Research Article

Plankton dynamics and photosynthesis responses in a eutrophic lake in Patagonia (Argentina): influence of grazer abundance and UVR

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ABSTRACT. A natural plankton population from the eutrophic lake Cacique Chiquichano, in the Argentine Patagonia, was monitored for one year to evaluate changes in photosynthetic parameters as a result of exposure to ultraviolet radiation (UVR, 280-400 nm), grazer abundance, and the taxonomic composition of the phytoplankton community. Both physical (temperature, solar radiation) and biological (grazers, taxonomic composition, photosynthetic parameters) variables fluctuated throughout the study. Crustacean zooplankton showed alternating dominance between cladocerans (*Daphnia spinulata*) and copepods (*Metacyclops mendocinus*). The phytoplankton community underwent concomitant changes throughout the year, with cyanobacteria and diatoms alternately dominating. In addition, although copepod abundance was not significantly related to changes in phytoplankton, the presence of *D. spinulata* was significant during periods of more transparent water; these periods were dominated by diatoms. On the other hand, cyanobacteria dominated the phytoplankton assemblage when the penetration of solar radiation into the water column was lower. Photosynthetic inhibition due to UVR decreased during the diatom-dominated periods. In contrast, inhibition increased along with the proportion of cyanobacteria, likely as a result of acclimation to low irradiance during the lake's phase of lower transparency. Moreover, the presence of *D. spinulata* was associated with the increased penetration of solar radiation into the water column, resulting in an indirect increment in the inhibition of cyanobacteria photosynthesis. The results suggest that both solar radiation and grazing abundance strongly influence the dynamics and photosynthetic activity of the phytoplankton in Lake Cacique Chiquichano.

Keywords: zooplankton, UVR, phytoplankton, photoinhibition, cyanobacteria, shallow lakes, Argentina.

Dinámica del plancton y respuestas fotosintéticas en una laguna eutrófica de Patagonia (Argentina): influencia de la abundancia de herbívoros y RUV

RESUMEN. Se estudió a lo largo del año una comunidad planctónica natural de la laguna eutrófica Cacique Chiquichano de Patagonia-Argentina, para evaluar los cambios en parámetros fotosintéticos como resultados de la exposición a radiación ultravioleta (RUV, 280-400 nm), abundancia de herbívoros y composición taxonómica de la comunidad de fitoplancton. Tanto las variables físicas (temperatura, radiación solar) como biológicas (herbívoros, composición taxonómica, parámetros fotosintéticos) variaron a lo largo del estudio. Los crustáceos zooplanctónicos mostraron dominancia alternada entre un cladócero (*Daphnia spinulata*) y un copépodo (*Metacyclops mendocinus*). La comunidad de fitoplancton sufrió cambios concomitantes durante el año, con cianobacterias y diatomeas alternando su dominancia. Sumado a esto, si bien la abundancia de copépodos no estuvo significativamente relacionada con cambios en el fitoplancton, la presencia de *D. spinulata* fue significativa en períodos de aguas más transparentes, durante los cuales las diatomeas fueron dominantes. Por otra parte, durante los períodos de menor penetración de la radiación solar en la columna de agua, las cianobacterias dominaron la comunidad fitoplanctónica. La inhibición fotosintética debido a RUV disminuyó durante los períodos dominados por diatomeas. En cambio, la inhibición aumentó al incrementarse la proporción de cianobacterias, probablemente como resultado de una aclimatación a la baja irradiancia durante la fase de menor transparencia de la laguna. Más aún, la presencia de *D. spinulata* estuvo asociada a una mayor penetración de la radiación en la columna de agua, por lo cual aumentó indirectamente la inhibición de la fotosíntesis en cianobacterias. Los resultados sugieren que tanto la radiación solar como la abundancia de

herbívoros tienen una fuerte influencia en la dinámica y actividad fotosintética del fitoplancton en la Laguna Cacique Chiquichano.

Palabras clave: zooplancton, RUV, fitoplancton, fotoinhibición, cianobacterias, lagos someros, Argentina.

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INTRODUCTION

The seasonal variations of environmental factors often result in changes of phytoplankton species composition, as each species has different requirements and characteristics (Flöder *et al.*, 2002). Since growth and production of phytoplankton are closely related to photosynthesis, temperature and solar radiation are two of the key physical variables controlling algal populations in a particular season in highly eutrophic waters (Schoor *et al.*, 2008). For example, it has been recently shown in a mesocosm study that while solar radiation has an important role in the initiation of the spring bloom, an increase in temperature may decrease the peak biomass, mean cell size and the share of diatoms in the phytoplankton community (Sommer & Lengfellner, 2008). The penetration of solar radiation into the water column depends on several factors, but in many cases particulate matter (such as phytoplankton) – POM and dissolved matter (such as chromophoric dissolved organic matter, CDOM) constitute the main attenuators (Morris *et al.*, 1995). Thus, light availability in the water column may have a strong influence in shaping the producers' community. For example in shallow water lakes of Argentina, light limitation contributes to a lower periphyton development as compared to that of phytoplankton (which can be take advantage of surface light while is transported by mixing) whereas in less light-limited waters periphyton may develop a high biomass (Sánchez *et al.*, 2010). Additionally, grazing plays a significant role in shaping the phytoplankton seasonal succession: In some lakes, for example, grazing pressure may reduce a considerable portion of phytoplankton biomass (Sommer *et al.*, 1993; Flöder & Sommer, 1999) sometimes inducing a "clear-water" phase (Lampert & Sommer, 1997). In these cases, phytoplankton cells need to cope not only with grazing pressure, but also with enhanced underwater radiation that can be potentially harmful (Williamson *et al.*, 2007).

In regard to solar radiation, a vast literature exists about the negative effects produced by ultraviolet wavelengths (UVR, 280-400 nm) on different cellular targets of plankton organisms: The DNA molecule, proteins and photosynthetic apparatus, among others - see review by Häder *et al.* (2007). In particular, the decrease of photochemical quantum yield, associated with photoinhibition (Osmond, 1994) has been extensively studied in diverse aquatic organisms such as macroalgae, phytoplankton and microphytobenthos (Villafañe *et al.*, 2003). While this photoinhibition is mostly caused by PAR, UVR can also have considerable effects (Sinha *et al.*, 2001a; Klisch *et al.*, 2005). UVR-induced photoinhibition is known to change seasonally depending on the composition of the natural assemblages, as seen in Patagonian marine coastal waters where the bloom (diaton-dominated) was relatively more sensitive that the pre- and post bloom communities (flagellate-dominated community) (Villafañe *et al.*, 2004a). In turn, the differential sensitivity of species could lead to changes in the taxonomic composition of the community (Worrest *et al.*, 1981) sometimes in a time-scale of days-weeks (Silva *et al.*, 2008). In sites with strong seasonal variations, such as mid-latitudes, the penetration of solar radiation will also change with time, thus producing a variable underwater radiation field throughout the year, depending on ambient physics, chemistry and biology (Bracchini *et al.*, 2004). Therefore it is relevant to consider all of these variables when experimentally studying the responses of the photosynthetic apparatus due to UVR exposure in different communities occurring throughout the seasonal cycle.

Additionally, a number of studies have investigated the effects of zooplankton feeding when algae cells were exposed to UVR. For example, De Lange *et al.* (2000) found that when grazers were absent, *in situ* UVA could stimulate algae growth, with UVB acting in the opposite direction. *Daphnia* clearance rates, however, showed large variations and were not significantly different in the radiation treatments used in the experiments. Short-term laboratory experiments showed that in general, females of the cladoceran *Daphnia magna* may ingest higher amounts of algae treated with high doses of artificial UVB (Germ *et al.*, 2004). This could be a way to compensate changes in cells, mediated by a UVB-induced reduction in digestibility (Van Donk & Hessen, 1995). Ultimately, grazers such as *Daphnia* may be affected by UVR-mediated changes in phytoplankton as a food source (Hessen *et al.*, 1997). This may influence life history traits and fitness of *Daphnia*, depending on which phytoplankton species are used as a food source (De

Lange & Van Reeuwijk, 2003). Additionally, short-term effects in laboratory may be different (and even contradictory) when considering different species of grazers and phytoplankton (De Lange & Lürling, 2003). Overall, most of these studies where performed with laboratory cultures and artificial UVR sources, while much less information is available for natural algae and grazers communities (i.e., which are exposed to strong seasonal changes in solar radiation, among other factors). In a recent paper Williamson *et al*. (2010) found that UVR did not influence zooplankton grazing in a mountain lake. However, the authors argued that considering the effects of UVR on the reproduction, plankton responses to UVR may be altered by changes in zooplankton population sizes as well as the direct response of phytoplankton to UVR and grazing. These long-term effects, however, were difficult to observe within the time frame of their experiments (i.e., 7 days) (Williamson *et al*., 2010).

It is clear that direct and indirect long-term effects of UVR on natural phytoplankton and zooplankton communities are difficult to predict. Moreover, studies with integrated observations and experiments on natural phytoplankton communities under solar radiation in a year-round sampling strategy are still scarce. Thus, the objective of this study is to evaluate the *in situ* seasonal dynamics of phytoplankton throughout the year and its relationships with both grazers and UVR, and how photochemical performance (in terms of photochemical quantum yield measurements) varies with taxonomic composition. Our working hypotheses were that: a) phytoplankton species composition and relative dominance throughout the year is shaped both by grazers and UVR, and b) photochemical performance is related to the taxonomic composition and thus it changes seasonally with phytoplankton community.

MATERIALS AND METHODS

Sampling site

In situ measurements and samplings were carried out at Lake Cacique Chiquichano, which is a eutrophic, shallow (max depth *ca*. 2 m), small lake/pond (0.18 km^2). The lake is located within the city of Trelew, Argentina (43º14.9'S, 65º17.9'W, population *ca*. 80,000 inh) and therefore it is exposed to some anthropogenic influence. Its waters often show a high chlorophyll concentration, in accordance with its high nutrient load throughout the year. The mean concentration of nutrients in the water during summer, autumn, winter and spring were: 30.9, 122.2, 192, and 44.3 μM of nitrogen (NO$_2^-$ + NO$_3^-$), 16.5, 8.8, 36.6,

and 58.8 μM phosphate (PO$_4^{-3}$), and 280, 506, 518, and 434 μM silicate (SiO$_4^{-2}$), respectively (Gonçalves, unpublished data). The absence of trees or tall buildings in its surroundings allows the water surface to be exposed to full solar radiation and also to the influence of strong winds that induce mixing in the whole water column. The lake is in a region subjected to a wide range of temperatures, solar radiation and wind intensity during the annual cycle (Helbling *et al.*, 2005). Some studies were previously carried out with natural phytoplankton communities from this lake to evaluate DNA damage caused by ultraviolet B (UVB, 280-315 nm) exposure (Klisch *et al.*, 2005) whereas the main grazers (cladocerans) were studied with respect of their tolerance (survival and motility) to UVR (Gonçalves *et al.*, 2002, 2007).

Water samples were collected throughout an annual cycle, from February 2005 to January 2006, with a frequency of once every 2-4 weeks. Sampling was conducted in the afternoon or evening before experimentation. Samples of zoo- and phytoplankton were collected from a fixed station on the shore (considered representative of the whole lake) and placed in 20-L plastic carboys previously cleaned with water and HCl (1 N). Water temperature was recorded *in situ* and the plankton samples were taken to the laboratory within 40 min. for subsequent determinations and experiments.

Zooplankton

We determined the abundance of copepods and cladocerans throughout the year and this data was used to later infer the grazing pressure of crustacean zooplankton on phytoplankton communities. Zooplankton samples were taken with a plankton net (200 µm) and fixed with formaline (~2% final formaldehyde concentration). The abundance of the cladoceran *Daphnia spinulata* Birabén, 1917 and the cyclopoid copepod *Metacyclops mendocinus* (Wierzejski, 1892) -the two species that characterized the zooplankton community throughout the year- were determined by considering the number of individuals (counted under a stereoscope) and the volume of water filtered.

Phytoplankton

Determinations of phytoplankton chlorophyll-*a* (chl-*a*) and cell concentration were conducted at the laboratory with traditional methods. Chl-*a* was determined *in vitro* for the total and < 20 µm (pico-nanoplankton) fraction after filtering 50-100 mL of sample (using Wathman GF/F filters, 25 mm in diameter) and extracting the pigments in 7 mL of

absolute methanol (Holm-Hansen & Riemann, 1978). The chl-*a* concentration was calculated from the fluorescence readings (before and after acidification with HCl) using a Turner Designs fluorometer (TD700). Pigments absorbance was also measured from these methanol extracts by doing a scan between 250 and 750 nm with an spectrophotometer (Hewlett Packard 8453E). Cell concentration and taxonomic composition was determined on preserved samples (0.4% formaldehyde) using the Uthermöl sedimentation method as described by Villafañe & Reid (1995) and with an inverted microscope. A drop of Rose Bengal was added to the Uthermöl chamber to better distinguish between small organic and inorganic particles. For each sampling date, phytoplankton species richness (*S*) was recorded as the total number of species present in a given sample. Each different species contributed to *S* even if it was not fully identified (e.g., *Scenedesmus* sp.1, *Scenedesmus* sp. 2, etc.).

Exposure experiments

These experiments were designed to evaluate the effects of solar radiation on photochemical performance of phytoplankton. A 10-L water sample was pre-filtered (200 μm, to remove larger grazers) and kept overnight inside a culture chamber at 18°C until the next day, when exposure experiments were conducted. The objective of keeping samples overnight at this temperature (close to the mean yearly value) was to maintain a 'common basis' for evaluating photochemical performance in the exposed samples. Otherwise, inter-seasonal comparisons would be complicated by different rates of enzymatic processes occurring under a broad range of *in situ* temperatures as commonly found in the study area. While this pre-acclimation might have slightly affected the initial fluorescence, it did not have any evident influence on the species composition (as observed microscopically) or on the photochemical responses at noon (which we used here). Therefore we reached a compromise between the comparability across seasons, and the representation of the natural community of plankton throughout the year, probably de-coupling to a certain extent our results from actual conditions in the lake. Exposure experiments were carried out as follows: Water samples were dispensed into 50-mL, UVR-transparent quartz tubes and exposed outdoors under solar radiation. Three radiation treatments were implemented (duplicate samples for each treatment): a) P (samples receiving only PAR, 400-700 nm): tubes wrapped with Ultraphan 395 filter (UV Opak, Digefra; 50% transmittance at 395 nm), b) PA (samples receiving

PAR + UVA, 320-700 nm): tubes wrapped with Ultraphan 320 (Montagefolie N° 10155099, Folex, 50% transmittance at 320 nm), and c) PAB (samples receiving full solar radiation, i.e., PAR + UVA + UVB, 280-700 nm): tubes without any filter. The spectra of the filters / materials are published in Figueroa *et al*. (1997). The samples were then incubated during the whole day, inside a water bath to keep homogenous temperature in all samples (18°C ± 2°C). Both at the beginning (9:00 h) and at the end of the exposure (17:00 h), and also every hour, fluorescence was measured to evaluate changes in the effective photochemical quantum yield (*Y*) as follows: a sub-sample was transferred to a 5-mL quartz cuvette, where fluorescence variables were measured *in vivo* using a portable pulse-amplitude modulated fluorometer (Water-PAM, Walz, Germany). To obtain the *Y* of cells, the instantaneous maximal fluorescence (F'_m) and the steady state fluorescence (F_t) of light-adapted cells were measured using a saturating white light pulse (~5300 mol photons m^{-2} s^{-1} in 0.8 s) in the presence of a weak actinic light. Then *Y* was calculated after Van Kooten & Snel (1990) and Genty *et al*. (1989) as:

$$Y = (F'_m - F_t) / F'_m = \Delta F / F'_m$$

The decrease of *Y* measured at 13:00 h (solar noon) was used here as a cumulative indicator of the effect of the exposure to solar radiation during the morning (i.e., between 9:00 and 13:00 h, local time) at each wavelength interval. This is presented here as an estimation of photoinhibition (i.e., *Y* in the PAB and PA treatments relative to that in the P control) over the incubation period, and it was calculated as:

Decrease of *Y* by UVB (%) = 100 [(Y_P - Y_{PAB}) - (Y_P - Y_{PA})] / Y_P = 100 (Y_{PA} - Y_{PAB}) / Y_P

Decrease of *Y* by UVA (%) = 100 [(Y_P - Y_{PA})] / Y_P

where Y_P, Y_{PA} and Y_{PAB} are the photochemical quantum yield of samples in the P, PA and PAB treatments, respectively.

Attenuation of solar radiation in the water column

Due to the lack of a small instrument to measure underwater radiation in such shallow eutrophic waters as those of Lake Cacique Chiquichano, the attenuation coefficient of PAR was estimated from chlorophyll and absorption measurements of the water column using published models derived from Branco & Kremer (2005) and Morris *et al*. (1995) for freshwater bodies with high chl-*a* and low penetration of radiation -see Gonçalves *et al*. (2007) for a detailed description. Total attenuation of PAR in this case can be considered to be a result of particulate (phytoplankton chlorophyll) and dissolved chromo-

phoric organic matter (CDOM). Each of these contributors may be estimated separately. Microscopic observations did not reveal any significant amount of inorganic particulate material in the samples, so we considered that their influence in the attenuation of solar radiation was negligible over the sampling period, and thus it was not included in the calculations. Briefly, the absorption coefficient at 320 nm (using distilled water as blank), a_{320}, was calculated following Osburn & Morris (2003) as:

$$a_{320} = 2.303 \, DO_{320} \, L^{-1}$$

where DO_{320} is the optical density at 320 nm of a 10-mL of filtered (Whatman GF/F) water sample, measured using a diode-array spectrophotometer (Hewlett Packard 8453E), and L is the optical path of the cuvette (0.05 m). The contributions of chl-a (μg L^{-1}) and CDOM (a_{320}) to the attenuation coefficient were then calculated as:

$$K_{\text{chl-}a} = 0.22 + 0.008 \text{ chl-}a + 0.054 \text{ chl-}a^{0.66}$$

$$K_{\text{CDOM}} = 0.1948 \, a_{320} - 0.9203$$

Finally, the attenuation coefficient for PAR (K_{PAR}) was estimated as the sum of the contributions of individual components in the water as $K_{\text{PAR}} = K_{\text{chl-}a} + K_{\text{CDOM}}$.

Solar radiation and other meteorological variables

Surface solar irradiance was obtained with a broad-band ELDONET filter radiometer (Real Time Computer, Möhrendorf, Germany) which measures (once a minute) energy in the UVB (280-315 nm), UVA (315-400 nm) and PAR (400-700 nm) wavebands. The radiometer is permanently installed on the roof of the Estación de Fotobiología Playa Unión (EFPU) where the exposure experiments were carried out. Daily solar exposure was calculated based on surface irradiance measurements. Meteorological variables such as wind intensity / direction and temperature were recorded automatically every 10 min using a meteorological station (Oregon Scientific WMR-918) permanently installed on the roof of the EFPU building. Surface water temperature was recorded *in situ* using a digital thermometer.

Data treatment and statistic analyses

For the determinations of Y, the average of five consecutive fluorescence measurements was used as representative of each sample. All radiation treatments were done in duplicate, so the mean and half-mean range was calculated for each treatment. A repeated ANOVA test was used to establish differences among radiation treatments. A multiple linear regression analysis was also used to explain the variability

observed in UVR-induced inhibition of Y. A significance level of 5% was used in all linear regressions and analysis.

RESULTS

Physical variables

Daily values of incident PAR varied throughout the year (Fig. 1a) between 0.1 and 12 MJ m^{-2}. Daily UVB varied accordingly, with relatively low values during winter and high during summer, ranging between 0.18 and 50 kJ m^{-2}. Average wind speed ranges (Fig. 1b) were within the historical values and trends (Helbling *et al.*, 2005) i.e., the historical daily mean speed values were (mean (SD)): 5.12 (0.70), 3.95 (0.41), 4.10 (0.53), and 5.16 (0.55) m s^{-1} for summer, autumn, winter and spring, respectively. Maximal wind speeds for the four days prior to sampling were, on average, between 7 and 20 m s^{-1}. The relative contributions of chromophoric dissolved organic matter (CDOM) and chl-a to the attenuation of underwater radiation in the water column are shown in Fig. 1c. The diffuse attenuation coefficient for PAR (K_{PAR}) varied between 2 and 13 m^{-1} with high values in February, April and August. It was thus determined that chl-a had stronger influence on K_{PAR} than CDOM, resulting in relatively high attenuation of solar radiation during autumn and at the end of winter. Based on these K_{PAR} values, the depth of the euphotic zone (i.e., 1% of surface PAR) varied between 0.35 and 2.3 m during the year (data not shown). It should be noted that inorganic particulate concentration was negligible in samples as assessed by microscopic observation of 24 h-settled samples. The lake also showed annual variations in surface temperature - between 24 and 4ºC (Fig. 1c) with a mean yearly value of ~16ºC.

Chlorophyll-*a* and zooplankton abundance

Total chl-a concentration during the study period (Fig. 2a) had three clear peaks - late February, April and August. The maximum chl-a (799 μg L^{-1}) was measured in August whereas relatively low values (4-55 μg L^{-1}) were determined during June and in the period September to December. The chl-a concentration in the pico-nanoplankton fraction accounted for the bulk of total chl-a during almost the whole year (Fig. 2a). From January to April however, microplankton cells were relatively more abundant but pico-nanoplankton still accounted for 50% or more of the total chl-a. In regard to zooplankton abundance, the cladoceran *D. spinulata* and the copepod *M. mendocinus* showed alternated dominance (Fig 2b) except during the autumn-winter period, when the
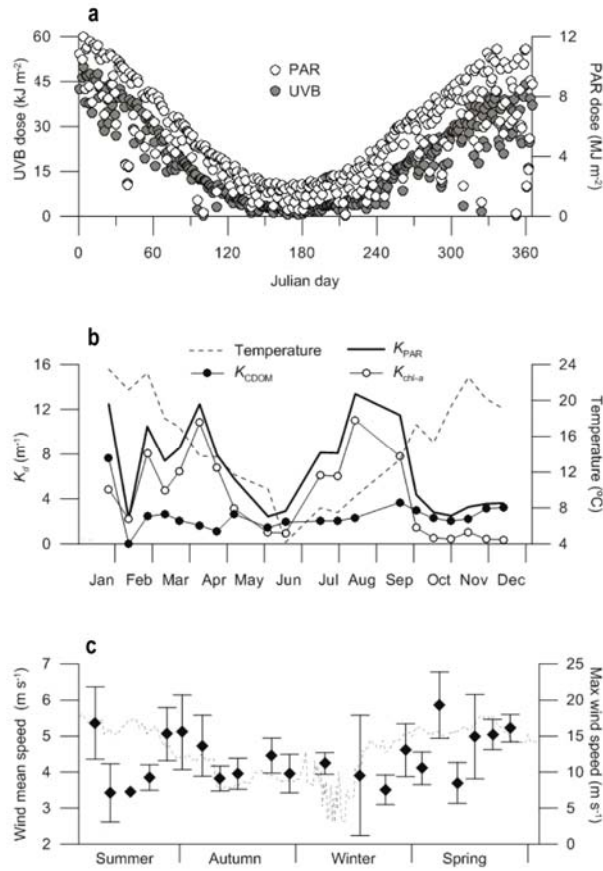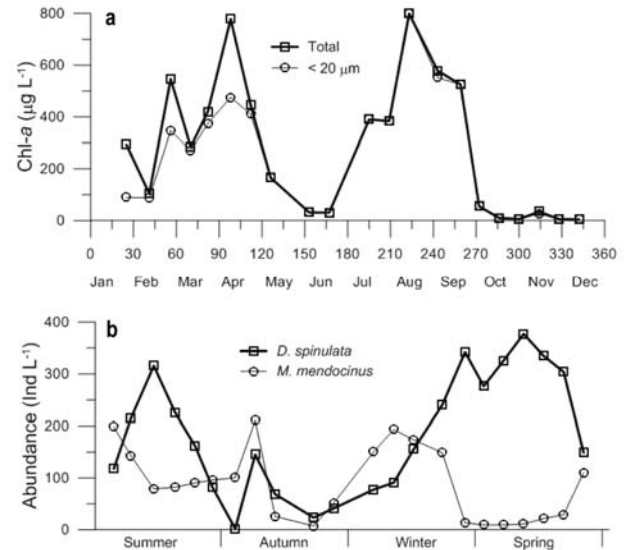
Figure 2. Variation of plankton abundances throughout the year. a) Chlorophyll-*a* concentration (μg L⁻¹) in the total and in the pico-nanoplankton (< 20 μm) fractions of phytoplankton during the study period, b) abundance of the crustacean zooplankton (*D. spinulata* and *M. mendocinus*, ind L⁻¹).

Figura 2. Variaciones anuales en la abundancia del plancton. a) Concentración de chlorofila-*a* (μg L⁻¹) en las fracciones total y pico-nanoplancton (< 20 μm) del fitoplancton durante el período de estudio, b) abundancia de crustáceos zooplanctónicos (*D. spinulata* y *M. mendocinus*, ind L⁻¹).

Figure 1. Physical variables over Lake Cacique Chiquichano. a) Daily incident PAR and UVB (in MJ m⁻² and kJ m⁻², respectively), b) mean wind speed (m s⁻¹) during the period 2001-2003 (from Helbling *et al.*, 2005) and maximal wind speeds (m s⁻¹) for the four days prior to sampling, c) temperature and estimated attenuators of solar radiation in the water column – K_{CDOM}, K_{chl-a}. Diffuse attenuation coefficient for PAR (K_{PAR}, in m⁻¹) during the study period, calculated as the sum of the contributions of CDOM and chl-*a*.

Figura 1. Variables físicas en la Laguna Cacique Chiquichano. a) Dosis diarias de PAR y UVB (en MJ m⁻² y kJ m⁻², respectivamente), b) velocidad media del viento (m s⁻¹) en el período 2001-2003 (de Helbling *et al.*, 2005) y velocidad máxima del viento (m s⁻¹) para los cuatros días previos al muestreo, c) temperatura y estimación de atenuantes de la radiación solar en la columna de agua – K_{CDOM}, K_{chl-a}. Los coeficientes de atenuación de PAR (K_{PAR}, en m⁻¹) durante el período de estudio se calcularon como la suma de las contribuciones de CDOM y chl-*a*.

Phytoplankton abundance and dominance

The phytoplankton community (Fig. 3) was charac-terized by periods of presence and absence of Cyanophyta groups (i.e., cyanobacteria). On the other hand, when cyanobacteria was absent or accounted for less than 2% of the total cells, Bacillariophyta (i.e., diatoms) appeared to dominate the community, while Chlorophyta (i.e., green algae) varied with no visible pattern. This allowed us to separate the year into four periods based on the taxonomic composition of the samples: Cya1-Cya2 (samples/periods with domi-nance of cyanobacteria) and Dia1-Dia2 (samples/ periods in which diatoms dominated), with cyanobacteria and diatoms dominating in total about half of the year each. For some variables, especially species richness, it was not possible to assign only one of these groups (e.g., when the contribution of cyanobacteria and diatoms was similar) therefore some degree of overlapping is present in our calculations (e.g., the last points of the period Cya1 are also the first points of the period Dia1). Moreover, cyanobacteria and diatom abundances were negatively correlated ($R^2 = 0.59$; $P < 0.01$). Cyanobacteria (Fig. 3a) dominated mostly during summer and winter

abundance of both groups was very low (e.g., Julian days 126 and 153). Highest abundances were ~400 and ~200 ind L⁻¹, for *D. spinulata* and *M. mendocinus*, respectively.

(although in some samples they co-occurred with green algae and diatoms). Diatoms (Fig. 3b) dominated during autumn and spring; in this group green algae (Fig. 3c) shared an important fraction in some samples, with cyanobacteria having little or no influence. Total cyanobacteria proportion (Fig. 3a) ranged from 0 to 99%, with two maximum values: 82% and 99% in Julian days 56 (summer, Cya1) and 259 (winter, Cya2) being *Microcystis* sp. and *Synechocystis* sp. the most important representatives in each period. Diatoms abundance varied with a similar null-to-full dominance of the community (Fig. 3b) and three times during this study they accounted for approximately 100% of cells: On Julian days 153 (autumn), 272 and 300 (spring). *Navicula* sp., *Cyclotella* sp., *Surirella* sp. and *Nitszchia* sp. varied throughout the year, although the two latest were most conspicuous in the autumn and spring maximum abundance of diatoms, respectively (Dia1 and Dia2). Green algae (Fig. 3c) did not exceed ~70% of the community, and their variations showed no clear pattern during the year. Maximal values of species richness (*S*, Fig. 3d) were observed in early autumn and in winter (*S* = 21 and *S* = 20 in April and August, respectively), whereas the minimum was observed during Autumn (*S* = 2 in June).

Representative phytoplankton taxa from Lake Cacique Chiquichano are shown in Table 1. Cyanobacteria-dominated samples were characterized by *Microcystis* spp., *Synechocystis* sp. and *Lyngbya* sp. whereas *Navicula* spp., *Nitzschia* sp., *Cyclotella* sp. and *Surirella* sp. were the most abundant species during diatom-dominated periods. Chlorophyceae (e.g., *Oocystis* spp. and *Scenedesmus* spp.) were frequently found year-round with different shares. The class Dinophyceae was poorly represented during the whole year and the only species present was *Peridinium* sp. Finally, only few Euglenophyta cells were observed during February and August (*Trachellomonas* sp. and *Euglena* sp., respectively).

Phytoplankton-zooplankton interactions

There were significant positive correlations between total chl-*a* concentration and the number of phytoplankton species present in the community during the Cya1 and Cya2 periods, meaning that the peaks of abundance were co-dominated by various species (Fig. 4a). No significant relationships (i.e., *S* versus chl-*a*) were observed during the periods of diatom dominance. In turn, during Cya1 and Cya2 periods, *S* was negatively correlated to the abundance of *D. spinulata* (Fig. 4b). Again, no significant correlations were established for the two periods of diatom domination. Finally, there were significant
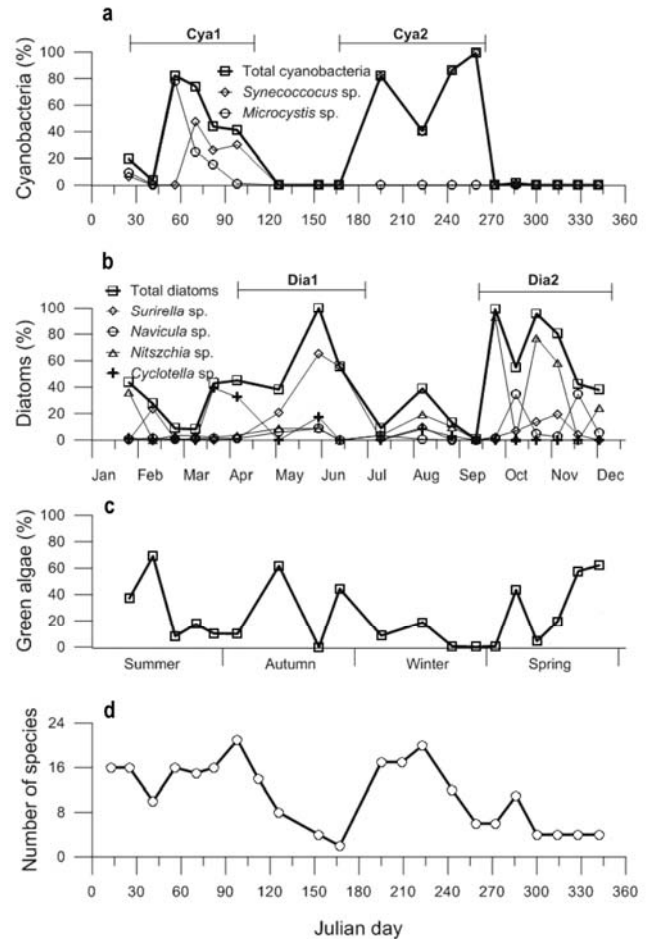
Figure 3. a-c) Relative proportion (% of total) of cyanobacteria, diatoms and green algae in Lake Cacique Chiquichano. Cyanobacteria- and diatom-dominated samples are marked as Cya1/Cya2 and Dia1/Dia2, respectively, d) changes throughout the year in phytoplankton species richness (*S*).

Figura 3. a-c) Proporción (% del total) de cianobacteria, diatomeas y algas verdes en la Laguna Cacique Chiquichano. Las muestras con dominancia de cianobacterias y diatomeas se señalan como Cya1/Cya2 y Dia1/Dia2, respectivamente, d) cambios a lo largo del año en la riqueza específica (*S*).

inverse correlations between *D. spinulata* dominance and both total phytoplankton chl-*a* and pico-nanoplankton chl-*a* fractions during the cyanobacteria-dominated periods (Fig. 4c). During Cya2 almost 100% of chl-*a* concentration was in the pico-nanoplankton fraction, so in Fig. 4c there is only one relationship for *D. spinulata* – chl-*a* during this period. On the other hand, no significant relationship was established between zooplankton abundance and chl-*a* during periods of diatoms dominance.
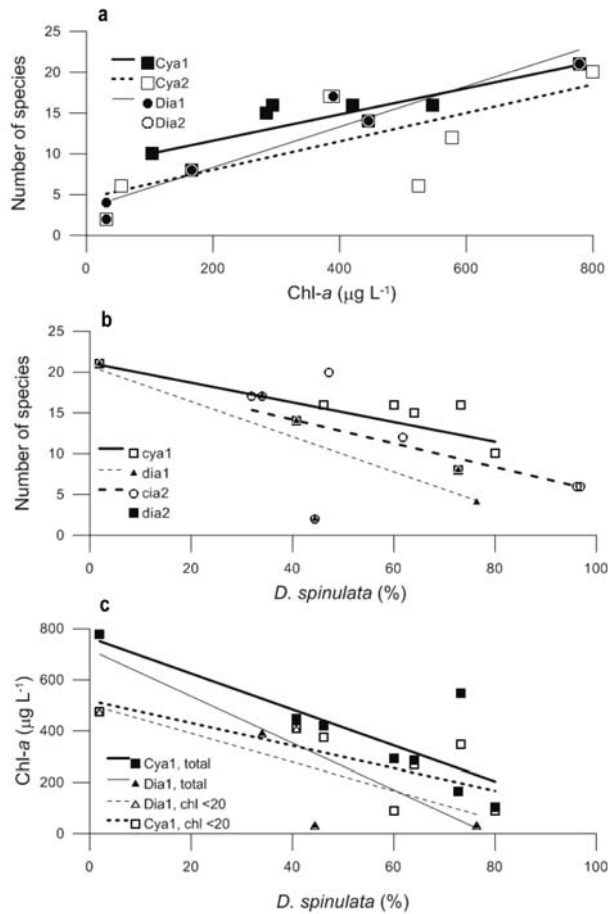
Figure 4. Relationship between species richness, chl-*a* concentration, and *D. spinulata* abundance pooling data from periods dominated by cyanobacteria (Cya1 and Cya2). a) Number of species versus chl-*a*, b) number of species versus relative abundance of *D. spinulata*, c) Chl-*a* concentration (total and pico-nanoplankton fractions) *versus D. spinulata* relative abundance. The lines denote linear significant fits ($P < 0.05$).

Figura 4. Relación entre la riqueza específica, concentración de clorofila-*a* y abundancia de *D. spinulata* agrupando los datos de períodos con dominancia de cianobacterias (Cya1 y Cya2). a) Riqueza específica versus chl-*a*, b) número de especies *versus* abundancia relativa de *D. spinulata*. c) concentración de clorofila-*a* (fracciones total y pico-nanoplancton) versus abundancia relativa de *D. spinulata*. Las líneas muestran el ajuste del modelo lineal ($P < 0,05$).

Photosynthetic responses of the phytoplankton communities

During all samplings, *Y* showed the typical daily pattern i.e., with *Y* decreasing significantly in all treatments towards noon and recovering partially or completely during the afternoon/evening (data not shown). However, when compared to samples receiving only PAR, *Y* at noon of those receiving full

solar radiation were inhibited in most of the experiments. According to the composition of the phytoplankton community, this decrease of *Y* due to UVR showed a different relationship (Fig 5a): UVR-induced inhibition of photosynthesis decreased with increasing proportion of diatoms whereas the opposite was found when cyanobacteria dominated the communities. In addition, UVR-induced inhibition was higher when more species were present during cyanobacteria-dominated periods (Fig. 5b) whereas the relationship was not significant when diatoms dominated.

Throughout the year, UVA and UVB inhibition varied significantly, with maximum values of 66 and 38% for UVA and UVB, respectively, but very low inhibition and even negative values were observed in some experiments (Fig. 6a). To further explore the variables accounting for UVR-induced inhibition, a relationship was established by means of a multiple linear
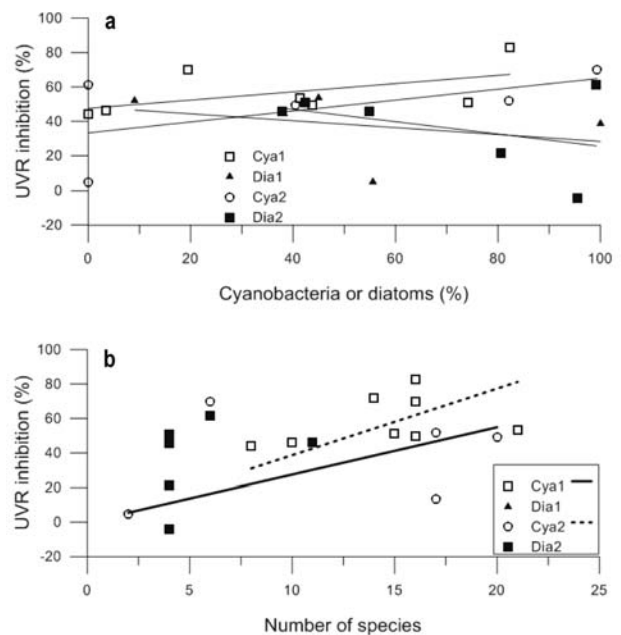

Figure 5. a) Percentage inhibition of photochemical quantum yield for samples exposed to UVR (relative to samples receiving only PAR) when cyanobacteria or diatoms dominated the community, b) relationship between the percentage of UVR-induced inhibition and species richness. The lines denote linear significant fits ($P < 0.05$).

Figure 5. a) Porcentaje de inhibición del rendimiento fotoquímico de las muestras expuestas a la RUV (con respecto a las muestras que recibieron solo PAR) en el momento de dominancia de diatomeas o cianobacterias, b) relación entre porcentaje de inhibición inducida por la RUV y la riqueza específica. Las líneas muestran el ajuste del modelo lineal ($P < 0,05$).

regression in which the attenuation coefficient for PAR (K_{PAR}), phytoplankton species richness (S), cyanobacteria abundance (Cya), *Daphnia* relative abundance (Daph) and UVR irradiance contributed for more than 92%:

Inh UVR = 2.109 K_{PAR} + 0.764 S + 0.13 Cya + 0.226 UVR + 0.203 Daph

The modeled and observed UVR inhibition throughout the year (Fig. 6b) showed a generally good agreement with the observed values (R^2 = 0.92; $P <$ 0.01), with UVR-induced inhibition increasing with increasing K_{PAR}, S, Cya, and UVR.
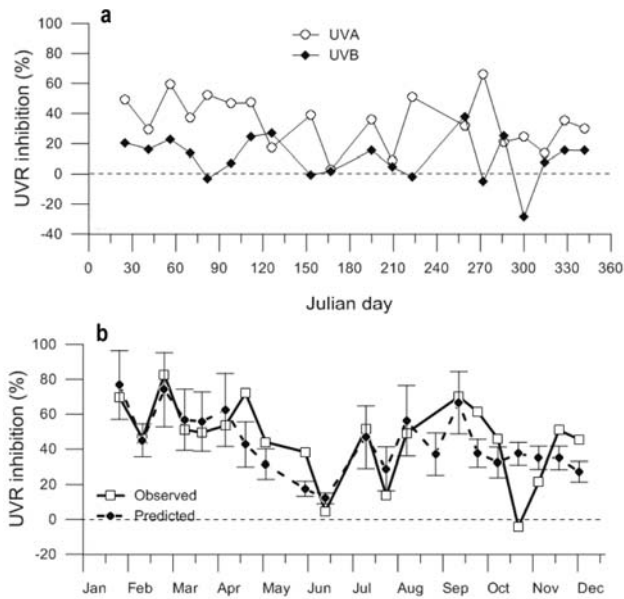

Figure 6. a) Inhibition of photochemical quantum yield due to UVA or UVB throughout the study period, b) modeled and observed values for total UVR-induced inhibition. Vertical bars indicate the 95% confidence level of the model.

Figure 6. a) Inhibición del rendimiento fotoquímico debido a UVA o UVB a lo largo del período de estudio, b) inhibición total debida a la RUV observada y predicha por el modelo. Las barras verticales indican los intervalos de confianza (95%).

DISCUSSION

Lake Cacique Chiquichano is clearly subjected to seasonal changes in physical (wind, temperature, solar radiation) and biological (plankton community composition and abundance) variables. According to our estimations, the underwater optical environment is characterized as one of reduced penetration of solar radiation, especially during periods of high chl-*a* (Fig. 1c) as seen in other studies carried out in eutrophic lakes on the northern Great Plains (Arts *et al.*, 2000). However, in this study we found that the periods of reduced penetration of solar radiation alternated with contrasting 'clear waters periods' (CWP, Fig. 1c) during which the euphotic zone encompassed almost the whole water column (Gonçalves *et al.*, 2007). It could be argued that seasonal changes in wind-driven re-suspension of benthic algae and inorganic particulate may be partially responsible for the observed seasonal patterns. However, microscopic observation did not reveal significant amounts of them in the samples. In addition, wind intensity (Fig. 1b) during the days previous to sampling was rather similar and high, and without a clear trend throughout the year. This allowed us to consider that the water column was well mixed all year round, and that if small amounts of wind-driven re-suspension of particulates occurred, this might have resulted in a very small underestimation of the attenuation of solar radiation. Furthermore, the magnitude of wind-induced re-suspension of particles is likely to be similar during the year, given the strong winds characteristic in the study area (Fig. 1c). We are aware that wind-driven re-suspension of particulates may be a factor to consider when studying shallow lakes, where it may cause regular 'injections' of particles from the bed (Arfi & Bouvy, 1995). For example, Carrick *et al*. (1993) found wind re-suspension to be an important variable influencing the phytoplankton chl-*a* changes of shallow, productive Lake Apopka, Florida (USA). They argued that wind-induced mixing brought up phytoplankton from the lake bottom to the surface water column where the irradiances of the surface layers stimulated growth. However, and although solar radiation and temperature were most likely not limiting factors the authors stated that grazing might had influenced phytoplankton dynamics.

Our results suggest a top-down control of phytoplankton, most likely with *D. spinulata* as the main grazer, capable of significantly decreasing the chl-*a* in the water column (Fig. 4c) and thus modifying the water column optical characteristics (Fig 1c). Although our experiments were not designed to calculate ingestion rates of *D. spinulata*, we inferred grazing pressure from *D. spinulata* abundance. This is supported not only by the well-known grazing capability of *Daphnia*, due to potential high clearance rates and fast population growth rates (Hebert, 1978; Boersma, 1997; Lampert & Sommer, 1997) but also by the negative relationship between this cladoceran and chl-*a* concentration (Fig. 4c). Although in this study we did not intend to obtain grazing rates, estimations of pigments from *Daphnia*

absorption spectra revealed high chl content (*ca*. 3 μg per mg of *Daphnia* dry weight) in the cladoceran, therefore supporting the idea of a strong feeding pressure of the cladocerans on phytoplankton. It is arguable that by removing chl-*a* from the water, *D. spinulata* may have contributed to induce periods of increased penetration of radiation into the water column (Lampert & Sommer, 1997; Williamson *et al.*, 2007). Indeed, this seems to be the case in our study site, as chl-*a* was the variable that contributed for most of the attenuation of solar radiation in the water column (Fig. 1c); thus when the cladoceran population exerted a strong grazing pressure over the phytoplankton community a CWP period was determined, as it seems to be a common pattern in many South American lakes (Echaniz *et al.*, 2006). Interestingly, plankton dynamics seemed to be regulated mainly by the presence and abundance of *D. spinulata* but instead, there was no clear relationship between *M. mendocinus* and the phytoplankton species present in the lake.

Not only phytoplankton abundance (estimated using chl-*a* concentration) but also *S* was significantly affected by the presence of *D. spinulata* (Fig. 4). However, its presence was significant only when cyanobacteria dominated the phytoplankton assemblages (i.e., Cya1 and Cya2, Figs. 4b, 4c) that were also the periods that coincided with high chl-*a* concentration (Fig. 4a). This is in agreement with the findings of Van Gremberghe *et al*. (2008) that reported that in general, the zooplankton community composition (especially the cladoceran community) was more important in structuring the cyanobacterial community than the total zooplankton biomass was. Particularly, previous studies (Oberhaus *et al.*, 2007) have reported the ability of the genus *Daphnia* to graze on cyanobacteria including *Microcystis* (Van Gremberghe *et al.*, 2008). Thus one can speculate that without the strong grazing pressure of *D. spinulata* on the phytoplankton community, Lake Cacique Chiquichano would mostly be a typical "ever-green" eutrophic lake during the whole year, with very low penetration of solar radiation into the water column and a high number of cyanobacteria species. However, this was not the case, and moreover, we postulate that the abundance of *D. spinulata* modulated not only the phytoplankton community, but also indirectly affected the observed photosynthetic responses of phytoplankton.

During the study period, cyanobacteria and diatoms alternated their dominance, as previously observed in other eutrophic waters (Watson *et al.*, 1997). Cyanobacteria dominated during the 'low transparency' phase in the lake, while diatoms dominated during the CWP (Figs. 1 and 3). The general photosynthetic response throughout the study period was of UVR-induced photoinhibition increasing with increasing proportion of cyanobacteria in the samples (Fig. 5a) and with increasing number of species (Fig. 5b) for both Cya1 and Cya2 periods. In principle, it may seem strange that inhibition increased when more phytoplankton species (and more chl-*a*) were present. However, this was most probably due to a selection towards few and more resistant species with increasing *D. spinulata* abundance and increasing UVR exposure. In both Cya-periods, *D. spinulata* may have grazed on pico-nanoplankton cells (Fig. 4c) decreasing also the number of species (Fig. 4b). It has been shown in previous studies (Helbling *et al.*, 2001b) that small cells are less sensitive to UVR than larger cells are, when addressing effects on photosynthesis. In our study, however, we can not rule out one of these possibilities (i.e., if the species were mostly affected by UVR) and then grazed by *D. spinulata,* or if the ingestion of these small cells was independent of UVR. *D. spinulata* might have fed less on relatively larger cells, which started to acclimate to higher irradiances (because of the increasing transparency of the water column) and thus became less sensitive. Therefore, and in this case, the process would be an indirect effect of grazing on the observed photoinhibition.

The fact that during periods of high proportion of cyanobacteria the community had higher photosynthetic inhibition values could be simply the result of cells being acclimated to low underwater radiation conditions during the "high attenuation" period. This is in agreement with a comparative study carried out in the Andean lakes that showed that phytoplankton in "high-attenuation" lakes had higher damage and inhibition of photosynthesis than in clear lakes (Villafañe *et al.*, 2004b) being the high sensitivity associated to their previous acclimation to low radiation levels. In fact, previous studies carried out in Lake Cacique Chiquichano (Klisch *et al*., 2005) have reported a high sensitivity towards UVR (as assessed through DNA damage) in summer populations (i.e., dominated by cyanobacteria). It should be noted though that some cyanobacteria are considered to be resistant to UVR because of their ability to synthesize UV-absorbing compounds (Sinha *et al.*, 2001b) that act as sunscreens and thus protect cells against UVR stress, while others seems to acquire resistance by changing their morphology (Wu *et al.*, 2005). Particularly, the genera *Synechocystis* and *Microcystis*–which were important members of the Cyanophyta community in Lake Cacique Chiquichano (Table 1), are known to produce UV-

Table 1. Representative phytoplankton groups on samples collected at Lake Cacique Chiquichano during the study period.

Tabla 1. Grupos representativos de fitoplancton presentes en la Laguna Cacique Chiquichano durante el período de estudio.

Cyanophyceae	Chlorophyceae	Bacillariophyceae	Dinophyceae
Anabaena sp.	*Actinastrum* sp.	*Achnanthes* sp.	*Peridinium* sp.
Lyngbya sp.	*Ankistrodesmus* sp.	*Cocconeis* sp.	
Merismopedia sp. 1, sp. 2	*Botryoccocus* sp.	*Cyclotella* sp.	
Microcystis sp. 1, sp. 2	*Closterium* sp.1, sp. 2	*Cymbella* sp.	
Pseudoanabaena sp.	*Coelastrum* sp.	*Gyrosigma* sp.	
Synechocystis sp.	*Kirchneriella* sp.	*Hantzchia* sp.	Euglenophyceae
Oscillatoria sp.	*Oocystis* sp.1, sp. 2	*Navicula* sp.1, sp. 2	*Euglena* sp.
	Pediastrum sp.1-sp. 4	*Nitzschia* sp.	*Trachellomonas* sp.
	Scenedesmus sp.1-sp. 9	*Surirella* sp.	
		Synedra sp.	
		Ulnaria sp.	

absorbing compounds (Liu *et al.*, 2004; Zhang *et al.*, 2007) however, in our study we did not register any significant amount nor a relationship between these potential protective compounds and cyanobacteria abundance throughout the year. In contrast to the response of cyanobacteria, UVR-induced inhibition decreased with increasing dominance of diatoms (Fig. 5a). In fact, diatoms seems to be more resistant to UVR as compared to other groups probably because of their silica "protection" as suggested by Wulff *et al*. (2008) or by the synthesis of UV-absorbing compounds as demonstrated for other environments (Helbling *et al.*, 1996), although this latter option did not appear to be the case. It could also be possible that diatoms displayed a high rate of repair, as also seen in marine environments of the Patagonian coast (Helbling *et al.*, 2001a) but this hypothesis remains to be tested for Lake Cacique Chiquichano assemblages.

In regard to the relative proportions of UVA- and UVB-induced photoinhibition, it was seen that although it was variable throughout the year, UVA accounted for most of the share (Fig. 6a) as it is the common response in natural waters (Villafañe *et al.*, 2003); however, there were some dates in which UVB-induced inhibition was similar to that of UVA, as also observed in marine assemblages of Patagonia (Villafañe *et al.*, 2004c). Moreover, there were some periods of complete diatom domination in which UVR even stimulated photosynthesis (Julian day 300, Fig. 6b) as also seen under low radiation (Barbieri *et al.*, 2002) or fast mixing conditions (Helbling *et al.*, 2003). Overall, the multiple linear regression model explained great part of the variability (i.e., 92%) based on *D. spinulata* and cyanobacteria abundances,

species and underwater attenuation of radiation. This latter parameter denotes the influence of CDOM and most importantly of Chl-*a* absorption in the final K_{PAR}. Weighting the causes of the species' replacements (e.g., radiation-induced photoinhibition versus the modification of community due to grazing) probably remains to be further elucidated. It is not clear if UVR affects phytoplankton and then it is easily eaten by *D. spinulata*, or if there is a selective feeding on some species leaving more resistant ones in the way.

Changes in phytoplankton structure or taxonomic composition have been investigated in other lakes before (considering grazing or not). However, little attention was paid in considering the indirect effects of common grazers on photosynthetic responses to solar radiation of natural communities occurring throughout the seasonal cycle, as we reported in this study. Overall, our results indicate that both solar radiation and grazing may affect the natural phytoplankton of Lake Cacique Chiquichano. Grazing pressure would contribute to shape the taxonomic composition of phytoplankton in this lake, driving different effects on the photosynthetic performance of the community according to the dominant group present at that time. It remains to be tested however, if the pattern observed in our results is typical for this kind of lakes (i.e., shallow, eutrophic waters but with a periodic occurrence of a CWP) and if our results can be generalized to water bodies with similar characteristics. Simultaneous, parallel experiments of grazing rates and selectivity would add more quantitative data on the role of grazing pressure, and it would be the next logical step triggered by the results of the present study.

ACKNOWLEDGMENTS

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