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Primary Research Paper

In situ responses of phytoplankton from the subtropical Lake La Angostura (Tucumán, Argentina) in relation to solar ultraviolet radiation exposure and mixing conditions

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Abstract

In situ experiments were conducted at various depths in the water column to determine the effects of solar ultraviolet radiation (UVR, 280–400 nm) on photosynthesis of natural phytoplankton assemblages from the subtropical Lake La Angostura (Argentina, 26°45′ S; 65°37° W, 1980 m asl.). Water samples were taken daily and incubated under three radiation treatments: (a) Samples exposed to UVR + Photosynthetic Available Radiation (PAR) – PAB treatment (280–700 nm); (b) Samples exposed to ultraviolet-A radiation (UV-A) + PAR – PA treatment (320–700 nm), and, (c) Samples exposed to PAR only – P treatment (400–700 nm). Additionally, depth profiles were done to determine different physical (i.e., temperature and underwater radiation field) and biological characteristics of the water column – photosynthetic pigments, UV-absorbing compounds, cell concentration, deoxyribonucleic acid (DNA) and cyclobutane pyrimidine dimers (CPDs). The effects of UVR on natural phytoplankton assemblages were significant only in the first 50 cm of the water column, causing a decrease in photosynthetic rates of 36 and 20% due to UV-A and ultraviolet-B radiation (UV-B), respectively; below this depth, however, there were no significant differences between radiation treatments. Concentration of CPDs per mega base of DNA in natural phytoplankton was low, < 27 CPDs MB^{-1} between 0 and 4 m. Data on net DNA damage, together with that on mixing conditions of the water column, suggest that mixing can favour phytoplankton by allowing cells to be transported to depths where active repair can take place. This mechanism to reduce UVR-induced DNA damage would be of great advantage for these assemblages dominated by small cyanobacteria and chlorophytes where UV-absorbing compounds that could act as sunscreens are virtually absent.

Introduction

One of the most important stress factors for plankton organisms is solar UVR (280–400 nm), as seen in several studies carried out in diverse environments of the World (see review of Helbling & Zagarese, 2003). Solar UVR causes important negative effects on phytoplankton, which include, on short-term basis, a reduction of photosynthesis rates and damage to the DNA molecule, among others (Buma et al., 2003; Villafañe et al., 2003). On the long run, reduction of growth rates and changes

in the taxonomic structure of the community are frequently cited (Villafañe et al., 2003). These effects in turn, can be translated to higher trophic levels of aquatic food webs, by affecting trophic interactions (Sommaruga, 2003) and carbon flux within the ecosystem (Mostajir et al., 1999).

The effects of solar UVR on plankton organisms have been extensively studied (see reviews of Hessen, 2003 and Villafañe et al., 2003), especially during the past two decades, after the discovery of the Antarctic ozone "hole" (Farman et al., 1985), which brought about a great concern about the implications of enhanced UV-B (280–315 nm) on aquatic life and ecosystems. Within this context, many studies dealing with the effects of solar UVR have been carried out in lakes, especially in North America (e.g., Furgal & Smith, 1997; Laurion et al., 1998; Williamson et al., 2001) and Europe (e.g., Vinebrooke & Leavitt, 1995; Laurion et al., 2000; Van Donk et al., 2001), which report a wide range of responses of phytoplankton, not only under enhanced radiation conditions (McNamara & Hill, 2000) but also under natural levels (Hill et al., 1997). The interaction of UVR with other environmental factors such as mixing has received less attention in lakes (Kohler et al., 2001), although the importance of variable irradiance regimes have been highlighted in the marine environment (Neale et al., 2003).

Although no comparative efforts have been put to evaluate the effects and impact of solar radiation on plankton from South American lakes, there are some studies carried out in specific locations of north Patagonia in Argentina (Helbling et al., 2001a; Villafañe et al., 2001, 2004 and references therein) and high-altitude lakes of Chile (Cabrera et al., 1997) and Bolivia – Lake Titicaca (Villafañe et al., 1999; Helbling et al., 2001b, 2002). We lack, however, of information on UVR effects on phytoplankton inhabiting subtropical lakes. These areas are important from a photobiological point of view, as organisms are exposed to relatively high radiation levels because of their geographical location (Madronich, 1993); additionally, some subtropical lakes are located at high altitudes, where relatively higher UVR levels are measured as compared with their counterpart latitude sites (Blumthaler & Rewald, 1992).

The aim of this study is to evaluate the *in situ* responses of phytoplankton organisms from Lake La Angostura (Tucumán, Argentina) to solar UVR exposure taking into consideration its natural mixing conditions. The lake presents important characteristics in relation to the radiation field under which organisms are exposed, not only because of its geographical location in a subtropical area (26° S), but also because of its elevation (1980 m a.s.l.). The approach used in our study was to determine the *in situ* photosynthetic inhibition of natural phytoplankton exposed to solar UVR, and to evaluate the causes and mechanisms that allow the observed responses of these organisms.

Materials and methods

Study site and collection of samples

Experiments were conducted during December 2004 with phytoplankton collected from the subtropical Lake La Angostura (Argentina, 26°45′ S; 65°37′ W, 1980 m asl., Fig. 1). The lake has an area of 8 km^2 and a mean depth of 20 m, and it constitutes a protected area that was created for watering, regulation of floods, fishery and touristic purposes. Although the lake is largely undersampled, some descriptive studies have been done about the physical characteristics and the taxonomy and distribution of phytoplankton and zooplankton species (Locascio de Mitrovich et al., 1997).

Experimental

Surface water samples were collected daily (early in the morning) with an acid-clean (1 N HCl) bucket to be used for experiments. At the beginning of each experiment, sub-samples were processed for the determination of photosynthetic pigments and UV-absorbing compounds concentrations and phytoplankton composition/quantification (see below). Additionally, physical characteristics of the lake (i.e., temperature, conductivity and underwater solar radiation) together with sampling at different depths of the water column (i.e., every meter from the surface down to 10 m depth) were done at noon to obtain profiles of different biological parameters such as chlorophyll-*a* (chl-*a*), UV-absorbing compounds

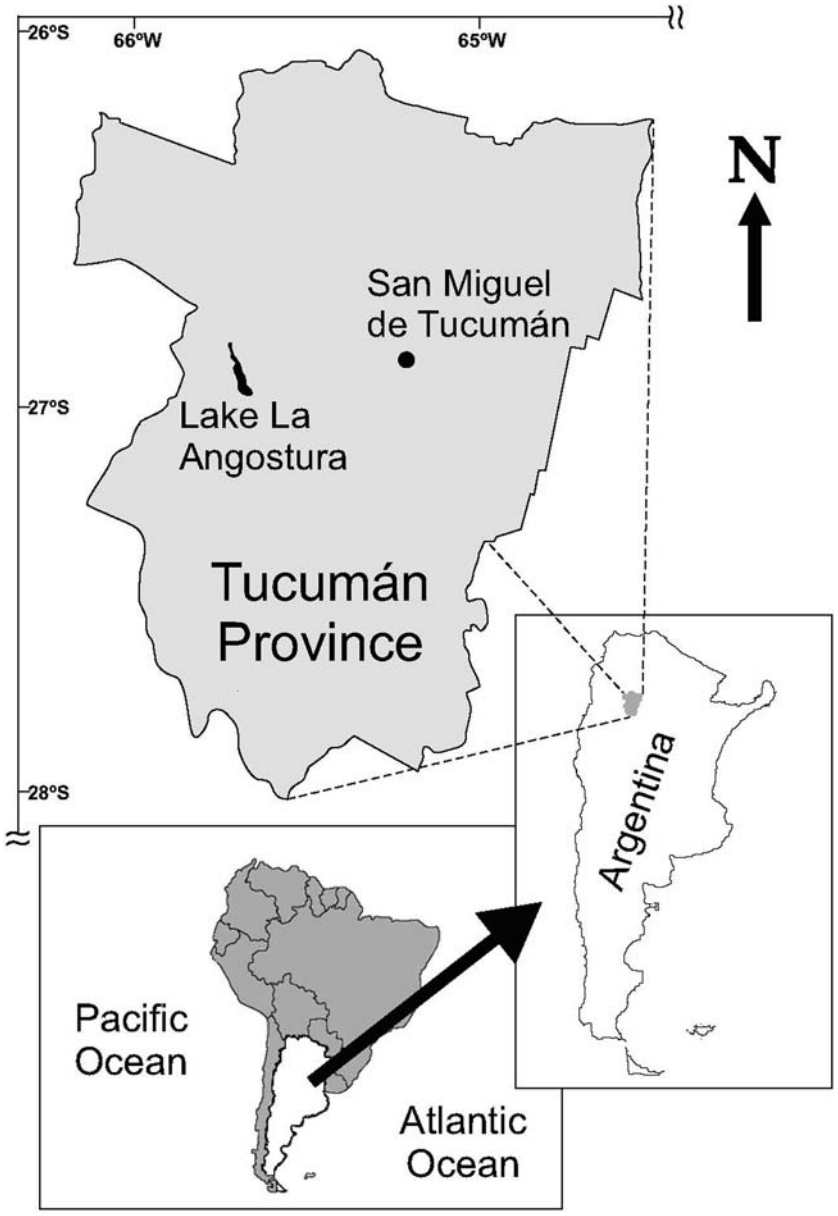

Figure 1. Map showing the study area and the relative position of Lake La Angostura in the Tucumán Province, Argentina.

concentrations, DNA concentration and amount of CPDs (see below).

Surface water samples were incubated *in situ* (0, 0.5, 1, 1.5 and 2 m depth) during 4 h centred on local noon (i.e. from 10 a.m. to 2 p.m.) to determine the effects of solar UVR on photosynthetic rates. For this, duplicate samples were placed in 50 ml quartz tubes and inoculated with labelled sodium bicarbonate (see below). Three different radiation treatments were implemented at each depth: (1) Duplicate samples that received full radiation (UVR + PAR, 280–700 nm) – uncovered quartz tubes; (2) Duplicate samples that received UV-A + PAR (320–700 nm) – tubes covered with UV cut-off filter foil (Montagefolie, No. 10155099, Folex) (50% transmission at 320 nm); and (3) Duplicate samples that received only PAR (400–700 nm) – containers covered with Ultraphan film

(UV Opak, Digefra) (50% transmission at 395 nm); the spectra of these materials are published in Figueroa et al. (1997). The tubes were then placed in anodized aluminium frames that were attached to a buoy. After the incubation period, the samples were processed to determine photosynthetic rates (see below). Four independent experiments (i.e., different dates) were performed with phytoplankton collected at Lake La Angostura.

Analyses and measurements

Photosynthetic rates

Samples for photosynthesis measurements were inoculated with 5 μCi (0.185 MBq) of labelled sodium bicarbonate (Steeman Nielsen, 1952). After the incubation period, 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, 2 ml of scintillation cocktail (Wallac Optiphase HiSafe 3) was added to the vials and the activity measured using a liquid scintillation counter.

CPDs determinations

Samples were filtered through 0.22 μm polycarbonate Millipore Isopore Membrane Filter (GTTP04700) and stored at −70 °C until analysis. DNA was extracted by incubating the filters for 30 min with 0.75 ml of hexadecyltrimethylammonium bromide (CTAB) extraction buffer (2 % [w/v] CTAB [Sigma], 1.4 M NaCl, 0.2 % [v/v] 2-mercaptoetanol, 20 mM of ethylenediaminetetraacetic acid disodium salt solution (EDTA), 100 mM Tris–HCl pH = 8). After extraction, samples were washed with 0.75 ml chloroform/isoamyl alcohol, 24:1 (v/v) and centrifuged (10 min, 12,000 rpm (17,000 g), 4 °C). DNA was precipitated with 0.5 ml of isopropanol (2 h, 4 °C) followed by centrifugation (30 min, 12,000 rpm, 4 °C). The pellet was washed with 80% cold ethanol, vacuum dried and dissolved in 0.1 ml TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). To remove ribonucleic acid (RNA), the extracts were incubated for 1 h with 75 μg ml^{-1} RNAse (Boehringer Mannheim) at room temperature. The samples were stored at −20 °C. The DNA concentration was quantified fluorometrically using Hoechst 33258 dye (Amersham) following the technique described in Paul & Myers (1982).

The amount of CPDs was determined using the method of Boelen et al. (1999) employing a primary antibody (H3, Affitech, Oslo) specific for thymine dimers. For this analysis, 100 ng of heat denaturated DNA samples were blotted onto nitrocellulose membranes (Schleicher & Schuell, pore size 0.1 μm) with a Minifold I SCR96D dot blot apparatus (Schleicher & Schuell). The membranes were dried in an oven (2 h, 80 °C) to immobilize the DNA. After a 30 min blocking step with 5 % (w/v) skimmed milk powder in phosphate buffered saline (PBS-T, PBS + 0.1% v/v Tween 20; Sigma), followed by three washing steps in PBS-T, the membranes were incubated overnight with the primary antibody H3 at 4 °C (1:2,000 in PBS-T and 0.5 % [w/v] skimmed milk powder). After repeated washing, incubation with horseradish peroxidase conjugated rabbit-anti-mouse serum (1:5000 in PBS-T and 0.5% [w/v] skimmed milk powder; Dako P0260) was done for 2 h at room temperature. CPDs were detected using ECL detection reagents (RPN2106 Amersham) in combination with photosensitive films (Hyperfilm ECL Amersham). Finally, the films were scanned and the quantification of dimers was done using Image Quant software (Quantity One 1-D Analysis Software Bio Rad). Each blot contained two dilution series of standard DNA with known amounts of CPDs (Boelen et al. 1999) which were compared to CPDs in natural phytoplankton.

Photosynthetic pigments

Chlorophyll-*a* (chl-*a*) concentration was determined fluorometrically by filtering 100 ml of sample onto a Whatman GF/F filter (25 mm); the filters were placed in 15 ml centrifuge tubes with 7 ml of absolute methanol (Holm-Hansen & Riemann, 1978). Then, samples were sonicated for 15 min, and the photosynthetic pigments extracted for at least 1 h. Chl-*a* concentration was calculated from the fluorescence of the extract before and after acidification with 1 N HCl (Holm-Hansen et al., 1965) using a fluorometer (Turner Designs model TD 700).

UV-absorbing compounds

UV-absorbing compounds were determined following the technique described in Helbling et al. (1996). Aliquots of 200 ml of sample (i.e., collected

at different depths) were filtered onto Whatman GF/F filters (25 mm). The filters were placed in 15 ml centrifuge tubes with 7 ml of absolute methanol. Then, the samples were sonicated for 15 min and pigments and UV-absorbing compounds were extracted for at least 1 h at 4 °C. After this, the samples were centrifuged, and a scan between 250 and 750 nm of the supernatant was done using a spectrophotometer (Hewlett Packard model HP-8453E). The peak height at 334 nm was considered as an estimator of UV-absorbing compounds concentration (Dunlap et al., 1995). In addition, HPLC (High Performance Liquid Chromatography) analyses were performed to identify UV-absorbing compounds as described in Sinha et al. (1999). Mycosporine like amino acids (MAAs) were extracted in 2 ml of 20 % methanol (v/v) and after centrifugation, the supernatant was evaporated in savant (Universal Vacuum System plus UVS 4000 A) and redissolved in 0.2 % acetic acid. An HPLC system (GILSON – equipped with a Sphery Sorb, model Ods2 C-18 column and guard (5 μm packing; 250×4 mm I.D.)) was used for analyses and purification of MAAs. Samples were injected with a Rheodyne syringe into the HPLC column. The wavelength for the detection was 310 nm; the phase mobile was 0.2 % acetic acid at a flow-rate of 1.0 ml min^{-1}. MAAs were identified by comparing the absorption spectra and the retention time with secondary standards (macroalgae of known MAAs content) available at in our laboratory.

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) and a drop of Rose Bengal was added to better distinguish between organic and inorganic material; after settling 10 ml of sample (diameter of the chamber: 25.9 mm), cells were analysed with an inverted microscope (Leica DM IL) following the technique described in Villafañe & Reid (1995).

Radiation measurements

Incident solar radiation was recorded continuously (one reading per minute) with 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. The penetration of solar radiation in the water column was measured at noon using the same instrument. In addition, DNA biodosimeters (i.e., calf thymus DNA) were incubated *in situ* at different depths (0–1 m) in the water column to determine the DNA effective dose (Buma et al., 2003). The amount of CPDs in the biodosimeters was determined as described above for natural phytoplankton assemblages.

Statistics

Duplicate tubes for each radiation treatment were implemented in each experiment. Four independent *in situ* incubations were done to allow the calculation of mean and standard deviations. To statistically test differences (e.g., between radiation treatments), the non-parametric Kruskal Wallis test (Zar, 1984) was applied to the data; a confidence level of 95% was used in all analyses.

Results

The underwater optical characteristics of Lake La Angostura are shown in Fig. 2. Lake La Angostura is a relatively "opaque" lake (Fig. 2a) with an attenuation coefficient (k_{PAR}) of 1.7 m^{-1}; the euphotic zone was measured down to 2.7 m depth. UVR was also greatly attenuated in the lake and neither UV-B nor UV-A were detected below 1 m (Fig. 2a); $k_{UV\text{-}A}$ and $k_{UV\text{-}B}$ were 5.6 and 9.1 m^{-1}, respectively. Vertical change of temperature in Lake La Angostura is shown in Fig. 2b, and it is seen that the water column was mixed down to 7.5 m depth, as inferred from this profile. Surface temperature was 17.7 °C, but at 10 m depth (i.e., below the epilimnion) water temperature was lower, 16.2 °C.

Biological characteristics (i.e., chl-*a*, cells and DNA concentration) of Lake La Angostura are shown in Fig. 3. Chl-*a* values were relatively high in the epilimnion, ranging from 12.5 to 10.5 $\mu g\ l^{-1}$, but these values dropped to 3.5 $\mu g\ l^{-1}$ at 10 m depth (Fig. 3a). Total cells concentration was also relatively high in the epilimnion (i.e., 4,000 cells ml^{-1}) except for the relatively low values (3300 cells ml^{-1}) found at 4 m depth. Microscopical analysis revealed the dominance of

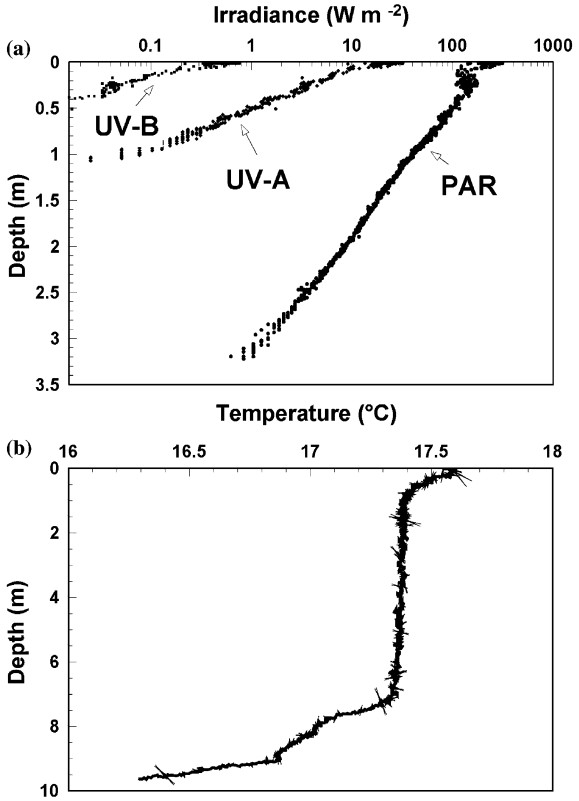

Figure 2. Representative profile showing the underwater radiation field (a) and temperature (b) in Lake La Angostura next to our *in situ* experiments; radiation units are W m^{-2}. The attenuation coefficients for PAR, UV-A and UV-B were 1.7, 5.6 and 9.1 m^{-1}, respectively. The profile was done on December 08, 2004 (12 h local time).

pico-nanoplankton cells (2–20 μm), represented by Chlorococcales chlorophytes (e.g., *Sphaerocystis* sp. <10 μm in diameter) and cyanobacteria colonies (Chroococcales), as well as by pennate diatoms – mainly *Navicula* spp. (<10 μm). Few large specimens were present in the samples, such as the chlorophytes *Closterium* sp., *Staurastrum* sp. *Scenedesmus* sp. and *Pediastrum* sp., and the diatom *Aulacoseira granulata*, which might contribute for an important part of total phytoplankton biomass in some samples. A similar pattern as that of chl-*a* and total cell concentrations was also observed for DNA (Fig. 3c), with relatively high values at surface (i.e., 20 μg l^{-1}) and low below the epilimnion (i.e., 6 μg l^{-1}), and with a relative minimum (16 μg l^{-1}) at 3–4 m depth.

A representative profile of *in situ* photosynthetic rates (as assessed through assimilation numbers) in samples exposed to the three different radiation treatments (i.e., PAB, PA and P) is shown in Fig. 4. Assimilation numbers in the P treatment were rather similar down to 0.5 m depth, 3 μg C (μg chl-*a*)$^{-1}$ h^{-1}, however, they decreased below this depth to 1 μg C (μg chl-*a*)$^{-1}$ h^{-1} at 2 m. There was a significant effect of the different wavebands ($p < 0.05$) at the surface, being UV-A and UV-B-induced photosynthetic inhibition of 36 and 20%, respectively; however, at 0.5 m, no significant differences between radiation

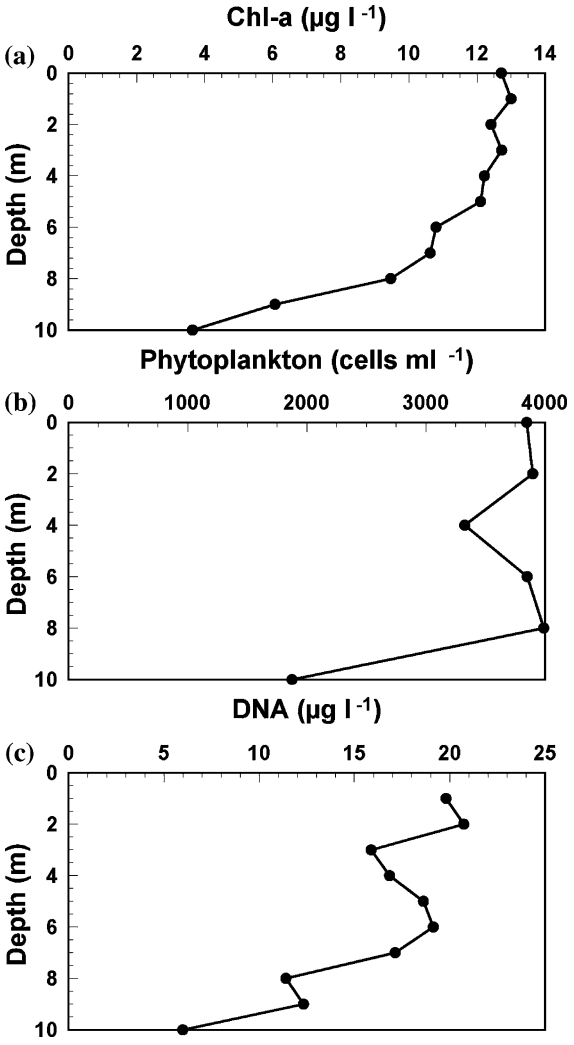

Figure 3. Representative biological characteristics as a function of depth of phytoplankton inhabiting Lake La Angostura. (a) Chlorophyll-*a* concentration (in μg l^{-1}), (b) Cell concentration (in cells ml^{-1}) and, (c) DNA concentration (in μg l^{-1}). The profile was done on December 08, 2004 (12 h local time).

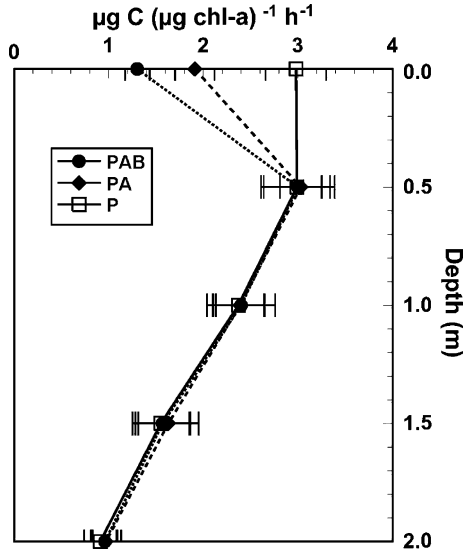

Figure 4. Depth distribution of assimilation numbers (in μg C [μg chl a]$^{-1}$ l h^{-1}) in natural phytoplankton assemblages from Lake La Angostura exposed to three radiation treatments – PAB (black circles), samples exposed to UVR + PAR; PA (black diamonds), samples exposed to UV-A + PAR and; P (white squares), samples exposed only to PAR. The data represent the mean and standard deviation (horizontal lines) of four experiments (done daily in the period December 06–09, 2004) using duplicate samples (n = 8).

treatments were detected. The mean irradiance received by the cells during the four experiments conducted was rather similar, with mean values of 240, 38 and 1.75 W m^{-2} for PAR, UV-A and UV-B, respectively.

The absorption characteristics of representative samples (0 and 7 m depth) collected at Lake La Angostura are shown in Fig. 5. The spectrum displayed the characteristic peaks of chl-a (440 and 665 nm) and carotenoids (470 nm); however, there was not a discernable peak of UV-absorbing compounds in the range 310–360 nm. HPLC analyses further corroborated the lack of mycosporine like aminoacids (MAAs) in waters collected at Lake La Angostura (data not shown), except for traces of shinorine found in the samples.

Data in Fig. 6 show the amount of CPDs present in both biodosimeters and natural phytoplankton assemblages. The amount of CPDs in the biodosimeters (Fig. 6a) incubated in surface waters was high, 1700 CPDs MB^{-1}, however, no CPDs were detected below the surface (i.e., at 5 cm); unfortunately, these data (only 2 points) did not provide enough resolution to calculate the attenuation of DNA effective doses ($k_{bd\text{-}eff}$). The amount of CPDs in natural phytoplankton exposed at

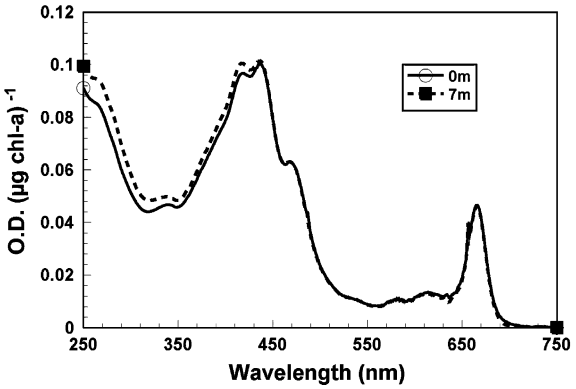

Figure 5. Representative absorption spectra – optical density (O.D.) as a function of wavelength (scan between 250 and 750 nm) of natural phytoplankton assemblages from Lake La Angostura. Samples were collected on December 08, 2004 (12 h local time).

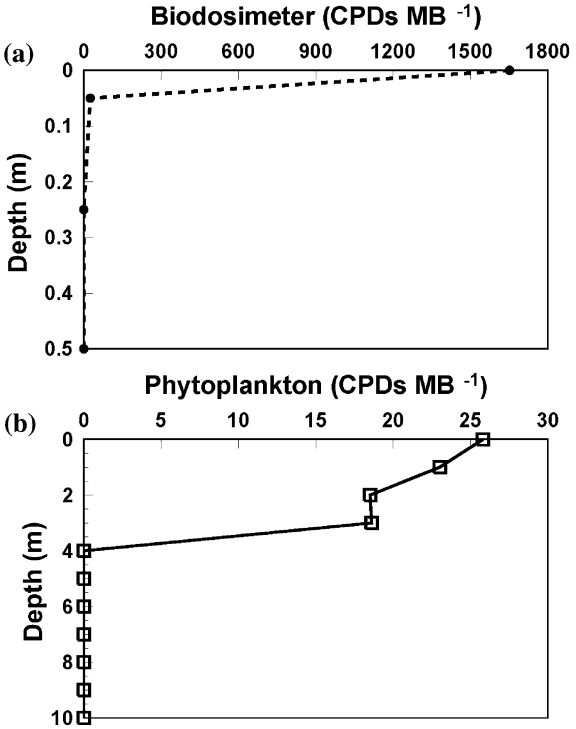

Figure 6. Amount of CPDs per mega base (MB) of DNA as a function of depth in: (a) Biodosimeter and, (b) Natural phytoplankton assemblages. Note the different scale in the y-axis in the two graphs. The profiles were obtained on December 09, 2004 (12 h local time).

different depths in the water column was much lower than that in the biodosimeters: Surface values were 27 CPDs MB^{-1} however, they gradually decreased down to 18 CPDs MB^{-1} at 3 m depth; below 4 m depth, CPDs were not detected.

Discussion

The geographical location of Lake La Angostura, in a subtropical region of Argentina, provides a very interesting scenario to carry out studies about the effects of solar UVR on phytoplankton, due to its particular characteristics that result in high fluxes reaching the ground, and hence potentially influencing the underwater radiation field. During the study period, mean UV-B irradiance at noon was >3 Wm^{-2} (data not shown), which is higher than the values determined in previous studies carried out in lakes of north Patagonia (i.e., Bariloche, Argentina) – 2 W m^{-2} during summer time (Villafañe et al., 2004). Particularly, the ratios of UV-B and UV-A to PAR were higher in Lake La Angostura, i.e., 0.73 and 15.8% of PAR, respectively, as compared to those obtained in Bariloche, 0.32 and 14.5% (Villafañe et al., 2004) at comparable times of the year. Part of the differences in PAR and UVR irradiance between these two places is due to their geographical location, as Bariloche is 15° South of Lake La Angostura. Additionally, differences in altitude between the two sites might play an important role: Lake La Angostura, located at 1980 m asl, may receive higher UV-B fluxes as compared with sea level counterparts, as seen in studies carried out in the Alps that reported a 10% increase in UV-B every 1000 m of altitude (Blumthaler & Rewald, 1992). However, one should be aware that the relationship between solar radiation levels and altitude is also dependent on the aerosol content in the atmosphere, so that this percentage might be slightly different in the Alps than in subtropical regions of Argentina. Finally, and due to its latitudinal location, ozone column concentrations over Lake La Angostura were relatively low during the study period, 260 Dobson Units (http://jwocky.gsfc.nasa.gov) which resulted in comparatively higher UV-B levels as compared with high latitude sites (Madronich, 1993) at the same time of the year.

Under such conditions of relatively high radiation fluxes reaching the surface of the Earth, it would be expected that organisms experience some degree of stress, especially due to UVR, as seen in many studies conducted in both terrestrial (Caldwell et al., 1995) and aquatic environments (Helbling & Zagarese, 2003). Particularly, one of the most noticeable effects caused by solar UVR exposure is photoinhibition – i.e., the reduction of photosynthesis rates, which occurs in most phytoplankton organisms (Villafañe et al., 2003). In Lake La Angostura, we have found significant inhibition of photosynthesis at the surface (Fig. 4) but it decreased very rapidly so that no effects were determined at 0.5 m. UV-A accounted for most of the UVR-induced photoinhibition – 36%, whereas UV-B added another 20%. Many studies have also found higher inhibition due to UV-A as compared to that due to UV-B (Kim & Watanabe, 1994, Pálffy & Vörös, 2003), just because the energy reaching the Earth's surface is higher within the UV-A range than in the UV-B waveband (Madronich, 1993). The integrated inhibition in the upper 2 m of the water column (almost the whole euphotic zone) was less than 5 and 2% for UV-A and UV-B, respectively, which are lower that those observed in other lakes of Argentina. For example, Villafañe et al. (2001) in their studies carried out in the Patagonia area determined that the integrated inhibition of photosynthesis in the euphotic zone due to UV-B was 6% at comparable times of the year. Thus the overall impact of UVR would be higher in temperate lakes of Patagonia than in the subtropical lake considered in our study. Similar results on the overall resistance of phytoplankton from subtropical areas were obtained in studies conducted in high altitude lakes – Lake Titicaca in Bolivia (16° S) (Villafañe et al., 1999; Helbling et al., 2001b) and Lake Waiau in Hawaii (19° N) (Kinzie III et al., 1998). In contrast, phytoplankton from Antarctic lakes were very sensitive to UVR even under the low irradiance levels that characterize the area (Neale et al., 1994).

It should be noted that in our incubations phytoplankton cells were exposed to fixed irradiances (i.e., at fixed depths in the water column) and thus they did not mix within the epilimnion – the layer of homogeneous physical/biological characteristics produced by wind stress (Neale et al., 2003) or by thermal stratification. Previous

studies have shown the importance of mixing at the time to evaluate the impact of UVR on phytoplankton so that it resulted in variable effects: Mixing either reduced UVR-induced photoinhibition (Barbieri et al., 2002; Helbling et al., 2003), enhanced it (Helbling et al., 1994; Neale et al., 1998) or caused no additional effects (Helbling et al., 1994); these variables responses are thought to depend on several factors, such as the radiation conditions imposed to the samples and the specific sensitivity of organisms under study. Particularly, mixing seems to be an important feature when considering photosynthetic inhibition in Lake La Angostura. In fact, and based on the temperature profile (Fig 2b), the distribution of various biological characteristics in the water column (Fig. 3) and the absorption spectra of phytoplankton at different depths (Fig. 5), we can assume that cells were mixing to at least 7 m depth. The overall result of mixing, added to the relatively high attenuation of solar radiation (k_{PAR} = 1.7 m^{-1}, Fig. 2a) in the water column, is that cells might have been exposed to lower irradiances than expected if considering only the surface radiation values (assuming that the optical characteristics of the water column were similar during the previous weeks to our sampling), obviously providing a potential advantage to cells inhabiting in such conditions. However, if attenuation of solar radiation in the water column varies significantly throughout the year (e.g., due to rainfall, changes in phytoplankton abundance, etc.) then, a different impact of UVR on the phytoplankton communities might be expected. In fact, the effect of UVR on phytoplankton communities under differential light climates have been addressed in other Andean lakes of Argentina and Villafañe et al. (2004) highlighted the importance of lake transparency in conditioning the impact of UVR on photosynthesis and DNA damage. In that study the authors found out that shading in opaque lakes resulted in a rather high vulnerability when organisms were transported to the high radiation levels characteristic of the photoactive zone and thus, higher photosynthetic inhibition and DNA damage were determined as compared to clear lakes. On the other hand, in phytoplankton from Lake La Angostura, we observed relatively low UVR-induced photoinhibition and DNA damage so we speculate that because of the relatively fast attenuation of potentially dangerous wavelengths together with mixing rate, phytoplankton cells were indeed ''protected''.

These differential responses could be associated, among other factors, to the turnover time (due to mixing) and the trophic state of phytoplankton: In the Bariloche area, the ''opaque'' lakes studied are shallow (< 2 m) and due to the strong winds they are well mixed resulting in a relatively fast turnover time within the epilimnion (Villafañe et al., 2004). Under these conditions, cells did not spend enough time outside the photoactive zone to allow efficient repair of any damage that might occur at the surface. On the other hand, we believe that mixing might favour phytoplankton under the physical and environmental conditions found during late spring in Lake La Angostura. Part of our speculation on these beneficial effects is based on the fact that, given the mixing conditions in Lake La Angostura – i.e., down to 7m and with less intense winds than those experienced at mid latitudes – the cells would be enough time outside the photoactive zone (where UV-B was not detected, Fig. 2) but they still receive enough radiation (i.e., PAR) to photorepair any damage that occurred at the surface. DNA data support this view, as CPDs in the biodosimeters were very high at the surface (i.e., 1700 CPDs MB^{-1}), and decreased to zero at 25 cm depth (Fig. 6a). However, natural phytoplankton CPDs levels at noon time were much lower (i.e., 25 CPDs MB^{-1}, Fig. 6b), even taking into account that part of them would be from bacteria retained in the filters (0.2 μm). These low CPDs values were determined down to ca. 3 m, suggesting that cells were carried out of the photoactive zone and thus they were repairing at depth. In fact, based on the CPDs levels in the biodosimeter (Fig. 6a) and the high UVR irradiance levels normally found in the study site at this time of the year (data not shown), we would expect higher CPDs levels in the phytoplankton assemblage, as seen in other water bodies dominated by small cells, i.e., the Caribbean Sea (Boelen et al., 2000) or temperate marine phytoplankton (Helbling et al., 2001c). Previous studies have shown that small cells are more susceptible to DNA damage than large cells (Helbling et al., 2001c) but they are able to acclimate very fast (within hours, Helbling et al., 2001a). However, fast repair mechanisms of any DNA damage

that occur at the surface, together with a dilution effect during mixing might account for the low CPDs naturally observed in Lake La Angostura.

It is obvious that such dynamics of actively repair DNA damage would result in an advantage for phytoplankton assemblages as those found in Lake La Angostura, especially considering that other potentially protective mechanisms against UVR stress might not be very effective. Such is the case of photoprotective UV-absorbing compounds (i.e., MAAs) that are widely known as sunscreens and are present in many marine and freshwater phytoplankton organisms (Banaszak, 2003). However, except for traces of shinorine, they were virtually absent in samples collected from natural phytoplankton assemblages from Lake La Angostura (Fig. 5). The lack of UV-absorbing compounds might be also partially related to the taxonomic composition of the assemblages, mostly dominated by chlorophytes, which is in agreement with previous findings reporting the lack of these compounds in most green algae (Banaszak, 2003). Additionally, the size structure of the community does not favour the accumulation of these UV-absorbing compounds, because in small-sized cells the useful concentration would be too high and osmotically disadvantageous (Garcia-Pichel, 1994). It should be noted, however, that self-shading (i.e., if phytoplankton biomass is rather high) could also result in a protection from solar UVR as determined by Wu et al. (2005). Further studies should concentrate in the understanding of possible mechanisms that allow the apparent high resistance of phytoplankton species found in Lake La Angostura.

Overall, our results suggest that phytoplankton cells at Lake La Angostura are well adapted to the radiation field under which they are exposed, as seen in the relatively low impact of UVR in photosynthetic rates, as well as in the low CPDs values registered in the water column. However, our data still open new questions about the different mechanisms of acclimation, together with annual changes in phytoplankton diversity that can result in variable UVR-protective strategies.

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