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Impact of Solar Ultraviolet Radiation on Marine Phytoplankton of Patagonia, Argentina[¶]

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ABSTRACT

Patagonia area is located in close proximity to the Antarctic ozone “hole” and thus receives enhanced ultraviolet B (UV-B) radiation (280–315 nm) in addition to the normal levels of ultraviolet A (UV-A; 315–400 nm) and photosynthetically available radiation (PAR; 400–700 nm). In marine ecosystems of Patagonia, normal ultraviolet radiation (UVR) levels affect phytoplankton assemblages during the three phases of the annual succession: (1) prebloom season (late summer–fall), (2) bloom season (winter–early spring) and (3) postbloom season (late spring–summer). Small-size cells characterize the pre- and postbloom communities, which have a relatively high photosynthetic inhibition because of high UVR levels during those seasons. During the bloom, characterized by microplankton diatoms, photosynthetic inhibition is low because of the low UVR levels reaching the earth's surface during winter; this community, however, is more sensitive to UV-B when inhibition is normalized by irradiance (*i.e.* biological weighting functions). *In situ* studies have shown that UVR significantly affects not only photosynthesis but also the DNA molecule, but these negative effects are rapidly reduced in the water column because of the differential attenuation of solar radiation. UVR also affects photosynthesis *versus* irradiance (P vs E) parameters of some natural phytoplankton assemblages (*i.e.* during the pre- but not during the postbloom season). However, there is a significant temporal variability of

P vs E parameters, which are influenced by the nutrient status of cells and taxonomic composition; taxonomic composition is in turn associated with the stratification conditions (*e.g.* wind speed and duration). In Patagonia, wind speed is one of the most important variables that conditions the development of the winter bloom by regulating the depth of the upper mixed layer (UML) and hence the mean irradiance received by cells. Studies on the interactive effects of UVR and mixing show that responses of phytoplankton vary according to the taxonomic composition and cell structure of assemblages; therefore cells use UVR if >90% of the euphotic zone is being mixed. In fact, cell size plays a very important role when estimating the impact of UVR on phytoplankton, with large cells being more sensitive when determining photosynthesis inhibition, whereas small cells are more sensitive to DNA damage. Finally, in long-term experiments, it was determined that UVR can shape the diatom community structure in some assemblages of coastal waters, but it is virtually unknown how these changes affect the trophodynamics of marine systems. Future studies should consider the combined effects of UVR on both phytoplankton and grazers to establish potential changes in biodiversity of the area.

INTRODUCTION

Extensive literature has reported the stressful effects of solar ultraviolet radiation (UVR; 280–400 nm) on marine and freshwater phytoplankton assemblages from diverse environments of the world: polar (1–6), temperate (7–14) and tropical (15–20). At the individual level, UVR-induced effects on phytoplankton include the reduction of growth and photosynthetic rates (21), damage to the DNA molecule (22) and proteins (23) and impairment of movement (24–26). At the community level, changes in biodiversity (27,28) and size structure, with concomitant alterations in the food web dynamics (29), have been reported because of UVR exposure.

Most studies about the effects and impact of UVR on phytoplankton have been carried out in polar areas (1–6), especially in Antarctica, where enhanced ultraviolet B (UV-B; 280–315 nm) has been reported because of the depletion of the ozone layer (30). In contrast, relatively less is known about the effects of solar UVR on organisms from temperate regions such as Patagonia. The Patagonia

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Abbreviations: α , light limited slope; β , photoinhibition parameter; BWF, biological weighting function; chl *a*, chlorophyll *a*; CPD, cyclobutane pyrimidine dimer; DOC, dissolved organic carbon; DU, Dobson unit; E_k , light saturation parameter; K_d , attenuation coefficient; MAA, mycosporine-like amino acid; PAR, photosynthetically available radiation; P_{max} , maximum rate of carbon fixation; P vs E, photosynthesis *versus* irradiance; TOMS, Total Ozone Mapping Spectrometer; μ , growth rate; UML, upper mixed layer; UV-A, ultraviolet A radiation; UV-B, ultraviolet B radiation; UVR, ultraviolet radiation; Z_{EU} , depth of the euphotic zone; Z_{UML} , depth of the upper mixed layer.

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Figure 1. Map of South America indicating the region of Patagonia (shaded area). The numbers indicate the locations where research was conducted to evaluate the effects of UVR on marine phytoplankton: (1) Bahía Nueva (42°42' S, 65° W); (2) Bahía Engaño (43°18' S, 65°02' W); (3) Bahía Camarones/Bahía Bustamante (44°54' S, 65°38' W) and (4) Beagle Channel (55°01' S, 68°18' W). The isoline of 275 DU is shown for reference.

area, located at the southern tip of South America and including part of Argentina and Chile (Fig. 1), has unique characteristics for carrying out environmental UVR studies for several reasons. First, the area is occasionally under the influence of the Antarctic polar vortex, thus receiving enhanced UV-B for some periods of time (31). Second, its great variability in cloudiness, from high cover over the Andes and sub-Antarctic regions to the relatively clear skies on the mid-latitude Atlantic coast (32), creates a range of environments with variable UVR climatology. Third, it presents a high variability in the nature and bio-optical characteristics of its water bodies (*e.g.* the upwelling deep waters in the Pacific and the shallow and very productive Atlantic waters). Finally, wind speed and frequency are rather high, especially during spring and summer (13,33), which strongly condition the depth of the upper mixed layer (UML) and hence the underwater radiation field.

In such interesting scenarios for the assessment of UVR impacts on organisms, a number of research groups have conducted studies with terrestrial plants (34,35), macroalgae (36–47) and plankton from several marine (10,12–14,48,49) and freshwater environments (11,50–59). However, because of the large differences and associated complexity in organisms/ecosystems, we only review data on the effects of UVR on marine phytoplankton from the Atlantic region of Patagonia. The assessment of the UVR impact on these organisms is essential, because they constitute the base of a very rich food web that includes fishes (*e.g.* hake) and invertebrate species (*e.g.* shrimp and mussels) of important commercial value (60).

ATMOSPHERIC CHARACTERISTICS

Solar radiation and ozone trends

Solar radiation is an environmental factor that strongly affects organisms living in aquatic ecosystems. The radiation levels at which aquatic organisms are exposed depend on several factors, *e.g.* the solar irradiance reaching the Earth's surface, type and

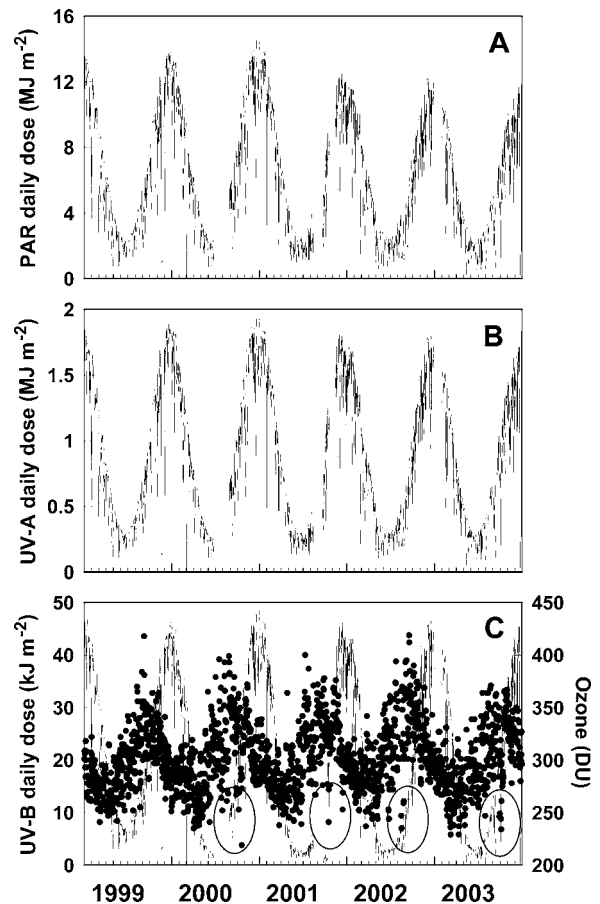


Figure 2. Daily doses of solar radiation and ozone concentration in the area of Bahía Engaño from 1999 to 2003. A: PAR (400–700 nm), in MJ m^{-2} . B: UV-A (315–400 nm), in MJ m^{-2} . C: UV-B (280–315 nm), in kJ m^{-2} and ozone concentrations (in DU, circles). Solar radiation data were obtained with a broadband ELDONET radiometer (www.eldonet.org) permanently installed on the roof of Estación de Fotobiología Playa Unión. Ozone concentrations data were obtained from NASA (<http://jwwocky.gsfc.nasa.gov>). Note the low ozone values during springtime (inside oval).

concentration of atmospheric gases (*i.e.* mainly ozone), altitude and particulate and dissolved material (61,62). At present, a number of research groups monitor solar radiation in different sites of Patagonia (see Villafañe *et al.* for detailed information of ground stations and instrumental [63]). As expected, there is a clear trend in Patagonia of high radiation values during summer and low during winter, with marked daily variability caused by cloud cover (13,31,49). As an example of annual trends for solar radiation levels over Patagonia, we present data collected at the Estación de Fotobiología Playa Unión (www.efpu.org.ar), Chubut, Argentina (43°18' S, 65°02' W) during the period 1999–2003 (Fig. 2). Daily doses of photosynthetically available radiation (PAR; Fig. 2A) vary from $\sim 14 \text{ MJ m}^{-2}$ in summer to $< 1 \text{ MJ m}^{-2}$ in winter; UVR daily doses have a similar pattern, with ultraviolet A (UV-A) ranging from ~ 2 to 0.15 MJ m^{-2} (Fig. 2B) and UV-B ranging from ~ 45 to $< 5 \text{ kJ m}^{-2}$ (Fig. 2C). However, variable irradiance and daily doses are determined in Patagonia because of the large latitudinal coverage (31), with concomitant changes in solar zenith angle, day length and atmospheric aerosols content, among other factors (64). More detailed information about latitudinal differences in radiation levels in different sites of Patagonia (*i.e.* temperate and sub-Antarctic) and along Argentina is presented in Orce and Helbling (31).

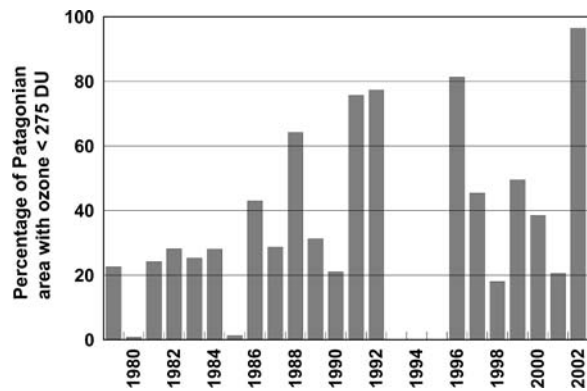


Figure 3. Maximum areal coverage (as percentage of the Patagonia area) of low-ozone air masses (<275 DU) during 1 day over Patagonia from 1979 to 2004. Data calculated from ozone images available at NASA (<http://jwocky.gsfc.nasa.gov>). No data are available for the period of 1993–1995.

Because UV-B radiation is additionally affected by ozone (61), data on the concentration of this gas over mid-Patagonia are shown in Fig. 2C. Ozone concentrations vary throughout the year (Fig. 2C), with low values (~220–230 Dobson units [DU]) in April to May and high ones (~400 DU) during September, which is in agreement with the reported dynamics of photochemical production of ozone over the stratosphere (65). During early spring, however, there are some days with relatively low ozone concentrations (*i.e.* 220–270 DU) that are associated with the Antarctic polar vortex and the ozone “hole.” In fact, several studies have shown the presence of low-ozone air masses over Patagonia, caused by the Antarctic polar vortex that covers the tip of South America for short periods of time (31,66–73) (Fig. 1) or ozone-depleted air masses that detach from the polar vortex (*i.e.* end of November to early December) and circulate northward (74,75). The extent of the Antarctic ozone “hole” over Patagonia has been determined by different techniques/methodologies such as launching balloons (76), estimations with satellite imagery (*i.e.* total ozone mapping spectrometer [TOMS] data from the Goodart Space Flight Center; <http://jwocky.gsfc.nasa.gov>) or using different types of radiometers (63), finding a signal that can be traced as north as 38° S (31,72,73). These studies have highlighted, however, the temporal and spatial variability of low-ozone air masses over Patagonia. We have used TOMS data to calculate the aerial coverage of low-ozone air masses (*i.e.* <275 DU; Fig. 3) to estimate the influence of the Antarctic ozone “hole” over Patagonia. We have chosen the limit of 275 DU based on data that fell out from the trend of ozone concentrations during spring (Fig. 2C). For this estimation, we also assumed that, because of the dynamics of the polar vortex, a comparable area over the sea would be affected by ozone depletion, which in turn would affect the underwater radiation field. There was a high year-to-year variability in the aerial coverage of low-ozone air masses, but in general, they covered ~20% of the Patagonia area during 1979 and increased up to ~95% during 2002 (Fig. 3). However, it should be stressed that this represents the maximum coverage of low-ozone air masses during 1 day, and the dynamics of the polar vortex is such that it influences Patagonia only a few days per year (Fig. 2C) (31).

Wind

Wind also strongly affects organisms living in aquatic systems, because it conditions the depth of the UML, which in turn

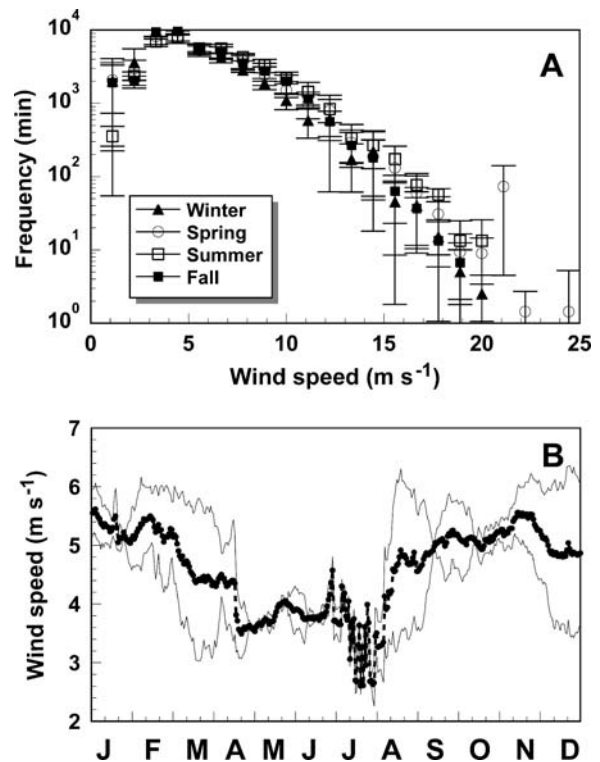


Figure 4. Wind speed data at Bahía Engaño. A: Mean seasonal frequency of wind speed (min) from 2001 to 2002. B: Mean daily wind speed (in m s^{-1}) and SD (thin lines) from 2001 to 2003. Partially adapted from Villafañe *et al.* (13).

modulates the mean irradiance received by phytoplankton (77,78). Additionally, wind is also important in resuspending particulate material, thus increasing the attenuation of solar radiation in the water column. Indeed, one of the most characteristic atmospheric features of Patagonia is the presence and duration of strong winds, as seen in Villafañe *et al.* (13). This study has determined a maximum wind frequency of 10^4 min at a mean seasonal speed interval of 3.5–4.5 m s^{-1} (Fig. 4A). The strongest winds (*i.e.* predominantly from the West) are usually found during spring, and to a lesser extent in summer, with mean seasonal values as high as ~25 m s^{-1} (Fig. 4A). The annual trend for mean daily wind speed is shown in Fig. 4B. The most interesting characteristic is the high variability of mean daily speed during the windy season (*i.e.* spring and summer); in contrast, fall and winter are characterized by low mean daily wind speed and little variability, indicating more stable conditions. This stability clearly influences the underwater radiation field caused by the presence of shallow (*i.e.* during winter) or deep (*i.e.* during spring and summer) UML (49), which are known to play an important role in the development of phytoplankton blooms in Patagonia coastal environments (13).

UNDERWATER RADIATION FIELD AND UVR-INDUCED PHOTOSYNTHETIC INHIBITION IN THE WATER COLUMN

Solar radiation is attenuated in the water column, and the depth penetration of the different wavebands depends on the surface irradiance and the attenuation coefficient (K_d) (62). The light-absorbing components in the aquatic system are the water, dis-

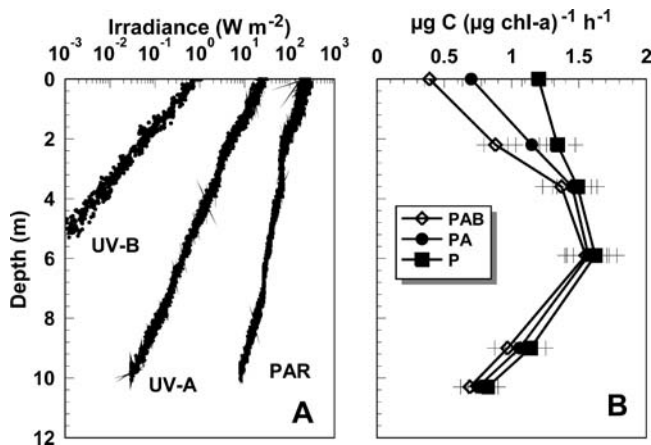


Figure 5. A: Underwater radiation field—PAR (400–700 nm), UV-A (315–400 nm) and UV-B (280–315 nm). B: Carbon fixation, *i.e.* assimilation numbers (in $\mu\text{g C } \mu\text{g chl } a^{-1} \text{ h}^{-1}$) for natural phytoplankton assemblages from Bahía Camarones exposed to three radiation treatments: PAR + UV-A + UV-B (PAB); PAR + UV-A (PA) and PAR only (P). The + symbols indicate 1 SD. Data obtained with an underwater broadband ELDONET radiometer that has sensors for UV-B, UV-A, PAR, temperature, conductivity and depth.

solved matter (especially organic carbon [DOC]), photosynthetic organisms and inorganic matter (79); in particular, the most important UVR absorbers in the water column are particles and dissolved organic matter (62). In general, the underwater radiation field in marine environments is highly variable, basically depending on location (*i.e.* coastal vs open ocean). For example, in the Chubut coast in Patagonia, K_{PAR} varies from 0.2 m^{-1} in seawater (*e.g.* Bahía Camarones, Fig. 1) to $>2 \text{ m}^{-1}$ in estuarine environments (*e.g.* Bahía Engaño; Fig. 1), which are strongly influenced by river discharge and thus receive large amounts of sedimentary material. Other factors that can also affect the penetration of solar radiation in coastal systems of Patagonia are the resuspension of sediments (caused by strong winds), run-off glacier water input (*i.e.* in sub-Antarctic environments such as the Beagle Channel; Fig. 1) or the presence of macroalgae that provide yellow-brown materials of humic type (*e.g.* Bahía Bustamante and Bahía Camarones; Fig. 1). The characteristics of the underwater radiation field, together with the specific sensitivity of species, are responsible for phytoplankton photosynthesis and solar UVR-induced inhibition in the aquatic environment.

In Fig. 5, we present an example of the underwater radiation field and UVR-induced photosynthetic inhibition of a natural phytoplankton assemblage characteristic from the area of Bahía Camarones. Solar radiation was differentially attenuated in the water column (Fig. 5A), with UV-B penetrating to $\sim 3.5 \text{ m}$ (1% of surface irradiance), whereas UV-A and PAR penetrated deeper, 7 and 15 m, respectively. The attenuation coefficients $K_{\text{UV-B}}$, $K_{\text{UV-A}}$ and K_{PAR} were 1.27, 0.66, and 0.31 m^{-1} , respectively, which are representative for most coastal areas of Patagonia during summer (*i.e.* postbloom conditions). When phytoplankton assemblages were incubated *in situ* in the water column under different radiation treatments (Fig. 5B), it was found that photosynthesis was significantly inhibited by UV-A and UV-B, with carbon fixation in surface waters reduced by 41% and 23%, respectively. The photosynthetic inhibition caused by UVR decreased with depth so that no significant differences ($P < 0.05$) among radiation treatments were found at 4 m depth. The higher carbon fixation

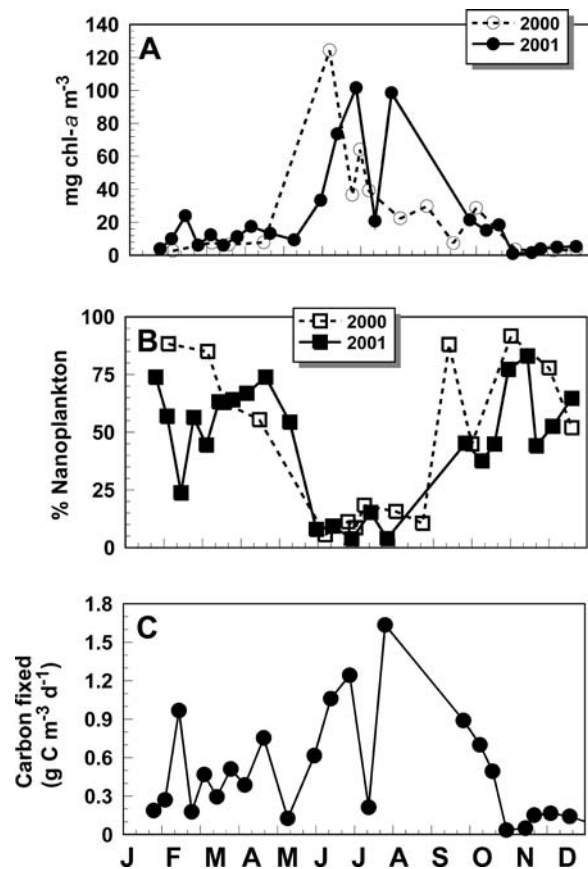


Figure 6. Temporal variability of phytoplankton biomass and primary productivity in the area of Bahía Engaño. A: Total chl *a* concentrations (in $\text{mg chl } a \text{ m}^{-3}$) for the years 2000 and 2001. B: Percentage of chl *a* in the nanoplankton fraction ($<20 \mu\text{m}$) for the years 2000 and 2001. C: Daily carbon fixation (in $\text{g C m}^{-3} \text{ day}^{-1}$) throughout the year 2001 for samples exposed to PAR. Partially adapted from Villafañe *et al.* (13).

rates at $\sim 6 \text{ m}$ depth, compared with those at the surface, also indicate that photoinhibition of phytoplankton photosynthesis at the surface was caused by high PAR levels. Similar profiles of photosynthesis under different radiation treatments have been obtained in other *in situ* studies conducted in diverse water bodies of the world (21), with UVR-induced inhibition decreasing with depth. It should be stressed that, in general, surface inhibition is not a good estimator of UVR-induced phytoplankton photosynthesis inhibition in the water column: Surface UVR-induced photosynthetic inhibition was rather similar in the Andean lakes, Beagle Channel and Bahía Bustamante; however, the integrated impact of UVR was much higher in sub-Antarctic waters, because a bigger portion of the euphotic zone was affected by this waveband (63).

SEASONAL CYCLE OF PHYTOPLANKTON AND DYNAMICS OF UVR-INDUCED PHOTOINHIBITION IN SURFACE WATERS

Phytoplankton abundance in coastal waters of Patagonia, as estimated by chlorophyll *a* (chl *a*) concentrations, vary seasonally (Fig. 6A), with high values ($\sim 100 \text{ mg chl } a \text{ m}^{-3}$) during winter, and relatively low ($<5 \text{ mg chl } a \text{ m}^{-3}$) during the rest of the year.

This seasonal pattern of a marked bloom period that occurs during late fall-winter has been found in studies carried out by Barbieri *et al.* (49) and Villafañe *et al.* (13) in the Chubut coast of Argentina. The bloom is mainly dominated by microplankton cells ($>20 \mu\text{m}$), whereas the pre- and postbloom are dominated by picoplankton ($<20 \mu\text{m}$; Fig. 6B). Taxonomic analyses reveal a general pattern of diatom-dominated microplankton populations, reaching values as high as $4 \times 10^3 \text{ cells mL}^{-1}$ during the bloom, with the diatom *Odontella aurita* being the dominant species. The prebloom samples are generally dominated by unidentified monads and flagellates ($\sim 0.5\text{--}3 \times 10^3 \text{ cells mL}^{-1}$); during this period, dinoflagellates (*e.g.* *Prorocentrum micans*, *Alexandrium tamar-ense*, *Protoperdinium* sp.) present their highest concentration ($\sim 60 \text{ cells mL}^{-1}$). Postbloom samples are generally dominated by unidentified monads and flagellates and also by small diatoms of the genus *Thalassiosira*. Following this seasonal pattern of phytoplankton abundance, carbon fixation rates in surface waters also present a clear trend throughout the year, with high values ($\sim 1.6 \text{ g C m}^{-3} \text{ day}^{-1}$) during the bloom and low ($<0.2 \text{ g C m}^{-3} \text{ day}^{-1}$) values during the postbloom period (Fig. 6C).

An interesting point in this seasonal trend refers to the occurrence of winter blooms (13,49,80), which are also observed in other coastal areas of Argentina near Patagonia (81). Our data suggest that the strong winds during spring and summer (Fig. 4A) favor the development of deep UML that preclude the bloom of large cells, resulting in the dominance of flagellates that can better use solar energy under relatively low irradiance conditions (77). In contrast, shallow mixing and more stable conditions (*i.e.* low wind speeds and less variability) characteristic of winter (Fig. 4B) allow the growth of large phytoplankton cells and result in high biomass. In fact, studies conducted in the Southern Ocean (82,83) also showed the importance of wind-driven UML and the associated light regimen in the development of phytoplankton blooms.

Studies conducted to assess the impact throughout the year of solar UVR on phytoplankton primary production (13) have determined that photosynthesis inhibition in surface waters is highly variable, with minimum values ($\sim 10\%$) during winter and maximum values ($\sim 60\%$) during late spring (Fig. 7A). The relative contributions of UV-A and UV-B to the total inhibition (Fig. 7B) also vary throughout the year, probably reflecting not only seasonal changes in irradiance (Fig. 2), but also the sensitivity of the different phytoplankton assemblages (Fig. 6A,B). During this time series study, the effects of UV-A were generally higher than that of UV-B (Fig. 7B) as also seen in many other aquatic environments, ranging from polar to tropical areas (21). This is generally attributed to the fact that the amount of UV-A energy that reaches the Earth's surface is much higher than that in the UV-B region. Despite this, it was found for phytoplankton from Patagonia that during some periods, the relative inhibition caused by UV-B and UV-A was rather similar or UV-B surpassed that of UV-A (Fig. 7B). In those occasions, especially during the prebloom, the increase of relative UV-B-induced photosynthetic inhibition (Fig. 7B) was associated with the dominance of small flagellates (Fig. 6B). During the bloom and postbloom, the increase in UV-B-induced inhibition of photosynthesis was associated with a relative increase of flagellates that occurred together with the large diatoms (Fig. 6B). This obviously hints at a taxonomic dependence on UVR effects where flagellates account for much of the observed UV-B inhibition. In fact, some studies have shown relatively high sensitivity of flagellates to UVR (27); however, small cells are generally more resistant than large cells when addressing

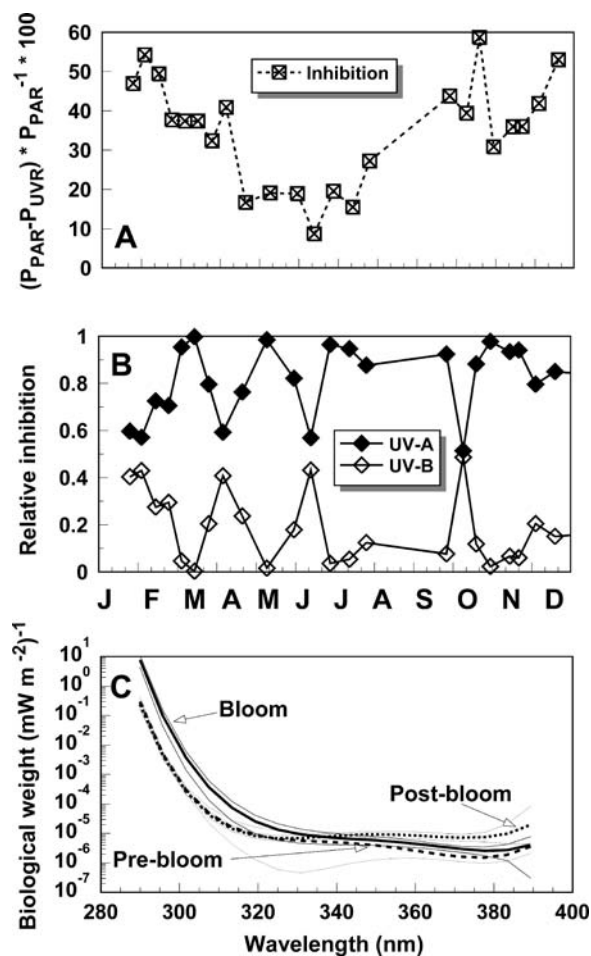


Figure 7. Inhibition of phytoplankton photosynthesis caused by UVR in the area of Bahía Engaño. A: Total inhibition (in percentage) by UVR throughout the year 2001. B: Relative contribution of UV-A and UV-B to the total UVR-induced inhibition of primary production. C: Mean biological weighting functions for prebloom, bloom and postbloom samples; the thin lines indicate 1 SD. Partially adapted from Villafañe *et al.* (13).

photosynthetic inhibition (1,84), most probably caused by a faster photoacclimation (11,84). We do not know, however, what are the reasons for such “pulses” of variable relative abundance of picoplankton cells, but changes in nutrients input, as normally occur in the study area (*i.e.* Bahía Engaño), that is under the influence of the Chubut river (85), might account for part of this variability.

A complementary view to understand the impact of UVR on phytoplankton from waters of Patagonia includes the assessment of a biological weighting function (BWF) (86), a function that relates the observed inhibition with the amount (and quality) of energy that caused the decrease in carbon fixation rates. The BWF calculated for the prebloom, bloom and postbloom periods (Fig. 7C) had significant differences in the UV-B range of the spectra. The biological weights ($[\text{mW m}^{-2}]^{-1}$) for wavelengths $<315 \text{ nm}$ were significantly higher ($P < 0.05$) in bloom samples, suggesting a higher sensitivity per unit energy of this assemblage compared with those of the pre- and postbloom. The biological weights for UV-A wavelengths, however, were not significantly different ($P > 0.05$) between the three periods, suggesting a similar response of phytoplankton throughout the year when exposed to this wave-

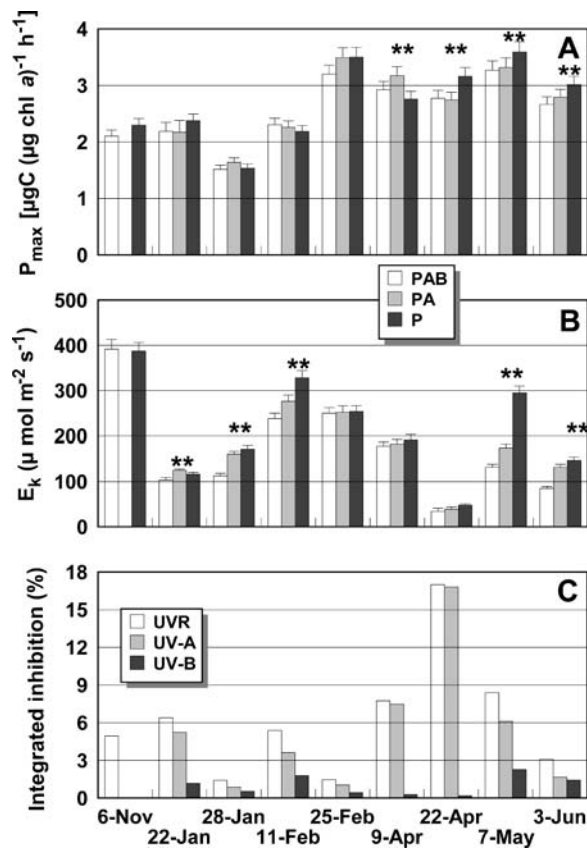


Figure 8. Mean photosynthetic parameters and total inhibition in natural phytoplankton assemblages collected from Bahía Nueva. A: Mean P_{max} (in $\mu\text{g C } \mu\text{g chl a}^{-1} \text{ h}^{-1}$). B: Mean E_k (in $\mu\text{mol m}^{-2} \text{ s}^{-1}$). White bars, samples exposed to the PAB treatment; gray bars, samples exposed to the PA treatment; black bars, samples exposed to the P treatment. *Significant differences ($P < 0.05$). C: Integrated photosynthetic inhibition (in %) in the euphotic zone caused by UVR (white bars), UV-A (gray bars) and UV-B (black bars). Adapted from Villafañe *et al.* (14).

band. At first sight, this would sound rather contradictory with the observed low photosynthetic inhibition during winter (Fig. 7A). However, low inhibition values were obtained because solar radiation levels during winter were low (Fig. 2). On the other hand, pre- and postbloom assemblages presented generally higher inhibition values (Fig. 7A) because of the high radiation levels during spring and summer (Fig. 2), but the BWF shows that cells were more resistant to UVR than those characterizing the bloom (Fig. 7C). Contrary to these findings in the Patagonia area, studies carried out with natural phytoplankton assemblages of Chesapeake Bay (9) did not determine significant interseasonal differences in the responses to UVR. Indeed, the authors found a significant intraseasonal variability when phytoplankton was exposed to similar artificial UVR, probably because of changes in species composition, light, temperature and nutrient availability. In the study carried out in Patagonia, it was found that cell size accounts for an important part of the variability observed in UVR-induced photosynthetic inhibition, with small cells being more resistant than large cells, perhaps because of their faster acclimation kinetics caused by a high surface-to-volume ratio (11,84). In contrast, picoplankton cells are generally more vulnerable to DNA damage, as determined in studies carried out by Buma *et al.* (10) and Helbling *et al.* (12).

Studies were also devoted to determine the impact of UVR in photosynthesis *versus* irradiance (P vs E) curves obtained from phytoplankton assemblages from surface waters off Bahía Nueva and Bahía Camarones (14). P vs E curves are useful tools to predict primary productivity and carbon fluxes over large areas of the world's oceans (87,88), and they also provide information on the photoacclimation status of cells at the time of sampling. P vs E curves are characterized by diverse parameters: 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 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) (79,89). The studies conducted in Patagonia waters throughout the year (14) have shown an important temporal variability of these parameters and related UVR effects (Fig. 8). P_{max} was significantly higher ($P < 0.05$) during the prebloom than during the postbloom period (Fig. 8A). In general, P_{max} values are lower than expected if only considering the effects of temperature (88); thus the variations in photosynthetic parameters found in Patagonia clearly hints for dependence with other environmental or biological parameters, as suggested by Côté and Platt (90) and Shaw and Purdie (91). For example, field experiments carried out in Patagonia (Marcoval *et al.*, unpublished) have shown that nutrient addition rapidly increase growth rates of summer phytoplankton communities, suggesting a nutrient-limited condition of 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 (92). Solar UVR had a significant impact on P_{max} during the prebloom but not during the postbloom, with UV-A accounting for most of the inhibition with the exception of some few samples. This variability in responses to UVR of P vs E parameters agrees with previous studies: Furgal and Smith (93) and Montecino *et al.* (94) have determined significant effects of UVR on P_{max} , whereas Montecino and Pizarro (8), working with phytoplankton off the Chilean coast collected at different seasons, did not find significant differences in P_{max} between radiation treatments.

There was no clear temporal trend of E_k determined in phytoplankton from Patagonia (Fig. 8B). E_k varied between high values ($>300 \mu\text{mol m}^{-2} \text{ s}^{-1}$) during the months of November, February to early April and May, and low values ($<200 \mu\text{mol m}^{-2} \text{ s}^{-1}$) during January and late April; the lowest E_k was determined in late April ($\sim 50 \mu\text{mol m}^{-2} \text{ s}^{-1}$). The study of Villafañe *et al.* (14) related E_k and wind speed (as an indirect measurement of UML depth) and established the negative relationship between these two parameters during the previous week of experimentation. As seen for P_{max} , the effects of UVR on E_k from natural phytoplankton were also variable: UVR had a significant impact on E_k during some periods (*i.e.* summer and late fall), whereas in others, there were no differences between samples exposed only to PAR and those that additionally received UVR (*i.e.* February, April). Photosynthetic parameters of the P vs E curves, as well as the attenuation coefficients and solar irradiance data, were used by Villafañe *et al.* (14) 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) of Bahía Nueva waters (Fig. 8C). UV-A accounted for most of UVR-induced photosynthetic inhibition, with maximum values of $\sim 16.5\%$, whereas UV-B-induced inhibition was comparatively small ($<2.5\%$ in all

experiments). This is also in agreement with previous data on UVR-induced photoinhibition, which indicate the importance of UV-A in contributing to the bulk of loss of carbon fixation in natural waters (21).

COMBINED EFFECTS OF UVR AND MIXING ON PRIMARY PRODUCTIVITY

Vertical mixing is one of the most important variables that conditions the performance of phytoplankton within the water column (95,96) because it exposes organisms to fluctuating (*i.e.* variable) radiation regimens (77). Recent studies have shown that the interaction of UVR with mixing (*i.e.* intensity and speed) can also significantly affect primary productivity in natural phytoplankton assemblages (5,20,49,77,78). The assessment of the combined effects of UVR and mixing are especially important in Patagonia because of the strong winds that characterize the area (Fig. 4). Particularly, Barbieri, *et al.* (49) carried out these types of studies during prebloom, postbloom and bloom conditions. To calculate photosynthetic inhibition caused by UVR (Fig. 9A), they simulated as if 60% of the euphotic zone (*i.e.* $Z_{UML}/Z_{Eu} = 0.6$, ~12 m depth for $K_{PAR} = 0.24 \text{ m}^{-1}$) was mixed. There were significant differences in UVR-induced photoinhibition between pre- and postbloom samples, with higher values in those exposed to a variable irradiance field and lower in those exposed to fixed irradiances (Fig. 9A). During the bloom, however, samples exposed to a fluctuating radiation regimen had significantly less carbon fixation than those exposed to fixed irradiances, supporting the idea that during this period cells take advantage of stable conditions, so that when exposed to simulated mixing, primary productivity was strongly inhibited by UVR. Similar conclusions were found in other studies carried out by Helbling *et al.* (77) and Neale *et al.* (5) in Antarctic waters that showed that fast mixing induced more photosynthetic inhibition because phytoplankton repair capacity was absent. In another set of experiments carried out by Barbieri *et al.* (49) in Patagonia during the postbloom (*i.e.* high irradiance and relatively strong winds, end of spring-summer), the authors further addressed the importance of mixing intensity (*i.e.* the portion of the euphotic zone that was mixed) and UVR on carbon fixation by phytoplankton (Fig. 9B). The integrated inhibition caused by UVR varied from a positive impact (*i.e.* less carbon fixation caused by exposure to UVR) under shallow mixing conditions to a negative impact (*i.e.* less carbon fixation when UVR was screened off) when $Z_{UML}/Z_{Eu} = 0.91$, thus showing the use of UVR as a source of energy for photosynthesis (Fig. 9B). In fact, the use of longer UVR wavelengths (*i.e.* UV-A) for photosynthesis has been also shown in other studies, especially when PAR levels are low (97,98) or when cells are mixing relatively fast in the water column (20). The study carried out by Barbieri *et al.* (49) also stressed the differences between fixed and variable irradiance conditions, with extreme photosynthesis inhibition in samples exposed to fluctuating irradiances (Fig. 9B).

LONG-TERM EFFECTS OF UVR IN NATURAL PHYTOPLANKTON ASSEMBLAGES

Although extensive research has been carried out to address the short-term effects of UVR on phytoplankton (*i.e.* with experiments lasting <1 day), the performance of communities over longer temporal scales (*i.e.* days/weeks) have been relatively less studied. In particular, very few studies have been carried out in Patagonia waters to evaluate the long-term responses of phytoplankton

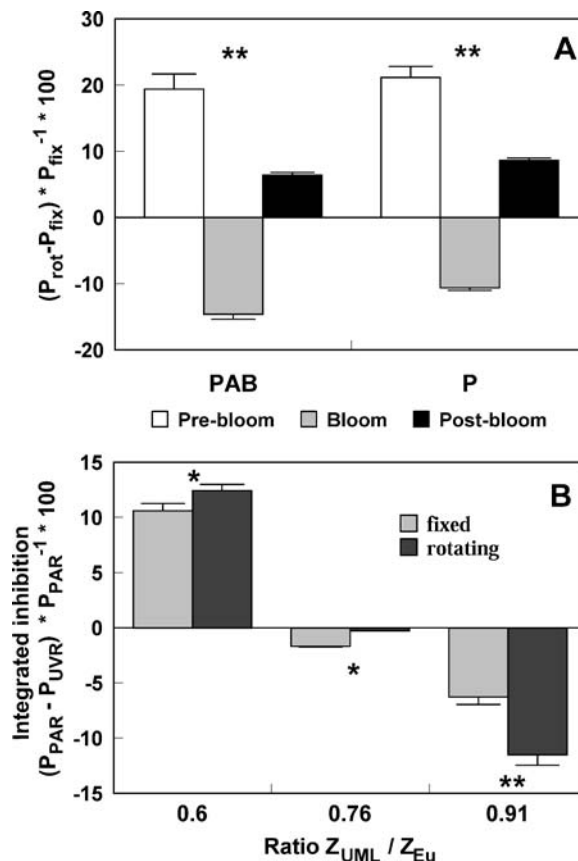


Figure 9. Combined effects of UVR and mixing in phytoplankton collected from Bahía Engaño. A: Comparison of depth integrated photosynthesis between fixed and rotating systems, calculated as $[(P_{rot} - P_{fix})/P_{fix}] \times 100$, for the PAB and P radiation treatments. White bars, prebloom samples; gray bars, bloom samples; black bars, postbloom samples. B: Comparison of integrated inhibition of photosynthesis [calculated as $(P_{PAR} - P_{UVR})/P_{PAR} \times 100$] when samples were exposed to different mixing conditions (*i.e.* ratio $Z_{UML}/Z_{Eu} = 0.6, 0.76$ and 0.91 , respectively). 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: * $P < 0.05$, ** $P < 0.01$. Adapted from Barbieri *et al.* (49) Copyright (2002) by the American Society of Limnology and Oceanography, Inc.

assemblages to solar UVR exposure (48,99) (Hernando *et al.*, unpublished and Marcoval *et al.*, unpublished). The common characteristic in these long-term studies is the variability in responses, clearly associated to the differential sensitivity to UVR of phytoplankton assemblages. In studies carried out with natural phytoplankton communities collected from waters off the Beagle Channel, Hernando and San Román (48) found significant effects of UVR on growth rates. Moreover, studies carried out with monospecific phytoflagellates cultures isolated from these sub-Antarctic communities showed the relatively high sensitivity of these organisms to solar UVR exposure (48). In contrast, no effects of UVR were found in a pennate diatom also isolated from the Beagle Channel.

Long-term experiments were also conducted by Villafañe (99) with three different phytoplankton assemblages off Patagonia to assess the combined effects of nutrient addition and UVR. In one set of samples (*i.e.* N_0), nutrients were added before exposure to solar radiation, and in another set (*i.e.* N_7), nutrients were added after 1 week. Within the same assemblage, growth rates (μ) were significantly lower in the N_0 than in the N_7 samples (Table 1). This

Table 1. Mean growth rates (day^{-1}) for Bahía Engaño, Bahía Camarones and Bahía Nueva experiments

| Treatment/site | Bahía Engaño | Bahía Camarones | Bahía Nueva |
|---------------------------------------|--------------|-----------------|-------------|
| N ₀ _PAB | 0.77 | 0.75 | 0.87 |
| N ₀ _PA | 0.86 | 0.62 | 0.96 |
| N ₀ _P | 0.83 | 0.65 | 0.99 |
| N ₇ _PAB | 0.95* | 1.53* | 1.40* |
| N ₇ _PA | 1.06* | 2.13* | 1.35* |
| N ₇ _P | 1.22* | 2.38* | 1.45* |
| P ($\mu\text{N}_0 = \mu\text{N}_7$) | 0.029 | 0.002 | 0.001 |

The radiation treatments were PAR + UV-A + UV-B (PAB); PAR + UV-A (PA), and PAR only (P). N₀ indicates addition of nutrients at the beginning of the experiment, whereas N₇ indicates the addition of nutrients after 7 days of exposure.

*Significant differences ($P < 0.05$) between N₀ and N₇ cultures (*i.e.* comparing the same radiation treatment). Growth rates were calculated during the exponential phase after nutrient addition.

was probably associated with the previous light history of these assemblages (*i.e.* collected during the strong mixing period and thus acclimated to low irradiance levels) so that cells in the N₀ cultures had a high energetic cost in adjusting to the new radiation conditions, *i.e.* similar of being at the surface. N₇ cultures had enough time to acclimate to the new (*i.e.* maximum) radiation conditions as imposed in the experiment, so that “selected” cells took full advantage of nutrient addition and hence had higher growth rates. In contrast, within the same nutrient condition from each phytoplankton assemblage, there were no significant differences in growth rates between radiation treatments (Table 1). Changes in the taxonomic composition of these assemblages, especially within the diatom community, were also evaluated in these long-term studies. Some assemblages responded to nutrient addition (*i.e.* Bahía Engaño; Fig. 10A), UVR exposure (*i.e.* Bahía Camarones; Fig. 10B), both or almost neither (*i.e.* Bahía Nueva; Fig. 10C). These variable responses have also been determined in long-term studies carried out in other environments: There were no changes in taxonomic composition caused by UV-B exposure in long-term experiments carried out in a high altitude (100), in a Canadian mesotrophic (101) and alpine lakes (102). However, the taxonomic composition changes observed in Bahía Engaño and Bahía Camarones agrees with other studies (27,28), where solar radiation seems to play a fundamental role in shaping phytoplankton community structure.

During these long-term experiments, acclimation to UVR exposure was also assessed by evaluating photosynthetic inhibition at the beginning and during the growth phase of N₀ and N₇ cultures (99). Photosynthetic inhibition caused by UV-B (Fig. 11A) was lower than that produced by UV-A (Fig. 11B). Within each experiment, there were no significant differences in photosynthetic inhibition caused by UV-B between N₀ and N₇ samples (Fig. 11A). However, in assemblages from Bahía Engaño and Bahía Camarones, photosynthetic inhibition caused by UV-A (Fig. 11B) was significantly higher at the beginning of the experiment, reflecting acclimation of phytoplankton to the new radiation conditions. This acclimation has been also observed in other studies carried out with monospecific cultures of Antarctic diatoms that also displayed lower photosynthetic inhibition at the end than at the beginning of experiments (103). A number of mechanisms are proposed to explain the acclimation of phytoplankton to solar UVR. One of these mechanisms includes the synthesis of photoprotective compounds, such as mycosporine-like amino acids (MAA) (104). However, no

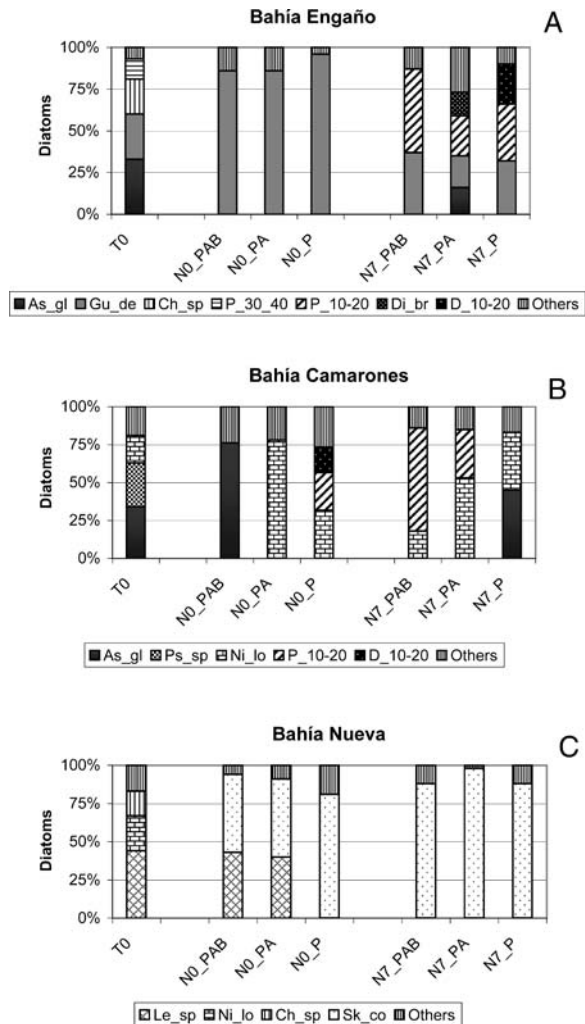


Figure 10. Relative contribution of diatoms species at the beginning (t_0) and during the exponential phase of the N₀ and N₇ cultures and for the three radiation conditions (*i.e.* PAB, PA and P). A: Bahía Engaño. B: Bahía Camarones. C: Bahía Nueva. D_{10–20}, discoids 10–20 μm in diameter; Di_{br}, *Ditylum brightwellii*; P_{10–20}, pennate 10–20 μm in diameter; P_{30–40}, pennate 30–40 μm in diameter; Ch_{sp}, *Chaetoceros* spp.; Gu_{de}, *Guinardia delicatula*; As_{gl}, *Asterionellopsis glacialis*; Ni_{lo}, *Nitzschia longissima*; Ps_{sp}, *Pseudonitzschia* spp.; Sk_{co}, *Skeletonema costatum*; Le_{sp}, *Leptocylindrus* sp.

significant amounts of these compounds were determined in natural samples of Patagonia waters at any time of the year (13,14,49) or during long-term experiments (99). Therefore the presence of MAA is probably not the main mechanism allowing phytoplankton from Patagonia to cope with natural solar UVR levels. One possible explanation to this lack (or eventually very small amount) of MAA is that during the high radiation period (*i.e.* spring-summer)—when MAA would be more important in protecting the cells—natural phytoplankton assemblages are dominated by nanoplankton (Fig. 6), whereas large microplankton cells dominate during winter. Previous studies (105) have found that it was not effective for small cells to synthesize and accumulate MAA; thus this protective mechanism is not an appropriate strategy for nanoplankton to cope with high UVR levels. Instead, other mechanisms such as active repair capacity of damage produced in the DNA molecule seems to be effective in phytoplankton assemblages from Patagonia, as seen in studies carried out by Buma *et al.* (10) and Helbling *et al.* (12).

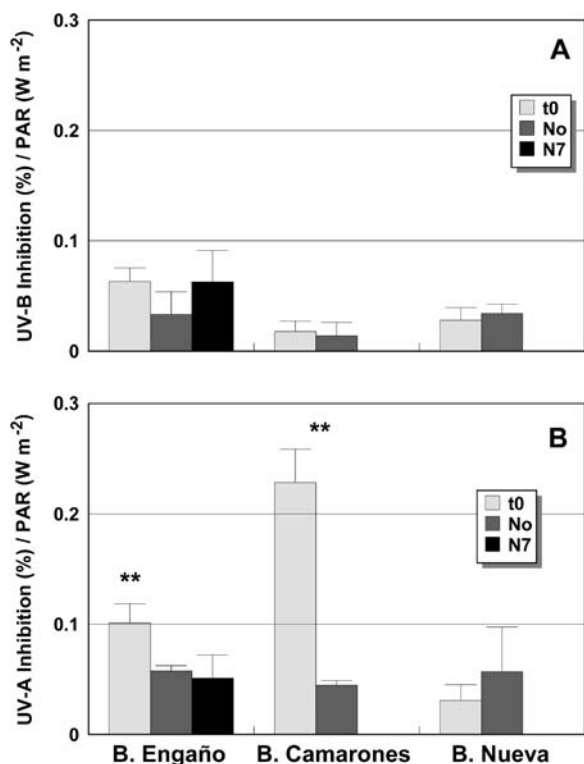


Figure 11. Photosynthetic inhibition (in %) caused by UV-B (A) and UV-A (B) during long-term experiments carried out with phytoplankton assemblages from Bahía Engaño, Bahía Camarones and Bahía Nueva exposed to different nutrient/radiation treatments. T_0 denotes the photosynthetic inhibition (UV-B and UV-A) at the beginning of the experiment, whereas N_0 and N_7 are the photosynthetic inhibition in the treatments in which nutrients were added at the beginning and after 1 week, respectively. *Significant differences ($P < 0.05$; no data were obtained in Bahía Camarones and Bahía Nueva at time N_7).

IMPACT OF UVR ON THE DNA MOLECULE

The DNA molecule is another target of UVR, with UV-B causing the formation of cyclobutane pyrimidine dimers (CPD) (10,12), which generally account for 80–90% of photoproducts formed (22); UV-A, in contrast, can induce indirect DNA oxidative damage (22). Studies were conducted by Buma *et al.* (10) and Helbling *et al.* (12) in waters off Patagonia (*i.e.* Bahía Bustamante) to evaluate the effects of UVR on phytoplankton assemblages. Biosidometer (*i.e.* bare DNA) data (Fig. 12A) allowed the evaluation of the penetration of UV-B in the water column, being $K_{UV-B} = 0.80 \text{ m}^{-1}$, with the 1% radiation level found at 5.8 m. In natural phytoplankton assemblages, and compared with other study sites, the initial CPD levels were very high (Fig. 12B) and higher than those found in other areas, *i.e.* marine tropical picoplankton (106), Antarctica (6) or Lake Titicaca, Bolivia (19). This hints to a prolonged history of previous UV-B exposure in the water column of phytoplankton from Patagonia; however, the decrease in CPD levels at 3 and 6 m depth clearly indicates that either repair takes place or damage is diluted by *de novo* DNA synthesis in viable cells. Figure 12C shows the size dependence of DNA damage in phytoplankton from Patagonia coastal waters, with small cells ($< 2 \mu\text{m}$) being more sensitive to UVR than large cells. This is in agreement with other studies carried out by Boelen *et al.* (106) and Karentz *et al.* (107), which also suggest that DNA damage is associated with the size structure of the community.

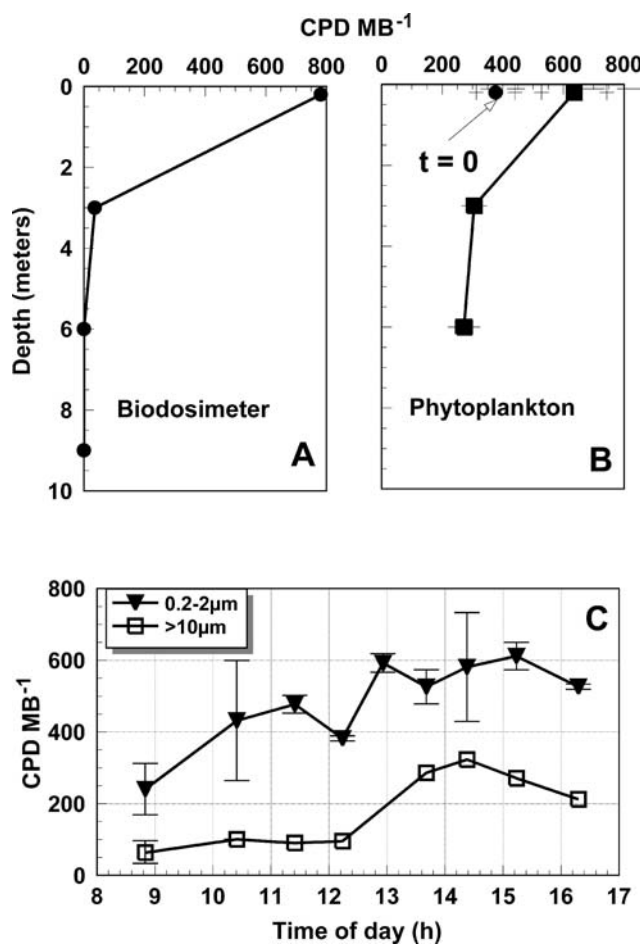


Figure 12. Depth profiles of CPD formation (CPD MB^{-1}) in (A) bare DNA samples and (B) natural phytoplankton populations collected from waters off Bahía Bustamante. The symbol at $t = 0$ indicates the amount of CPD at the start of the experiment. The + symbols indicate 1 SD. After Helbling *et al.* (12). C: CPD accumulation patterns in two phytoplankton size fractions: G, $> 10 \mu\text{m}$; 6, $0.2\text{--}2 \mu\text{m}$ fraction. The vertical lines indicate 1 SD. Adapted from Buma *et al.* (10).

SUMMARY

Patagonia is a fascinating area to study the responses of marine organisms to UVR, because it offers unique characteristics to carry out environmental UVR research. A clear trend of increasing aerial coverage of low ozone air masses over Patagonia is evident from 1979 to 2004 during the austral spring, and this occurred at least once a year during the studied period. In addition, wind forcing vertical mixing is responsible for relatively shallow UML during winter and deeper UML during spring and summer. The investigations carried out in Patagonia point toward a wind-driven mixing, which in turn affects the irradiance field (intensity and variation regimen) at which organisms are exposed, thus affecting phytoplankton responses to UVR, taxonomic composition and community size structure. Overall, there is a very clear seasonal cycle (in accordance with wind data and UML simulations) with high phytoplankton biomass in late fall-winter (*i.e.* bloom period), with large microplankton diatoms dominating the community. In contrast, during the rest of the year, low phytoplankton biomass is determined, mainly because of the presence of pico-nanoplankton. Photosynthesis inhibition is high in late spring-summer and low in

winter, and the contribution of UV-B and UV-A to this inhibition is variable, mainly because of the differential sensitivity of phytoplankton assemblages throughout the year. Nevertheless, the inhibition caused by UV-A is generally higher than that caused by UV-B. However, when considering the energy received by the cells, as derived from the BWF, bloom phytoplankton assemblages are more sensitive to UV-B than are the pre- and postbloom. Cell size, together with vertical mixing, seems to be the variable that accounts for most of the variability of the photoinhibition of phytoplankton in Patagonia, with small cells being more resistant to photosynthetic inhibition because of faster acclimation kinetics (related to high surface/volume ratio), but more vulnerable to DNA damage. Vertical mixing simulations during winter (*i.e.* with bloom species) highlighted the importance of water column stability for the development of the bloom because cells under variable irradiance were inhibited compared with fixed irradiance. In contrast, and during pre- and postbloom conditions, vertical mixing enhanced carbon fixation by small cells, and they were able to use UVR as a source of energy for photosynthesis. In addition, long-term experiments showed the importance of the combined effects of pulses of nutrients (such as those occurring in the Chubut river estuary) and UVR in selecting species, thus shaping the phytoplankton community structure. How these changes in phytoplankton assemblages would affect biodiversity of higher trophic levels of the area is virtually unknown, and future studies should include these determinations.

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