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Photosynthesis versus irradiance characteristics in phytoplankton assemblages off Patagonia (Argentina): temporal variability and solar UVR effects

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ABSTRACT: From November 2002 to June 2003, we carried out experiments to determine the temporal variability of *P* vs. *E* curves and the effects of solar ultraviolet radiation (UVR, 280–400 nm) on photosynthetic parameters of natural phytoplankton assemblages from Patagonia. Samples were collected at 2 sites, Bahía Nueva and Bahía Camarones (Chubut, Argentina), and exposed to solar radiation for 4 to 6 h under 3 quality radiation treatments (i.e. PAR + UVR, 280–700 nm; PAR + UV-A, 320–700 nm; and PAR only, 400–700 nm), and under 6 to 8 levels of ambient irradiance (i.e. by using 0 to 5–7 layers of neutral density screens). Samples collected at Bahía Nueva had relatively high P_{max} values (~3–4 µg C (µg chl *a*)$^{-1}$ h^{-1}) during the pre-bloom period (i.e. late summer to late fall) and relatively low values (~1.5–2.5 µg C (µg chl *a*)$^{-1}$ h^{-1}) during the post-bloom period (i.e. late spring to early summer); similar results were observed in samples collected at Bahía Camarones. The light saturation parameter E_k, on the other hand, did not show a clear pattern and values ranging from 50 to 400 µmol m^{-2} s^{-1} were observed throughout the study period. High P_{max} values were significantly correlated with the concentration of large diatoms (R^2 = 0.6, $p < 0.05$), the taxonomic group that characterized the pre-bloom period. UVR significantly reduced P_{max} values ($p < 0.05$) during the pre-bloom but not during the post-bloom period. UVR also significantly affected E_k ($p < 0.05$) in all Bahía Camarones samples but only in some from Bahía Nueva. UV-A was responsible for the bulk of photosynthetic inhibition throughout the study period; the highest UV-A-induced integrated inhibition values in the water column were ~16.5 and 7% for Bahía Nueva and Bahía Camarones, respectively. On the other hand, UV-B-induced photosynthetic inhibition reached maximum values of 2.3 and 3.9% for Bahía Nueva and Bahía Camarones, respectively. Since under certain environmental conditions *P* vs. *E* parameters can be significantly reduced by UVR, we suggest that remote sensing algorithms using these parameters should also consider the impact of UVR in their estimates of primary production.

KEY WORDS: Patagonia · Photosynthesis · *P* vs. *E* · Phytoplankton · UVR

INTRODUCTION

Photosynthesis versus irradiance (*P* vs. *E*) curves are very useful tools to predict primary productivity and carbon fluxes over large areas of the world's ocean (Platt & Sathyendranath 1988, Behrenfeld & Falkowski 1997); they also provide information on the photoacclimation status of cells at the time of sampling. *P* vs. *E* curves are characterized by diverse parameters, i.e. α (the light limited slope of the *P* vs. *E* curve), E_k (the light saturation parameter, i.e. the intercept between the initial slope of the *P* vs. *E* curve and P_{max}), β (the

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photoinhibition parameter, i.e. the negative slope of the curve at high irradiances) and P_{max} (the maximum rate of carbon fixation, i.e. maximum production) (Kirk 1994, Sakshaug et al. 1997). These parameters are dependent on several factors such as the irradiance levels at which samples are exposed and the incubation period, species composition, physiological status of cells, previous light history, as well as the temperature and CO_2 concentration (Kirk 1994, Sakshaug et al. 1997, Macedo et al. 2002). Studies have also demonstrated that the interaction of solar radiation with other factors (e.g. mixing) may also affect *P* vs. *E* relationships (Marra 1978, Yoder & Bishop 1985).

Ultraviolet radiation (UVR, 280–400 nm) is a stress factor that can considerably reduce phytoplankton photosynthetic rates (see review of Villafañe et al. 2003) and thus, it is plausible that UVR might also affect *P* vs. *E* relationships. Since UVR effects on aquatic autotrophs are dependent on factors such as the irradiance/dose levels at which cells are exposed, as well as their specific sensitivity and acclimation to these short wavelengths, it is obvious that it is not possible to generalize on how UVR affects *P* vs. *E* relationships in any aquatic body on the basis of studies performed in other locations or under other conditions. Thus, rigorous studies considering the radiation climate as well as the taxonomic structure of natural communities have to be performed before any model can be applied to determine productivity from *P* vs. *E* curves.

The purpose of this work is to evaluate the temporal variability of photosynthetic parameters and the effects of solar UVR on *P* vs. *E* relationships of coastal phytoplankton communities from Patagonia. To achieve this objective, we collected phytoplankton samples at different times of the year (i.e. during the post- and pre-bloom seasons) from 2 contrasting sites of the Chubut coast (Argentina) and we exposed cells to ambient radiation levels to measure photosynthetic rates. So far, very few studies have addressed phytoplankton primary productivity in the Argentinean Sea, especially at the Patagonia coast (Charpy & Charpy-Rubaud 1980, Buma et al. 2001, Helbling et al. 2001a, Villafañe et al. 2004a). In particular, research about the UVR effects on phytoplankton of the area started relatively recently (Buma et al. 2001, Helbling et al. 2001a, Villafañe et al. 2001, 2004a, Barbieri et al. 2002). Hence, the results of this work will add important and useful information about primary productivity under natural radiation levels of this still much under-sampled region, which can be later extrapolated to larger areas when appropriate bio-optical models are applied.

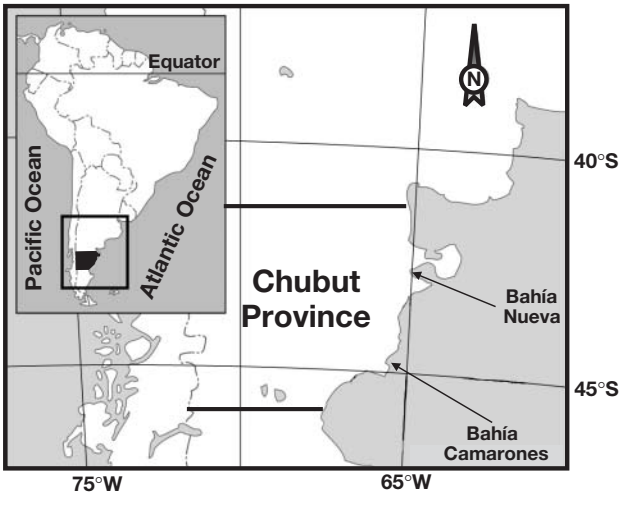

Fig. 1. Map showing the sampling sites Bahía Nueva and Bahía Camarones, and the relative position of the Chubut Province in South America

MATERIALS AND METHODS

Study area. Nine experiments were performed at different times of the year to determine *P* vs. *E* relationships of samples collected at Bahía Nueva (42.7° S, 65° W) (Fig. 1). The sampling site is located within Golfo Nuevo, an enclosed system with relatively little exchange with open waters from the Atlantic Ocean (Rivas & Beier 1990). For comparative purposes, we also carried out 3 experiments at Bahía Camarones (44.9° S, 65.6° W) (Fig. 1), a bay with open waters to the Atlantic Ocean located about 250 km south of Estación de Fotobiología Playa Unión (EFPU). The experiments were carried out in the period late spring 2002 to fall 2003 (i.e. late November 2002 to early June 2003). Rough weather conditions prevented us from obtaining temperature and light profiles during all samplings but, based on wind speed data, the upper mixed layer (UML) depth was estimated to be down to at least 10 m. In both sampling areas, the depth of the UML is highly dependent on wind speed and duration; spring and summer are the windy seasons (Villafañe et al. 2004a). The attenuation coefficient (K_d) for photosynthetically active radiation (PAR) varied from 0.2 to 0.35 m^{-1} in Bahía Nueva waters and from 0.25 to 0.31 m^{-1} in Bahía Camarones; water temperature was similar at both sites, varying from 8 (in June) to 19°C (in January) (E. W. Helbling unpubl. data).

Experiments and determinations. Surface water samples were collected at 500 to 1000 m off the coast using an acid-cleaned (1 N HCl) polycarbonate carboy. Samples from Bahía Nueva were collected early in the morning and immediately taken to the EFPU where *P* vs. *E* experiments were performed on the same day; on

the other hand, experiments with waters collected in Bahía Camarones were conducted on site. The samples were put in quartz tubes to determine photosynthetic rates (see 'Analyses and measurements') under 3 quality radiation treatments and under 6 to 8 levels of ambient irradiance (quantity radiation treatments). The radiation quality treatments were as follows: (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, N°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 quantity (i.e. irradiance) treatments were obtained by covering the tubes with none or an increasing number of neutral density screens up to 5 or 7 layers, thus obtaining a total of 6 or 8 quantity treatments (i.e. from 100 to <2% of total irradiance). A tray containing the tubes (i.e. a total of 36 or 48 tubes) was then put in a water bath with running water as a temperature control (in Bahía Nueva experiments) or off shore with 1 to 2 cm of seawater covering the tubes (in Bahía Camarones experiments) and exposed to natural radiation for 4 to 6 h, the incubations being centered on local noon. We chose an incubation time long enough so that any repair mechanism would be at steady state. At the beginning of the experiments, samples were taken to determine chlorophyll *a* (chl *a*) concentration, UV-absorbing compounds and floristic composition (see 'Analyses and measurements'). In addition, different atmospheric parameters (see 'Analyses and measurements') were continuously monitored throughout the study period.

Analyses and measurements. The analytical procedure for each determination/measurement was as follows:

Photosynthetic rates: Samples were put in 20 ml quartz tubes and inoculated with 5 µCi (0.185 MBq) of labeled ($NaH^{14}CO_3$) sodium bicarbonate (Steeman Nielsen 1952). After the incubation period, the samples were filtered onto Whatman GF/F glass fiber filter (25 mm). The filters were then placed in 7 ml scintillation vials, exposed to HCl fumes overnight, dried and counted using standard liquid scintillation techniques (Holm-Hansen & Helbling 1995).

Chl a and UV-absorbing compounds: Chl *a* concentration was measured by filtering 100 ml of water sample onto a Whatman GF/F glass fiber filter (25 mm) and extracting the photosynthetic pigments in absolute methanol (Holm-Hansen & Riemann 1978). Chl *a* concentration was determined by fluorometric techniques (Holm-Hansen et al. 1965) using a Turner Designs fluorometer (model TD700). The fluorometer was calibrated using pure chl *a* from *Anacystis nidulans* (Sigma #C 6144). UV-absorbing compounds were estimated by filtering 1 to 3 l of water sample onto a Whatman GF/F glass fiber filter (47 mm) and extracting these compounds in absolute methanol overnight. Scans (250–750 nm) were obtained using a Hewlett Packard spectrophotometer (model 8453E) and from these data, the concentration of UV-absorbing compounds was estimated by peak analysis (Helbling et al. 1996).

Floristic analysis: Water samples were fixed with buffered formalin (final concentration in the sample = 0.4% of formaldehyde). Quantitative analysis of phytoplankton cells was carried out using an inverted microscope (Utermöhl 1958). The samples (25 ml) were settled for 24 h and then counted under 200× magnification for microplankton (>20 µm) and under 40× magnification for pico-nanoplankton cells (<20 µm). A drop of Rose Bengal was added to the sample in the settling chamber to better distinguish between organic and inorganic material (Villafañe & Reid 1995).

Radiation and other atmospheric measurements: Incident solar radiation was continuously measured using a broad band ELDONET radiometer (Real Time Computers) that measures UV-B (280–315 nm), UV-A (315–400 nm) and PAR (400–700 nm) with a frequency of 1 reading min^{-1}. In addition, continuous monitoring of other atmospheric parameters (i.e. temperature, humidity, wind speed and direction, barometric pressure and rain) was carried out using a meteorological station Oregon Scientific (model WMR-918).

Statistics: The parameters of the *P* vs. *E* curves were obtained using the model of Eilers & Peeters (1988) and fitting the data by iteration:

$$P = E/(aE^2 + bE + c)$$

where *P* is the production (µg C (µg chl *a*)$^{-1}$ h^{-1}), *E* is the irradiance (µmol m^{-2} s^{-1}), and *a*, *b* and *c* are the adjustment parameters. The initial slope (i.e. α), the maximum production rate (P_{max}) and the light saturation parameters (E_k) are expressed as a function of the parameters *a*, *b* and *c* as follows:

$$E_k = (c/a)^{1/2}$$
$$\alpha = 1/c$$
$$P_{max} = 1/[b + 2(ac)^{1/2}]$$

The parameter '*a*' is considered the photoinhibition term but, according to modifications of Eilers & Peeters (1988), it can also be interpreted as a function of the exposure time above E_k (see Macedo et al. 2002 for further details). A Kruskal-Wallis non parametric test (Zar 1984) was used to determine significant differences between the estimated parameters (confidence level = 0.05); the correlation between variables was established using a Kendall's τ test.

RESULTS

Atmospheric conditions

Solar radiation and ambient temperature data during the period November 1 2002 (Day of Year 305) to June 30 2003 (Day 181) are shown in Fig. 2. There was a day-to-day variability in daily doses due to cloud cover and a clear trend for decreasing doses was observed after Day 50. Maximum daily doses were measured during the period December to January, reaching values of ~12 MJ m^{-2}, 1.8 MJ m^{-2} and 50 kJ m^{-2} for PAR, UV-A and UV-B, respectively (Fig. 2A–C). On the other hand, maximum daily doses during early winter were <1 MJ m^{-2}, <0.2 MJ m^{-2} and ~1.5 kJ m^{-2} for PAR, UV-A and UV-B, respectively (Fig. 2A–C). Ambient temperature (Fig. 2D) also had high variability during the study period, with mean values ranging from ~5 to 27°C that fell within the range –2 to 35°C (no data were collected during Days 50 to 70).

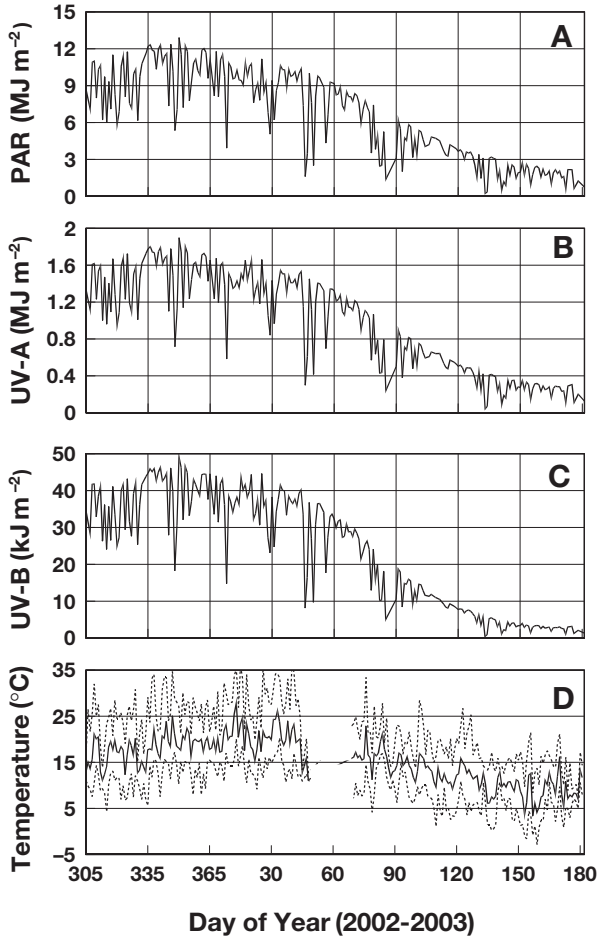

Fig. 2. Atmospheric conditions for the period November 1, 2002 (Day 305), to June 30, 2003 (Day 180). (A) Daily doses of photosynthetically active radiation (PAR), 400–700 nm (in MJ m^{-2}); (B) daily doses of UV-A, 315–400 nm (in MJ m^{-2}); (C) daily doses of UV-B, 280–315 nm (in kJ m^{-2}); (D) mean (——), maximum and minimum (----) daily temperature (in °C). No temperature data were collected during Days 50 to 70

Bahía Nueva experiments

The *P* vs. *E* curves obtained for the different phytoplankton assemblages collected from Bahía Nueva are shown in Fig. 3. There was a range of responses depending on the time of the year when the experiments were conducted. In some experiments (Fig. 3A,E), no photoinhibition was determined, whereas in others (Fig. 3G), it was very important or evident at irradiances >300 µmol m^{-2} s^{-1} (e.g. Fig. 3H,I) or >600 µmol m^{-2} s^{-1} (e.g. Fig. 3B–D). The impact of UVR on *P* vs. *E* was important in pre-bloom samples (i.e. fall samples), causing an additional decrease in CO_2 fixation at high irradiances (Fig. 3F–I). *P* vs. *E* parameters (Fig. 4) showed variable responses in assemblages collected at different times of the year. P_{max} values (Fig. 4A) were significantly higher ($p < 0.05$) in the pre-bloom period (i.e. late summer to fall; 2.6 to 3.6 µg C (µg chl *a*)$^{-1}$ h^{-1}, see also Fig. 3E–I) than during the post-bloom (i.e. late spring to early summer; 1.5 to 2.4 µg C (µg chl *a*)$^{-1}$ h^{-1}, see also Fig. 3A–D). There was a significant impact of UVR on P_{max} in samples collected during the pre-, but not during the post-bloom (Fig. 4A, Table 1). This negative effect was mostly due to UV-A, but on the same samples (e.g. 9 April), exposure to UV-A resulted in a significantly higher P_{max} (Fig. 4A). There was no clear trend in the light saturation parameter E_k associated with the sampling period (Fig. 4B). E_k varied with high values (i.e. >300 µmol m^{-2} s^{-1}) occurring in November, February, early April and May, and low values in January and in late April (i.e. <200 µmol m^{-2} s^{-1}); the lowest E_k (~50 µmol m^{-2} s^{-1}) was determined in late April. A significant effect of solar UVR on E_k was found in some experiments (i.e. summer and late fall), with high values in samples exposed only to PAR and low in those that additionally received UVR wavelengths. Since we did not have detailed data on the UML depth (a variable that might affect E_k), but rather on wind speed, we established the relationship between E_k and the mean wind speed for the week prior to our experiments (Fig. 4C). We used wind speed in the previous week as an indirect measurement of UML depth, expecting that with increasing wind speed, this depth would increase. A significant negative correlation ($R^2 = -0.65$, $p < 0.001$) was established between these 2 variables, with a decrease in E_k with increasing mean wind speed for all radiation treatments (i.e. PAB: PAR + UV-A + UV-B,

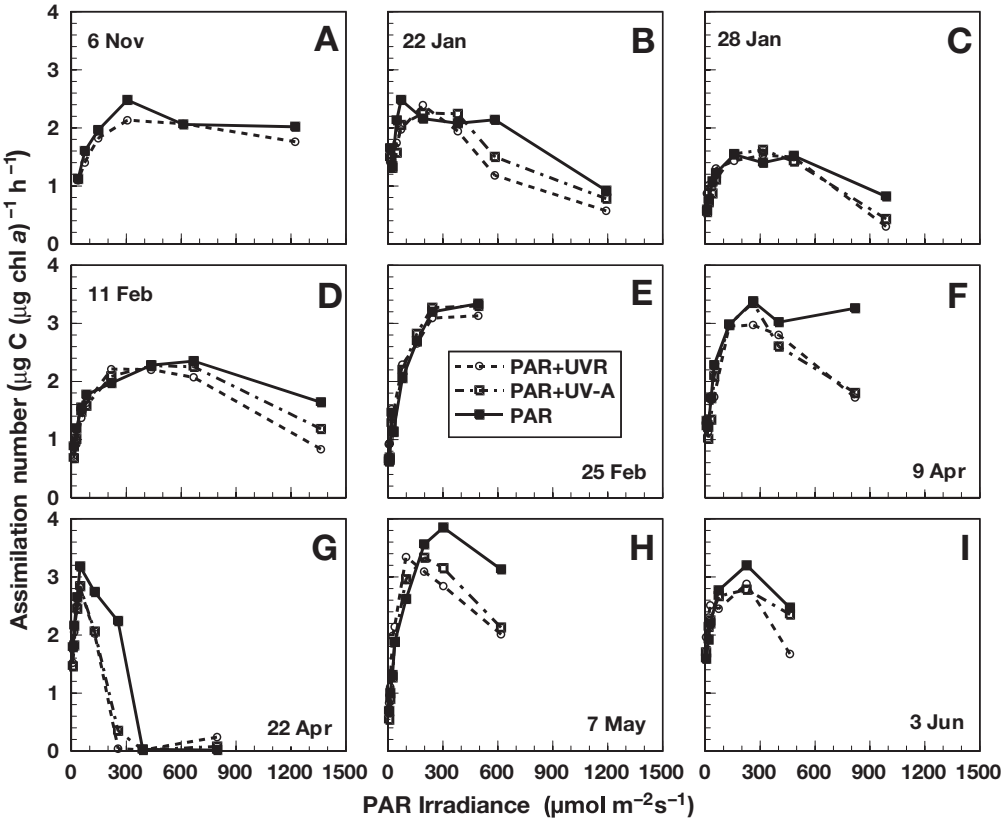

Fig. 3. Phytoplankton assimilation numbers as a function of the mean PAR irradiance to which samples from Bahía Nueva were exposed. Samples exposed to PAR + UV-A + UV-B (○); samples exposed to PAR + UV-A (□); samples exposed to PAR only (■). Experiments were carried out on: (A) November 6, 2002; (B) January 22, 2003; (C) January 28, 2003; (D) February 11, 2003; (E) February 25, 2003; (F) April 9, 2003; (G) April 22, 2003; (H) May 7, 2003; and (I) June 3, 2003

Table 1. Relative effects (in %) of UV-A and UV-B on P_{max} and E_k during experiments carried out in Bahía Nueva and Bahía Camarones. Positive values indicate a decrease in the value of the parameter considered either due to UV-A or UV-B (as compared to PAR). *$p < 0.05$. –: data not available for November 6 and 23 experiments

	P_{max}		E_k	
	UV-A	UV-B	UV-A	UV-B
Bahía Nueva				
6 Nov	–	–	–	–
22 Jan	8.52	–0.60	–7.33*	18.47*
28 Jan	–6.81	8.25	6.20	28.16*
11 Feb	–3.41	–2.13	15.85*	11.71*
25 Feb	0.20	8.37	0.79	0.79
9 Apr	–14.97*	9.10*	4.19	2.33
22 Apr	13.20*	–0.96	9.23	4.18
7 May	7.63*	1.36	41.01*	14.41*
3 Jun	7.36*	4.12	10.37*	31.79*
Bahía Camarones				
23 Nov	–	–	–	–
3 Feb	–7.01	–3.31	32.77*	11.16*
2 Jun	–1.79	11.27	2.72	24.48*

280–700 nm;, PA: PAR + UV-A, 315–700 nm; and P: PAR, 400–700 nm).

To assess the overall impact of solar UVR on our samples, we used data obtained from the *P* vs. *E* curves together with that of attenuation coefficients and solar irradiance to calculate the daily integrated loss of carbon fixation due to UVR, UV-A and UV-B in the euphotic zone (i.e. down to 1% of surface irradiance) (Fig. 5). We also considered the mean monthly irradiance as well as the mean irradiance during the day of experimentation to account for any variability in solar radiation (e.g. if the day of the experiment was the brightest of the month). In general, it was observed that UV-A was responsible for the bulk of UVR-induced photosynthetic inhibition, with maximum values of ~16.5% (i.e. from a total of 16.9%, 22 April); in other experiments; however, (e.g. 7 May), UV-A accounted for a smaller portion of total inhibition, ~6 out of 8.4%. The integrated inhibition due to UV-B was comparatively small (<2.5% in all experiments).

The biological characteristics of the area were different throughout the study period. Chl *a* (Fig. 6A)

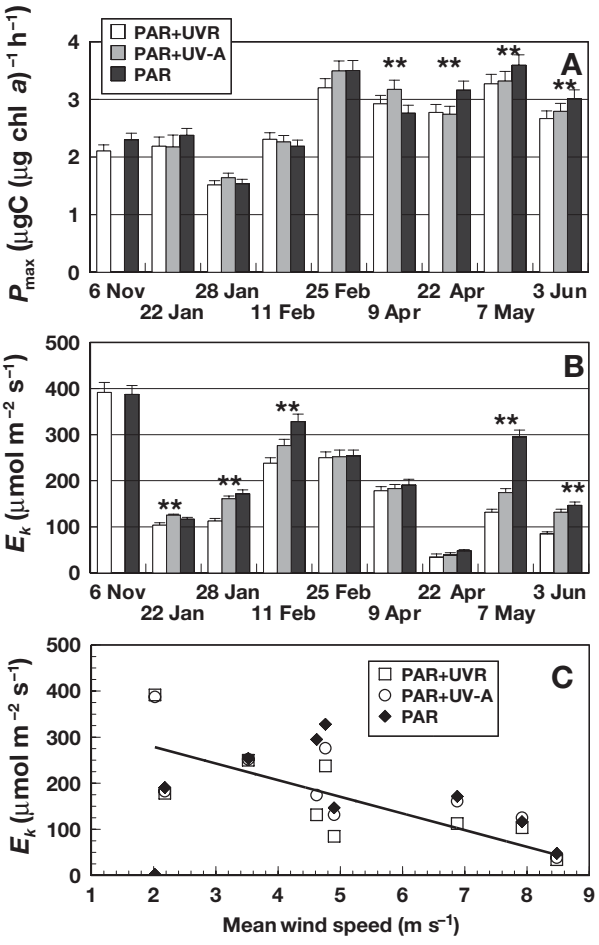

Fig. 4. Mean photosynthetic parameters for the 9 experiments carried out with waters collected from Bahía Nueva. (A) Mean P_{max}; (B) mean E_k. White bars: samples exposed to PAR + UVR; gray bars: samples PAR + UV-A; black bars: samples exposed to PAR only. **Significant differences ($p < 0.05$). (C) Relationship between E_k for the 3 radiation treatments (i.e. PAR + UVR, PAR + UV-A and PAR only) performed in each experiment and the mean wind speed of the previous week of experimentation. Line represents the best fit ($R^2 = -0.65$, $p < 0.001$)

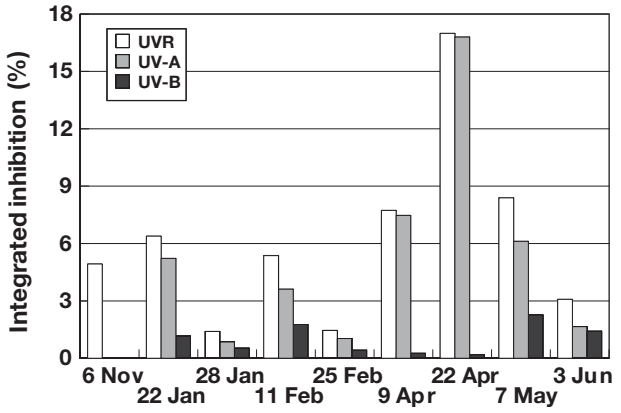

Fig. 5. Integrated photosynthetic inhibition in the euphotic zone due to UVR (white bars), UV-A (gray bars) and UV-B (black bars) during the 9 experiments carried out with Bahía Nueva waters

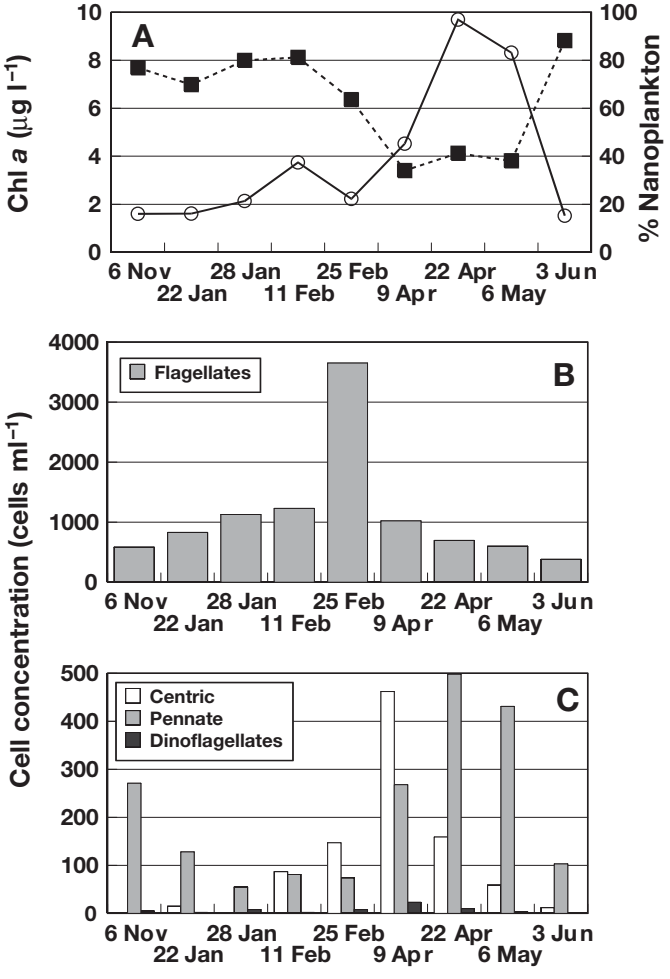

Fig. 6. Biological characteristics of samples collected at Bahía Nueva. (A) Total chl *a* concentration (○) and percentage of chl *a* in the nanoplankton fraction (<20 µm) (■); (B) unidentified monad/flagellate concentration; (C) centric diatom, pennate diatom and dinoflagellate concentration

reached maximum values during late April and May (i.e. ~8–10 µg l^{-1}), whereas during late spring, early summer and late fall, chl *a* values were <2 µg l^{-1}. Microplankton characterized the high chl *a* period, whereas pico-nanoplankton dominated the rest of the time, accounting for approximately 80% of chl *a* allocation. Unidentified monads/flagellates dominated in all experiments (Fig. 6B) and, with the exception of samples collected in late February (i.e. when they reached a concentration of ~3800 cells ml^{-1}), the concentration of these organisms always varied between 500 and 1000 cells ml^{-1}. The concentration of dinoflagellates (Fig. 6C) was low in all samples (i.e. <25 cells ml^{-1}), whereas that of diatoms varied between 55 and 730 cells ml^{-1}, with pennates generally

dominating over centric diatoms, with the exception of February and early April samples (Fig. 6C). There was a pattern of relatively high abundance of pennate diatoms during November, decreasing during the summer and increasing again towards fall (their highest concentration was ~500 cells ml^{-1} in late April). Centric diatoms reached a maximum concentration of ~470 cells ml^{-1} in early April. During late April and May, the most important diatom species was the pennate *Nitzschia longissima*, whereas in early April, the centrics *Skeletonema costatum* and *Chaetoceros* spp. were abundant, although *N. longissima* also made up an important fraction of the diatom community. Finally, it is interesting to note that we found a significant positive correlation between the integrated inhibition due to UVR and UV-A, and the concentration of pennate diatoms, with a correlation coefficient (Kendall's τ) of 0.929 ($p = 0.001$) and 0.857 ($p = 0.003$) for UVR- and UV-A-induced inhibition, respectively.

Bahía Camarones experiments

Fig. 7 shows the *P* vs. *E* curves, photosynthetic parameters and taxonomic composition in the 3 experiments carried out with samples collected at Bahía Camarones. *P* vs. *E* curves (Fig. 7A,D,G) were different in these experiments: The highest P_{max} values were determined in June (i.e. ~6 µg C (µg chl *a*)$^{-1}$ h^{-1}) (Fig. 7G,H), while the lowest values were determined during February (i.e. <1.5 µg C (µg chl *a*)$^{-1}$ h^{-1}) (Fig. 7D,E); the experiments carried out with samples collected in November displayed intermediate values (Fig. 7A,B). E_k values in these 3 experiments varied within the range 125–400 µmol m^{-2} s^{-1}. The impact of UVR on photosynthetic parameters was also different: UVR only had a significant effect on P_{max} in late November (Fig. 7B), whereas no significant differences between treatments were found in February and June experiments (Fig. 7F,I). On the other hand, UVR had a

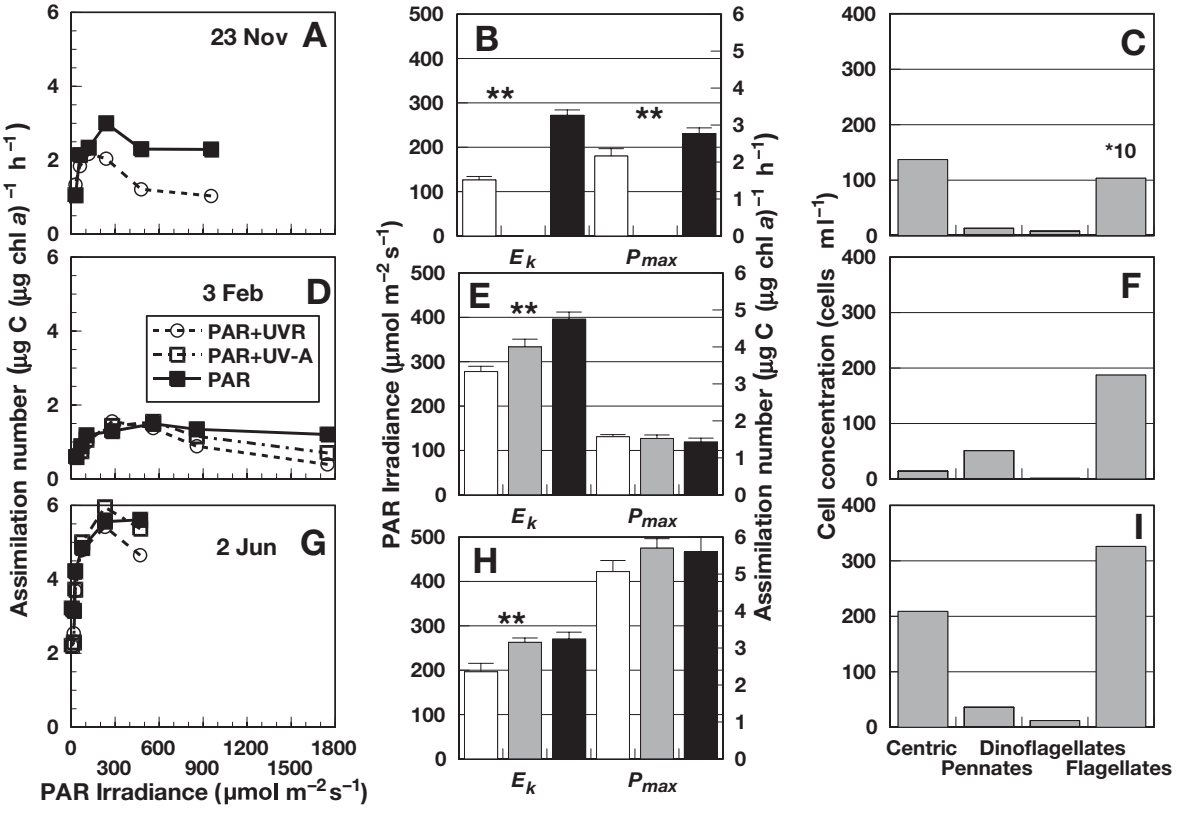

Fig. 7. *P* vs. *E* curves, photosynthetic parameters and species composition of samples collected at Bahía Camarones. (A,D,G) Phytoplankton assimilation numbers as a function of the mean PAR irradiance to which samples were exposed. The experiments were carried out on (A) November 23, 2002, (D) February 3, 2003, and (G) June 2, 2003. (B,E,H) Mean photosynthetic parameters, E_k and P_{max}, for the experiments carried out on (B) November 23, 2002, (E) February 3, 2003, and (H) June 2, 2003. Open bars: samples exposed to PAR + UVR; gray bars: samples PAR + UV-A; black bars: samples exposed to PAR only. (C,F,I) Phytoplankton concentration discriminated in centric and pennate diatoms, dinoflagellates and unidentified monads/flagellates for the experiments carried out on (C) November 23, 2002 (note the scale used for quantification of monads/flagellates), (F) February 3, 2003, and (I) June 2, 2003

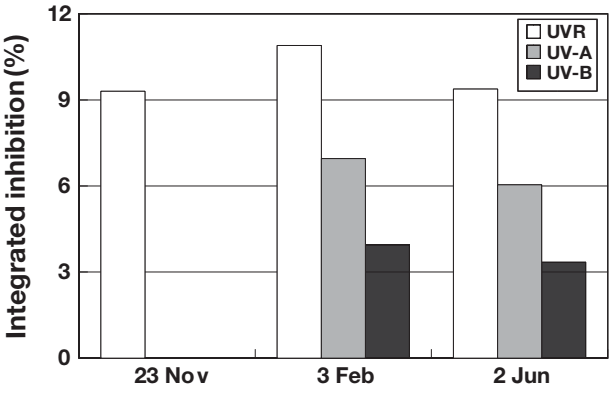

Fig. 8. Integrated photosynthetic inhibition in the euphotic zone due to UVR (white bars), UV-A (gray bars) and UV-B (black bars) during the 3 experiments carried out with Bahía Camarones waters

significant impact on E_k in the 3 experiments (Fig. 7B,E,H). In addition, UVR-induced photoinhibition was determined in all experiments (Figs. 7A,D,G). The biological characteristics of these samples were as follows: Chl *a* concentrations were 2.58, 1.10 and 2.54 µg chl *a* l^{-1} for November, February and June experiments, respectively, with phytoplankton cell concentrations varying between ~250 and 1200 cells ml^{-1}. The assemblages were always dominated by unidentified monads/flagellates (Fig. 7C,F,I); however, the diatom community structure was different in the 3 experiments, with a variable proportion of centrics/pennates. During November, the most important diatom species was the centric *Guinardia* sp., whereas during February, small pennates (10–30 µm in diameter) dominated the assemblage. On the other hand, small discoids diatoms (10–20 µm in diameter) characterized the diatom community during June. Dinoflagellates concentration was very low (<15 cells ml^{-1}) in the 3 samples collected at Bahía Camarones. We calculated the integrated UVR-induced photosynthetic inhibition within the euphotic zone (Fig. 8) and UV-A accounted for more than half of the total UVR-induced photosynthetic inhibition (i.e. with values up to 7%), while the integrated photosynthetic inhibition due to UV-B was lower, <4% in all experiments.

DISCUSSION

Temporal variability of *P* vs. *E* curves

The response of natural phytoplankton communities to solar radiation is highly variable, not only because of changes in the underwater radiation field (i.e. in turn due to variations in the zenith angle and in the absorption characteristics of the water body, Hargreaves 2003), but also because of changes in nutrient status, temperature and species composition occurring throughout the seasonal cycle. The Patagonia region, in the southern tip of South America (Fig. 1), presents a characteristic seasonal cycle in atmospheric conditions, with relatively high PAR and UVR levels during summer, which decrease towards winter (Orce & Helbling 1997, Barbieri et al. 2002, Villafañe et al. 2004a). The same pattern is also observed for ambient temperature, with mean values up to 31°C during summer and down to −3°C during winter (Villafañe et al. 2004a). In the present work, we have found that both atmospheric parameters, incident radiation and surface temperature (i.e. during our study period from late spring to late fall) (Fig. 2) are within the normal ranges previously determined in the area (Orce & Helbling 1997, Barbieri et al. 2002, Villafañe et al. 2004a). In addition, previous studies have determined a seasonal cycle in phytoplankton community structure and nutrient concentrations in the area of Golfo Nuevo (Gayoso 2001). We are, however, not aware, of similar studies carried out in the area of Bahía Camarones.

In the context of a system characterized by variable biological, chemical and physical parameters, we focused on the temporal variability of *P* vs. *E* relationships of phytoplankton assemblages of 2 near-shore sites of Patagonia. As expected, a range of responses to solar radiation, particularly in relation to the photosynthetic parameters P_{max} and E_k were found in assemblages sampled at different times of the year (Figs. 3, 4 & 7). For Bahía Nueva experiments, we determined relatively high P_{max} during the pre-bloom (i.e. late summer to late fall) (Figs. 3E–I & 4A), whereas low values were measured during the post-bloom (i.e. late spring to late summer) (Figs. 3A–D & 4A). It was not possible to establish a seasonal trend for P_{max} in Bahía Camarones due to the limited number of experiments performed, but we, nevertheless, also observed the highest value during fall (Fig. 7H) and the lowest in summer (Fig. 7E). The differences in P_{max} at different times of the year have been associated with variations in radiation levels and temperature (Shaw & Purdie 2001) and with changes in nutrients supply and taxonomic structure of the community (Côté & Platt 1983, Tillmann et al. 2000).

The range of P_{max} values are in the order of those found in previous studies carried out in other places along the Patagonia coast (i.e. Bahía Bustamante, Chubut) where summer (i.e. post-bloom) assemblages had P_{max} values <1.5 µg C (µg chl *a*)$^{-1}$ h^{-1} (Helbling et al. 2001a). These P_{max} values and the values reported here (Figs. 3, 4 & 7) are much lower than expected if only considering the effects of temperature (Behrenfeld & Falkowski 1997). Thus, the variations in photosynthetic parameters found in our study clearly hint at

a dependence on other environmental or biological parameters, as determined by Côté & Platt (1983) and Shaw & Purdie (2001). In fact, the low P_{max} determined in our post-bloom assemblages of late spring–early summer (Figs. 3A–D, 4A & 7A,D) appears to be closely related to nutrient limitation. Field experiments carried out by us (V. E. Villafañe unpubl. data) have shown that nutrient addition rapidly increased growth rates of summer phytoplankton communities in the study area, suggesting a natural nutrient-limited condition in these assemblages. Additionally, the variability of P_{max} in relation to temporal changes in the community structure has been thought to occur because the optical absorption cross-section of the photosynthetic apparatus, and hence P_{max}, varies between species (Falkowski et al. 1985); in fact, Finkel (2001) found a high correlation between P_{max} and the optical absorption cross-section of marine diatoms.

With regard to the biological structure of phytoplankton assemblages, our study is in good agreement with previous findings for the Bahía Engaño area (also on the Chubut coast), in which 3 'seasons' were clearly distinguished (Barbieri et al. 2002, Villafañe et al. 2004a): a post-bloom season (i.e. spring–summer), a pre-bloom season (i.e. fall) and a bloom period during winter, each one characterized by different chl *a* levels and taxonomic composition. Microplankton diatom concentrations increased during the pre-bloom season and small cells were especially abundant in the post-bloom period, as also seen here with phytoplankton assemblages in Bahía Nueva (Fig. 6). High chl *a* values towards the cold season seem to be a rather common feature in Patagonia (Gayoso 2001, Barbieri et al. 2002, Villafañe et al. 2004a), suggesting that these phytoplankton dynamics are not local or restricted to a small area, but rather occur along the coast. One common variable among the different sites seems to be the shallow UML conditions that favor the growth of microplankton diatoms and hence, the development of a winter bloom (Villafañe et al. 2004a). Moreover, relatively large diatoms dominated the pre-bloom and bloom periods, when wind speed was relatively low (Villafañe et al. 2004a). In this study, we calculated the mean wind speed during the week prior to our experiments (Fig. 4C) and we found that with low wind speeds, P_{max} increased significantly ($R^2 = -0.67$, $p < 0.05$), as did the concentration of centric diatoms ($R^2 = -0.72$, $p < 0.01$), which is also in agreement with previous findings in which we observed that centric diatoms bloomed and dominated during calm periods (Villafañe et al. 2004a).

Variations in irradiance levels are often related to changes in mixing conditions produced by wind, so that for the same irradiance conditions, phytoplankton cells within shallow UMLs are exposed to relatively higher levels than those circulating within deeper UMLs (Neale et al. 2003). Coastal areas of the Patagonia region are indeed characterized by a wide range of wind speeds, with high values during spring–summer, whereas wintertime is a relatively calm period (Villafañe et al. 2004a). Besides this seasonal pattern, there is also a high intra-seasonal variability in wind speeds; thus, natural phytoplankton populations may be exposed to relatively large fluctuations in irradiance as a consequence of variable UMLs (Barbieri et al. 2002). As E_k can be considered as an indicator of the photoacclimation status of cells, it is expected that fluctuating light regimes have a significant impact on E_k. In fact, E_k changes according to the irradiance fluctuations in the water column but there is never a complete match between E_k and the instantaneous irradiance because light acclimation takes some time (Sakshaug et al. 1997). However, it should be noted that different time scales of photoacclimation are possible, according to the process being evaluated. Short-term scales (i.e. minutes) can be expected when evaluating electron transport; a period <1 h would be required if the xantophyll cycle is studied in relation to the photosystem II (PSII), or it would take hours–days for redox processes leading to changes in chl *a* concentration (Sakshaug et al. 1997). In our study, we found that E_k was very variable and did not show a seasonal pattern either in Bahía Nueva (Fig. 4B) or in Bahía Camarones experiments (Fig. 7B,E,H) and, moreover, no apparent relationship between E_k and taxonomic composition (Fig. 6 & 7) was determined. However, we did find a significant inverse relationship between wind speed and E_k (Fig. 4C), suggesting an acclimation to the new mixing conditions, as with high wind speeds, the UML depth would deepen, and thus, the mean irradiance received by the cells would be lower, consequently resulting in a relatively small E_k value.

The effects of solar UVR

Among the many effects induced by exposure to UVR, one of the most studied has been the reduction of photosynthetic rates in phytoplankton cells (see review of Villafañe et al. 2003). Many studies have shown a relatively high surface inhibition due to UVR, with this inhibition decreasing with depth. However, it has been found that surface inhibition is not a good estimator of water column integrated inhibition, and indeed, the inhibition at different irradiances (i.e. depths) should be considered when assessing the overall impact of UVR in a water body (Villafañe et al. 2001). The maximum UVR-induced integrated inhibition in our study area (i.e. <17%) is much less than that estimated for both polar areas, ~20% each for UV-A and UV-B

(Helbling & Villafañe 2002). Hence, the impact of UVR on Patagonian waters would probably be much less than that estimated for polar areas, but on the other hand, higher when compared to tropical sites, where UVR-induced photosynthetic inhibition was reported to be comparatively low (Helbling et al. 1992, 2003).

The different photosynthetic responses of phytoplankton to UVR have been frequently associated with species-specific sensitivity, with the concomitant differences in their acclimation and repair mechanisms once the effect (or damage) has occurred (Roy 2000, Banaszak 2003, Buma et al. 2003, Villafañe et al. 2003). Such acclimation/repair mechanisms essentially include: (1) avoidance (through movements away from the source of radiation, habitat selection, etc.), (2) screening, either by extra cellular features (cuticles, sheaths, etc.) or intracellular protective compounds (e.g. mycosporine-like amino acids, MAAs), (3) repair of both direct and indirect UVR damage (DNA and protein repair, antioxidant enzymes, etc.), and (4) short- and long-term acclimation (Roy 2000). For example, reversible changes (which take minutes to hours) such as those of fluorescence or heat dissipation (via the xanthophyll cycle, a major photoprotective process) or energy redistribution between photosystems may occur (Roy 2000). Although we did not specifically test differences in acclimation/repair among the assemblages, we evaluated one of them, by measuring the amount of UV-absorbing compounds (i.e. MAAs) in each sample. The presence of such compounds is one of the mechanisms that organisms may have to protect themselves against harmful levels of UVR. These mechanisms, hence, have a favorable effect on their growth and general performance (Banaszak 2003), including primary productivity (Neale et al. 1998). UV-absorbing compounds, however, were not present in significant amounts in any of the samples collected in Bahía Nueva and Bahía Camarones (data not shown), suggesting that this was not the most important photoprotective mechanism that phytoplankton used (at least during the study period) to cope with potentially damaging UVR levels. Indeed, other protective mechanisms might be of importance for phytoplankton in this area and they should be addressed in future studies aiming to determine the overall impact of UVR on phytoplankton from Patagonia. So far, we have evidence that DNA repair mechanisms are active in phytoplankton assemblages in the Patagonia area, as seen in studies carried out by Helbling et al. (2001a), Buma et al. (2001) and Villafañe et al. (2004b).

Although our knowledge of UVR-induced effects on phytoplankton has increased significanty, few studies have specifically addressed the impact of UVR on *P* vs. *E* parameters. This is surprising, as many models used together with remote sensing information are based on these parameters (e.g. Behrenfeld & Falkowski 1997); thus, if UVR effects are not taken into account, primary production might occasionally be overestimated. The reported effects of UVR on *P* vs. *E* parameters are varied. Studies carried out by Furgal & Smith (1997) and Montecino et al. (2001) have determined significant effects of UVR on P_{max}; Montecino & Pizarro (1995), on the other hand, working with phytoplankton communities off the Chilean coast collected during different seasons, did not find significant differences in P_{max}, regardless of the radiation treatment to which the cells were exposed. We are not aware of any study that specifically addressed the effects of UVR on the light saturation parameter. In our study, we determined different responses in *P* vs. *E* parameters when phytoplankton cells were exposed to different radiation treatments. Significant UVR effects on P_{max} were mostly due to UV-A (i.e. in the pre-bloom assemblages from Bahía Nueva [Fig. 4A, Table 1] and in the late spring assemblages from Bahía Camarones [Fig. 7B, Table 1]), as also seen in studies evaluating the impact of UVR on primary production rates (see review of Villafañe et al. 2003). Differences in the impact of UVR on *P* vs. *E* parameters may also be related to the taxonomic structure of the assemblages. In fact, we determined (at least in Bahía Nueva experiments) that those assemblages characterized by relatively more pennate diatoms were significantly affected by UVR (Figs. 4A & 6C). This is in agreement with previous research (Helbling et al. 1996), where it was reported that pennate diatoms are highly sensitivity to UVR. In regard to the timing of the UVR impact on photosynthetic parameters, it is somehow contradictory that although radiation levels during fall are relatively low (as compared to those in summer), P_{max} was significantly affected by UVR. However, as reported by Villafañe et al. (2004a) in a time series study conducted at Bahía Engaño, phytoplankton receiving high radiation levels during summer were relatively less inhibited by UVR as assessed by Biological Weighting Functions (BWF), probably due to their acclimation to higher radiation levels. Our data, which show a significant impact of UVR on photosynthetic parameters during fall, also agree with previous findings (Helbling et al. 1994), in which photosynthesis of microplankton diatoms was more inhibited by UVR (provided that cells did not synthesize UV-absorbing compounds) than in nanoplankton cells.

With regard to the light saturation parameter, in Bahía Nueva samples, E_k values were only significantly reduced by UVR in some experiments (Fig. 4B), whereas in Bahía Camarones samples, UVR significantly reduced E_k in all samples (Fig. 6B,E,H). Since E_k is related to the previous light history of cells, it is

expected that UVR should have a differential impact when cells come from a relatively deep UML (i.e. during the windy season) as seen in summer samples (Fig. 4B), compared to cells from shallow UMLs. Previous studies (Helbling et al. 2001b) have also shown that cell size is very important in determining the acclimation of cells to the new irradiance conditions, with nanoplankton cells acclimating much faster than microplankton cells. Thus, the significant decreases in E_k observed during late fall is probably related more to species composition than to UML depth.

Our study thus indicates, on the one hand, that fall/winter, environmental conditions in the area (i.e. low wind speeds together with relatively high nutrient concentrations and shallow UMLs) favor the development of microplankton diatoms with a relatively high P_{max}; such assemblages might, however, be more affected by natural UVR levels. On the other hand, our data also suggest that any model using *P* vs. *E* parameters to estimate global primary production or carbon fluxes should consider the impact (or lack thereof) of UVR on these parameters to more accurately estimate CO_2 uptake by phytoplankton.

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