

Experimental assessment of UV effects on temperate marine phytoplankton when exposed to variable radiation regimes

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Abstract

Phytoplankton samples were collected at Bahía Engaño, Chubut, Argentina (43°S, 65°W) at different times of the year to assess the combined effect of ultraviolet radiation (UVR, 280–400 nm) and vertical mixing (i.e., the depth of the upper mixed layer, UML) on photosynthesis. Samples were exposed to fixed and fluctuating radiation regimes in an illuminated chamber at 15°C (photosynthetically available radiation [PAR] = 66 W m⁻²; UV-A = 15.3 W m⁻²; UV-B = 0.7 W m⁻²), receiving either PAR + UVR or PAR only. A comparison between fixed and rotating systems showed that when $Z_{\text{UML}}/Z_{\text{Eu}} = 0.6$ (i.e., 60% of the euphotic zone [Eu] was mixed), only postbloom assemblages (codominated by nanoplanktonic flagellates and diatoms [*Chaetoceros* spp.]) were affected significantly by UVR. Integrated carbon fixation values during pre- and postbloom periods were higher under mixed conditions than under fixed irradiances. However, during the bloom (dominated by the microplanktonic diatom *Odontella aurita*), phytoplankton exposed to fluctuation radiation regimes had lower integrated carbon fixation. When post-bloom samples were exposed to different mixing conditions, integrated UVR-induced inhibition reduced carbon fixation by 11–13% when $Z_{\text{UML}}/Z_{\text{Eu}} = 0.6$, whereas when $Z_{\text{UML}}/Z_{\text{Eu}} = 0.91$, carbon fixation increased by 7–12%. The differences in responses observed between prebloom, bloom, and postbloom samples can be attributed to a number of factors, such as the light history of cells, taxonomic composition, and size structure of the community and most probably reflect the different inhibition kinetics of these assemblages.

Phytoplankton are normally exposed to fluctuating radiation regimes in their natural environment. In a specific geographic location, radiation levels reaching the Earth's surface, both photosynthetically available radiation (PAR, 400–700 nm) and ultraviolet radiation (UVR, 280–400 nm), vary throughout the year, mainly because of changes in solar zenith angle (Madronich 1993). Natural radiation fluctuations also occur within temporal scales ranging from days to minutes because of changes in cloudiness and ozone conditions (Lubin and Jensen 1995) as well as mixing depth (Denman and Gargett 1983), potentially affecting organisms in different ways. Phytoplankton, whose movement in the water column mostly depends on turbulent motions, experience significant fluctuations in radiation regimes because of changes in the depth of the upper mixed layer (UML), which take place mainly as a result of variations in weather conditions, such as sun heating, wind stress, or storm activity (Neale et al. in press).

The most obvious effects of fluctuating radiation regimes on phytoplankton include changes in their photoacclimation (Falkowski and Wirick 1981; Cullen and Lewis 1988), with

concomitant variations in P-E parameters, fluorescence yield, or cellular chemical composition (Marra 1978; Denman and Gargett 1983; Cullen and Lewis 1988). Although most of these studies have considered the responses of organisms under variable PAR regimes, it is now recognized that fluctuating UVR levels such as those produced by mixing can also affect the performance and fitness of aquatic organisms (Helbling et al. 1994; Neale et al. 1998a,b, in press; Zagarese et al. 1998; Huot et al. 2000; Xenopoulos et al. 2000; Köhler et al. 2001). Very few studies have addressed the combined effects of variable UVR regimes and vertical mixing on phytoplankton photosynthesis (Helbling et al. 1994; Neale et al. 1998a,b, in press; Köhler et al. 2001).

In the Atlantic coastal area, very few studies have determined on-site phytoplankton marine primary productivity rates (Charpy and Charpy-Roubaud 1980; Buma et al. 2001; Helbling et al. 2001a). On the Patagonia coast, research has just started to evaluate the effects of UVR on phytoplankton (Villafañe et al. 2001) and determining its effect on photosynthesis and DNA material (Buma et al. 2001; Helbling et al. 2001a); however, none of these studies have specifically addressed the effect of fluctuating radiation regimes on these processes. These types of studies are of great importance for this region, where aquatic organisms are normally exposed to changes in radiation regimes, especially by the alternation of strong winds with calm periods, which ultimately affects the depth of the UML. In this area, dense phytoplankton blooms develop during winter, characterized by the dominance of the diatom *Odontella aurita*, that sustain a very rich coastal fishery (Villafañe et al. 1991). Therefore, the aim of this study is to determine the effects of variable UVR and PAR on the integrated primary production of natural phytoplankton populations characteristic of coastal Patagonian waters.

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Materials and methods

Sampling site—Studies were carried out with natural phytoplankton populations collected at Bahía Engaño, Chubut, Argentina (43°S, 65°W) from March to December 2000 at a coastal station denominated Egi. This station, located at the mouth of the Chubut River estuary, was found to be highly variable with regard to its physical and biochemical characteristics (Villafañe et al. 1991; Helbling et al. 1992a); thus, a continuous monitoring of phytoplankton species composition, chlorophyll *a* (Chl *a*), UV-absorbing compounds, surface water temperature, and conductivity was carried out every 10–20 d since 1999. In addition, vertical profiles of temperature and irradiance were determined during several cruises using a submersible ELDONET filter radiometer (Real Time Computers), with sensors for UV-B (280–315 nm), UV-A (315–400 nm), PAR (400–700 nm), temperature, and depth. Data from these profiles were used to calculate attenuation coefficients for solar radiation and to estimate the depth of the UML (i.e., Z_{UML}) based on the temperature data from the profiling radiometer and conductivity data obtained with a Horiba probe. Experiments to determine the effects of UVR on phytoplankton photosynthesis under a variable radiation field were carried out during prebloom, bloom, and postbloom conditions. Samples were collected during high tide with an acid-clean (1 N HCl) carboy, dispensed into a plastic container, and immediately taken to the laboratory at Estación de Fotobiología Playa Unión (EFPU), where experiments were conducted as described below.

Experimentation—Because the study area is highly variable in terms of wind speed and duration (thus conditioning the depth of the UML), two different approaches were implemented to study the combined effects of UVR and vertical mixing on phytoplankton photosynthesis. In the first type of experiments, samples collected at different times of the year were exposed to a variable irradiance field, as if 60% of the euphotic zone (Eu) was mixed (i.e., $Z_{\text{UML}}/Z_{\text{Eu}} = 0.6$). In these experiments, we wanted to establish a common level of comparison (i.e., similar mixing and irradiance conditions) for natural phytoplankton populations that had different light histories and acclimations to solar radiation. These experiments were done during March (prebloom), June (bloom), and December (postbloom) 2000. In another set of experiments, samples with the same light history and acclimation (i.e., the same phytoplankton population) were exposed to increasing levels of mixing ($Z_{\text{UML}}/Z_{\text{Eu}} = 0.6, 0.76,$ and 0.91), thus simulating a deepening of the UML. These experiments were conducted during spring (early December 2000), which is the windy season in the study area.

An experimental device, similar to that described in Helbling et al. (1994) with two systems, one fixed and one rotating, was used in these experiments. Both systems had various layers of neutral-density screens that allowed attenuation of incident radiation (from 100% to either 6, 3, or 1.5%), thus approximately simulating the irradiance field received by cells in different portions of the euphotic zone (although they did not mimic the differential attenuation of UVR and PAR in the water column). Both systems were placed inside an illuminated chamber (*see below*); the sam-

ples in the fixed system were incubated at the same irradiance level during the whole incubation period, whereas the samples in the rotating system were gradually moved to the next irradiance level every 30 min. The experiments with $Z_{\text{UML}}/Z_{\text{Eu}} = 0.6$ lasted 5 h for a complete rotation (five levels of irradiance), whereas the experiments with $Z_{\text{UML}}/Z_{\text{Eu}}$ ratios of 0.76 and 0.91 lasted 6 and 7 h (six and seven levels of irradiance, respectively). Samples in each irradiance level were exposed to either UVR + PAR (samples in quartz tubes) or to PAR only (samples in quartz tubes covered with Ultraphan UV Opak [Digefra]), 50% transmission at 395 nm. A total of four tubes (duplicate samples for PAR + UVR and PAR) were exposed in each irradiance level in each system.

For all experiments, surface phytoplankton samples were placed in 50-ml quartz tubes, inoculated with labeled sodium bicarbonate (*see below*), and incubated at 15°C in an illuminated chamber (Sanyo model MLR 350) with five Q-Panel UVA 340 lamps and 10 Phillips daylight lamps, which provided irradiances of 66, 15.3, and 0.7 W m⁻² for PAR, UV-A, and UV-B, respectively. In our experimental device, these irradiances represented the 100% level.

Analysis and measurements—Photosynthetic rates were determined using the technique described in Holm-Hansen and Helbling (1995). Briefly, samples were incubated with 5 μ Ci of NaH¹⁴CO₃ (0.185 MBq), and after the incubation period, they were filtered onto Whatman GF/F glass fiber filters (25 mm diameter), put in 7-ml scintillation vials, and exposed to HCl fumes overnight. Then, the samples were dried and counted using standard liquid scintillation techniques.

For Chl *a* analysis, an aliquot of 100 ml of sample was filtered onto Whatman GF/F filters (25 mm) and the photosynthetic pigments extracted in absolute methanol (Holm-Hansen and Rieman 1978). Chl *a* determination was done by fluorometric methods (Holm-Hansen et al. 1965). Chl *a* concentration in the pico-nanoplankton fraction (<20 μ m diameter) was determined as previously described, except that the sample was pre-filtered through a 20- μ m Nitex® mesh. The fluorescence of the methanolic extract was measured in a Turner Designs fluorometer (model TD 700) before and after acidification, and Chl *a* concentration was calculated from these readings. The fluorometer was calibrated using pure Chl *a* from *Anacystis nidulans* (Sigma C6144).

We also determined the presence of UV-absorbing compounds in the samples. For this analysis, an aliquot of 500–1,000 ml of water sample was filtered onto Whatman GF/F filters (47 mm), and the photosynthetic pigments and UV-absorbing compounds were extracted overnight at 4°C in 7 ml of absolute methanol. The estimation of concentration of these UV-absorbing compounds (Helbling et al. 1996) was done by peak analysis (Microcal Origin Software) of the scans (250–750 nm) with a Hewlett Packard spectrophotometer (model 8453E).

Floristic analyses were made of phytoplankton samples fixed with buffered formalin (final concentration in the sample, 0.4%). Samples were settled overnight using 10- or 25-ml cylinders, and qualitative and quantitative analyses were done with an inverted microscope.

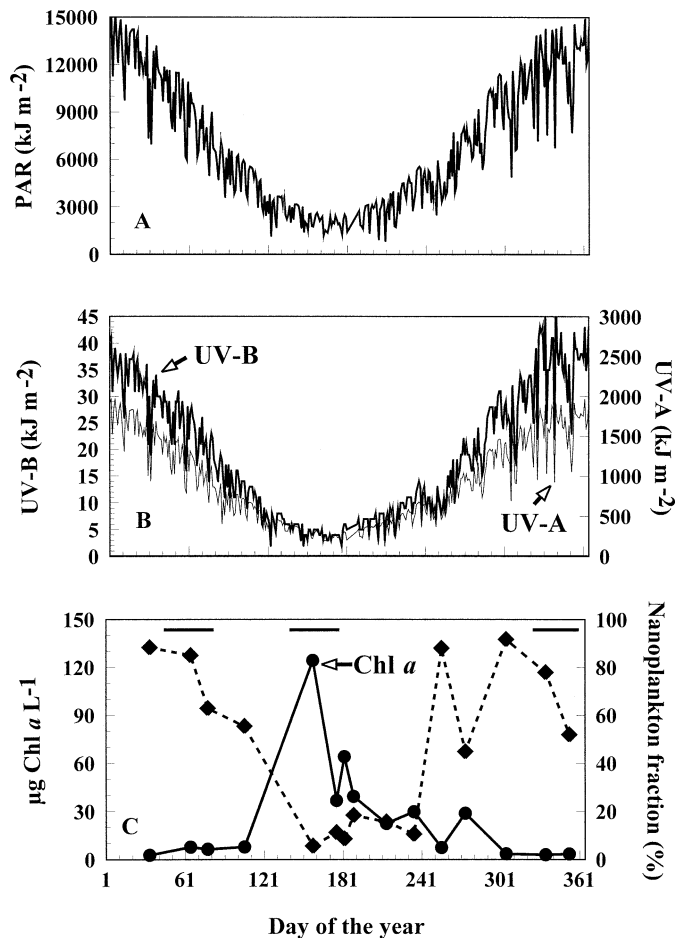


Fig. 1. General characteristics of the study area during the year 2000 as a function of time (day of the year). (A) Daily doses of PAR (400–700 nm); (B) daily doses of UV-A (315–400 nm) and UV-B (280–315 nm). Units are kJ m^{-2} . (C) Phytoplankton concentration, as estimated by Chl *a* ($\mu\text{g L}^{-1}$), and percentage of nanoplanktonic cells. The three horizontal lines indicate the time of sampling of prebloom, bloom, and postbloom assemblages and experimentation.

Radiation levels in the illuminated chamber were measured using a Groβel portable cosine sensor (model GMBH) that was previously intercalibrated with a terrestrial ELDONET radiometer (Real Time Computers). This latter instrument, which is permanently installed on the roof of the EFPU, collects data on incident solar radiation in broad bands for PAR, UV-A, and UV-B at a frequency of once per minute (Villafañe et al. 2001).

Results

The general conditions (solar radiation, Chl *a* concentration) of the study area are presented in Fig. 1. Incident solar radiation throughout the year (Fig. 1A,B) had a high day-to-day variability because of changes in cloud cover. Daily doses of PAR (Fig. 1A) varied between $15,000 \text{ kJ m}^{-2}$ and $1,500 \text{ kJ m}^{-2}$ for summer and winter, respectively. UVR daily doses had a similar pattern of high values during summer and

low during winter, with UV-A varying between $\sim 2,000$ and 250 kJ m^{-2} (Fig. 1B) and UV-B from ~ 45 to 5 kJ m^{-2} (Fig. 1B). Phytoplankton abundance, as estimated by Chl *a* concentrations, also varied seasonally (Fig. 1C), with high values ($\sim 125 \mu\text{g Chl a L}^{-1}$) during winter and low during summer ($< 5 \mu\text{g Chl a L}^{-1}$). There was an inverse relationship (i.e., first-order exponential decay, $r^2 = 0.82$) between the total Chl *a* concentration and the allocation of Chl *a* in the pico-nanoplankton fraction ($< 20 \mu\text{m}$), so that those samples collected during winter (with high Chl *a* content during the bloom condition), were dominated by microplanktonic cells ($> 20 \mu\text{m}$), whereas samples with low Chl *a* concentration (pre- and postbloom conditions) were dominated by pico-nanoplankton. Floristic analysis revealed a general pattern of diatom-dominated microplanktonic populations, with *Odontella aurita* being the most important species, accounting for $\sim 82\%$ of total cells during the winter bloom (mean = $4,625 \text{ total cells ml}^{-1}$). Samples dominated by small cells were generally characterized by the presence of monads and flagellates during the prebloom period, whereas those collected at the end of December (postbloom) also had an important contribution of nanoplanktonic diatoms ($\sim 39\%$ of total cells) of the genus *Chaetoceros* (mean = $2,236 \text{ total cells ml}^{-1}$).

We compared the combined effect of UVR and mixing on these phytoplankton populations (prebloom, bloom, and postbloom samples) when exposed to the same irradiance condition simulating $Z_{\text{UML}}/Z_{\text{Eu}} = 0.6$ (Fig. 2). In the fixed system, UVR-induced photoinhibition depended on the irradiance level received by cells, so that at high relative irradiances (i.e., 66 W m^{-2}), pre- and postbloom samples (Fig. 2A,C, respectively) were significantly inhibited at $\sim 25\%$, whereas those collected during the bloom were inhibited at 16% (Fig. 2B). This inhibitory effect decreased with decreasing irradiance level, so in those samples that received 4.12 W m^{-2} (i.e., the lowest level of irradiance used in these experiments), the effect was reversed and a negative value was obtained, indicating more carbon fixation when cells received UVR. This higher carbon fixation in the samples exposed to UVR + PAR, at irradiances $< 16.5 \text{ W m}^{-2}$, was significantly evident ($p < 0.05$) in prebloom samples collected in March (16 and 41% for the 8.25 and 4.12 W m^{-2} irradiance levels, respectively; Fig. 2A). The samples collected in December (postbloom) and exposed to UVR + PAR had a 13% increase in carbon fixation at the 4.12 W m^{-2} irradiance level (Fig. 2C), as compared to samples receiving only PAR, whereas bloom samples had an enhancement of 13 and 30% for the 8.25 and 4.12 W m^{-2} irradiance levels, respectively (Fig. 2B). On the other hand, samples incubated under fluctuating irradiances (i.e., a rotating system) that received a mean irradiance of 25.6 W m^{-2} were not significantly inhibited by UVR (Fig. 2A,B), except for postbloom samples that had a significant photoinhibition of $\sim 10\%$ (Fig. 2C). The maximum photosynthetic rates during prebloom, bloom, and postbloom were 27 , 210 , and $32 \text{ mg C m}^{-3} \text{ h}^{-1}$, respectively.

Data from these experiments (i.e., with a simulated $Z_{\text{UML}}/Z_{\text{Eu}} = 0.6$) were used to calculate the effects of variable irradiance (i.e., mixing) on the integrated primary productivity of the top 60% of the euphotic zone (Fig. 3). This sim-

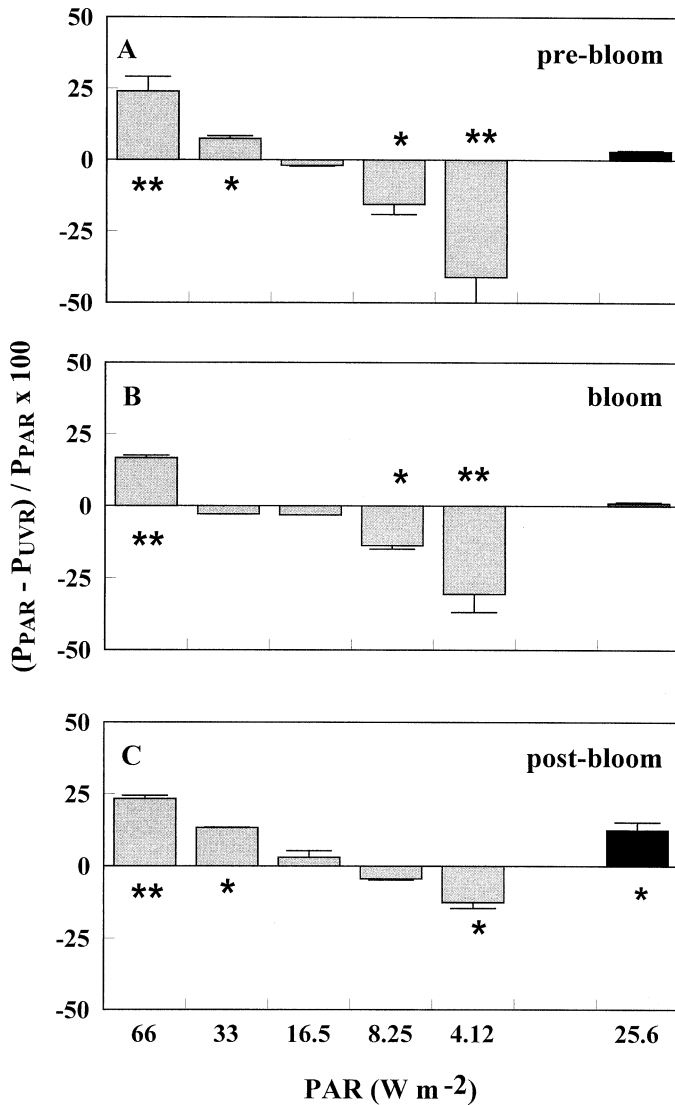


Fig. 2. Data on the inhibition by UVR as a function of irradiance (calculated as $[(P_{\text{PAR}} - P_{\text{UVR}})/P_{\text{PAR}}] \times 100$) for different phytoplankton assemblages. (A) Prebloom samples collected during March 2000; (B) bloom samples collected during June 2000; (C) postbloom samples collected during December 2000. The gray bars represent samples incubated in the fixed system at different irradiances, and the black bars represent the sample in the rotating system. Kruskal-Wallis test: * $p < 0.05$, ** $p < 0.01$.

ulated upper 60% of the euphotic zone corresponded to a depth of about 12 m, for an attenuation coefficient $K_{\text{PAR}} = 0.24 \text{ m}^{-1}$ during prebloom and postbloom conditions. There were striking differences, between both pre- and postbloom samples, with higher values of carbon fixation in the samples exposed to a variable irradiance field and lower values in the samples exposed to fixed irradiances. Bloom samples, on the other hand, presented lower values of integrated photosynthesis when exposed to simulated mixing conditions, as compared to those in the fixed system. The integrated carbon fixation (i.e., 60% of the euphotic zone) of the samples exposed to UVR + PAR was 715 and 838 $\text{mg C m}^{-2} \text{ h}^{-1}$ for the rotating and fixed systems, respectively. There were also

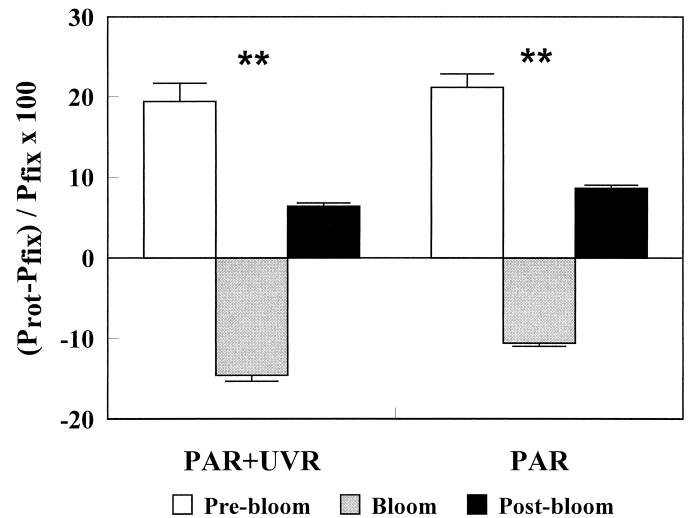


Fig. 3. Comparison of integrated photosynthesis between fixed and rotating systems (calculated as $[(P_{\text{rot}} - P_{\text{fix}})/P_{\text{fix}}] \times 100$) for the PAR + UVR and PAR-only radiation treatments. Kruskal-Wallis test: * $p < 0.05$, ** $p < 0.01$.

variations between samples exposed to UVR + PAR and PAR only, with the relative difference between systems (i.e., $P_{\text{rot}} - P_{\text{fix}}$) being smaller in pre- and postbloom samples and slightly higher during the bloom.

Phytoplankton assemblages collected during the post-bloom (i.e., December) were exposed to a variable irradiance field simulating an increase in $Z_{\text{UML}}/Z_{\text{Eu}}$ ratios from 0.6 to 0.91 (Fig. 4). All samples incubated at fixed irradiances exhibited significant UVR-induced photoinhibition (~ 13 – 25%) at irradiance levels $> 16.5 \text{ W m}^{-2}$. Samples receiving intermediate irradiances did not exhibit significant inhibition because of UVR, whereas samples exposed to low irradiances ($\leq 4.12 \text{ W m}^{-2}$) did have significant negative values, as much as 25% in samples receiving $\sim 2 \text{ W m}^{-2}$ (Fig. 4B,C). On the other hand, in the samples exposed to a fluctuating irradiance field, UVR either photoinhibited or increased carbon fixation according to the portion of the euphotic zone that was being mixed (i.e., depending on the $Z_{\text{UML}}/Z_{\text{Eu}}$ ratio). There was a significant UVR-induced photoinhibition of 13% (Fig. 4A) when the samples were in a simulated $Z_{\text{UML}}/Z_{\text{Eu}} = 0.6$ (i.e., shallow mixing). The photoinhibition was not significantly different from zero when the $Z_{\text{UML}}/Z_{\text{Eu}}$ relation increased to 0.76 (Fig. 4B); however, carbon fixation increased significantly (12%) in the samples exposed to UVR + PAR (negative values in Fig. 4C) when the $Z_{\text{UML}}/Z_{\text{Eu}}$ ratio further increased to 0.91.

The integrated inhibition of the postbloom samples for the three mixing conditions is shown in Fig. 5. The integrated inhibition due to UVR varied from a positive effect (i.e., inhibition of photosynthesis) under a shallow mixing condition to a negative effect (i.e., enhancement of photosynthesis) when $Z_{\text{UML}}/Z_{\text{Eu}} = 0.91$. Although this was observed in both fixed and rotating systems, there were significant differences among them, with a higher effect (either positive or negative) in those samples exposed to a variable radiation field. The maximum difference between the two systems was $\sim -5\%$ under deep mixed conditions (i.e., $Z_{\text{UML}}/Z_{\text{Eu}} = 0.91$).

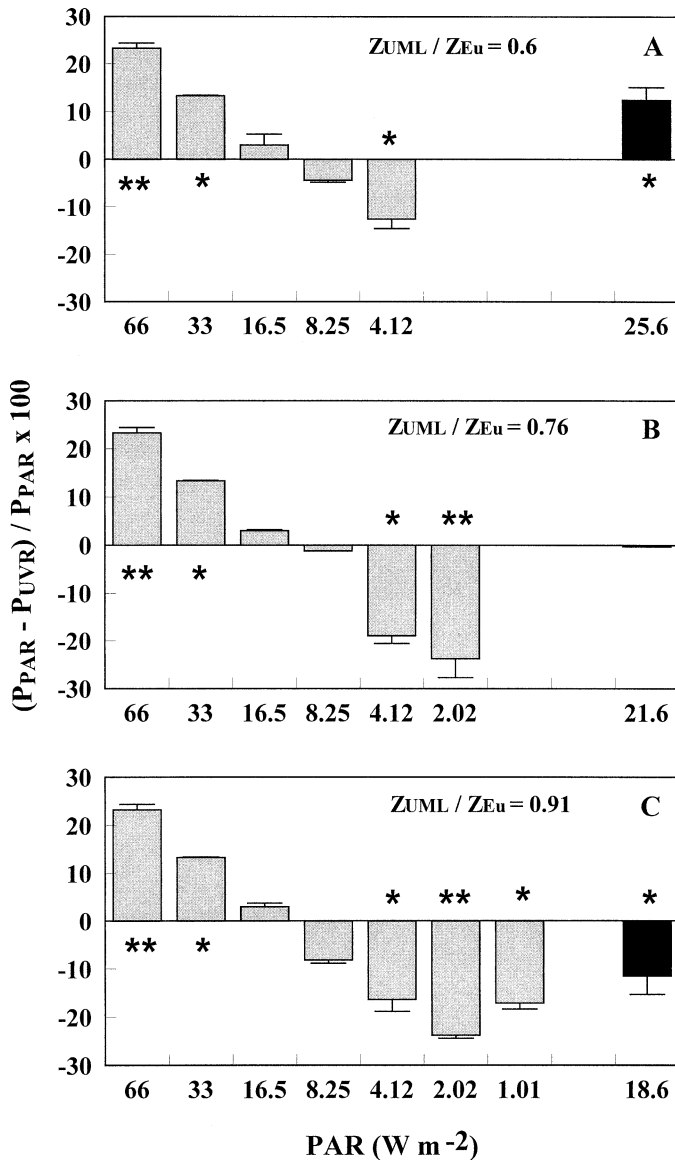


Fig. 4. Data on inhibition by UVR as a function of the irradiance (calculated as $[(P_{PAR} - P_{UV})/P_{PAR} \times 100]$) for a postbloom phytoplankton assemblage exposed to different mixing conditions. (A) $Z_{UML}/Z_{Eu} = 0.6$. (B) $Z_{UML}/Z_{Eu} = 0.76$. (C) $Z_{UML}/Z_{Eu} = 0.91$. The gray bars represent the samples incubated in the fixed system, whereas the black bars represent the sample in the rotating system. Kruskal-Wallis test: * $p < 0.05$, ** $p < 0.01$.

Discussion

Solar radiation, especially UVR, is a very important environmental variable that affects photosynthesis of aquatic autotrophic organisms, inhibiting or even enhancing carbon fixation by cells (Villafañe et al. in press, and references therein). Radiation levels received by aquatic organisms depend on several factors, such as the concentration of particles and dissolved organic and inorganic material (Kirk 1994). Vertical mixing is also especially important, considering that, under the same incident radiation and particle concentration, cells moving within a shallow Z_{UML} will be exposed to a

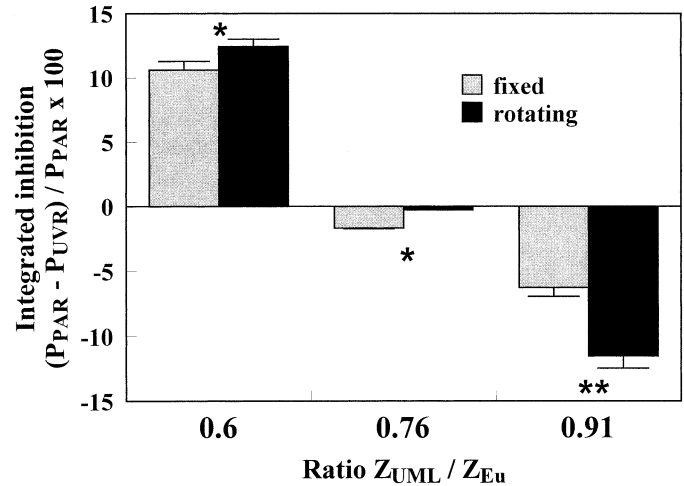


Fig. 5. Comparison of integrated inhibition of photosynthesis (calculated as $[(P_{PAR} - P_{UVR})/P_{PAR} \times 100]$) when samples were exposed to different mixing conditions. The gray bars represent the samples incubated in the fixed system, whereas the black bars represent the samples in the rotating system. Kruskal-Wallis test: * $p < 0.05$, ** $p < 0.01$.

higher mean irradiance than those within a deep Z_{UML} (Helbling et al. 1994; Neale et al. in press). A number of studies have considered the relationship between fluctuating radiation regimes (as produced by mixing) and aquatic primary productivity. For example, the pioneer work of Sverdrup (1953) highlighted the importance of not only vertical mixing per se, but also the portion of the euphotic zone that was being mixed. Early studies of Marra (1978) showed that vertical mixing enhanced primary production in the water column. Furthermore, Mitchell and Holm-Hansen (1991) concluded that the depth of the UML was a key factor for the initiation of blooms in Antarctica, so that with shallow Z_{UML} (< 25 m), blooms could develop. Helbling et al. (1994) considered the combined effects of solar UVR and vertical mixing on Antarctic phytoplankton photosynthesis, showing for the first time that Z_{UML} was very important in conditioning UVR inhibition. In their study, they demonstrated that with shallow Z_{UML} , the short-term effect of UVR greatly enhanced photoinhibition as compared to samples under deep Z_{UML} conditions. Neale et al. (1998a,b), working with phytoplankton assemblages from the Weddell Scotia Confluence, showed the combined effects of mixing and column ozone concentrations, obtaining similar results to those of Helbling et al. (1994).

We demonstrated in this study that UVR-induced photoinhibition depends on several factors, with the time of sampling, and hence the light history of cells (Fig. 1), being especially important. The differential UVR responses that we found between seasons were expected, in part because of the different taxonomic composition and size structure of the phytoplankton assemblages. The bloom consisted of microplanktonic cells (i.e., centric diatoms), whereas the prebloom samples were dominated by nanoplanktonic monads and flagellates (Fig. 1C); postbloom samples, on the other hand, were codominated by nanoplanktonic flagellates and diatoms of the genus *Chaetoceros*. Many studies have demonstrated

differential sensitivity of phytoplankton to UVR. For example, studies carried out with Antarctic assemblages have shown that, in general, flagellates are more vulnerable than diatoms (Villafañe et al. 1995a). Studies carried out by Kopczyńska (1992) and Villafañe et al. (1995b) have determined the ubiquitous presence of flagellates in areas of deep mixing; hence, it is expected that these organisms are rather sensitive to UVR because of their history of acclimation to low mean irradiances. It was also seen that the diatom *Chaetoceros* was especially vulnerable to solar radiation (Helbling et al. 1992b), with UVR wavelengths being responsible for inducing the formation of resting spores. It is interesting to note that the bloom was dominated by the centric diatom *Odontella aurita* and that the absorption spectrum of this assemblage showed only a small “shoulder” of UV-absorbing compounds (data not shown). Thus, the presence of these compounds, which could account for variations in inhibition values because of their protective characteristics (Roy 2000), might not play a significant role in the assemblages studied here. One should be aware, however, that in this study, at least two factors are important to consider concerning the synthesis and presence of UV-absorbing compounds. (1) The bloom occurred during winter, when incident solar radiation was low (Fig. 1). (2) The methodology used by us to estimate the presence of UV-absorbing compounds is not as sensitive as high-pressure liquid chromatography analyses, so it is possible that some compounds were indeed present in the samples, but not in high concentration. We believe that variations in values of photoinhibition between different types of assemblages are probably more related to differential vulnerability to DNA damage. Studies carried out in a nearby area (Buma et al., 2001; Helbling et al. 2001a) have shown the formation of a relatively high number of cyclobutane pyrimidine dimers (CPDs) in postbloom assemblages.

Part of the variability can also be attributed to differences in kinetic responses to UVR of the microplanktonic and nanoplanktonic assemblages. For example, in a study carried out in the Andean lakes (Helbling et al. 2001b), it was found that small cells had faster inhibition kinetics as compared to larger cells because of their different surface to volume ratios and, consequently, their ability to use solar radiation. Variable sensitivity to UVR of phytoplankton assemblages can also be attributed to differential nutrient status, as seen in studies carried out by Litchman et al. (2002), who showed that UVR sensitivity increased in nutrient-limited cultures of dinoflagellates. In fact, because our samples were collected at different times of the year, a different nutritional status (e.g., depleted nutrients during postbloom) can be expected between the samples.

The fluctuating irradiance regime adds a new complication in assessing the overall sensitivity to UVR of our phytoplankton assemblages. It is seen that when considering UVR-induced photoinhibition, prebloom and bloom samples subjected to a variable irradiance field did not show any significant inhibition when exposed to $Z_{\text{UML}}/Z_{\text{Eu}} = 0.6$ (Fig. 2A,B), whereas those from the postbloom period had a significant inhibition due to UVR (Fig. 2C). However, a rather different but complementary picture is seen when addressing the effect of UVR on the integrated productivity (Fig. 3). We found that for pre- and postbloom nanoplanktonic pop-

ulations, mixing had an overall positive effect (i.e., an increase of integrated production as compared to fixed conditions) but had a negative effect (i.e., decrease of integrated production) on microplanktonic cells, which characterize the bloom period (Figs. 1C, 3). These differences in integrated production seem to be more related to PAR and, to a lesser extent, to UVR (i.e., difference of both treatments). The large cells characteristic of the bloom period, with relatively low surface-to-volume ratio, likely have a lower kinetic response to fluctuating irradiances, which results in lower integrated production than samples in a more “stable” radiation regime that had a longer time to acclimate to the irradiance conditions.

A variable UML depth, as considered in this study, is a usual feature because of variations in solar heating and climatological conditions, such as those produced by changes in wind speed and direction (reviewed by Neale et al. in press). In addition, an important point that we considered refers to the mixing rate at which samples were exposed. Denman and Gargett (1983) estimated that a vertical displacement of 10 m could occur in a period from minutes to hundreds of hours. From previous measurements of the UML depths and wind speed, mixing rates were estimated for the Patagonia area (Helbling et al., unpubl. data). Based on these calculations, we have chosen 5 h for a complete rotation when $Z_{\text{UML}}/Z_{\text{Eu}} = 0.6$. Other studies, though, have used a faster speed of rotation, as for example, that carried out in Alps lakes (Köhler et al. 2001), where a rotation of 4 min for a vertical displacement of 2 m has been implemented. In our study, the chosen $Z_{\text{UML}}/Z_{\text{Eu}} = 0.6$ used to compare the responses of phytoplankton during different seasons represents a displacement of ~12 m for the pre- and postbloom situations ($K_{\text{PAR}} = 0.24 \text{ m}^{-1}$) and much lower during the bloom season. This speed of rotation, however, might be too low for the windy season (postbloom condition), when the cells are likely moving at a higher rate of mixing in the water column. However, calm periods with virtually no winds and strong solar heating occurring during December might dampen the circulation of the cells. We decided to use the same speed of rotation to be able to compare the effect of UVR during different seasons. In our case, vertical mixing can completely reverse the effect due to UVR, so that although mixing enhanced UVR inhibition when $Z_{\text{UML}}/Z_{\text{Eu}} = 0.6$ (Figs. 2C, 4A), this effect decreased and was reversed with increasing depth of the UML. Moreover, an enhancement of photosynthesis (i.e., a negative integrated inhibition) was found when $Z_{\text{UML}}/Z_{\text{Eu}} = 0.91$ (Fig. 5). This higher carbon fixation in the samples exposed to UVR + PAR, as compared to the ones exposed to PAR only, suggests that phytoplankton cells might be light-limited; hence, they were able to use UVR as an energy source for photosynthesis, as seen in the study carried out by Prézélin et al. (pers. comm.), who showed that UVR wavelengths can in fact enhance phytoplankton photosynthesis.

Our data stress the importance of the $Z_{\text{UML}}/Z_{\text{Eu}}$ ratios in conditioning not only the effect of UVR but also the difference in integrated carbon fixation between samples exposed to variable or to fixed irradiances. A continued increase of solar UVR due to ozone depletion during the austral spring–summer would have then a greater effect on postbloom as-

semblages that are in relatively shallow UMLs. However, the combined effect of UVR and mixing also likely depends on cell size, previous light history (i.e., acclimation processes), and the nutrient status. We do not know, however, how different speeds of rotation, in conjunction with $Z_{\text{UML}}/Z_{\text{Eu}}$ ratios and UVR, would affect the phytoplankton assemblages in the area and whether this might be one of the variables that allows blooms to occur during winter. Future studies should concentrate on these topics to establish the overall effect of UVR on phytoplankton from the Patagonian coast.

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