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Effect of UV-B and different PAR intensities on the primary production of the mixotrophic planktonic ciliate *Stentor araucanus*

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Abstract

Stentor araucanus is a mixotrophic ciliate that, in Andean lakes, inhabits the upper epilimnetic levels, which are commonly avoided by other planktonic organisms. This freshwater heterotrich has dark pigmented cortical granules and lives autotrophically with endosymbiotic algae. The effect of photosynthetically active radiation (PAR) and ultraviolet (UV)-B radiation on primary production was analyzed during summer 2003–2004 in Lake Moreno Oeste, a highly transparent ultraoligotrophic lake (mean summer K_d = 0.16 m^{-1}). Primary production (PP) was measured in the field in the euphotic zone during both static and variable-depth incubations. Static exposure of the organisms was examined at different depths (0.30, 10, and 20 m), and the variable depth exposure involved experimental containers moved continuously up and down the epilimnion (0–15 m). In the static exposure closest to the surface and in the mobile incubation, quartz tubes were incubated with and without a UV-B screen (Mylar™). Additionally, PP was measured in the laboratory with and without previous exposure to a UV-B lamp (290–315 nm). *S. araucanus* was present throughout the summer with highest abundances at or above 15 m in depth. A high proportion of the ciliate population (80%) was, therefore, exposed to UV radiation, and between 30% and 60% of the population occupied depths at which UV-B (305 nm) exceeded 1% of surface incidence. PP values were higher in the epilimnion than below it and were not reduced by exposure to high irradiances of PAR+UV-A and PAR+UV-A+UV-B. The laboratory experiments showed no difference between UV-B and PAR preexposure treatments. The variable-depth epilimnetic incubations gave similar PP values and did not differ from the static incubations. The average PAR irradiance of the epilimnion was high, around 600 μmol photons m^{-2} s^{-1}, which was the value at which *S. araucanus* reached a saturation level in the laboratory. In contrast, the incubations at 20 m differed significantly from those in the epilimnion, exhibiting lower values, except when PAR irradiance was higher than 100 μmol photons m^{-2} s^{-1}. These results indicate that pigmented mixotrophs like *S. araucanus* achieve high population densities in the epilimnion because they receive sufficient irradiance (PAR between 100 and 1,600 μmol photons m^{-2} s^{-1}) to allow endosymbiotic algae to produce.

Photosynthesis in aquatic organisms is highly affected by light intensity and particularly by high irradiances of photosynthetically active radiation (PAR: 400–700 nm) and ultraviolet radiation (UVR: 290–400 nm) in the upper layers of oligotrophic systems. Excessive PAR, as well as UVR, can inhibit photosynthesis (Cullen and Lesser 1991; Villafañe et al. 1995, 1999), damage intracellular components such as DNA (Karentz et al. 1991; Prezelin et al. 1994; Helbling et al. 2001), and inactivate certain key photosynthetic proteins (Greenberg et al. 1989; Schofield et al. 1995).

Ultraoligotrophic temperate Andean lakes of Argentina (around 41°S) are highly transparent to solar UV radiation because of low dissolved organic carbon (DOC) concentrations (DOC $\leq$ 0.6 mg L^{-1}) (Morris et al. 1995). Consequently, high irradiances are a potential risk for planktonic organisms inhabiting the upper layers of the water column in these lakes (Zagarese and Williamson 2000). This may explain the deep vertical distribution of the calanoid copepod *Boeckella gracilipes* (Alonso et al. 2004) and the deep chlorophyll maxima observed at 30 m in depth formed by a mixotrophic ciliate (*Ophrydium naumanni*), autotrophic picoplankton, and dinoflagellates (Queimaliños et al. 1999; Modenutti and Balseiro 2002; Modenutti et al. 2004). In these clear lakes, photosynthesis inhibition by UV-B radia-

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Table 1. Light conditions in Lake Moreno Oeste during December 2003–February 2004 (austral summer). Values are given as average ± standard error. References: K_d, extinction attenuation coefficient (m^{-1}); I_0, irradiance at 0-m depth measured with a PUV-500B Biospherical Instruments (305–380 nm in μW cm^{-2} nm^{-1}, PAR in μmol photons m^{-2} s^{-1}); $Z_{1\%}$, depth at 1% of surface irradiance for different wavelengths (m); I_m, mean irradiance of the epilimnion; and I_{MIRI}, mean irradiance of the epilimnion variable-depth incubations (0–15 m depth) (305–380) nm in μW cm^{-2} nm^{-1}, PAR in μmol photons m^{-2} s^{-1}). I_m and I_{MIRI} were calculated according to Helbling et al. (1994). SII, surface-integrated irradiance (during the 4 h of incubations) measured with a GUV-510 Biospherical Instruments (305–380 nm in W m^{-2}, PAR in mol photons m^{-2}).

	305 nm	320 nm	340 nm	380 nm	PAR
K_d	0.759±0.010	0.649±0.009	0.497±0.008	0.301±0.005	0.160±0.004
I_0	3±0.2	37±1.7	64±3.0	86±4.0	1,648±90.0
$Z_{1\%}$	6±0.08	7±0.10	9±0.14	15±0.27	29±0.90
I_m	0.24±0.023	3.34±0.268	7.49±0.53	16.8±1.30	585±46.40
I_{MIRI}	0.23±0.022	2.68±0.661	6.06±1.52	13.2±3.44	544±26.40
SII	736±40	4,447±151	7,599±470	10,530±277	30.3±0.97

tion was determined in winter phytoplankton communities (Helbling et al. 2001), and the net primary production of two protists (*O. naumanni* and *Gymnodinium paradoxum*) was reduced at the upper layers by PAR+UVR (Modenutti et al. 2004). However, this situation may not apply to the UV-B–resistant mixotrophic heterotrich *Stentor araucanus.* This species was found in the upper levels of clear-water lakes (Modenutti et al. 1998; Woelfl and Geller 2002) and is highly resistant to UVR: it survived after 72 h of exposure to solar and artificial UV-B (Modenutti et al. 1998), doses lethal to other organisms such as *Daphnia pulicaria* (Zagarese et al. 1994).

S. araucanus is a planktonic endemic ciliate species described by Foissner and Woelfl (1994). The cells are conical shaped, lack sessile stages, and appear as dark dots to the naked eye as a result of their intense pigmentation. This dark appearance is caused by the presence of cortical blue–green granules, which contain stentorin and are located between ciliary rows. The cytoplasm has many symbiotic algae of a *Chlorella* type with cup-shaped chloroplasts. There have been no reports of unpigmented individuals or cells without symbiotic algae (Foissner and Woelfl 1994; Modenutti et al. 1998; Foissner et al. 1999). As is the case with other symbiont-bearing species of *Stentor, S. araucanus* was observed to have a positive phototaxis (Foissner and Woelfl 1994; Modenutti et al. pers. obs.).

Because *S. araucanus* has endosymbiotic algae and dominates the epilimnion of Andean lakes, it may be efficient in fixing carbon through photosynthesis in highly illuminated layers of the water column, even in the presence of UVR. Therefore, the aim of this study was to investigate, through field and laboratory experiments, the effect of PAR and UVR irradiance gradients on the cell-specific primary production of *S. araucanus.* In the field we determined the proportion of the natural population exposed to different types of potentially hazardous radiation. We measured inorganic carbon (C) uptake by *S. araucanus* in light gradients, both in the field and laboratory, to determine how light intensity controls endosymbiotic algae photosynthesis.

Materials and methods

Study area—Lake Moreno Oeste (41°5′S and 71°33′W, 758 meters above sea level) is located within Nahuel Huapi National Park (Patagonia, Argentina). Its surface area is 6 km^2 and its maximum depth is 90 m, with a warm monomictic thermal regime (epilimnion 15–17°C; hypolimnion 7°C) (Queimaliños et al. 1999; Modenutti et al. 2000). Lake Moreno is ultraoligotrophic, with a low dissolved carbon concentration (Morris et al. 1995) and corresponding high PAR and UVR transparency (Table 1). The euphotic zone extends down to 35 m, and blue–green light prevails in deep waters (Pérez et al. 2002).

Sampling and data collection—The lake was sampled at noon during summer (December 2003–February 2004) on seven occasions. Studied variables included (1) light vertical profiles (0 to 50 m) of UV bands (305, 320, 340, and 380 nm) and PAR (400–700 nm); (2) temperature profiles; and (3) *S. araucanus* vertical distributions.

Light and temperature profiles were measured using a PUV 500B submersible radiometer (Biospherical Instruments). Daily ground irradiances were also recorded by a Radiometer GUV-510 (Biospherical Instruments), located 5 km from the sampling site. Water samples were obtained at 0, 5, 10, 15, 20, and 30 m in depth with a 2-liter Ruttner bottle. To avoid sampling mixing of water column, samples were taken consecutively and separately from 0 to 30 m. A volume of 250 ml was sampled for ciliate enumeration and was preserved in Lugol's iodine solution in the boat just after sampling.

In the laboratory, *S. araucanus* individual chlorophyll *a* (Chl *a*) content was estimated. Six groups of 100 *S. araucanus* were carefully picked up with a micropipette under a stereomicroscope, rinsed in 0.2 μm–filtered lake water, and placed on a GF/F filter. Chlorophyll *a* was extracted with hot ethanol (Nusch 1980) and measured with a 10-AU fluorometer (Turner Designs).

Enumeration of ciliates was performed following the Utermöhl technique with an inverted microscope (Olympus IX70) with 50-ml chambers and was carried out by scanning the entire surface chamber at ×200 magnification. Ciliate identification was performed following the methods of Foissner and Woelfl (1994) and Foissner et al. (1999).

Primary production measurements—Primary production (PP) was measured with the ^{14}C technique (Steeman Nielsen 1951, 1952). Dark bottle measurements were substituted by

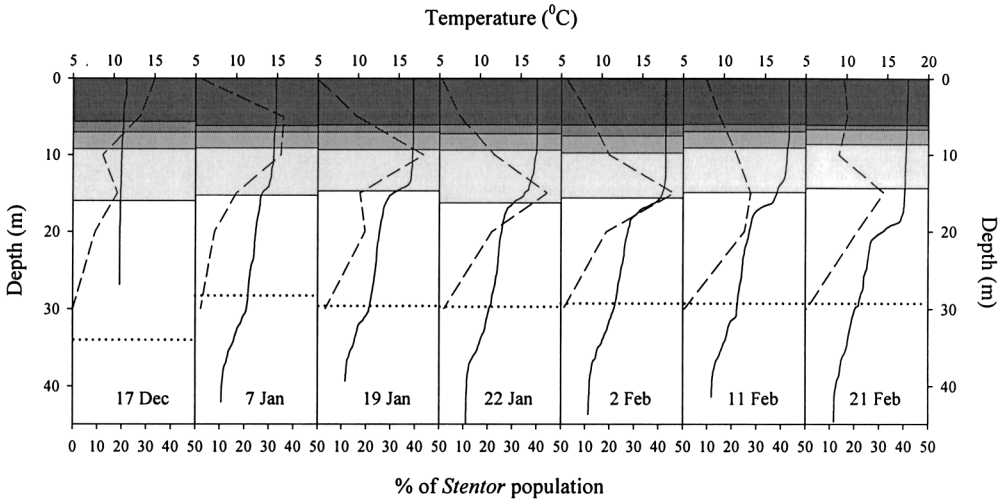

Fig. 1. Vertical profiles of the relative abundance of *S. araucanus* (dashed line), temperature (solid line) and depth of 1% of surface PAR (dotted line), and UV (305, 320, 340, and 380 nm, gray scale) in Lake Moreno Oeste during summer 2003–2004.

the ''time 0'' organic ^{14}C measurement by adding the isotope to the dark bottle and immediately filtering and analyzing (Fanhenstiel et al. 1994). For measuring cell-specific PP of *S. araucanus*, we performed field incubations in 12-ml quartz tubes filled with filtered lake water (0.2-μm Millipore™ filter). Twenty *S. araucanus* were separated under a stereomicroscope, rinsed twice in filtered lake water, and added to the tubes carefully. No increase in mortality was induced by this procedure (Modenutti et al. 1998). To each tube, 1.22 kBq $NaH^{14}CO_3$ ml^{-1} (Amersham) was added and then incubated in situ for 4 h centered around the noon hour.

After incubation, 500-μl aliquots were taken to check total activity. The samples were filtered with plastic disposable syringes and plastic filter holders containing 0.2 μm Nucleopore™ polycarbonate filters. Filters were acidified with 200 μl of 1 mol L^{-1} HCl for 60 min in 20-ml scintillation vials. After addition of 10 ml of scintillation liquid, the vials were counted in a Wallac 1414 scintillation counter. Photosynthetic carbon assimilation was calculated based on the proportion between ^{14}C uptake and total inorganic C availability measured on filtered lake water (glass fiber; GF/F) (Steeman Nielsen 1951, 1952).

Field study—During summer 2003–2004, six field experiments were carried out (17 December; 19 and 22 January; and 2, 11, and 21 February). Lake water and protists were sampled at 0 m, 10 m, and 20 m in depth on the same day, 2 h before starting the incubations. Incubations were carried out in 12-ml quartz tubes held in a frame at different levels of the euphotic zone: 0.30 m, 10 m, and 20 m in depth, with individuals collected from the same depths. The upper-level incubation (0.30 m) was run in two treatments, one exposed to full sunlight (0.3 mQ: quartz tubes at 0.30 m) and the other to PAR+UV-A (0.3 mM: quartz tubes wrapped with Mylar™ film with a cut-off at 320 nm at 0.30 m). The 10-m depth treatment was exposed to PAR+UV-A (380 nm + PAR, Fig. 1), whereas the 20-m depth treatment received only PAR (Fig. 1). Each treatment (0.3 mQ, 0.3 mM, 10 m, and 20 m) consisted of four replicates. Each tube contained 20 ciliates and was incubated for 4 h centered around solar noon.

On five occasions (the experiment on 19 January failed), additional 4-h epilimnetic incubations were performed with a variable depth incubation line. This incubator consists of a frame fixed to a moving device that runs downward and upward along a rope between two fixed depths (mixing layer running incubator [MIRI], Bertoni and Balseiro unpubl. data). Incubation tubes were moved through the epilimnion (0 to 15 m) at a speed of 8 cm s^{-1}. Mixing rate was estimated based on the depth of the mixing layer, water and air temperature, and wind speed (Bertoni and Balseiro unpubl. data). These incubations let us determine the actual PP of *S. araucanus* in a turbulent epilimnion, which is typical in Andean lakes. Under these conditions, we incubated two treatments with four replicates each (full sunlight and PAR+UV-A) utilizing the same method used in the 0.30-m fixed-depth incubation, but in this case ciliates were collected in a composite sample from 0 to 15 m in depth.

Laboratory experiments—We conducted laboratory incubations to obtain the photosynthesis-irradiance (P/E) response curve of *S. araucanus.* Ciliates were collected in a composite (0–15-m) sample. The experiments were carried out at seven light intensities (from 17 to 1,500 μmol photons m^{-2} s^{-1}) in a light gradient incubator filled with (15 $\pm$ 0.1°C) circulating water. The incubator tubes were fixed to a rotating frame (0.25 rpm), and light was provided by halogen lamps (two each of 1,000 W and 500 W bulbs). We used 12-ml quartz tubes with 0.2 μm–filtered lake water and 20 *S. araucanus.* Each light treatment was conducted in four replicates immediately after the individuals were placed in the different light levels. In addition, we carried out another experiment with acclimatized organisms. These individuals were exposed for 15 h prior to the PP measurement to PAR (800 μmol photons m^{-2} s^{-1}) and PAR+UV-B (800 μmol photons m^{-2} s^{-1} + 6 μW cm^{-2} of UV-B). A TL20/12 fluo-

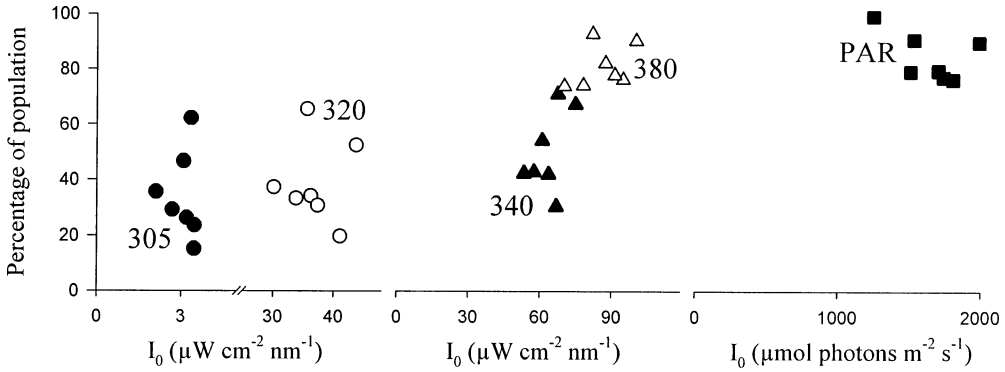

Fig. 2. Percent of *S. araucanus* population (as an integration of abundances from 0 to $Z_{1\%}$ of the corresponding wavelength) exposed to more than 1% of surface irradiance (I_0) of the different wavelengths (filled circles: 305 nm; open circles: 320 nm; filled triangles: 340 nm; open triangles: 380 nm; and squares: PAR).

rescent lamp (Philips) was the source of UV-B radiation (280–315 nm). The lamp was wrapped with acetate film to prevent any output less than 290 nm. The spectral output of the lamp (defined by the manufacturer) has maximum emission at 313 nm, with negligible energy above 320 nm (Bertoni and Callieri 1999). We added 1.22 kBq $NaH^{14}CO_3$ ml^{-1} to the tubes, and the incubation was run for 4 h. In the experiment carried out with acclimatized individuals we used five light intensities (1,506, 861, 536, 101, and 8.2 μmol photons m^{-2} s^{-1}). Light intensity was measured with a Biospherical Instrument QSL 2101 sensor inside a tube. After the incubation, we followed the same protocol used to measure photosynthesis in the field.

Data analysis—The P/E data were normalized to Chl *a* and then fitted to the Eilers and Peeters (1988) model:

$$P = \frac{I}{aI^2 + bI + c} \quad \text{and}$$

$$\alpha = \frac{1}{c} \quad \text{and} \quad P_{\max} = \frac{1}{b + 2\sqrt{ac}}$$

where α is the initial slope and $P_{\max}$ is the maximal production rate. To fit the data, we used Sigma Plot 2001 to perform nonlinear least-squares regression. Data analysis was performed with Sigma Stat 2.03.

For each waveband (305, 320, 340, and 380 nm and PAR), the mean irradiance within the epilimnion was computed following Helbling et al. (1994).

$$I_m = I_0 \frac{1 - e^{(-K_d Z)}}{K_d Z}$$

where I_0 is the irradiance at the surface, K_d is the diffuse attenuation coefficient (for the corresponding wavelength band), and Z is the depth of the mixed layer.

Photosynthetic inhibition (P_{inh}) by UV-B radiation was determined by comparing the PP in the two treatments (PP in quartz tubes (P_Q) and PP in quartz tubes wrapped with Mylar™ (P_M)) of the upper level incubation (0.30 m), as follows:

$$P_{\text{inh}}(\%) = \frac{P_M - P_Q}{P_M} \times 100$$

Results

Field study—During summer 2003–2004, Lake Moreno Oeste was thermally stratified (beginning in December). At the time of the December experiment, the thermocline was at 15 m; however, it became deeper at the end of the sampling period (February 2004) (Fig. 1). The euphotic zone (the depth at which 1% of surface PAR occurred) included the whole epilimnion, the metalimnion, and the upper portion of the hypolimnion (Table 1; Fig. 1). All sampling and incubations were performed on clear and sunny summer days with very high irradiances (PAR $I_{0\max}$ averaged 1,600 μmol photons m^{-2} s^{-1}, and surface-integrated irradiance during the 4-h incubations was around 30 mol photons m^{-2}) (Table 1). The coefficient of variation of these means was less than 10%, indicating very low variation in irradiance between days. The average PAR irradiance of the epilimnion was high, around 600 μmol photons m^{-2} s^{-1}, and almost the whole layer was exposed to UV-A radiation ($Z_{1\%}$ for 380 nm was down to 15 m), whereas the upper ~40% was exposed to the 305-nm UV-B band (Table 1). On the experimental dates there were no remarkable differences in stratospheric ozone (260–290 Dobson Units) (NASA Ozone Processing Team pers. comm.).

S. araucanus was present in all samples, with highest abundances observed in the epilimnion at or above 15 m (Fig. 1). Thus, a high proportion of the ciliate population (75–95%) was exposed to UVR; furthermore, between 25% and 60% of the population endured high UV-B levels (Fig. 2). On four occasions, *S. araucanus* maximum abundance was in the epilimnion above the thermocline, and on three midsummer occasions (late January and early February) it was in the thermocline (Fig. 1). This vertical distribution was not related to differences in I_0 or in surface-integrated irradiance.

Individual ciliate Chl *a* content reached 1.06 (±0.06) ng Chl $cell^{-1}$, and no marked changes in chlorophyll cell con-

Table 2. *S. araucanus* PP_{cell} (ng C ciliate^{-1} h^{-1}) in the static incubations (0.30 m, 10 m, and 20 m in depth) and calculated photosynthetic inhibition (P_{inh}). Q, quartz tubes; M, quartz tubes wrapped with Mylar™. Values of each treatment are given as average of the four replicates ± standard edrror.

Date	P_{inh} (%)	PP, 0.3 m, Q	PP, 0.3 m, M	PP, 10 m	PP, 20 m
17 Dec 03	19.0	1.077±0.020	1.333±0.088	2.575±0.013	1.204±0.248
19 Jan 04	−1.9	1.669±0.197	1.637±0.141	1.947±0.136	0.387±0.037
22 Jan 04	15.7	2.157±0.175	2.561±0.264	1.800±0.245	0.304±0.095
2 Feb 04	−3.5	1.614±0.346	1.559±0.368	1.011±0.040	0.112±0.040
11 Feb 04	−5.6	1.432±0.194	1.356±0.084	1.357±0.156	0.211±0.028
21 Feb 04	11.1	1.006±0.093	1.132±0.250	1.019±0.186	0.192±0.059
Mean	5.8	1.493±0.173	1.597±0.206	1.618±0.248	0.402±0.165

tent were observed in the vertical profiles or over the summer. Cell-specific PP varied with the incubation depth, averaging 0.402 ng C ciliate^{-1} h^{-1} at 20 m in depth, 1.618 ng C ciliate^{-1} h^{-1} at 10 m, and 1.597 ng C ciliate^{-1} h^{-1} at 0.3 m in depth (Table 2). No marked trend in the PP per cell was observed over time (Table 2). Photosynthetic inhibition due to full UVR exposure during the summer season was low, averaging 5.8% (maximum of 19% in early summer; Table 2).

The comparison of individual PP of *S. araucanus* in the different static incubation treatments showed significant differences (two-way ANOVA, $p < 0.001$). However, the only treatment with significant differences (Tukey test, $p < 0.001$) was that of 20 m in depth, which was lower (Fig. 3). These results indicated that the epilimnetic irradiance levels (0.3-m and 10-m depths) were favorable for photosynthetic activity; in all cases PP was at or above 1 ng C ciliate^{-1} h^{-1} (Fig. 3, dotted line). On the other hand, the low values obtained at 20 m, with PAR irradiances less than 100 μmol photons m^{-2} s^{-1}, indicate that the endosymbiotic algae were light limited at this level. On 17 December, the 20-m treatment differed from the other five experiments (Tukey test, $p < 0.001$), with PP above 1 ng C ciliate^{-1} h^{-1} (Fig. 3). It is notable that, on this date, the 20-m depth irradiance was higher than 100 μmol photons m^{-2} s^{-1} as a result of a low K_d value ($K_{d\,(PAR)} = 0.13$ m^{-1}) and not because of changes in weather conditions.

At 0.3 m, PP was similar in the presence or absence of UV-B (i.e., quartz tubes versus quartz tubes wrapped with Mylar™). Furthermore, both treatments were not significantly different than that incubated at 10 m in depth (Tukey test, $p > 0.05$), indicating that endosymbiotic algae did not experience photoinhibition from high irradiances (both PAR and UVR) present in the upper layers (Fig. 3).

The epilimnetic variable-depth incubations gave similar results; no differences between the two treatments (presence or absence of UV-B) were observed (two-way ANOVA, $p > 0.05$) (Fig. 4a). In these incubations, the calculated photosynthetic inhibition averaged 0%. As a result of the different extinction coefficients of each wavelength (Table 1), the amount of irradiance (as percentage of surface irradiance) received by the organisms increased progressively from 7% for 305 nm to 35% for PAR. During the cycle of the moving incubation, the UVR, in particular wavelengths shorter than 340 nm, dropped below the 1% of surface irradiance (Fig. 4b), though the device remained within the euphotic zone. The mean irradiances of our variable-depth incubations were close to the mean epilimnetic ones (Table 1; I_m vs. I_{MIRI}). Individual primary production of *S. araucanus* incubated under these conditions varied between 1 and 2 ng C ciliate^{-1} h^{-1} (Fig. 4a), and these values did not differ from those of 0.30-m (quartz and Mylar™) and 10-m static incubation treatments (two-way ANOVA, $p > 0.05$).

Laboratory experiments—In P/E response curves, PP increased with irradiance up to saturation at 600 μmol photons m^{-2} s^{-1} (Fig. 5). Interestingly, this value was very similar to the mean epilimnetic irradiance obtained during our summer

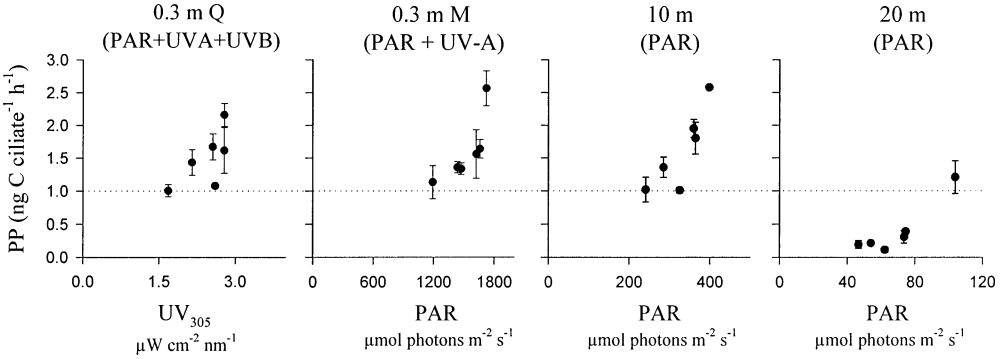

Fig. 3. Primary production in *S. araucanus* in relation to UV-B (305 nm) and PAR wavelengths during static incubations (Q: quartz tubes for full sunlight; M: quartz tubes wrapped with Mylar™). Dotted line represents the level of 1 ng C ciliate^{-1} h^{-1}.

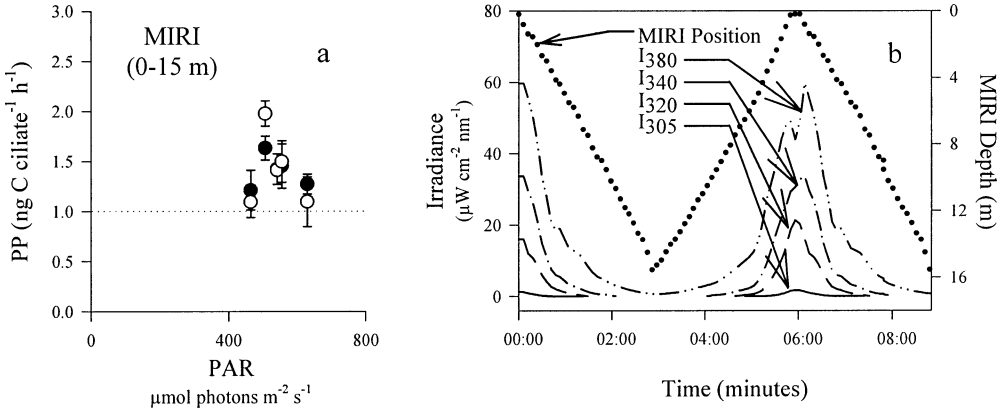

Fig. 4. (a) Primary production in *S. araucanus* during the epilimnetic variable-depth incubations (MIRI) from 0 m to 15 m in depth. Filled circles: quartz tubes for full sunlight; and open circles: quartz tubes wrapped with Mylar™. Light intensity was calculated according to Helbling et al. (1994). Dotted line as in Fig. 3. (b) UVR received in a simulated cycle of the MIRI, measured with the PUV 500B.

study (Table 1). Also, the calculated P_{max} was around the mean values of the epilimnetic field incubations. I_k (P_{max}/α) was around 100 μmol photons m^{-2} s^{-1}, which is relatively high in comparison with other phytoplanktonic taxa, although not unusual for surface-acclimated phytoplankton assemblages (Neale and Richerson 1987).

In the second laboratory experiment, samples were exposed to PAR or PAR+UV-B for 15 h prior to the photosynthesis measurements. Subsequent PP values (Fig. 6) did not differ between the two acclimatized treatments (two-way ANOVA, $p > 0.05$), indicating that photosynthesis was not inhibited as a result of the previous UV-B exposure. In addition, we found significant differences in PP between the five different light intensities (two-way ANOVA, $p < 0.001$). PP was significantly different only in the lowest irradiance treatment (8.2 μmol photons m^{-2} s^{-1}), regardless of the presence or absence of previous UV-B exposure (Tukey test, $p < 0.01$).

Discussion

Photosynthesis is more sensitive to UV-B in phytoplankton than in terrestrial plants, likely as a result of the less effective protective pigmentation in phytoplankton (Day and Neale 2002). Previous studies have shown that the upper levels (0 to ~10 m) of Lake Moreno have high levels of harmful UVR that cause photosynthetic inhibition in phytoplankton and other mixotrophic ciliates (Helbling et al. 2001; Modenutti et al. 2004). For example, the mixotrophic ciliate *Ophrydium naumanni* shows a considerable reduction (up to 80%) in PP when incubated at 5 m in depth (PAR irradiances higher than 550 μmol photons m^{-2} s^{-1} and UV-B wave band 0.037 μW cm^{-2} nm^{-1}) (Modenutti et al. 2004). This scenario changes completely when the pigmented mixotrophic ciliate *S. araucanus* is considered. Substantial fractions (up to 60%) of this ciliate population were exposed to

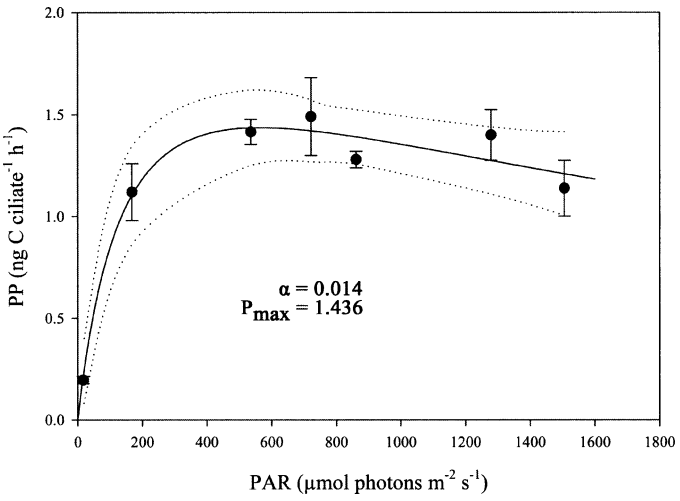

Fig. 5. Laboratory P/E curve of *S. araucanus.* Dotted line represents 95% confidence limits.

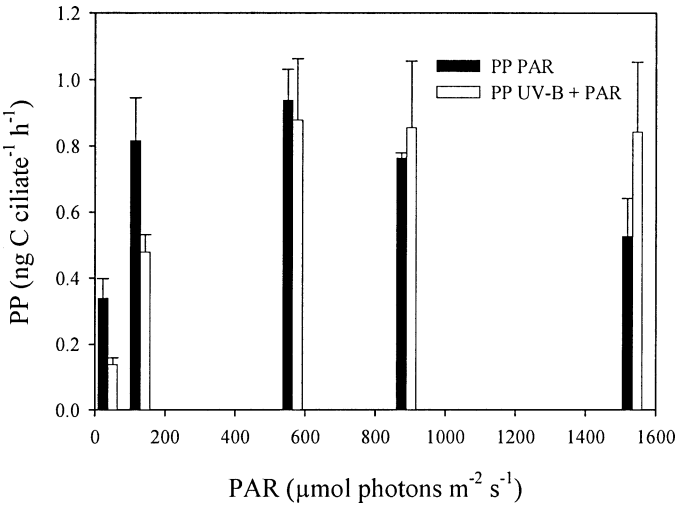

Fig. 6. Primary production of *S. araucanus* in laboratory experiments carried out with acclimatized organisms (15 h exposure to UV-B+PAR and PAR).

high levels of UVR. Laybourn-Parry et al. (1997) stated that locomotory activities of large ciliates (e.g., *Stentor*) are likely to be overridden by turbulent mixing of the water column. Therefore, the organisms present above the thermocline are carried close to the surface. Our results showed that the vertical distribution of *S. araucanus* was not related to weather conditions. Although the ciliate maximal abundance was often not immediately beneath the lake surface, raising the possibility of avoiding the highest UV-B intensities, the field incubation demonstrated high tolerance to full solar irradiance. A previous study showed that *S. araucanus* survival and growth were not affected by UV-B exposure either in laboratory or field experiments (Modenutti et al. 1998). In addition, our data show that endosymbiotic algae of *S. araucanus* achieved similar values of PP when incubated with or without exposure to UV-B in the field, and even after previous UV-B exposure in the laboratory. In our experiments there was no negative correlation between PP and harmful radiation (Fig. 3). In this sense, this mixotrophic association is clearly favorable for the endosymbiotic algae.

Photosymbiosis is an ecological strategy for expanding into habitats previously unavailable to one or both symbionts (Haussmann et al. 2003). Andean lakes are nutrient-poor, high-light environments in which mixotrophic ciliates are especially favored (Queimaliños et al. 1999; Modenutti et al. 2000; Woelfl and Geller 2002). In Lake Moreno, nutrient concentrations are low (total dissolved phosphorus less than 2 μg L^{-1}) and do not change in the water column from 0 to 52 m (Modenutti et al. 2000). Light is clearly not limiting in the lake, since the euphotic zone reaches the hypolimnion (Fig. 1), but the upper epilimnetic levels are potentially harmful. Symbiosis can be of great ecological importance in this kind of situation. In the planktonic environment, this strategy provides greater flexibility (Jones 1994), since it may improve access to scarce nutrients (Nygaard and Tobiesen 1993). Thus, the endosymbiotic algae are favored by the nutrient cycle inside the ciliate. In addition, the present results provide evidence of an advantage in colonizing the upper levels of the water column without photosynthesis inhibition.

The role of the cortical pigment is still under discussion (Foissner and Woelfl 1994). Stentorin belongs to the mesonaphtodianthrone group of compounds, which includes the photodynamic pigments hypericin and phagopyrin (Tartar 1961; Moeller 1962). The fact that stentorin absorbs near UV as well as visible wavelengths (Moeller 1962) may indicate that this pigment provides protective screening of UV. Up until now, no evidence of other photoprotective compounds, such as mycosporine-like aminoacids, has been obtained for *S. araucanus* (Moeller pers. comm.). It is possible, however, that the presence of these dark granules acts as a light umbrella for the endosymbiotic algae, since stentorin absorbs PAR mainly between 400 and 600 nm (Moeller 1962). In this sense, the irradiance below 100 μmol photons m^{-2} s^{-1} appeared to be insufficient to allow endosymbiotic algae to produce under such a dark pigmented umbrella.

S. araucanus appears to be well adapted to high light levels; in fact, in the laboratory experiments we obtained a rather high I_k around 100 μmol photons m^{-2} s^{-1}. This level is not unusual for surface-acclimated phytoplankton assemblages (Neale and Richerson 1987) and is consistent with *S. araucanus* being a species acclimated to the high-light epilimnion of Andean lakes. This is further supported by the observation that its PP decreases sharply when light falls beyond 100 μmol photons m^{-2} s^{-1}. At this level of irradiance, other phototrophic organisms of Lake Moreno Oeste exhibited high positive PP values (Modenutti et al. 2004). Our field and laboratory experiments also showed that *S. araucanus* PP values were not significantly different for incubations from 100 to 1,600 μmol photons m^{-2} s^{-1} in fixed- or in variable-depth incubations. This constancy in PP along the light gradient of the epilimnion implies that photosynthetic efficiency (PP per energy unit) decreases as light increases. This capability of varying photosynthetic efficiency would be an advantage for organisms that can be exposed to high light intensities by mixing. Moreover, the laboratory experiments comparing UV-B+PAR and only PAR and the field incubations with and without UV-B exposure (quartz and Mylar™ in the 0.3-m treatments) showed no differences, indicating that this species is able to produce even when it is near the surface on clear and sunny days with very high irradiances including UV.

The red tide dinoflagellate *Gymnodinium breve* exhibits positive phototaxis, and during blooms these organisms tend to be concentrated near the surface, where cells are exposed to high levels of PAR and UVR (Kamykowski et al. 1998). During an experimental study, *G. breve* photosynthetic efficiency exhibited distinct diurnal patterns corresponding to varying surface irradiances, but differences in oxygen production rates were not directly attributable to UV (Evens et al. 2001). These results indicate that *G. breve* possesses an inherent UV resistance and a robust photosynthetic capability (Evens et al. 2001). *S. araucanus* also exhibited a UV resistance, but the mid-day vertical distribution was not observed to be related to changes in surface irradiance.

In general, pigmented mixotrophs such as *S. araucanus* may achieve high population densities in epilimnetic depths because there they receive enough light energy (I_m ~600 μmol photons m^{-2} s^{-1}) to allow endosymbiotic algae to produce. *S. araucanus* is virtually the only photosynthesizing organism in the epilimnion of clear Andean lakes, so it must have effective mechanisms of dealing with high irradiances. In addition, the low sensitivity of *S. araucanus* to UVR allowed us predict that this organism will increase in abundance in response to further ozone depletion.

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