having a stronger effect than UVBR. At the same time CPD accumulation was related only to UVBR exposure. In addition, daily patterns of photosynthetic inhibition and DNA damage accumulation were far from similar (Figs 5B, 6C). Evidently, DNA damage accumulation and photosynthetic inhibition patterns reveal effects on at least two cell

targets, i.e. nuclear DNA and the photosystems located on the thylakoid membranes in the chloroplast. As has been suggested earlier (Buma *et al.*, 1996b, 2000), DNA damage mainly accumulates in nuclear DNA and not in chloroplast DNA, because chloroplasts still multiply in cells where cell division is blocked. Several mechanisms can be imagined as to how these targets may influence each other, when UVR affects them simultaneously. First, a decrease in photosystem performance will lower the energy flow required for the induction and execution of DNA repair processes. Secondly, nuclear DNA damage may inhibit *de novo* synthesis of proteins, including enzymes such as RUBISCO, required for adequate functioning of the photosynthetic process.

Finally, the extent to which both targets are affected will be determined by irradiance conditions and/or species-specific differences in vulnerability. Cell size may play a crucial role in determining the effects of UVR on DNA damage, photosynthetic inhibition and acclimation processes. From the data we have obtained it is expected that Lake Titicaca phytoplankton will not suffer a dramatic impact in the case of an ozone depletion event (i.e. an increase of solar UVBR).

Acknowledgements

We especially thank S. Morales and the personnel at the Centro de Investigación y Desarrollo Piscícola del Altiplano (CIDPA) who kindly provided support and materials; C. Mendoza helped during field work and carried out the taxonomic analysis of phytoplankton samples, L. Taibo helped with computer work and L. Sala performed ^{14}C analysis. We also thank the personnel at the Ministry of Foreign Relations (International Cooperation) in Buenos Aires, the Argentinian Embassy in Bolivia, and Fundación Playa Unión for logistic support. We thank P. Neale for helpful suggestions on the BWF calculations and for providing BWF data. The comments and suggestions of two anonymous referees and the journal editor are greatly appreciated. This work was partially supported by Fondo Argentino de Cooperación Horizontal - FO-AR (Project ARG/92/004) and Agencia Nacional de Promoción Científica y Tecnológica (BID 802/OC-AR-PICT 97 no. 01-00046-00955). This is Contribution no. 14 of Estación de Fotobiología Playa Unión.

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