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Solar UVR-induced DNA damage and inhibition of photosynthesis in phytoplankton from Andean lakes of Argentina

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With 7 figures and 2 tables

Abstract: During January 1999, studies were carried out in temperate lakes of the Andean region of Argentina (41° S, 71° W) to determine the short-term effects of solar ultraviolet radiation (UVR, 280–400 nm) upon natural phytoplankton assemblages. Organisms from one 'clear' (Lake Moreno) and two 'opaque' lakes (Morenito and El Trébol) were exposed to different radiation regimes to assess photosynthesis inhibition and cyclobutane pyrimidine dimers (CPDs) accumulation/repair. UV-B caused significant DNA damage in organisms from 'opaque' lakes, especially those from Lake Morenito. Organisms from the 'clear' Lake Moreno, on the other hand, presented lower CPDs accumulation rates. UV-B had relatively low effects inhibiting photosynthesis in these opaque lakes (2 and 9.5 %, for lakes Morenito and El Trébol, respectively) and most of the inhibition was due to UV-A (75 and 71 % inhibition for lakes Morenito and El Trébol, respectively). In Lake Moreno, photosynthetic inhibition was 35 and 15 % for UV-A and UV-B, respectively. A number of causes seems to account for the different responses observed among phytoplankton assemblages, being one of the most important underwater radiation fields, and hence for the light acclimation history of cells. In addition, factors such as differences in type and effectiveness of the strategy used by the organisms to cope with solar UVR, as well as differences in the size structure and taxonomic composition of the community, are also important at the time to evaluate the overall impact of solar UVR in these lakes.

Key words: Andean lakes, CPD, DNA, Patagonia, photosynthesis, phytoplankton, UVR.

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Introduction

The effects of solar ultraviolet radiation (UVR, 280–400 nm) upon aquatic photosynthetic organisms have been extensively reported in the literature (see reviews books by De Mora et al. 2000, and Helbling & Zagarese 2003). On one hand, by damaging essential molecules such as proteins and DNA (Buma et al. 1996, Garde & Gustavson 1999), UVR can alter cellular processes such as the uptake of nutrients (Behrenfeld et al. 1995), photosynthesis (Villafañe et al. 2003) or DNA transcription/replication (Setlow et al. 1963), which can finally result in an overall reduction of phytoplankton fitness. On the other hand, UVR wavelengths can be beneficial, as they may enhance photosynthetic rates (Barbieri et al. 2002, Helbling et al. 2003) or they can photodegrade chromophoric dissolved organic matter (CDOM) producing thus the photomineralization of CDOM with the consequently release of inorganic nutrients that are available for photosynthetic organisms (De Lange et al. 2003).

Whether UVR presents negative or advantageous force for phytoplankton will ultimately depend on a combination of factors, among which the penetration of biologically effective radiation in the water column, and hence the amount of UVR received by the cells, plays a determinant role. The penetration of biologically effective radiation is in turn determined by UVR levels reaching the water surface (Madronich 1993) as well as by the optical absorption of different components – the water itself, particulate (both organic and inorganic) and dissolved matter (Hargreaves 2003). Studies carried out in freshwater environments have shown that CDOM is a good estimator of solar UVR transparency of the water column (Scully & Lean 1994, Morris et al. 1995, Laurion et al. 2000).

Patagonian Andean lakes of Argentina are very different in terms of their biological (Díaz & Pedrozo 1993, 1996, Helbling et al. 2001 a) and optical characteristics (Morris et al. 1995, Helbling et al. 2001 a, Alonso et al. 2004). This variability in both the community structure and in the underwater radiation field certainly offers a unique opportunity to evaluate UVR effects upon aquatic organisms inhabiting these lakes. In this region, several studies have described the responses to solar UVR of zooplankton organisms (Zagarese et al. 1997 a, b, 1998 a, b, Alonso et al. 2004), fish larvae (Battini et al. 2000), biological interactions in a mesocosm (Pérez et al. 2003) and photochemical aspects (Zagarese et al. 2001). However, and with the exception of the work of Helbling et al. (2001 a) carried out with winter communities exposed to artificial radiation conditions, we are not aware of studies specifically addressing the effects of UVR upon phytoplankton organisms of temperate lakes of Patagonia.

The objective of this study is to determine the effects of solar UVR upon photosynthesis and DNA in phytoplankton from three Andean lakes of Argentina that have marked differences in water transparency. The approach was to determine photosynthetic rates and DNA damage when natural phytoplankton assemblages were exposed to different solar radiation wavebands. It should be noted that while both UV-A (315–400 nm) and UV-B (280–315 nm) can significantly reduce photosynthetic rates, only UV-B causes the formation of cyclobutane pyrimidine dimers (CPDs); UV-A, on the other hand, can induce indirect DNA oxidative damage (BUMA et al. 2003). In this work we will estimate DNA damage through the formation of CPDs, which accounts for about 80–90 % of photoproducts formed (BUMA et al. 2003). It should be considered though, that other UVR-induced photoproducts, such as pyrimidine (6–4) pyrimidone photoproducts [(6–4) PDs] can be as 300 times effecting in blocking DNA polymerase, being therefore more cytotoxic than CPDs (MITCHELL & NAIRN 1989).

Materials and methods

Study site and collection of samples

Experiments were conducted during January 1999 with phytoplankton collected from three Andean lakes of the Patagonia region of Argentina: Moreno, Morenito and El Trébol (41° S, 71° W, 800 m a.s.l.). Lakes Morenito and El Trébol are small lakes with a surface area < 1 km^2 (max. depth ~10 m), whereas Lake Moreno is a rather large lake with a surface area of ~6 km^2 (mean depth ~50 m). Surface water samples were collected daily (early in the morning) using a clean bucket (1 N HCl) and transported immediately to the laboratory (approx. 20 minutes away from the sampling sites) where several experiments were carried out as described below.

Experimental design

Experiments were done to determine the effects of solar UVR upon phytoplankton photosynthesis rates and DNA under simulated *in situ* conditions in a large pool with running surface water (15–17 °C) used as temperature control. At the beginning of each experiment sub-samples were processed for the determination of initial cyclobutane pyrimidine dimers (CPDs) levels, pigment concentration and phytoplankton composition/quantification (see below). Three types of experiments were carried out (all on different dates) to determine UVR effects as follows:

UVR-induced photosynthetic inhibition (all lakes)

Samples were transferred to 50 ml quartz tubes, inoculated with labelled radiocarbon (see below) and incubated for 6–8 hours around local noon under three radiation treatments (quadruplicates for each treatment): a) Tubes covered with Plexiglas UF-3 (cut-

off at 400 nm) so that the samples received only PAR (P treatment); b) Tubes covered with a sharp cut-off Schott filter (WG320) so that the samples received UV-A + PAR (PA treatment); and, c) Tubes without any filter to receive full solar radiation (PAB treatment). The transmission spectra of filters and materials have been published elsewhere (BUMA et al. 2001 a, VILLAFAÑE et al. 2003). Two independent experiments (i.e., different dates) were conducted with waters collected from each lake.

CPDs induction and repair (all lakes)

Samples were dispensed in 10 L high-UVR transmission polypropylene bags (the spectral characteristics of these bags have been published in BUMA et al. 2001 a) to study CPDs accumulation and repair in several microbial size fractions (i.e., 0.2 µm, 2 µm and 10 µm) when exposed to different radiation conditions. The radiation treatments were the following: a) Two bags incubated under full solar radiation and harvested around noon or at the end of the afternoon (PAB treatment); b) Two bags incubated under UVR opaque PMMA that received only PAR (the spectral characteristics of this material have been published in BUMA et al. 2001 a) for the morning or whole day period – P treatment; c) Two bags incubated under full solar radiation during morning hours, after which the bags were covered by either UVR opaque PMMA or 3 mm glass plates to remove total UVR or UV-B, respectively (PAB-P and PAB-PA treatments, respectively), d) One bag incubated under UVR opaque PMMA during morning hours, after which UV-A was allowed to pass during afternoon hours by replacing the PMMA screen by a glass screen (P-PA treatment). Each bag had two DNA biodosimeter tubes attached to the side to allow for DNA effective dose assessment during the experiments. The DNA biodosimeters consisted of a small quartz tube filled with a solution of bare DNA – 10 µg/ml calf thymus DNA in TE-buffer (10 mM Tris-HCl; pH = 8.0; 1 mM EDTA) (BOELEN et al. 1999).

Daily course of CPDs accumulation and photosynthetic inhibition (Lake Moreno only)

Experiments were performed to determine the accumulation of CPDs during the day in the three size fractions (i.e., 0.2–2, 2–10, > 10 µm) from Lake Moreno incubated under full solar radiation. A total of nine bags and eighteen DNA biodosimeter tubes were placed in the temperature-controlled water pool early in the morning. Then, one bag and duplicate biodosimeter tubes were removed one by one at successive PAR doses of 5.5 E/m^2 and processed for CPDs determination. Simultaneously, the daily course of UVR inhibition of photosynthesis was followed in these natural phytoplankton assemblages. For this measurement, three radiation treatments were implemented with eighteen quartz tubes (50 ml) exposed to full solar radiation (i.e., UVR + PAR – PAB treatment), eighteen quartz tubes (50 ml) covered with Mylar-D film (i.e., UV-A + PAR-PA treatment), and eighteen quartz tubes (50 ml) covered with Plexiglas UF-3 (i.e., PAR only – P treatment); the transmission spectra of these materials are published in HELBLING et al. (1992). Two tubes from each treatment were removed, together with a bag and the biodosimeters (i.e., early in the morning, and at equal PAR doses).

Analyses and measurements

Photosynthetic rates

Samples for photosynthesis measurements were inoculated with 5 µCi (0.185MBq) of labelled sodium bicarbonate (STEEMANN NIELSEN 1952). After the incubation period, the samples were filtered onto Whatman GF/F filters (25 mm), placed in 7 ml scintillation vials and exposed to HCl fumes overnight. After drying the filters, scintillation cocktail (Wallac Optiphase HiSafe 3) was added to the vials and the activity measured using a liquid scintillation counter (HOLM-HANSEN & HELBLING 1995).

CPDs formation

Each sample (i.e., in each bag) was size-fractionated by filtration and the filter frozen and stored (−80 °C) until analysis, which was carried out at the University of Groningen (The Netherlands). DNA was extracted from the filters using the procedure described in BUMA et al. (2001 a). To remove RNA, the extracts were incubated for 1 h with 75 µg/ml RNAse (Boehringer Mannheim) at room temperature. The DNA concentration of the extracts was determined fluorometrically using Picogreen dsDNA quantitation reagent (dilution 1 : 400, Molecular Probes) on a 1420 Victor multilabel counter (EG & G 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 (H3, Affitech, Oslo) directed mainly to thymine dimers. Briefly, 100 ng of heat denaturated DNA samples were blotted onto nitrocellulose membranes (Schleicher and Schuell, Protran 0.1 µm) which were then baked at 80 °C to immobilize the DNA. After a 30-minutes blocking step, followed by three washing steps, the membranes were incubated with the primary antibody H3 (overnight, 4 °C). After repeated washing, incubation with the secondary antibody (HRP rabbit-anti-mouse, Dako P0260) was done for two hours at room temperature. CPDs were detected 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). The vulnerability for CPDs induction was assessed by calculating the Mean Damage Ratio (MDR) (BUMA et al. 2003) by normalizing CPDs values in microorganisms to the CPDs values obtained in the biodosimeter.

Photosynthetic pigments

Chlorophyll-a (chl-a) concentration was determined fluorometrically by filtering 100 ml of sample onto a Whatman GF/F filter (25 mm) after which the photosynthetic pigments were extracted in absolute methanol during 1 h (HOLM-HANSEN & RIEMANN 1978). Chl-a concentration was then calculated from the fluorescence of the extract before and after acidification with 1 N HCl (HOLM-HANSEN et al. 1965) using a Turner Designs fluorometer (model TD 700).

Cell counts and taxonomic analyses

Samples for identification and enumeration of phytoplankton were placed in 125 ml brown bottles and fixed with buffered formalin (final concentration of 0.4 % in the sample); after settling 25–50 ml of the sample, cells were analyzed with an inverted microscope (Leica DM IL) following the technique described in VILLAFAÑE & REID (1995). In addition, size distribution and mean cell area of phytoplankton species were determined by attaching a video camera (Philips LDH 0462/00) to the inverted microscope and by using image analysis (Wintrack Software, Real Time Computers Inc.). For this measurement, an aliquot of 25 ml of the sample was settled overnight; 10–20 fields were analyzed and at least one hundred cells were measured.

Radiation measurements

During the whole experimentation period incident solar radiation was recorded continuously (one reading per minute) with a GUV 511 radiometer (Biospherical Instruments, Inc.) that has four channels in the UVR region of the spectra (305 nm, 320 nm, 340 nm and 380 nm) as well as a broad band PAR channel (400–700 nm). The penetration of solar radiation in the water column was measured at the same dates when CPDs induction and repair were done using an ELDONET broad band filter radiometer (Real Time Computers Inc.) that has sensors for UV-B (280–315 nm), UV-A (315–400 nm) and PAR (400–700 nm) and temperature and depth channels. In addition, DNA biodosimeters were used throughout in simulated *in situ* experiments and incubated in situ at different depths in the water column to determine the DNA effective dose ($k_{bd\text{-}eff}$) as described in BUMA et al. (2003).

Statistics

A non parametric Kruskal-Wallis analysis (ZAR 1984) was used to establish differences among treatments and/or lakes; a confidence level of 95 % was used in all analyses.

Results

The water column in the three studied lakes was well mixed, as inferred from the temperature profiles (Fig. 1). Because of their shallowness, mixed conditions were determined down to the bottom in lakes Morenito and El Trébol (Figs. 1 B, C); in the deep Lake Moreno, well mixed conditions were found at least in the upper 12 m of the water column (Fig. 1 A). The three lakes had differences in temperature, with values of ~22 °C in Lake Morenito (Fig. 1 B), ~17 °C in Lake El Trébol (Fig. 1 C), and ~15 °C in the large Lake Moreno (Fig. 1 A). The underwater optical characteristics of these lakes were also markedly different (Fig. 1, Table 1). Lake Moreno (Fig. 1 A) was a clear lake with a relatively deep penetration of solar radiation (Table 1), and the euphotic zone (1 % of surface PAR irradiance) comprised the upper 30 m of the water col-

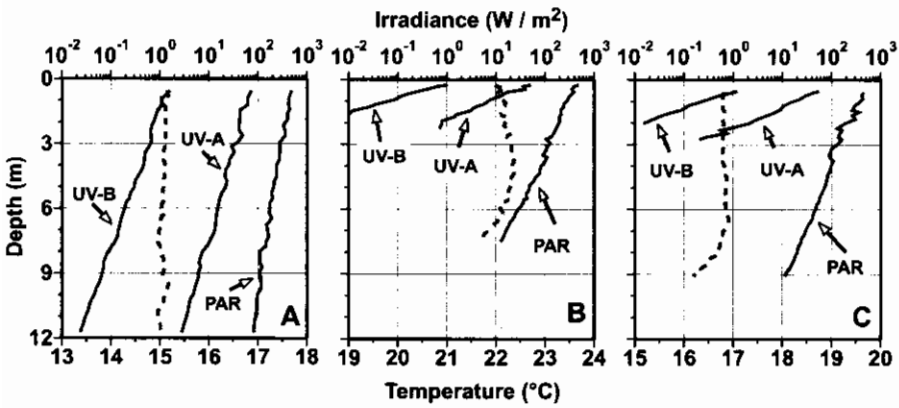

Fig. 1. Solar UV-B, UV-A and PAR irradiances and temperature as a function of depth and for the three Andean lakes sampled. **A)** Lake Moreno; **B)** Lake Morenito and, **C)** Lake El Trébol. The broken lines in the figure indicate the underwater temperature (in °C). Solar irradiance for PAR, UV-A and UV-B is expressed in W/m^2.

Table 1. Attenuation coefficients for UV-B, UV-A and PAR in the three lakes studied in the Patagonia Andes.

Lake	UV-B (280–315 nm)	UV-A (315–400 nm)	PAR (400–700 nm)
Moreno	0.36 m^{-1}	0.28 m^{-1}	0.15 m^{-1}
Morenito	2.8 m^{-1}	2.18 m^{-1}	0.46 m^{-1}
El Trébol	2.54 m^{-1}	2.39 m^{-1}	0.4 m^{-1}

umn. UVR also penetrated relatively deep, and the 1 % of surface UV-B and UV-A were found at 12.8 and 16.4 m, respectively. Lakes Morenito (Fig. 1 B, Table 1) and El Trébol (Fig. 1 C, Table 1) were considered as 'opaque' lakes as solar radiation was attenuated much faster than in Lake Moreno; the euphotic zone in lakes Morenito and El Trébol was measured down to 10 and 11.5 m, respectively. In these two 'opaque' lakes UVR was greatly attenuated and neither UV-B nor UV-A were detected below 3 m (Figs. 1 B, C). The biodosimeter profiles from lakes El Trébol and Moreno (Fig. 2) also highlight the different penetration of UV-B in the water column. CPDs values were high (~2800 CPDs/MB at the sub-surface) and accumulated in the upper 8 m of the water column in the clear Lake Moreno. In Lake El Trébol, on the other hand, CPDs values at surface were lower than in Lake Moreno (~1600 CPDs/MB), and no CPDs accumulation was detected below 0.4 m. The data obtained with the biodosimeters also allowed us to calculate the attenuation of DNA effective doses ($k_{bd\text{-}eff}$), which were 6.24 and 0.74 for lakes El Trébol and Moreno, respectively.

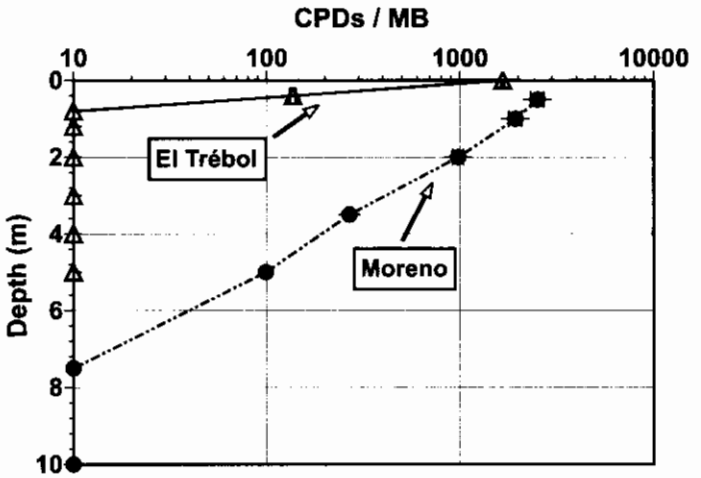

Fig. 2. Depth profiles of penetration of biologically effective dose as determined with biodosimeters incubated in lakes El Trébol and Moreno. The (+) symbols indicate the standard deviation.

The lakes also differed in size structure and composition of the phytoplankton communities. Although the phytoplankton communities in the three lakes were characterized during the sampling period by small cells (i. e., $<20\,\mu m$ in effective diameter), image analyses showed differences in the size distribution of cells (data not shown), with Lake Moreno presenting a slightly higher proportion of large cells as compared to that of lakes El Trébol and Morenito. Microscopical analysis also revealed differences among the lakes in regard to the taxonomic composition: small pennate diatoms characterized Lake El Trébol, whereas unidentified monads/flagellates and chlorophyte colonies dominated in lakes Morenito and Moreno, respectively. Other groups were also present – e. g., large pennate diatoms, dinoflagellates – but never accounted for a significant proportion of the phytoplankton community. During the sampling period, total cell values were low in the three lakes (<250 cells/ml) as well as chlorophyll a concentrations ($<1\,mg/m^3$).

A comparison between lakes in regard to CPDs accumulation for the most abundant phytoplankton size group, i. e., the 2–10 μm cell size fraction is shown in Fig. 3. Initial CPDs values were 52, 10 and 23 CPDs/MB in lakes Moreno, Morenito and El Trébol, respectively. During the morning, CPDs values increased significantly ($P < 0.05$) in the two opaque lakes in samples exposed to full solar radiation (i.e., PAB) (Figs. 3 B, C) whereas in Lake Moreno CPDs values remained relatively constant (Fig. 3 A); CPD values in samples exposed to PAR only also remained constant during the morning. During the afternoon, all samples exposed to full solar radiation significantly accumulated CPDs ($P < 0.05$) from its noon value, being the damage rate (i.e., damage ac-

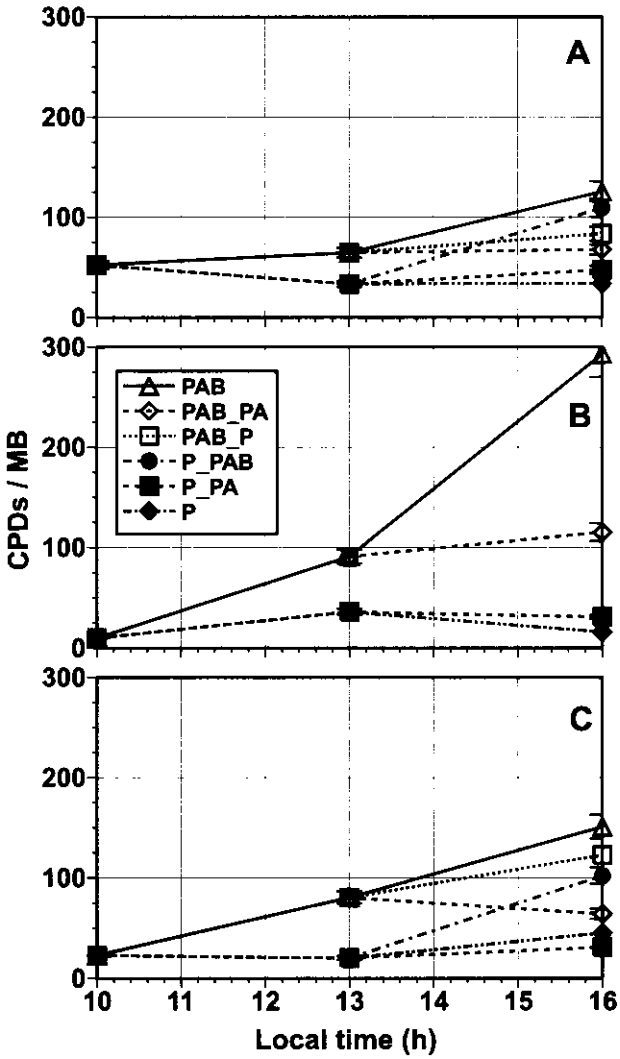

Fig. 3. Accumulation of CPDs and repair of DNA damage in phytoplankton (2–10 µm size fraction) incubated under various irradiance treatments. **A)** Lake Moreno; **B)** Lake Morenito and, **C)** Lake El Trébol. Cut-off screens were placed above the samples at 13 h to differentiate DNA damage occurring during morning and afternoon as well as to evaluate photorepair (full explanation in the text). PAB indicates samples exposed to full solar radiation; PA indicates samples exposed to PAR + UV-A and P indicates samples exposed only to PAR. The symbols (⊤) indicate the standard deviation.

cumulation during 3 h) highest in Lake Morenito (i.e., final mean values ~300 CPDs/MB, Fig. 3 B). In Lake El Trébol the damage rate was constant throughout the experiment (Fig. 3 C) whereas in Lake Moreno (Fig. 3 A) it was signifi-

Table 2. Mean solar radiation incident upon the experiments conducted to determine CPDs accumulation and repair (Fig. 3). Morning denotes incubations from 10 to 13 h; afternoon denotes incubations carried out from 13 to 16 h. PAR irradiances are expressed in $\mu E\ cm^{-2}\ s^{-1}$ and UVR irradiances (i.e., 305, 320, 340 and 380 nm) in $\mu W\ cm^{-2}$.

	Lake Moreno		Lake Morenito		Lake El Trébol	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
PAR	0.174	0.213	0.172	0.215	0.174	0.214
305 nm	4.97	8.44	4.55	7.87	5.42	9.06
320 nm	26.9	36.1	26.2	35.8	27.3	36.9
340 nm	48.5	62.3	47.6	62.4	48.7	62.9
380 nm	63.2	81.1	62.3	81.2	63.3	81.3

cant in the afternoon but not during the morning. As expected, samples exposed in the afternoon to either PAR + UV-A or PAR only did not show significant CPDs accumulation.

The higher CPDs formation during afternoon hours might reflect the impact of higher irradiances received then as compared to those of morning hours (Table 2). Thus, in order to assess this effect, CPDs formation data from biodosimeters is presented in Fig. 4. The CPDs accumulation at the end of the experiments was significantly higher ($P < 0.05$) in the biodosimeter exposed to full solar radiation in Lake Moreno experiments – ~5000 CPDs/MB (Fig. 4 A) than in those carried out in lakes Morenito and El Trébol (~3000 CPDs/MB, Figs. 4 B, C). With the exception of Lake El Trébol, there was higher CPDs accumulation in biodosimeter samples collected during the afternoon than in the morning. To evaluate the vulnerability of phytoplankton assemblages of the three lakes in terms of DNA damage, we calculated the mean damage ratio (i.e., MDR) with data from Figs. 3 and 4. Lake Morenito had the highest MDR values ~ mean of 0.17 (SD 0.06), whereas lakes Moreno and El Trébol had MDR mean values of 0.04 (SD 0.02) and 0.06 (SD 0.01), respectively.

Photosynthetic rates were rather similar when exposed to full solar radiation (i.e., $P > 0.05$) in the three phytoplankton assemblages (Fig. 5). There was a slight increase in carbon fixation when UV-B was excluded from the samples, but it was significantly higher ($P < 0.05$) only in Lake El Trébol. In the three lakes though, phytoplankton had a significant increase in carbon fixation ($P < 0.05$) when UV-A was additionally filtered out, with the highest values being also observed at Lake El Trébol. The PAR-only treatment also presented significant differences in carbon fixation among the three lakes, being assemblages from Lake Moreno those with the lowest photosynthetic rates (~4 $\mu g\ C\ l^{-1}\ h^{-1}$) whereas those from El Trébol had the highest values (~11 μg

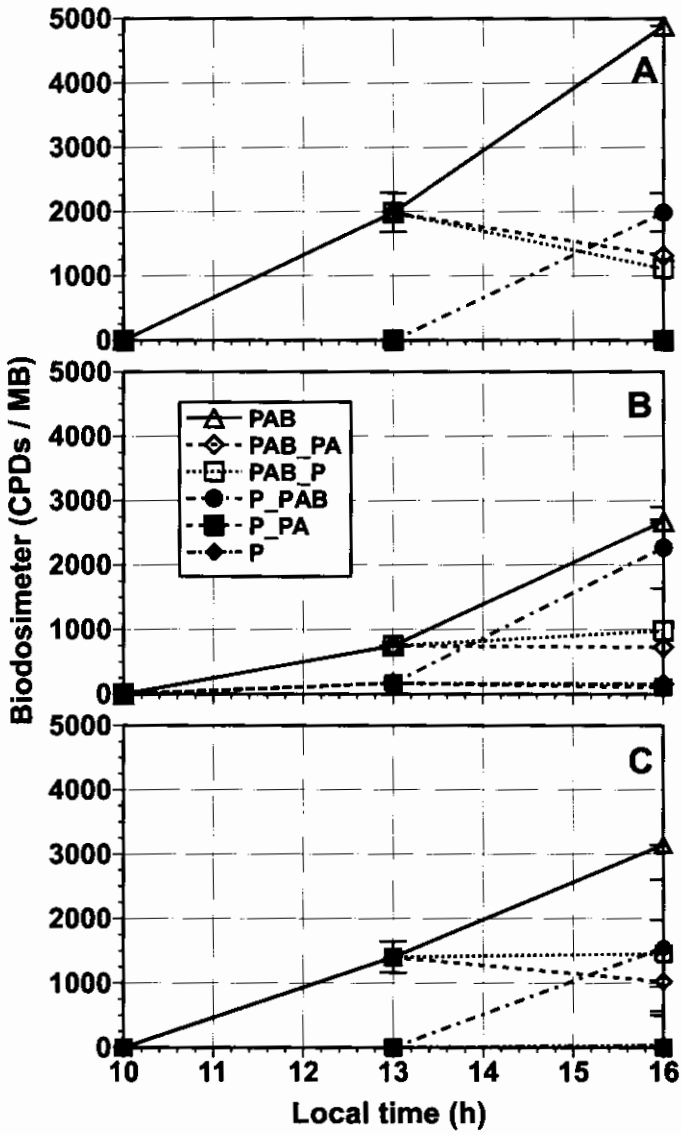

Fig. 4. Accumulation of CPDs in biodosimeters incubated under various irradiance treatments (see Fig. 3). **A)** Lake Moreno; **B)** Lake Morenito and, **C)** Lake El Trébol. PAB indicates samples exposed to full solar radiation; PA indicates samples exposed to PAR + UV-A and P indicates samples exposed to only PAR. The symbols (⊤) indicate the standard deviation.

C $l^{-1} h^{-1}$). The mean irradiance received by the cells during the experiments was rather similar – 0.19 µE $cm^{-2} s^{-1}$ for PAR, and 6.49, 30.9, 54.5 and 70.9 µW $cm^{-2} nm^{-1}$ for 305, 320, 340 and 380 nm, respectively.

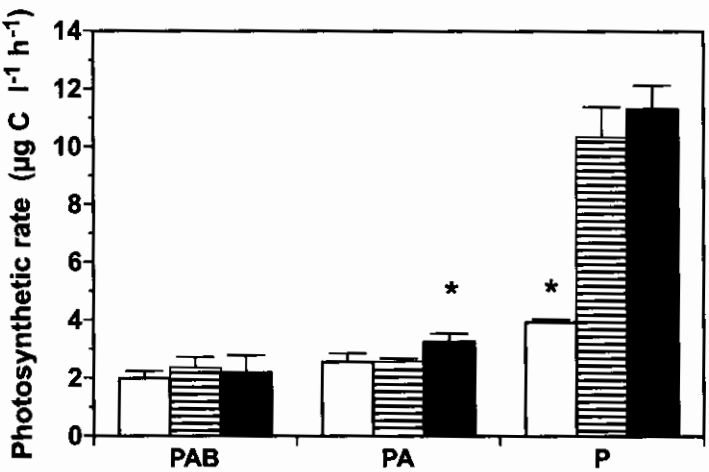

Fig. 5. Photosynthetic rates as a function of different quality radiation treatments in phytoplankton assemblages from lakes Moreno (white bars), Morenito (hatched bars) and El Trébol (black bars). The asterisk on top of the bars indicates significant differences ($P < 0.05$).

Daily inhibition of photosynthesis in parallel to CPDs accumulation was followed in Lake Moreno assemblages (Fig. 6). As seen before, carbon incorporation was significantly higher ($P < 0.05$) in samples exposed only to PAR as compared to the treatment exposed to full radiation. There was a slight but significant increase in photosynthetic inhibition between these treatments, reaching a difference of 25 µg C/l (Fig. 6 A). UV-B was responsible for more than 65 % of the total photosynthetic inhibition (i. e., difference between the values obtained in PAR + UVR and PAR + UV-A treatments divided the total inhibition) during afternoon hours. CPDs accumulation in the 0.2 µm size fraction also increased with time from the initial value of 50 CPDs/MB to ~200 CPDs/MB at the end of the experiment (Fig. 6 B). In the 2–10 µm and > 10 µm cell size fractions CPDs accumulation was significantly lower ($P < 0.05$), but also slightly increased throughout the experiment, reaching values of 45 and 23 CPDs/MB (i. e., in the 2–10 µm and > 10 µm cell fractions, respectively). Finally, accumulation of CPDs in the biodosimeter was rather low during morning hours but increased steadily in the afternoon, reaching values of ~3000 CPDs/MB at the end of the experiment.

Discussion

In this study we have shown that natural assemblages from temperate Andean lakes of Patagonia respond in different ways to solar UVR. We particularly fo-

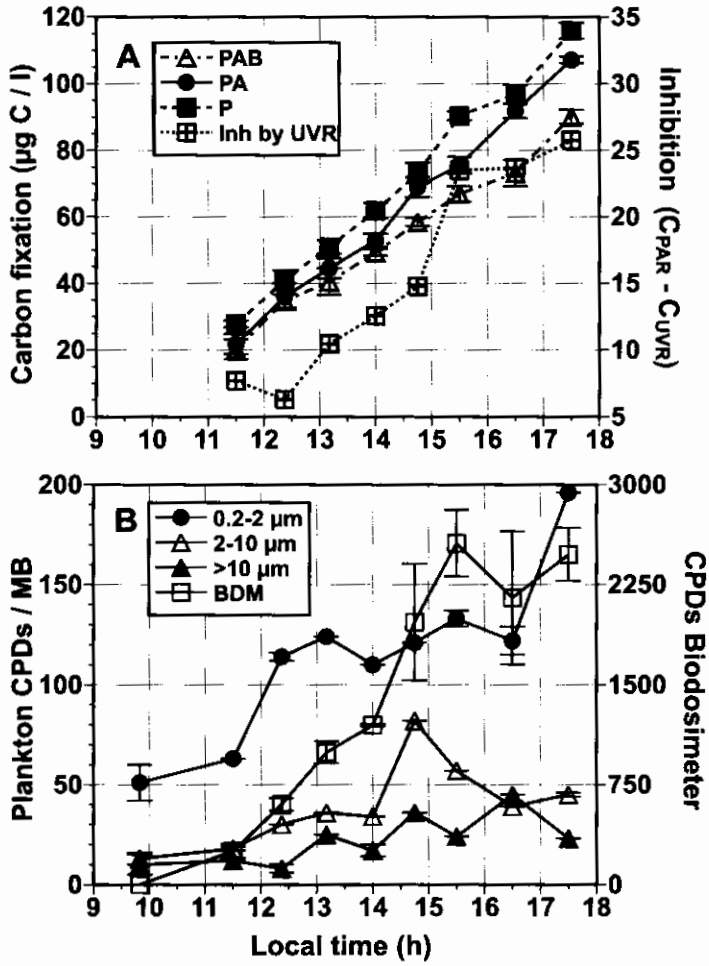

Fig. 6. Daily course of UVR photosynthetic inhibition and CPDs accumulation for phytoplankton from Lake Moreno. **A)** Carbon fixation as a function of different radiation treatments and inhibition due to UVR. **B)** CPDs accumulation in three different phytoplankton size fractions and DNA effective dose, as measured with the biodosimeter (BDM). The symbols (⊤) indicate the standard deviation.

cused on two of the most important effects of UVR upon phytoplankton organisms: photosynthetic inhibition (see review by VILLAFAÑE et al. 2003) and DNA damage (see review by BUMA et al. 2003). So far, many studies have evaluated the role of UVR in inhibiting photosynthesis and damaging the DNA molecule in various regions of the world, i. e., polar (HELBLING et al. 1992, SMITH et al. 1992, NEALE et al. 1998 a, BUMA et al. 2001 b, MEADOR et al. 2002), temperate (HELBLING et al. 2001 a, b, BUMA et al. 2001 a, BANASZAK & NEALE 2001), and tropical marine and freshwater environments (KINZIE III

et al. 1998, VILLAFAÑE et al. 1999, HELBLING et al. 2001 c, 2003, BOELEN et al. 2000, 2001, 2002).

We can summarize the overall effects of solar UVR upon phytoplankton of temperate lakes of Patagonia as follows: In terms of DNA damage (Fig. 3), organisms from Lake Morenito presented the highest CPDs accumulation rates (Fig. 3 B) and MDR values, followed by those from Lake El Trébol (Fig. 3 C). When considering photosynthesis (Fig. 5) these two lakes also presented high UV-A-induced inhibition (mean of 75 and 71 % for lakes Morenito and El Trébol, respectively) and much lower due to UV-B (2 and 9.5 % for lakes Morenito and El Trébol, respectively). Samples from Lake Moreno, on the other hand, had the lowest CPDs accumulation (Fig. 3 A) and MDR values; photosynthetic inhibition was also low (35 and 15 % for UV-A and UV-B, respectively) compared to the other two lakes (Fig. 5). Many causes might account for these differential responses, such as the characteristics of the underwater radiation field, the type and effectiveness of the strategy used by the organisms to cope with solar UVR, and differences in the size structure and taxonomic composition of the community. Optical characteristics in the three lakes were different, and based on underwater radiation measurements (Fig. 1, Table 1) and $k_{bd\text{-}eff}$ calculated from the biodosimeters (Fig. 2) we could clearly distinguish two types of environments: One was the 'clear' waters of Lake Moreno, and the other the 'opaque' waters of lakes Morenito and El Trébol. These two types of environments represent two extreme conditions for the area in terms of underwater radiation; however, other studies (e. g., MORRIS et al. 1995 and LAURION et al. 2000) have determined extreme k_{PAR} values of 5.21 and 0.08 m^{-1} in American lakes and in the Tyrolean Alps, respectively. However, the differences in penetration of solar radiation in our study sites are large enough to allow a comparison of the effects of natural radiation upon phytoplankton assemblages exposed and acclimated to two extreme regimes. A major part of the variability of UVR transparency (i. e., $k_{UV\text{-}B}$ from 0.36 to 2.8 m^{-1} in lakes Moreno and Morenito, respectively, Fig. 1, Table 1) seems to be related to variations in DOM, especially DOC compounds (e. g., fulvic acids, tannic acids and lignins) as determined in many studies in other parts of the world (SCULLY & LEAN 1994, MORRIS et al. 1995, LAURION et al. 2000). Although we did not specifically address the variability in DOC concentrations in these lakes, previous studies in the area have determined DOC values ranging from 0.65 to 1.70 g/m^3 in lakes Moreno and El Trébol, respectively (MORRIS et al. 1995). Our irradiance data as well as the $k_{bd\text{-}eff}$ values suggest that the cells in the 'opaque' lakes could be more protected than those in the 'clear' lake. One can argue however, that because of the lower water transparency, cells are exposed to a low mean irradiance and thus are 'dark' adapted. This in turn would potentially result in high damage rates if cells are brought to the surface by mixing (NEALE et al. 2003). In addition, a recent study

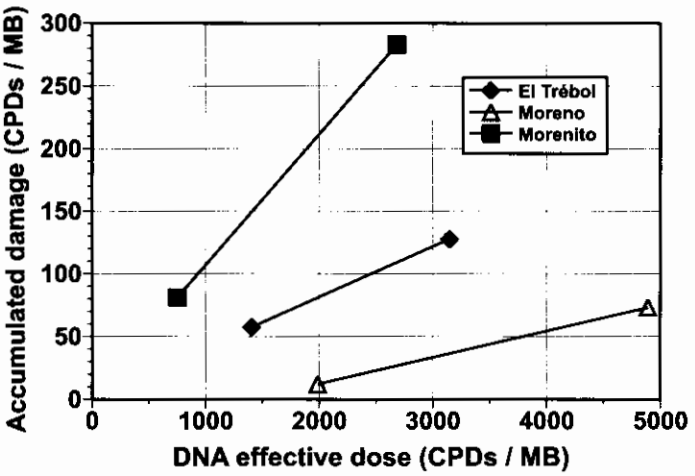

Fig. 7. Dose response relationship between CPDs accumulation in the 2–10 µm phytoplankton size fraction as a function of the DNA effective dose for lakes Moreno, Morenito and El Trébol.

(HELBLING et al. 2003) has shown that the intensity of mixing (i. e., the turnover speed within the UML) was critical for the acclimation of cells, and phytoplankton photosynthesis could be either enhanced or reduced by solar UVR depending on the mixing rate. In our case, it was seen that phytoplankton from Lake Morenito was the most sensitive to solar radiation, having the highest CPDs accumulation even at lower DNA effective doses (Fig. 7). In the 'clear' Lake Moreno, phytoplankton had the lowest DNA damage even though they received the highest DNA effective doses.

It is evident though, that although differences in the underwater radiation field may contribute to different responses from phytoplankton organisms to solar UVR, the optical characteristics would not solely be responsible for the observed responses. The taxonomic composition may account for part of the variability in responses as different assemblages were present in the lakes: small pennate diatoms characterized Lake El Trébol, whereas unidentified monads/flagellates dominated in lakes Morenito and Moreno. In fact, several studies have determined that under similar radiation conditions a wide range of responses can be observed within different taxonomic groups (VERNET et al. 1994, HELBLING et al. 1996, SOMMARUGA & BUMA 2000) but so far no generalizations can be made in regard to the particular sensitivity to UVR of each taxon. These differences in responses can be attributed not only to intrinsic factors of genetic origin, but also to the presence of photoprotective compounds (i. e., mycosporine like aminoacids – MAAs – or carotenoids) (VERNET et al. 1994, HELBLING et al. 1996) that might allow organisms to improve their overall fitness under UVR stress. For example NEALE et al. (1998 b) have

determined a reduction of UVR-induced photosynthesis inhibition in a dinoflagellate strain, which was attributed to the presence of several MAAs (i. e., mycosporine-glycine, palythine, porphyra-334 and palythene). In our study we did not determine the presence of UV-absorbing compounds in natural phytoplankton assemblages, but the absorption characteristics (data not shown) did not indicate the presence of these compounds; future studies, however, should consider this aspect in greater detail.

We also considered the size structure of the community as potential source for the variability in the responses of phytoplankton to solar UVR. Several studies have demonstrated the size-dependence of UVR effects (KARENTZ et al. 1991, LAURION & VINCENT 1998, HELBLING et al. 2001 a, b) with small cells (i. e., high surface to volume ratio) being more resistant when addressing photosynthesis inhibition, but more vulnerable to DNA damage (HELBLING et al. 2001 b). On the other hand, large cells (providing that they do not have high concentrations of UV-absorbing compounds) are more sensitive to UVR when considering photosynthetic inhibition, but they are more resistant for DNA damage (KARENTZ et al. 1991, HELBLING et al. 1992, 1994, BUMA et al. 1997, BOELEN et al. 2000, HELBLING et al. 2001 b). In all three lakes the smaller size fraction (0.2–2 µm, i. e. heterotrophic bacteria mainly) exhibited more rapid CPDs accumulation as compared with the larger size fractions (i. e. 2–10 µm and > 10 µm, Fig. 6 and data not shown, for lakes Morenito and El Trébol). This corresponds with studies performed in several regions, where generally higher vulnerability for CPDs induction is found in bacteria as compared with larger, eukaryotic cells (reviewed in BUMA et al. 2003). Moreover, a comparison of vulnerability for CPDs induction between these regions clearly showed a very low CPDs induction rate (i. e., normalized to incident DNA effective UV-B) for organisms from Andean lakes as compared with microorganisms from lower altitudes or latitudes. This indicates that physiological and/or ecological acclimation to the prevailing (high irradiance) UV-B regime may occur, but that nevertheless CPDs accumulation cannot be prevented (BUMA et al. 2003). Image analyses as well as microscopic observations of our samples indicated that the phytoplankton communities of the three lakes were mainly characterized by small cells (< 20 µm in effective diameter), with a very slight difference in the proportion of large cells (i. e., microplankton) in Lake Moreno. Hence we do not think that these size structures of cells within the communities would account *per se* for the observed differential effects of solar UVR, especially because the differences are found in the 2–10 µm size fraction only (Fig. 3).

Previous studies have revealed the presence of two main mechanisms by which phytoplankton organisms can repair the UVR-induced DNA damage (SANCAR & SANCAR 1988, ROY 2000, BANASZAK 2003): a) Photoreactivation, which utilizes long UV-A and blue light energy and, b) Nucleotide excision re-

pair, also known as dark repair, because it does not require photoreactivating light. Of both mechanisms, photoreactivation seems to be far more common in phytoplankton (KARENTZ et al. 1991, BUMA et al. 2001 a) than dark repair. Our data however, indicate that photoreactivation, if present, was not enough to cope with DNA damage as seen by the continuous increase in CPDs (Fig. 3). Also, there was null or slight decrease in CPDs during the afternoon in the samples were UV-B was filtered out, also suggesting low photoreactivation activity in these lakes. Even though we did not perform experiments to specifically test dark repair, there are some hints that might support the view that dark repair was important for these phytoplankton Andean communities, or at least in the clear Lake Moreno. The cells in our experiments were exposed to the maximum radiation conditions (i. e., surface radiation), but DNA damage in the clear Lake Moreno occurred in the upper 7 m of the water column (Fig. 2), with CPDs formation increasing significantly during the day (Figs. 3 A, 6 B). Early morning CPDs determinations however, were significantly low probably due to dark repair occurring at night. One cannot rule out, however, that part of the decrease could be accounted by a potential dilution of the DNA damage either by synthesis of *de novo* DNA or vertical mixing in the water column. In addition to differences in taxonomic characteristics, differences in temperature could account for part of the variability in responses between lakes. For example, studies have revealed the importance of temperature in determining the effectiveness of the photorepair mechanism (ROCCO et al. 2002). Here we have found relatively large temperature differences, especially between Lake Morenito (i. e., 22 °C) and the other two lakes (i. e., ~15 °C), which may result in the higher effectiveness in repair as determined in Lake Morenito.

In conclusion, this study shows that several factors account for the variability in responses of phytoplankton organisms of temperate Andean lakes when exposed to solar radiation. Taxonomic composition, as well as different strategies of protection and repair between organisms from 'opaque' and 'clear' lakes might take place to mitigate UVR-induced damage to acclimate natural assemblages to solar radiation. Our study also highlighted the importance of DOM in conditioning the underwater radiation field and thus DNA-damage in the phytoplankton assemblages. Even though phytoplankton cells might find 'protection' in 'opaque' waters, this would result in a disadvantage in water bodies exposed to windy conditions such as those in Patagonia, with an overall result of higher DNA damage as compared to 'clear' lakes.

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