Ultraviolet radiation and primary productivity in temperate aquatic environments of Patagonia (Argentina)

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Proefschrift

ter verkrijging van het doctoraat in de
Wiskunde en Natuurwetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. F. Zwarts,
in het openbaar te verdedigen op
vrijdag 15 oktober 2004
om 16.15 uur

door

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geboren op 20 juni 1964
te Capital Federal, Argentina

Contents

Chapter 1

General introduction: Solar ultraviolet radiation and its impact on aquatic systems of Patagonia, South America

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ABSTRACT

Solar ultraviolet radiation (UVR, 280-400 nm) is known to cause a number of detrimental effects in aquatic organisms. The area of Patagonia, which is sometimes under the influence of the Antarctic ozone "hole", occasionally receives enhanced levels of ultraviolet B radiation (UV-B, 280-315 nm). Great efforts have been put into creating a database for UVR climatology by installing a variety of instruments in several localities in the region. However, no comparable effort has been made to determine the impact of normal and enhanced levels of solar UVR upon organisms. Most of the photobiological research in aquatic systems of Patagonia has focused on determining the effects of solar UVR in phytoplankton photosynthesis, DNA damage, and mortality, fecundity and repair mechanisms in zooplanktonic species. Some work has also been done with fish larvae and interactions between species at low trophic levels of the aquatic food web. The results of these studies indicate that in order to assess the overall impact of UVR in a certain waterbody, it is also necessary to consider other variables, such as changes in cloudiness, ozone concentrations, differential sensitivity of organisms, and depth of the upper mixed layer / epilimnion, all factors that can preclude or benefit the acclimation of species to solar radiation.

INTRODUCTION

Stratospheric ozone depletion, e.g., the ozone "hole", with the concomitant increase of the most energetic and potentially damaging daylight component UV-B, 280-315 nm (Madronich 1993), is a cause of great concern in the scientific community. Consequently, many studies carried out during the last decade (1900 – 2000) have focused on determining ozone concentrations (Madronich 1993, Kirchhoff et al. 1997a, b), solar ultraviolet (UVR, 280-400 nm) fluxes reaching the Earth's surface (Madronich 1993, Booth et al. 1994, Frederick et al. 1993, 1994), and effects upon various organisms (e.g., Young et al. 1993, Weiler & Penhale 1994, Figueroa et al. 1996, De Mora et al. 2000). In addition to the enhanced solar UV-B radiation caused by the stratospheric ozone depletion, it has now been recognized that normal solar UVR can also cause stress. In fact, aquatic organisms are very sensitive to ambient levels of UVR. The deleterious effects produced by the short wavelength components of the solar spectrum include, among others, damage to the genetic material – DNA, inhibition of photosynthetic rates, increased rates of mortality, inhibition of growth rates and changes in motility (Holm-Hansen et al. 1993a, Cullen & Neale 1994, Buma et al. 1995, Häder et al. 1995, Shick et al. 1996, Sommaruga et al. 1996, Kiffney et al. 1997b).

The Patagonia region, located at the southern tip of South America, includes part of Argentina and Chile (Fig. 1), has two large cities (Punta Arenas, Chile, 53°S, 70.9°W, and Ushuaia, Argentina, 54.5°S, 68° W) and some other less dense populations in close proximity to Antarctica, and may occasionally be under the direct influence of the ozone "hole". Several institutions have concentrated on determining ozone column concentrations and measuring

incident solar radiation over this area. However, it is surprising that no comparable efforts have been devoted to evaluating and understanding the biological effects of normal and enhanced levels of solar UVR. In fact, at present, very few research groups are carrying out activities in relation to the impact of solar radiation on the biota of Patagonia.

The aquatic environments of Patagonia present a very interesting scenario that would warrant this type of photobiological studies, for several reasons. First, the area is occasionally under the influence of the Antarctic polar vortex, thus receiving enhanced levels of UV-B radiation for some periods. Second, there is a great variability in cloudiness, from high cover in the Andes and sub-Antarctic regions, to the relatively clear skies on the mid latitude Atlantic coast (Lubin & Jensen 1995, Helbling et al. 1998) thus creating a range of environments with very different UVR climatology. Third, there is a great variability in the nature, and bio-optical characteristics of waterbodies, including the upwelling deep waters in the Pacific, the shallow and very productive Atlantic waters, and a large number of lakes from the Andes to both oceans. Finally, wind speed is rather high for most part of the year in the Patagonia region (mean of 32 km h^{-1} during spring and summer) (Baigún & Marinone 1995) conditioning the depth of the upper mixed layer / epilimnion in the water column and hence the underwater radiation field.

Due to the substantial differences and associated complexity in terrestrial and aquatic ecosystems, we review only existing data on the effects of UVR upon various waterbodies of Patagonia, as well as data on ozone and solar radiation measurements carried out in this area. We are aware, though, that some groups have carried out research activities in Patagonia in relation to the effects of solar radiation on terrestrial plants (Rousseaux et al. 1998, Searles et al. 1999), as well as to physiological aspects of human beings (Ladizesky et al. 1995).

MEASUREMENTS OF OZONE AND SOLAR ULTRAVIOLET RADIATION

Atmospheric conditions

In order to develop a database for the UVR climatology in the Patagonia region, a number of instruments have been installed at different locations (Fig. 1, Table 1), with some of them integrated in networks. The instruments range from the sophisticated, high-resolution scanning spectroradiometers (SUV-100, Biospherical Instruments Inc.) from the NSF/USAP-UV Monitoring Network, to the low cost, easy manipulation DOAS detectors. In between, there are narrow band (e.g., GUV-510 and GUV-511, Biospherical Instruments Inc.), and broad band radiometers (e.g., ELDONET sensors, Real Time Computers Inc.). This variety of instruments, which complement each other with the information they provide, has improved our understanding of the dynamics of the ozone "hole" over Patagonia. However, some difficulties have arisen in comparing data from different sites; nevertheless, it is possible to outline general characteristics of the UVR climatology and ozone conditions over this area.

<u>Figure 1</u>: Map of Patagonia showing the location sites of instruments collecting data on incident solar radiation.

<u>Table 1</u>: Instruments recording incident solar radiation in Patagonia.

Instruments	Type of data	Locations in Patagonia
SUV-100 scanning spectroradiometers (Biospherical Instruments Inc.)	Spectral scans hourly in the range 280-620 nm.	Ushuaia (Argentina) Valdivia (Chile)
GUV-510 and 511 radiometer (Biospherical Instruments Inc.)	Measurements at 305, 320, 340, 380 nm and PAR (400-700 nm).	Trelew, Bariloche and Ushuaia (Argentina) Valdivia (Chile)
DOAS – UV-B detectors	Data at two channels centered at 300 nm and 313 nm.	Río Grande, Bariloche (Argentina) Punta Arenas (Chile)
ELDONET radiometer (Real Time Computers Inc.)	Broad bands for UV-B (280-315 nm), UV-A (315-400 nm) and PAR (400-700 nm)	Playa Unión (Argentina)
Brewer MK-IV spectrophotometer	Specific wavelengths in the UV-B region: 306.3, 310.0, 313.4, 316.7, and 319.9 nm.	Ushuaia (Argentina) Punta Arenas (Chile)
UV-Biometer (Solar Light Co.)	Erythema dosis	Punta Arenas, Puerto Natales, Porvenir and Puerto Williams (Chile)

In agreement with model outputs (Holm-Hansen et al. 1993a, Madronich 1993), data   obtained by the UVR -PAR Argentinean Monitoring Network (Orce & Helbling 1997, Orce et al. 1997) indicate that solar UV-B irradiance increases steadily from south to north, with a

marked day-to-day variability. Measurements of UV-A and PAR irradiances also showed a south to north increase with significant variability due to cloud cover. The integrated daily values were high at mid-latitudes (summer values of 1500 KJ m^{-2} for UV-A – 315-400   nm-and 90 KJ m^{-2} for UV-B), due to a combination of relatively high irradiances and long day-light periods (Orce & Helbling 1997). The daily variability observed in the data has two main causes. First, cloudiness plays the most important role in the determination of ambient solar radiation levels reaching the Patagonia region, and it is more evident at the southernmost locations and over the Andes mountains (Frederick et al. 1993, 1994, Díaz et al. 1994, 1996, Lubin & Jensen 1995, Orce & Helbling 1997). The other source of variability in the UVR data (principally in the UV-B region) is atmospheric ozone column concentrations, causing enhanced levels of UV-B at the time of low ozone concentrations. In Patagonia, ozone column concentrations have been measured by launching balloons at specific sites such as Punta Arenas, Chile (Kirchhoff et al. 1997a), inferred from satellite data obtained by the Total Ozone Mapping Spectrometer (TOMS) (Booth et al. 1994, Frederick et al. 1993, 1994, Bojkov et al. 1995, Kirchhoff et al. 1997b, Orce & Helbling 1997), or from solar UVR ground-based measurements (Tocho et al. 1994, 1996, Bojkov et al. 1995, Orce & Helbling 1997, Perez et al. 1998). Ozone depleted air masses from the polar region reach lower latitudes of Patagonia during October – November, in events with variable duration. This can be visualized from TOMS maps (Fig. 2A) or inferred from ground-based solar UVR measurements (Fig. 2B, C). This was also demonstrated in other studies carried out at several locations of Patagonia (Frederick et al. 1993, 1994, Díaz et al. 1994, 1996, Jaque et al. 1994, Tocho et al. 1994, 1996, Orce & Helbling 1997, Orce et al. 1997, Perez et al. 1998). For example, the analysis of 1978-1991 TOMS dataset at Ushuaia showed the lowest ozone value (189 Dobson Units, D.U.) during October of 1991 (Booth et al. 1994). At this locality, Frederick et al. (1993) estimated that the largest local noon radiation levels at 306.5 nm during an ozone depletion event were equivalent to that expected at the summer solstice in Buenos Aires, about 20 degrees northwards. In addition, Bojkov et al. (1995), also working at Ushuaia, have reported   increases of 80% and 35% at 300 nm and 305 nm, respectively, during a 15% ozone decline in October; the measured irradiance at 300 nm was similar to the value recorded at the same site at the summer solstice. Studies carried out at Punta Arenas have shown that the largest relative enhancement in UV-B radiation, during an ozone depletion event, occurred   between 296-297 nm, with the spectral irradiance at 297 nm being at least 10 times higher than during a "normal" ozone day (Kirchhoff et al. 1997b). At this site, and using   ground-based UVR measurements, Kirchhoff et al. (1997a) estimated a drop in ozone   concentrations from 325 to 200 D.U. in October of 1995. The DOAS Network instruments have also noticed several ozone depletion events during the period 1993–1995. Although the intensity of these low-ozone episodes decreased northwards, they have been detected at latitudes as low as 37°S (Tocho et al. 1996, Perez et al. 1998).
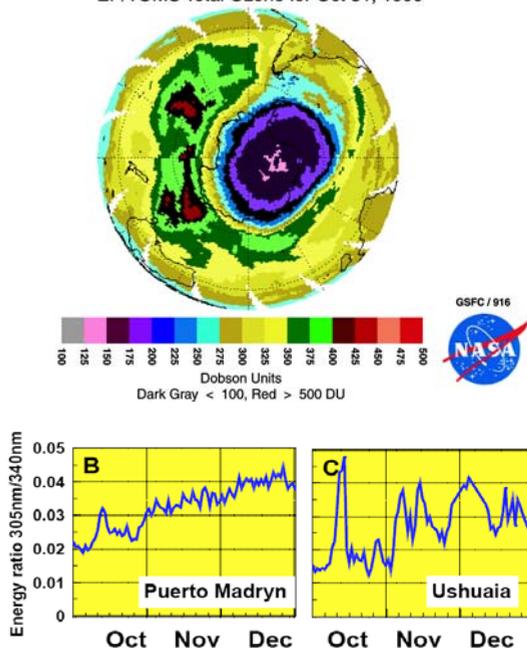
EP/TOMS Total Ozone for Oct 31, 1999

Figure 2: **A)** Polar view of the ozone "hole" over Patagonia on October 31, 1999, obtained from TOMS data from Goodart Space Flight Center (http://jwocky.gsfc.nasa.gov).
Ratio of energies at 305 and 340 nm obtained with a GUV 510 spectroradiometer (Biospherical Instrument Inc.) as a function of day during the austral spring, for: **B)** Puerto Madryn, and **C)** Ushuaia. High values of the 305 / 340 ratio indicate low ozone concentrations (Figs. B and C reprinted from Global and Planetary Change, 15, Orce & Helbling, Latitudinal UVR-PAR measurements in Argentina: Extent of the 'ozone hole'. Copyright (1997) with permission of Elsevier Science).

Orce & Helbling (1997) and Orce et al. (1997) have used the ratio of energies at 305 nm and 340 nm as an estimator of ozone concentrations. They detected significant enhanced levels of UV-B radiation not only in sub-Antarctic environments (Ushuaia, Fig. 2C), but also at mid-latitudes (Puerto Madryn - 43°S, Fig. 2B). These studies have also shown that although the largest relative enhancement of spectral irradiance at 305 nm occurred during the month of October, the absolute irradiance values for UV-B were still lower than those found during the summer solstice (Orce & Helbling 1997). Moreover, Díaz et al. (1994) concluded that the combination of low solar zenith angles and modest mid-summer ozone depletion produces more biologically damaging UV radiation than strong early-spring ozone depletion when the solar zenith angle is large. Ozone-poor air masses over Patagonia probably occur in relation to an extension of the Antarctic polar vortex over lower latitudes (Orce & Helbling 1997), but the possibility of a detachment from this system (Kirchhoff et al. 1996) should also be considered.

Underwater radiation field

The water column attenuates solar radiation, and the amount of energy received at any depth depends not only on the surface irradiance, but also on the attenuation coefficient K_d. The light-absorbing components in the aquatic system are the water, dissolved matter especially dissolved organic carbon (DOC), photosynthetic biota, i.e., phytoplankton and macroalgae, and inorganic matter (Kirk 1994). The DOC pool is a heterogeneous mixture of chemical species with a relatively high degree of aromaticity, which is collectively referred to as humic acids. Absorption of UVR by the water itself is very weak, and attenuation of PAR is important only above 500 nm (Kirk 1994). The most important UVR

absorbers in the water are particles and DOM.

The variables conditioning the attenuation coefficients in waterbodies of Patagonia vary with location and type of environment as seen in Fig. 3 and Table 2. There is a high variability in the attenuation of UV-B (Fig. 3A) and UV-A (Fig. 3B) in the different environments of Patagonia. The attenuation of PAR (Fig. 3C), on the other hand, is less variable than UVR. In general, the attenuation of light in marine systems seems to be more affected by the presence of organisms than by inorganic matter, but in sub-Antarctic environments, e.g., coastal waters of the Beagle Channel, Tierra del Fuego, runoff glacier water plays an important role in absorbing solar radiation (Helbling et al. 2001a, Hernando, pers. com.). At mid-latitude coastal marine environments, phytoplanktonic organisms are the great absorbers, but in some localities like Bahía Bustamante, in the Atlantic Ocean, certain types of macroalgae (e.g., *Macrocystis pyrifera* and *Gracilaria verrucosa*) may provide a significant amount of yellow-brown materials of the humic type (Helbling et al. unpub. data). Other coastal sites, such as estuaries, are affected by river discharges, which are very important in contributing sedimentary material (e.g., Bahía Engaño, Atlantic Ocean).
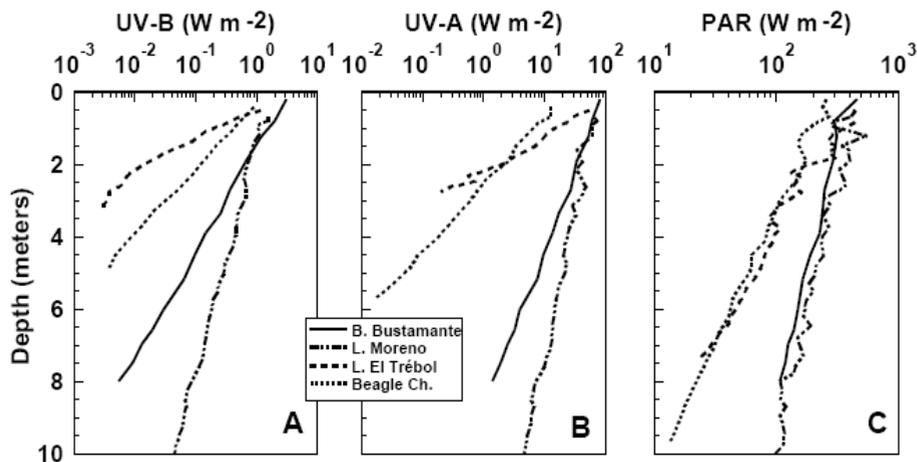

Figure 3: Underwater radiation field for different freshwater (Lakes Moreno and El Trébol) and seawater (Bahía Bustamante and Beagle Channel) sites of Patagonia. Radiation units are in W m^{-2}. **A)** UV-B (280-315 nm), **B)** UV-A (315–400 nm), and **C)** PAR (400-700 nm).

Site	Latitude and longitude	$K_{(UV-B)}$	$K_{(UV-A)}$	$K_{(PAR)}$
Lake Moreno, Río Negro	41°S, 71°W	0.36	0.28	0.15
Lake El Trébol, Río Negro	41°S, 71°W	2.54	2.39	0.40
Bahía Bustamante, Chubut	45°S, 66.5°W	0.80	0.46	0.16
Beagle Channel, Tierra del Fuego	54.4°S, 68°W	1.36	1.20	0.31

Table 2: Optical characteristics of waterbodies sampled in Patagonia. Attenuation coefficients (K_d) for UV-B, UV-A and PAR are in m^{-1}.

In oligotrophic freshwater lakes, UVR is mostly attenuated by DOC of terrestrial origin. Compared to seawater, lakes differ widely in the DOC concentration for several reasons: (*i*) The relationship between lake volume and watershed area varies enormously among lakes, (*ii*) lakes occur in regions that are highly variable in hydrological balance and vegetation cover , and (*iii*) lakes are at variable altitude above sea level, with many of them located above the tree line. In addition, the lakes of Patagonia occur across one of the steepest gradients of rainfall - over 4000 mm year^{-1} in the Andes to about 250 mm year^{-1} in the steppe - and along an altitudinal gradient from sea level to about 2000 m a.s.l. Such gradients determine dramatic changes in DOC and alkalinity that, in turn, affect DOC solubility and optical characteristics. There is evidence that photochemical transformation of DOM is wavelength dependent, with different processes being favored by different spectral composition of the radiation (Zepp et al. 1995). In natural waters, the spectral composition is largely controlled by depth and DOC concentration; therefore, vertical mixing determines the type of photochemical reactions that take place within a water parcel (Zagarese et al. 1998a, b).

The underwater radiation field is affected by the depth of the upper mixed layer (Z_{UML}) or epilinmion (Z_{ep}), and the relationship between this depth and that of the euphotic zone (Z_{eu}, the depth of 1% of surface irradiance) will determine the mean radiation received by the cells (Helbling et al. 1994, Neale et al. 1998c, Zagarese et al. 1998a, b). The Z_{UML} in marine systems is conditioned by both vertical mixing produced by wind stress, as well as by temperature and / or salinity, i.e., density, σ_t. In lakes, wind stress and thermal stratification play the major role in determining Z_{ep}. Studies carried out in the Patagonia region indicate that Z_{UML} tends to be deep at mid–latitudes due to the frequent strong winds. Wind is also important in resuspending particulate material, thus increasing the attenuation of solar radiation in the water column (Zagarese et al. 1998a, b, Helbling et al. 2001a, b).

EFFECTS OF SOLAR UVR ON AQUATIC ORGANISMS

Solar UVR is known to cause a number of adverse effects on living organisms. Most studies have been oriented towards evaluating the impact at lower trophic levels of the food web (Cullen et al. 1992, Helbling et al. 1992b, 1994, Neale et al. 1998c). Aquatic primary producers (phytoplankton and macroalgae) incorporate solar energy into the aquatic ecosystem through the photosynthetic process, and many studies have shown that they could be especially sensitive to UVR (Helbling et al. 1994, Häder et al. 1995, Neale et al. 1998c). Therefore, it is expected that any negative effect of UVR upon autotrophic organisms would

also directly or indirectly affect higher trophic levels. In the Patagonia region most   photobiological studies of aquatic systems have focused on the impact of UVR upon phytoplankton (Helbling et al. 2001a, b), zooplankton (Zagarese et al. 1997a, b, 1998a, b) fish larvae (Battini et al. 2000), and macroalgal-invertebrate interactions (Hernández et al. 1999, Menchi, unpub. data). A research project has just started in which the effects of solar UVR upon marine macroalgae will be evaluated. We are not aware of any studies performed with other components of the aquatic food web. In the following paragraphs we will highlight the key results obtained in regard to the effects of solar UVR on aquatic organisms of Patagonia waters.

Phytoplankton

The studies carried out with phytoplanktonic organisms have focused on determining the impact of solar radiation in the photosynthetic process and damage to genetic material (DNA). Several sites have been selected for these studies, including sub-Antarctic waters - Beagle Channel (Tierra del Fuego) and temperate marine environments - Playa Unión and Bahía Bustamante, Chubut (Helbling et al. 2001a), as well as Andean lakes in the Bariloche area, Patagonia (Helbling et al. 2001b).
Photosynthetic inhibition in phytoplankton, using *in situ* techniques, varied considerably in the different environments (Fig. 4).
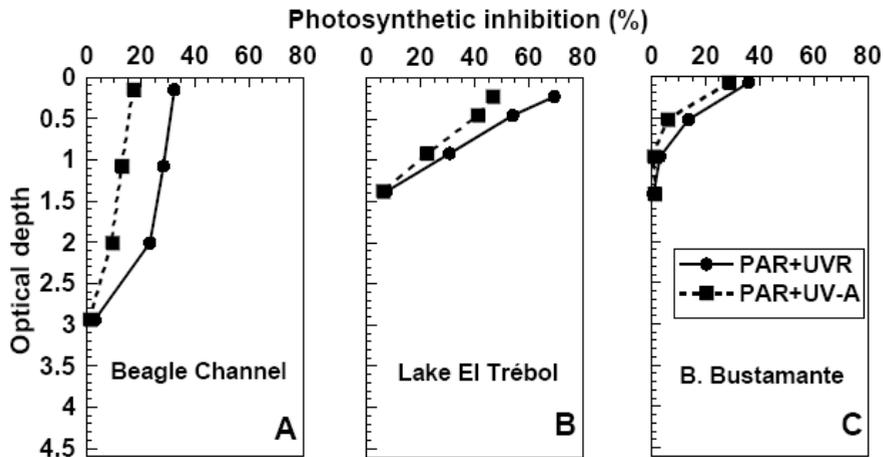
Figure 4: Percentage of photosynthetic inhibition due to solar UVR as a function of the optical depth in waterbodies of Patagonia. The inhibition (%) was calculated as $[100 * (P_{PAR}-P_{UV})/P_{PAR}]$, where P_{PAR} indicates the carbon fixed in the PAR treatment and P_{UV} the carbon fixed either in the PAR + UV-A or PAR + UVR treatments. **A**) Beagle Channel, **B**) Lake El Trébol and, **C**) Bahía Bustamante.

The optical depth was used in order to compare the waterbodies sampled - the optical depth of 4.6 is equal to $K_d * Z_{eu}$. Sub-Antarctic phytoplankton (Fig. 4A) were inhibited by ambient

UVR levels in a considerable portion of the euphotic zone (about 3 optical depths). The partial contributions of UV-B and UV-A to the total inhibition were approximately equal and the maximum inhibition (at the surface) was of about 35% (Helbling et al. 2001a). In mid-latitude waterbodies (Figs. 4B, C), inhibition of phytoplanktonic photosynthesis was significant only in a small portion of the euphotic zone (1.5 optical depths). At the surface, freshwater phytoplankton (Fig. 4B) seemed to be more inhibited - 70% (Helbling et al. 2001b) than marine phytoplankton - 40% (Fig. 4C, (Helbling et al. 2001a)). In these mid-latitude areas, most of the photosynthetic inhibition was due to UV-A radiation (over 60%), as was also observed in freshwater and marine ecosystems elsewhere (Helbling et al. 1992b, Smith et al. 1992, Kim & Watanabe 1993, Villafañe et al. 1999).

When comparing the worst case scenario for marine phytoplankton, i.e., incubations at the surface receiving the maximum irradiance, there were no significant differences in the total UVR inhibition for mid-latitude (e.g., Bahía Bustamante) and sub-Antarctic environments (Fig. 4A, C). However, as the depth distribution of photosynthetic inhibition was different, i.e., 1.5. and 3 optical depths at Bahía Bustamante and Beagle Channel, respectively, the integrated loss of carbon fixation due to UV-B in the euphotic zone would be higher for the sub-Antarctic site (24%) than for Bahía Bustamante (3%). In a mid-latitude freshwater site (Lake El Trébol, Fig. 4B) carbon fixation loss due to UV-B was calculated to be 6%. Based on these results, and considering the decrease in integrated primary production, it is expected that sub-Antarctic phytoplankton would be the most vulnerable in the case of an increase in solar UV-B radiation due to ozone depletion.

Genetic material of temperate marine phytoplankton is also affected by ambient levels of solar UVR, especially to UV-B, as evaluated through the formation of cyclobutane pyrimidine dimmers, CPDs. The studies carried out with natural populations (Helbling et al. 2001a) have shown that there is a significant damage to the DNA in surface waters when exposed to summer ambient levels of solar radiation. In fact, CPD levels were higher than those measured in other waterbodies, such as tropical seawater (Boelen et al. 2001) or Lake Titicaca (Helbling et al. 2001c), suggesting a prolonged history of previous UV-B exposures combined with low repair capacity in these cells.

Other studies (Helbling et al. 2001b) have addressed the sensitivity to UV-B of winter phytoplankton of the Andean lakes. This study has pointed out the importance of size structure of the community when evaluating inhibition of photosynthetic rates; microplanktonic cells (> 20 µm in diameter) seem to be more affected by ecologically relevant levels of artificial UV-B than nanoplanktonic cells (< 20 µm).

Overall, the results obtained indicate that at the time of assessing the impact of solar radiation in the water column, it is also necessary to take into account other variables, such as changes in cloudiness, species composition, including cell size structure of the community, and depth of the upper mixed layer / epilimnion, all factors that can preclude or benefit the acclimation of phytoplankton to solar radiation.

Zooplankton

Zooplanktonic organisms are also affected by solar UVR, and some of the effects reported are increasing rates of mortality, reduced fertility and impairment of movement (Holm-Hansen et al. 1993a, Häder et al. 1995). The studies carried out in the lakes of the Andes area showed that there

were large differences in UV tolerance even between species belonging to the same genus (Zagarese et al. 1997a). In a comparative study carried out to evaluate the differential responses to UVR of copepod species from Patagonia lakes (Zagarese et al. 1997a), it was found that *Boeckella gibbosa* and *B. brevicaudata* were more resistant to ambient UV-B levels, in terms of mortality, as compared to *B. gracilipes*, with this latter species being affected also by ambient UV-A levels (Zagarese et al. 1997a, b). Its biological weighting function, i.e., the effectiveness of different wavelengths to cause a particular damaging effect, is notably higher than that of DNA damage (Setlow 1974) especially in the UV-A region, suggesting that other molecules or structures (proteins, membranes) may also be impaired. The differential tolerance to UVR in the 3 species studied has been associated to differences in their photoprotection potential (i.e., the resistance to UV-B in the absence of recovery radiation - PAR) as well as to their photoreactivation capacity (i.e., higher resistance to UV-B in the presence of PAR). In that study, it was found that the photoprotection potential was lower in the translucent *B. gracilipes* and red colored *B. gibbosa* as compared to *B. brevicaudata* (dark pigmented). On the other hand, photoreactivation was observed in *B. brevicaudata* and *B. gibbosa*, but not in *B. gracilipes*. Thus, different strategies allow these organisms to cope with the damaging UV-B levels found in the nature: *B. gracilipes* depends exclusively on the attenuation by the external media, so that it can be found in relatively dark waters, deep in the water column or in relatively turbid shallow lakes, whereas *B. brevicaudata,* thanks to a combination of photoreactivation and photoprotection mechanisms, can be found in lakes with high irradiance levels of visible and ultraviolet radiation.

The importance of water mixing produced by wind has been addressed for some freshwater zooplanktonic species, such as *Ceriodaphnia dubia* and *B. gracilipes* (Zagarese et al. 1998a). The effect of water mixing seems to be dependent on the capacity of the organism for photorepair, so that reciprocity - the dependence of effects only on dose, regardless the irradiance - was satisfied in species with little photorepair capacity (such as *B. gracilipes*), but failed in species with a high photorepair capacity (*C. dubia*).

Fish larvae

Early life stages of fish may be damaged by solar UVR (Hunter et al. 1979, Zagarese & Williamson 2000). In Patagonia there is only one study on the effects of UV on fish larvae, such as on eggs of *Galaxias maculatus* (Battini et al. 2000), a landlocked form of a small catadromous fish. Battini et al. (2000) estimated the depth at which 50% of the exposed eggs would die (LD_{50} depth, Fig. 5) so that, depending on water transparency, the LD_{50} ranged from a few centimeters to over 12 meters. Interestingly, in some high elevation lakes, the LD_{50} is greater than the lake depth, strongly suggesting that UV radiation may be a sufficient cause to explain the lack of fish in such lakes.
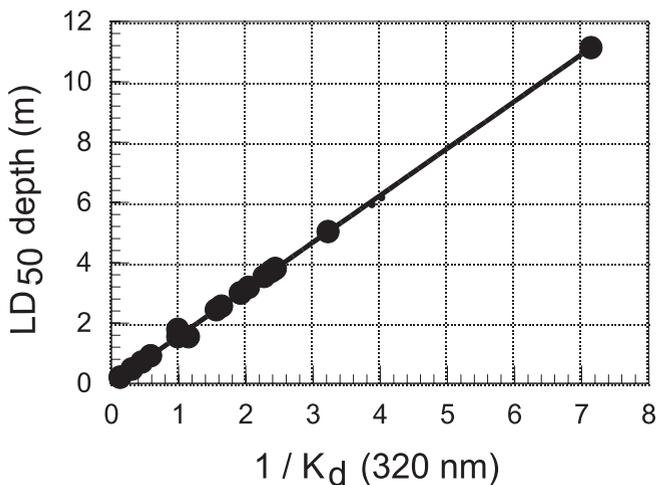
Figure 5: Depth at which 50% of fish eggs die (LD_{50}) as a function of the inverse of the attenuation coefficient at 320 nm ($1/k_{320}$) (redrawn after Battini et al. 2000).

Trophic interactions

The effects of UVR on trophic interactions have been evaluated through the synthesis of UV-absorbing compounds in marine macroalgae and subsequent bioaccumulation by invertebrate consumers (Hernández et al. 1999, Menchi, unpub. data). It has long been hypothesized that UV-absorbing compounds, especially mycosporine-like aminoacids (MAAs) might act as sunscreens and thus conferring protection against damaging levels of UVR (Shick et al. 1996). These compounds, that absorb UVR between 310 and 360 nm wavelength, are synthesized by organisms having the shikimate pathway, hence they are absent in metazoans; however they can be obtained through diet (Shick et al. 1996). In a study carried out with temperate marine organisms, it was found that the isopod *Idotea* sp., a key organism in the diet of pelagic fishes, was able to accumulate UV-absorbing compounds after being fed with a MAA reach diet provided by the red algae *Polysiphonia* sp. (Hernández et al. 1999). Furthermore, these UV-absorbing compounds were localized in specific organs of the crustacean body, such as gonads, but they were absent from the carapace (Menchi, pers. comm.).

FUTURE NEEDS FOR UVR STUDIES IN AQUATIC SYSTEMS OF PATAGONIA

A considerable number of instruments have been installed in Patagonia, and great efforts have been exerted to maintain and collect UV data. These types of measurements are essential for understanding the biological impact of UVR. However, some of these data are not easily obtained or they have not been published. Furthermore, no comparable efforts have been put to integrate these measurements, including an intercalibration process among the various types of instruments.

Regarding the impact of UVR on aquatic systems, more detailed studies are needed in order to understand the interactions between different variables, e.g. water mixing, cloudiness, ozone concentration, increasing temperature, in the observed effects. It is imperative to understand the autoecology of key species to better evaluate their potential to acclimate in

a scenario of increasing solar UV-B, and how this might affect the biodiversity and trophodynamic of the aquatic systems.

Long-term ecological monitoring of the impact of solar radiation on selected habitats, such as (*i*) mountain lakes (above the tree line); (*ii*) set of lakes across a DOC gradient , and (*iii*) mid-latitude areas with low cloud covering, would provide an unprecedented data base for the Patagonia region. These data will provide unique information to understand and predict changes in the aquatic biota due to solar UVR.

THE AIM OF THIS STUDY – THESIS OUTLINE

Although most studies about the effects of UVR on phytoplankton have been carried out in polar areas including the Arctic, where ozone depletion events have been also registered (Müller et al. 1997), we now know that normal UVR levels can produce significant effects in temperate and tropical phytoplankton (Lesser & Lewis 1996, Banaszack & Neale 2001, Helbling et al. 1992b, 2001a, b, c, Villafañe et al. 1999). However, we have little information about the effects of UVR on phytoplankton of the Patagonia region which, from a photobiological perspective, is a very interesting area to carry out these types of studies. The importance of this geographic region lies on two main reasons: (*i*) Phytoplankton species live under a highly variable radiation regime due to marked seasonal shifts and to sporadic ozone depletion events as result from its proximity to the Antarctic ozone "hole" (Orce & Helbling 1997) and, (*ii*) The large continental shelf area of the Atlantic coast in Patagonia is very productive, sustaining high stocks of commercial fish and invertebrates species. Hence, any potential deleterious effects of UVR on the base of the trophic food web in Patagonian waters could significantly alter the dynamics and biodiversity of the system as a whole. In addition, lakes of Patagonia are also very interesting sites to evaluate the effects of solar UVR, because they present a wide range of DOM concentrations (Morris et al. 1995) that ultimately affect waterbody transparency. Under such variety of environments (i.e., different underwater light climate), a number of responses to UVR of phytoplankton organisms inhabiting these lakes are expected to occur. Within this context, the aim of this thesis is *to assess the effects of solar ultraviolet radiation on primary productivity in temperate aquatic environments of Patagonia.* The general outline of the thesis is presented below.

From the scattered information that was available (i.e., as stated in the *General Introduction*) there was an obvious need of complementing and emphasizing studies addressing the effects of solar radiation (especially UVR) on phytoplankton from Patagonian waters. The effects of solar radiation on phytoplankton are generally assessed with different methodologies and under different perspectives (i.e., short vs. long term experiments) including important variables for the studied environment (e.g., wind and nutrients in the case of aquatic ecosystems of Patagonia). As this thesis particularly focuses on the effects of UVR on aquatic primary productivity, ***Chapter 2*** reviews the current methodologies / techniques to assess UVR effects on primary producers (i.e., phytoplankton, microphytobenthos and macroalgae) and provides information on how photosynthesis in these organisms is affected by UVR.

To asses the effects of solar UVR on phytoplankton photosynthesis in Patagonia at different times of the year, a year-long study is presented in ***Chapter 3***. This chapter provides information about the seasonal cycle of phytoplankton as well as on the variable climatology throughout the year, and it determines an annual pattern of UVR-induced effects on phytoplankton photosynthesis under various biological and meteorological conditions as found in the study site.

Once the seasonal variations of phytoplankton and the concomitant UVR-induced effects were determined, detailed experimentation was performed during the high radiation periods (i.e., spring-summer). These experiments were done in three different places of Patagonia: A rather closed system (i.e., Bahía Nueva) and two open sea sites, one of them in close proximity of the Chubut River discharge (i.e., Bahía Engaño), and the other not influenced by freshwater runoff (i.e., Bahía Camarones). At these places the research focused on: *i*) Short-term experiments (***Chapter 4***) to evaluate UVR effects on P vs E (i.e., productivity versus irradiance) characteristics

as well as their temporal variability and, *ii*) Long-term experiments (***Chapter 5***) to assess the combined effects of UVR and nutrient addition on growth and taxonomic composition of phytoplankton assemblages.

Since the DNA molecule is another cellular target that is affected by UVR, two chapters of the thesis are devoted to jointly assess DNA damage and photosynthesis inhibition. In particular, ***Chapter 6*** addresses the *in situ* impact of UVR on both targets in a marine phytoplankton community, whereas ***Chapter 7*** is a study carried out in three freshwater lakes of the Andes region having extreme conditions of solar radiation penetration due to differential DOM content.

Finally, the ***Summary*** presents the general conclusions of this work, as well as future potential lines of research in Patagonia.

Chapter 2
Photosynthesis in the aquatic environment as affected by UVR

Villafañe VE, Sundbäck K, Figueroa FL, Helbling EW

ABSTRACT

Since the discovery of the Antarctic ozone 'hole', many studies have been conducted to determine the effects of enhanced UV-B (280-315 nm) on photosynthetic rates of autotrophic organisms. It is accepted now that even natural levels of UVR (280-400 nm) are stressful for some autotrophic organisms. In this chapter we will summarize what we know about the effects of UVR on photosynthesis of aquatic organisms. Here we consider three major groups – phytoplankton, microphytobenthos (MPB), macroalgae / marine angiosperms – which differ in many ways, especially in regard to their habitats. While phytoplankton live in the water column, MPB and macroalgae occupy the benthic environment. This creates substantial differences with respect to the amount and quality of radiation that they receive. Thus, although there is a common and general response to UVR of these autotrophic organisms – i.e., inhibition of photosynthesis - there are differences among the groups studied. These are mainly due to differences in the radiation conditions to which cells are exposed, as well as to the specific sensitivity / acclimation of the organisms under study. To evaluate the overall response of aquatic primary producers to UVR, it is crucial to consider the temporal scale of experimentation, to allow enough time for repair mechanisms and acclimation to UVR. Thus, short–term experiments frequently give an insight about the worst – case scenario for UVR effects on photosynthesis. We also review in this chapter the effects of UVR upon some related physiological processes (e.g., nutrient incorporation / assimilation, pigment synthesis / bleaching) and morphology (e.g., cell size) that may in turn affect the photosynthetic performance. Finally, to determine the impact of natural and increased levels of UVR upon aquatic ecosystems, we consider the interactive effects of other variables (pH, carbon dioxide concentrations, temperature, etc.) with UVR. Consequences upon aquatic autotrophic organisms of increased UV-B levels due to ozone depletion events are still uncertain, but changes in biogeochemical cycles, community structure, and trophic web dynamics can be expected.

INTRODUCTION

The photosynthetic process in aquatic ecosystems is responsible for fixing approximately 40% of our planet´s yearly amount of carbon available for the production of new living matter, with about 48.5 Pg C yr^{-1} fixed in the aquatic ecosystems (Falkowski 1994, Behrenfeld & Falkowski 1997, Field et al. 1998). Carbon fixation in the aquatic environment, mediated by the utilization of solar radiation, takes place in both the water column and the benthos. While water-column autotrophic organisms (mainly phytoplankton) are responsible for most of the share in carbon fixation, benthic organisms (i.e., macrophytes and microalgal communities) are involved in about 10% of the total production (Mann & Chapman 1975, Häder et al. 1995). Although this latter amount is globally less than that due to phytoplankton, marine macrophytes also provide food (directly or through detritus) to a wide variety of invertebrates and fish in the coastal ecosystems (Duggins et al. 1989). Benthic microalgal

communities, on both hard and soft substrata, also serve a crucial ecological function in shallow freshwater and marine habitats. They constitute the local basis of the food webs in shallow areas, which are recognized as having high secondary production (e.g., of fish and their prey). In these areas, the microphytobenthic – MPB - communities may account for 50% or more of the total primary production, equaling or exceeding the productivity of the water column (Underwood & Kromkamp 1999).

Even though solar radiation is attenuated in the water column (Hargreaves 2003), it penetrates to a depth that will vary, among other things, according to the location (e.g., oceanic vs. coastal), latitude and concentration of particulate and dissolved matter. The euphotic zone in the water column (i.e., 1% of surface PAR, 400-700 nm) can vary from few centimeters in estuarine waters or lakes with a heavy load of DOM (Morris et al. 1995, Neale 2001) to more than 100 m in the open ocean (Kirk 1994). Hence ultraviolet radiation (UVR, 280-400 nm) can penetrate accordingly to comparable depths (Smith & Baker 1979, Hargreaves 2003). In coastal waters, biologically effective ultraviolet B radiation (UV–B, 280-315 nm) reaches only to 1 m depth, as in the Baltic Sea (Piazena & Häder 1997), whereas in the Mediterranean it can penetrate as deep as 20 m (Figueroa 1998). This variability is also observed in other environments: For example, in a study carried out in freshwater Japanese ponds and lakes, Hodoki & Watanabe (1998) determined that the 1% of surface UV-B varied from 0.3 to 2 m, depending mostly on the concentration of chlorophyll-*a* (chl-*a*) and particulate organic carbon present in the water body. The photic zone in the benthic environment extends to ca. 3 mm into the sediment. Fiber optic microsensor measurements have shown that UVR can penetrate down to at least 1.25 mm of this zone, and through scattering it can even exceed the incoming UVR by up to 50% (Bebout & Garcia-Pichel 1995, Garcia-Pichel & Bebout 1996). In addition, as the water column in estuaries and embayments is often shallow, and regularly absent in intertidal areas, UVR can reach high levels at the sediment surface. Thus, and in view of this background, UVR should be considered a very important environmental factor that can affect different metabolic and physiological processes in autotrophic organisms living in the water column and in the benthos.

In this chapter we will discuss the role of UVR in affecting the photosynthetic process in phytoplankton, MPB, and macroalgae. This is especially important as the effects of UVR on the photosynthesis of these organisms may have a considerable impact on higher trophic levels of the aquatic ecosystem (Leech & Johnsen 2003), as well as in climate change (Williamson & Zagarese 2003) and biogeochemical cycles (Zepp 2003).

METHODOLOGY TO ASSESS UVR EFFECTS ON PHOTOSYNTHESIS

Exposure of samples

In order to assess UVR effects on photosynthesis, three approaches for exposing algae to UVR are used. These include (1) natural solar radiation, modified by various filters that selectively screen off certain wavebands of radiation; (2) natural solar radiation which is supplemented with artificial UVR from lamps, and (3) fully artificial radiation, implying laboratory experiments. UVR experiments at their best require both that the target organisms are exposed to as realistic a light field as possible, and that high - quality measurements of radiation are obtained. The most realistic results are probably gained from experiments

performed under natural solar radiation; artificial radiation sources, however, have also been shown to be very useful for studying mechanistic aspects of UVR responses.

 In situ incubations

In this type of incubations, the samples are exposed to solar radiation in their natural habitat and at their natural *in situ* depth. To assess the effects of ambient UVR, this approach often involves three types of radiation fields, achieved by filters, i.e. PAR + UV-B + UV-A, PAR + UV-A, and only PAR (see section *Variables measured and experimental approaches*). Although *in situ* incubations will result in the most realistic responses, they certainly have the constraint of being conditioned by weather conditions. Therefore, comparatively few *in situ* studies on the effects of UVR on algal photosynthesis have been conducted, particularly in rough-weather areas, such as the Arctic (Helbling et al. 1996b) and Antarctica (Smith et al. 1992, Holm-Hansen et al. 1993b).

Phytoplankton can be exposed to an *in situ* field of radiation by using UV-transparent (see section *Variables measured and experimental approaches*) bottles hanging from a line or tubes placed in trays (Fig. 1) which are incubated at different depths in the water column (Smith et al. 1992, Holm-Hansen & Helbling 1995). One disadvantage with this approach is that phytoplankton cells are kept at a fixed depth for the entire incubation period (e.g., few hours), thus receiving a constant proportion of the surface incident radiation. In the water column, however, cells are moving within the upper mixed layer (UML) and thus exposed to a variable field of irradiance (Helbling et al. 1994, Neale et al 2003). So far, few studies have addressed the importance of mixing rates on the phytoplankton photosynthesis (Helbling et al. 1994, Neale et al. 1998c, Köhler et al. 2001), and with the exception of the experiments performed by Marra (1978) on the effects of PAR, we are not aware of such studies done under *in situ* conditions.

Fixed screens with different filtering capacities have been frequently used to study the *in situ* effect of ambient UVR on shallow - water benthic microalgae in streams (Kiffney et al. 1997a), lakes (Vinebrook & Leavitt 1999) and marine habitats (Francoeur & Lowe 1998, Reizopoulou et al. 2000). In a four-month *in situ* experiment on UVR effects on MPB communities of a microtidal bay, Wulff et al. (1999) used 80 x 80 cm screens placed in wooden frames that were pressed into the sediment. This type of field set-up, however, requires frequent cleaning and careful monitoring of the radiation field below the screens. In the case of marine macrophytes (macroalgae and marine angiosperms), most of the *in situ* experimentation has been conducted in the intertidal zone, where access to growing plants is relatively easy (Figueroa et al. 1997b, Bischof et al. 1998a, Gómez & Figueroa 1998, Flores-Moya et al. 1998). Subtidal populations have received less attention due to the complications of working *in situ* at different depths, especially in high latitude zones (Bischof et al. 1998a, b). Several authors (Figueroa et al. 1997b, Gómez & Figueroa 1998, Flores-Moya et al. 1998, 1999) have investigated effects of UVR on macroalgal photosynthesis by incubating algae in their natural environment and monitoring daily variation in photosynthesis and irradiance under different radiation treatments using a similar set up as those described for MPB experiments (Fig. 1). More recently, however, efforts have been devoted to analyze *in situ* photosynthetic activity of subtidal algae. This experimental design has consisted of determining the effective quantum yield by using an

underwater fluorometer (Beer et al. 1998, Ralph et al. 1998, Beer & Björk 2000, Figueroa & Gómez 2001). An alternative approach has been to incubate marine plants for several days at their natural growth site, and after that period the algae were collected and the quantum yield measured using a non - submersible fluorometer (Hanelt et al. 1997b, Häder et al. 1998, Flores-Moya et al. 1999, Figueroa et al. 2002).
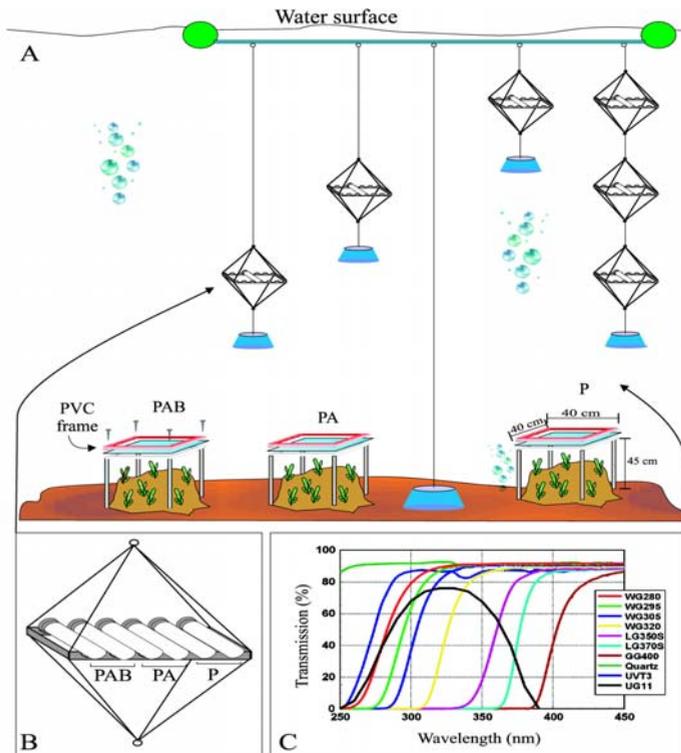

Figure 1: Schematic representation of *in situ* incubation for phytoplankton and benthic algae. **A)** General disposition of trays with tubes and filters for cutting off different portions of the solar spectrum; in the bottom a set up for benthic algae incubation is presented. **B)** Close up of one tray containing duplicate quartz tubes for three different radiation treatments: PAB, unfiltered solar radiation; PA, PAR + UV-A and P, only PAR. **C)** Transmission characteristics of various materials and filters used in photobiological experimentation.

Simulated in situ incubations

Considering the practical difficulties of *in situ* incubations, outdoor incubations in temperature - controlled containers (e.g., on deck of research vessels, or in flow - through systems on land sites) have been used as an alternative approach. This incubation method is suitable for both short-term (hours) and long-term (days - weeks) experiments carried out with microalgae (Helbling et al. 1992b, Holm-Hansen & Lubin 1994, Vernet et al. 1994, Sundbäck et al. 1996, Odmark et al. 1998), as well as with macroalgae (Häder et al. 1996a, b, 1997a, b, Franklin & Forster 1997, Flores-Moya et al. 1998, Grobe & Murphy 1998, Altamirano et al. 2000). This set up is often used for determining a worst-case scenario, as samples are exposed to surface (i.e., maximum) incident irradiance. Therefore, neutral density filters are often used to approximately simulate the attenuation of solar radiation in the water column. These filters, however, do not mimic the differential spectral attenuation that actually occurs in the water column (Hargreaves 2003), and samples are generally exposed to higher UV-B / UV-A / PAR ratios than they would normally experience. It is particularly

important to approach realistic ratios between UV-B, UV-A and PAR, as DNA repair mechanisms depend on those ratios (Karentz 1994, Quesada & Vincet 1997, Buma et al. 2003).

In contrast to phytoplankton, simulated *in situ* incubations imply fairly realistic light conditions for MPB in the intertidal or littoral zone. This is particularly true when incubating intact sediment cores, as the sediment will provide natural refuges for benthic microalgae, such as motile diatoms and cyanobacteria (Garcia-Pichel & Castenholz 1994, Sundbäck et al. 1996, Odmark et al. 1998). Similar approaches have also been used for hard substrata, often involving colonization of artificial substrata (Bothwell et al. 1993).

Supplemented UV-B or UVR

As with several UV experiments carried out with terrestrial organisms (Caldwell et al. 1995, Björn et al. 1999), experimental treatments on aquatic organisms have included the enhancement of ambient UV-B. In some cases, these treatments simulate ozone depletion events. Such experiments, in which natural solar radiation is enhanced by artificial UVR, have been done with phytoplankton (El-Sayed et al. 1990, Mostajir et al. 1999, Wängberg et al. 2001) and microphytobenthos (Sundbäck et al. 1997). A few studies have included simultaneously exclusion and enhancement of UV-B (Odmark et al. 1998, Underwood et al. 1999). A shortcoming, however, in the majority of experiments using elevated levels of UV-B, has been the use of fixed levels of UV-B for few hours per day. Moreover, the levels of enhancement have varied greatly, from moderate (~ 20%) to ca. 100% above ambient, often resulting in unnatural ratios between PAR and UVR, thus making comparisons between experiments difficult. As mentioned before, it is crucial for ecologically relevant studies that the spectral composition of the radiation is realistic (Santas et al. 1997). One way to achieve this is to provide additional UV-B so that it mirrors the natural curve, as has been used in terrestrial studies (McLeod 1997). This is possible with a system in which the intensity given is controlled by a computer system linked to a UV-B sensor that continuously measures ambient UV-B levels. This type of set-up allows the simulation of low levels of enhanced UV-B (5% - 20%) as observed during ozone depletion events, and has been used to study the UV-B response of both MPB (Underwood et al. 1999, Wulff et al. 2000) and phytoplankton (Wängberg et al. 2001).

Artificial radiation

Various artificial radiation sources have been used to assess UVR effects on aquatic autotrophic organisms. An assorted number of them are commercially available, such as fluorescent and halogen lamps. So far, most studies carried out with artificial radiation sources have been done with the main objective to determine the impact of UVR at fixed irradiances (Figueroa et al. 1997a), or in combination with neutral density screens and cut - off filters to obtain biological weighting functions (BWFs) (Cullen & Neale 1994, Neale & Kieber 2000). In order to determine the sensitivity of intertidal and subtidal algae, Dring et al. (1996a, b) used a solar simulator, in which different levels of ozone reduction can be arranged, and Röttgers (1999) had used a similar system to address the response of phytoplankton cultures to changes in UVR. However, it has been found very difficult to mimic the solar radiation spectrum in these types of experiments; in fact very few of the

light sources can give reliable results in photobiological research (Brown et al. 2000). Moreover, one should be extremely cautious when extrapolating the results obtained in this way to the natural environment.

MATERIALS AND FILTERS

A combination of different materials and filters are normally used to separate different wavebands of the incident irradiance spectrum. In most of the experiments conducted either in the field or in the laboratory, it is customary to use tubes or vessels made of a material transparent to UVR, such as Quartz, Plexiglas, or Teflon. There are many types of filters that are broadly used in photobiological research, ranging from 'film type' filters, such as Ultraphan, Folex, Mylar-D, and acetate, to 'glass type' filters such as Schott, Hoya and Oriel. Representative spectra of the transmission characteristics of commonly used filters and materials are shown in Fig. 1. In general, the materials are long pass filters, and thus they screen off the energy of the lower wavelengths. However, there are filters that allow the energy of just a portion of the spectrum to pass, as is the case of the UG11 filters (see Fig. 1).

VARIABLES MEASURED AND EXPERIMENTAL APPROACHES

Various experimental approaches have been used to evaluate the impact of UVR on different cell processes (Fig. 2). The evolution of oxygen (Aalderink & Jovin 1997, Hanelt et al. 1997a) and incorporation of radiocarbon (Steemann Nielsen 1952, Holm-Hansen & Helbling 1995) have been widely used not only to determine the productivity of a water body, but also to assess the impact of UVR (Helbling et al. 1992b, 2001a, b, c, Smith et al. 1992, Kim & Watanabe 1994, Häder et al. 1996a, b, Beardall et al. 1997, Figueroa et al. 1997a, b, Neale et al. 1998b, c). In addition, oxygen microsensors (Revsbech 1989) have been shown to be practical tools for high-resolution measurements of UVR effects in sediments and microfilms (Sundbäck et al. 1996, 1997), particularly in combination with optical microsensors measuring UVR (Garcia-Pichel & Bebout 1996).

In recent years, pulse amplitude modulated (PAM) chlorophyll fluorescence associated with the photosystem II (PSII) has become a useful tool for evaluation of photosynthesis (Henley et al. 1991, Hanelt 1992, 1996, Schreiber et al. 1995, Häder & Figueroa 1997). In fact, chlorophyll fluorescence can function as an indicator of different functional levels in photosynthesis, such as photon capture by light-harvesting pigments, primary light reactions, thylakoid electron transport reactions, dark-enzymatic stroma reactions and slow regulatory feedback processes (Schreiber et al. 1986). The relationship between oxygen evolution and chlorophyll fluorescence in different organisms has also been demonstrated (Genty et al. 1989, Flameling & Kromkamp 1998). Photosynthetic activity has been estimated as chlorophyll fluorescence in macroalgae growing in a variety of water bodies, as in the Arctic (Hanelt et al. 1997b, Hanelt 1998), Antarctic (Bischof et al. 1998b, Hanelt et al. 1994a), North Sea (Dring et al. 1996b), Chinese Sea (Hanelt et al. 1994b), Mediterranean Sea (Häder et al. 1997a, b, Gómez & Figueroa 1998, Jiménez et al. 1998), tropical (Franklin et al. 1996) or Patagonian (Häder et al. 2000, 2001). Taking into account the differences in photosynthetic organization between macroalgae and higher plants, an optimization of the PAM instrumentation has been needed to meet accurately the low chlorophyll fluorescence

emission of macroalgae (Büchel & Wilhelm 1993, Hanelt 1996). Furthermore, the presence of phycobilisomes in the light-harvesting system of red algae results in generally lower fluorescence values than that measured in green - and brown algae (Büchel & Wilhelm 1993). Due to the increased sensitivity of the PAM fluorescence instrumentation in recent years, this technique has been also used to study UV-B effects on MPB (Underwood et al. 1999), as well as to address UVR effects on phytoplankton (Marwood et al. 2000, Schofield et al. 1995, Röttgers 1999).
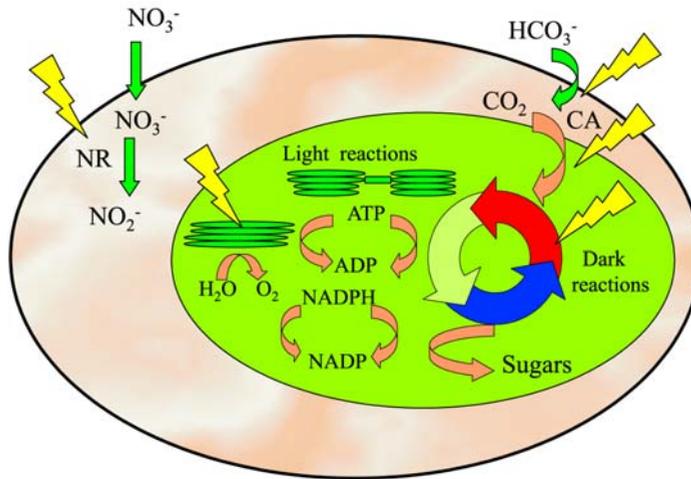

Figure 2: Diagram of a eukaryotic algal cell indicating different processes that could be influenced by UVR and that directly or indirectly affect the photosynthetic process.

Studies on the effects of UVR upon phytoplankton have been conducted using both natural communities and monospecific cultures (Bühlmann et al. 1987, Cullen & Lesser 1991, Cullen et al. 1992, Helbling et al. 1992b, 1994, 1996a, b, 2001a, b, c, Smith et al. 1992, Behrenfeld et al. 1993, Neale et al. 1994, 1998b, c, Vernet et al. 1994, Villafañe et al. 1995b, 1999, Laurion & Vincent 1998, Marwood et al. 2000, Banaszack & Neale 2001). The exposure of samples has included *in situ* (Holm-Hansen et al. 1993b, Vernet et al. 1994, Villafañe et al. 1999, Helbling et al. 2001a) and simulated *in situ* incubations (Helbling et al. 1992b, 1994, 2001c, Vernet et al. 1994), as well as the use of artificial radiation (Bühlmann et al 1987, Helbling et al. 2001b). Short-term studies have been generally performed in periods of less than one day, implying that no acclimation is generally allowed, and hence, some of the observed effects represent the worst-case scenario. Still, the majority of UVR studies on phytoplankton photosynthesis have been done using this approach, and they provide a base of comparison among species and different ecosystems. Long-term experiments (i.e., days, weeks), on the other hand, are a preferable choice when making predictions about the effects of UVR on an ecological scale; however, relatively few studies have been performed using this approach (Worrest 1982, Villafañe et al. 1995b, Helbling et al. 1996b, 2001a, Lesser et al. 1996, Wängberg et al. 1996, Hazzard et al. 1997, Holm-Hansen 1997).

The response of benthic microalgae to UVR has mainly been assessed by studying natural or semi - natural communities *in situ*, or in outdoor experimental flumes (see references below),

although laboratory experiments have also been made (Peletier et al. 1996, McNamara & Hill 2000). Basically, two types of studies have been conducted: (1) experiments where communities have been allowed to colonize on hard substrata (Bothwell et al. 1993, Vinebrook & Leavitt 1996) and, (2) experiments where intact natural communities in sediments have been studied (Odmark et al. 1998, Wulff et al. 1999). These two approaches differ in the aspect that the former allows UVR to exert a selective pressure during early growth and succession, which is not the case when studying already established, dense communities with no or little net growth. These two approaches also differ in the choice of target variables. While photosynthetic rate (^{14}C incorporation, oxygen microprofiles), and photochemistry (PAM) have been monitored for MPB in sediments, accrual of biomass (as chl *a*, or algal cells) has been the most commonly measured variable, particularly in long-term experiments on periphyton on hard substrata (see references in *Effects of UVR on microphytobenthos photosynthesis*).

Finally, the impact of UV-B radiation on marine macrophytes has been mostly conducted on individual species and not on the whole community. The criteria to select species for experimentation / analyses have varied: (a) they are key species due to their contribution in primary production, or because they create a habitat for other marine plants and invertebrates, as the seagrass *Posidonia oceanica* in the Mediterranean Sea (Pergent et al. 1994, Figueroa et al. 2002), *Laminaria* beds in the North Sea (Lüning 1990), or *Macrocystis* on the Pacific coast of California (Mann & Chapman 1975), (b) they represent a high share of macroalgal biomass in the ecosystem, as *Ulva* in eutrophic coastal waters (Schramm & Nienhuis 1996) and, (c) they are commercially important as *Porphyra* sp., *Gelidium sequipedale*, *Macrocystis pyrifera* or *Chondrus crispus* (Figueroa et al. 1997b, 2002, Karsten et al. 1998a, Gómez et al. 2001).

EFFECTS OF UVR ON PHYTOPLANKTON PHOTOSYNTHESIS

In the following paragraphs we summarize the status of our knowledge about both, short–term and long–term effects upon phytoplankton photosynthesis. In the past years, however, several reviews dealing with the impact of UVR on phytoplanktonic organisms have been published (Vincent & Roy 1993, Holm-Hansen & Lubin 1994, Vernet & Smith 1997, Wängberg & Selmer 1997, Vernet 2000), so we encourage the reader to refer to them for more specific details that are not addressed here.

Short-term effects

One of the best-known effects of solar radiation upon phytoplanktonic organisms is photoinhibition, which refers to the reduction of photosynthetic rates at relatively high irradiances (Osmond 1994). Many studies have used production – irradiance (P-E) curves to determine phytoplankton photoinhibition due to high PAR levels (Neale & Richerson 1987, Helbling et al. 1995); in addition, research has been carried out to determine the additional effects of UVR, not only in tropical (Villafañe et al. 1999) but also in temperate (Helbling et al. 2001a) and polar regions (Holm-Hansen et al. 1993b, Helbling et al. 1995). Interestingly, it has been shown that the relative effect of UVR (i.e., as compared to the PAR control) is sometimes higher at lower irradiances. On bright days, when high PAR levels already inhibit the photosystem, UVR produces a relatively lesser effect. This observation, however, depends on many variables, such as the light history of the cells and species composition. In addition, when phytoplankton cells are exposed to increased levels of solar

radiation, they may show a threshold for inhibition, which is followed by a steep increase in photosynthetic inhibition at mid – irradiances, levelling off at higher irradiance values (Helbling et al. 1992b, 2001c). However, in some cases no discernible threshold was determined (Behrenfeld et al. 1993, Helbling et al. 1996b).

In general, when *in situ* incubations are done, UVR causes a sharp decrease in photosynthetic rates (as compared with the PAR – only treatment) especially in surface waters (Fig. 3). Even though UV-B radiation is more effective per unit energy (Blumthaler & Webb 2003), and hence potentially more damaging than those at longer wavelengths, many studies conducted in different locations have showed that UV-A is responsible for most of the photosynthetic inhibition, just because their natural levels are much higher (Holm-Hansen et al. 1993b, Kim & Watanabe 1994, Villafañe et al. 1999). Photosynthesis inhibition decreases with depth, depending, among other things, on water transparency, presence of microorganisms, as well as on incident radiation. The depth distribution of photosynthesis inhibition is highly variable and hence, surface values are not good indicators of the total inhibition in the water column as it has been demonstrated in a comparison between freshwater and seawater environments from mid-latitudes and sub-Antarctic areas (Villafañe et al. 2001). Furthermore, when evaluating the integrated photosynthesis inhibition, it is more important to consider the extent of the euphotic zone that is inhibited (e.g., optical depth), rather than the physical depth at which the inhibition is observed.

Inhibition of photosynthesis due to UVR is highly variable, depending on the irradiance / doses received by the cells, their specific sensitivity and acclimation potential, as well as the interaction with other variables that can mask the observed effects (mixing, temperature, pH, etc). The daily integrated loss of carbon fixation in the euphotic zone in Antarctic waters was calculated to be about 4.9 %, under normal ozone column concentrations (Holm-Hansen et al 1993b). At the time of ozone depletion events, which are responsible for a relative increase in incident solar UV-B (Blumthaler & Webb 2003), there was a greater photosynthetic inhibition – reducing daily aquatic primary production by an additional ~ 4-12% (Smith et al. 1992, Holm-Hansen et al 1993b). However, taking into consideration the magnitude and timing of ozone depletion events, the yearly loss of carbon fixation in the Southern Ocean due to these processes was estimated to be < 0.15% (Helbling et al. 1994). In addition, some studies (Helbling et al. 1994, Neale et al. 1998c) have demonstrated that the effects of mixing, i.e., fluctuating radiation regimes (Neale et al. 2003), are more important in affecting photosynthesis than the variations in ozone levels. Studies conducted with temperate phytoplankton (Barbieri et al. 2002), simulating mixing conditions for Patagonian waters, showed an UVR - induced reduction of photosynthetic rates when the UML extended to a relatively small portion of the euphotic zone (Z_{UML} / Z_{Eu} <0.5). When mixing was deep (Z_{UML} / Z_{Eu} >0.8), and mean PAR levels were low, phytoplankton was able to use UV-A radiation for carbon fixation. The use of solar UV-A at low PAR irradiances has also been observed in Californian waters, with a 10-20 % increase in photosynthesis due to this effect (Prézelin et al. 2000).
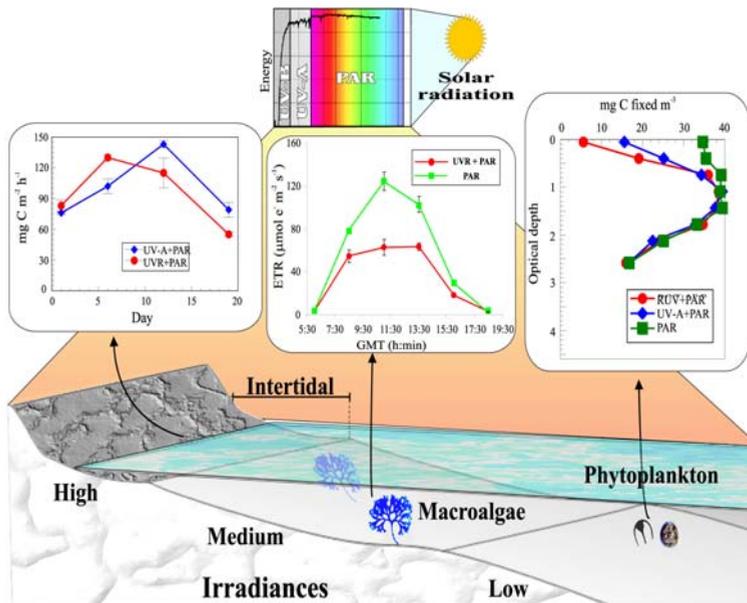
Figure 3: Schematic illustration of potential effects of solar radiation on phytoplankton, MPB and macroalgae photosynthesis. For benthic algae, three zones are defined in term of the irradiance received: high, medium and low. While the high irradiance zone is clearly defined as the intertidal zone, the boundaries between medium and low radiation are uncertain and will vary among different water bodies, species considered, etc. The graphs are examples of UVR – induced photoinihibition in the three groups of algae considered here.

In terms of photosynthesis, studies have demonstrated that tropical phytoplanktonic species are more resistant to UVR than those from polar environments (Helbling et al. 1992b, 2001c, Villafañe et al. 1999), probably due to their evolutionary light history with naturally high radiation levels. In addition, tropical organisms had a higher irradiance threshold for photosynthesis inhibition (Helbling et al. 2001c) than polar species (Helbling et al. 1992b, Helbling & Villafañe 2002), thus providing an additional evidence of their resistance to high UVR levels. Solar radiation increases with altitude (Blumthaler & Rehwald 1992) and thus photosynthesis in lakes located at high altitudes might exhibit enhanced inhibition. The inhibition of photosynthesis, however, depends not only on the irradiance received at the lake surface, but also on the differences on water temperature, attenuation coefficients and phytoplankton composition among other variables (Neale et al. 2001). Biological Weighting Functions (BWFs) (Cullen et al. 1992, Neale & Kieber 2000) had also implied the higher resistance of tropical organisms to UVR (Helbling et al. 2001c) as compared to those from polar environments (Neale et al. 1994, Helbling & Villafañe 2002). Fig. 4 shows a comparison of different BWFs calculated for different geographic locations – Arctic, Antarctica, tropical lakes and temperate latitudes.
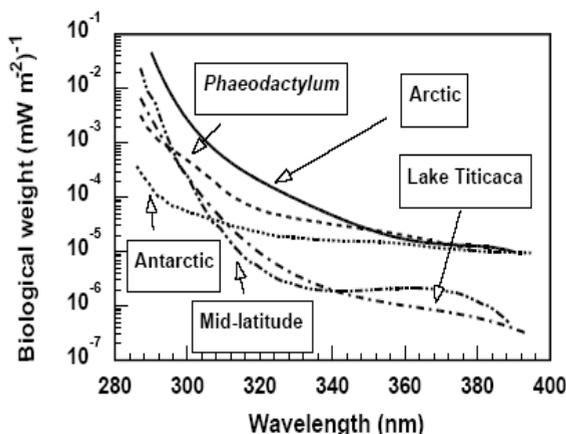
Figure 4: Representative biological weighting functions of a laboratory culture and phytoplankton assemblages from different environments.

Long-term effects

During long-term experiments, species have the potential to acclimate to new radiation conditions, and processes such as DNA repair and synthesis of photoprotective compounds may occur (Helbling et al. 1996a, Holm-Hansen 1997, Roy 2000, Zudaire & Roy 2001, Banaszak 2003). One of the best ways to test UVR effects on aquatic autotrophic organisms on a long–term basis is by using a "model ecosystem" or mesocosms (Wängberg & Selmer 1997), in which a parcel of the aquatic body is isolated and allowed to progress under similar conditions as in the natural environment. The main restriction of these experiments is that it is not possible to completely simulate natural conditions – e.g., water movements are restricted and larger organisms are normally excluded. Hence, one should be cautious when interpreting results obtained in these experiments, as other factors (e.g., immigration) are important components when addressing UVR effects from an ecological point of view (Neale et al. 2003). Experiments carried out in polar areas (Helbling & Villafañe 2002) showed that, at the beginning of experimentation, both Arctic and Antarctic phytoplankton cells were significantly inhibited by UVR. This inhibition, however, did not increase as the experiment progressed, and growth rates (based either on chl *a* content or carbon incorporation) were not significantly different between the UVR+PAR and the PAR treatments (Helbling & Villafañe 2002). Kim & Watanabe (1994) found that even though short-term exposure to UVR provoked a significant decrease of chl *a* and photosynthetic rates in two freshwater phytoplankton species, *Melosira* sp. and *Chlorella ellipsoidea*, under prolonged UV-A exposure, however, the algae acclimatized by reactivation of the photosystem and enhanced cellular chlorophyll synthesis. Results from long-term exposure of freshwater phytoplankton are also very variable with no effects determined in an Alpine location (Halac et al. 1997), low impact of UVR in a community from a Canadian lake (Laurion et al. 1998) and significant changes in phytoplankton composition in a lake from the Andes region (Cabrera et al. 1997).

Interactive effects of UVR with other ecological variables are important when addressing

photosynthetic inhibition on a long–term basis. In particular, temperature seems to play an important role. For example, the temperate dinoflagellate *Prorocentrum micans* had a maximum decrease in photosynthetic rates after 21 days of exposure to solar UVR (Lesser 1996), whereas Antarctic phytoplankton had this maximum inhibition after 9 days (Lesser et al. 1996). In addition, research has been conducted to address the interactive effects of UVR and nutrient limitation. There was variability in the responses, with studies that revealed that nutrient – limited cultures were more sensitive to UV-B than nutrient – replete cultures (Cullen & Lesser 1991; Lesser et al. 1994); however, Behrenfeld et al. (1994) did not find growth inhibition produced by UV-B in nitrogen – limited cultures. Bergeron & Vincent (1997) determined growth rates in different phytoplankton size categories present in a P - enriched system in a Subarctic lake and found different responses according to the wavebands to which cells were exposed.

EFFECTS OF UVR ON MICROPHYTOBENTHOS PHOTOSYNTHESIS

Although benthic microalgal communities include the same major algal taxa as the pelagic communities, there is a crucial difference. The density of autotrophic, as well as heterotrophic microorganisms is several orders of magnitude higher in benthic communities, resulting in microbial mats or biofilms. These are characterized by steep physical, chemical and biological gradients, leading to a close spatial and temporal coupling of turnover processes within the mat system (Paerl & Pinckney 1996). Thus, it can be expected that the responses to UVR of these communities are rather complex.

Short-term effects

Decreased photosynthetic rates (measured as ^{14}C uptake, oxygen production, or chlorophyll fluorescence) appear to be the most frequently observed short-term effect for MBP, particularly at enhanced UV-B levels (Garcia-Pichel & Castenholz 1994, Bebout & Garcia-Pichel 1995, Sundbäck et al. 1997, Odmark et al. 1998, Underwood et al. 1999, McNamara & Hill 2000, Wulff et al. 2000). These results are, however, ecologically relevant only when realistic, moderate increases of UV-levels are used. Wulff et al. (2000) found a 50% decrease in ^{14}C - uptake of MPB on sand when UV-B was increased by 15% above ambient (23% when biologically weighed according to Cullen et al. (1992), though only under nutrient depleted conditions. Using oxygen microsensors, Bebout & Garcia-Pichel (1995) found a dramatic (50-90%) decrease in gross photosynthesis of the surface layers of a cyanobacterial mat (*Microcoleus chthonoplastes*) under moderate UV-B irradiances (0.35-0.79 W m^{-2}). This decrease was also related to an active downward migration in response to UV-B. Non - invasive fluorescence measurements on natural diatom biofilms (dominated by *Gyrosigma balticum*) exposed to supplemented UV-B (7 and 15% above ambient), resulted in a sequence of responses, starting by significantly increased effective quantum yield - Φ_{PSII} (probably reflecting downward migration), followed by a reduction in maximum quantum yield of PSII (F_v/F_m) and minimal fluorescence (F_o) (Underwood et al. 1999).
Observed short-term responses of MPB photosynthesis to ambient UV levels are less clear-cut, when compared with supplemented UV-B, and appear to vary with substrate type and community density. For a muddy sediment, no significant effects of ambient UV-B on either carbon uptake or oxygen microprofiles of a diatom mat (dominated by large motile

species) were found (Sundbäck et al. 1996). In a sandy sediment, on the other hand, both carbon uptake and allocation of a community dominated by small (mainly non - motile) diatoms and cyanobacteria, decreased significantly under ambient UV-B, although only at the end of a 3 - week experiment (Odmark et al. 1998). Finally, no effects of ambient UV-B on photosynthetic rates were observed in freshwater stream periphyton (Hill et al. 1997).

Long-term effects

Growth, measured as the accrual of biomass or chl *a*, is the variable most often studied for long-term UV-B effects on MPB. The clearest negative effects of UV-B on MPB growth have been observed for periphyton colonizing artificial substrata (Bothwell et al. 1993, Vinebrook & Leavitt 1996, Francoeur & Lowe 1998, Santas et al. 1998a, Reizopoulou et al. 2000). However, in the majority of these experiments, significant negative effects on growth (30-100% decrease) were only found during the first few weeks. After this UV - inhibition phase, statistically significant negative effects disappeared, or were even reversed (Bothwell et al. 1993, Kiffney et al. 1997a, Santas et al. 1998b, Vinebrook & Leavitt 1996, 1999) (note that some of these studies excluded both UV-A and UV-B). In one case, the explanation for the reversed effect was the higher sensitivity of grazers than their prey (Bothwell et al. 1993, 1994, Sommaruga 2003). When experimenting with already established periphyton communities, however, no detrimental effects were observed (Hill et al. 1997, Vinebrook & Leavitt 1998).

The effect of UV-B on the growth of natural, established MPB communities inhabiting marine sediments shows a different general pattern than the above-mentioned colonization experiments. In sediments, significant effects appear to be fewer, they are more frequently found for rate variables (photosynthesis, C-allocation) than for state variables (biomass, pigment and species composition), and they occur later during the experiment (after 1-2 weeks) (Fig. 3) (Sundbäck et al. 1997, Odmark et al. 1998, Wulff et al. 1999). The delay of effects may partly be due to increasing nutrient limitation in the course of the experiments caused by the experimental set-up, particularly when working with sandy sediments, which are generally poorer in nutrients than fine sediments (see Odmark et al. 1998 and section *Nutrient incorporation / assimilation and enzyme activities*). However, an intriguing question is why the observed effects on rate variables were not reflected more clearly in the state variables? Besides biological reasons, there could be methodological reasons. For example, if a deeper sediment layer than the actual layer affected by UV-B is sampled, there might be a 'dilution' of effects (see further details in Wulff et al. 1999).

Peletier et al. (1996) concluded, from laboratory experiments with diatom species isolated from intertidal sediments, that ambient (or even future increased) UV-B is unlikely to affect sediment - inhabiting MPB. Although it may appear that experiments on intact sediment MPB do at least partly support this conclusion (Sundbäck et al. 1996), they do not fully rule out the role of (even ambient) UV-B as a controlling factor (Odmark et al. 1998, Underwood et al. 1999, Wulff et al. 2000). Odmark et al. (1998) found that, while the removal of UV-B created a response, moderate enhancement of UV-B had less obvious effects. This suggests that ambient UV-B can indeed be a factor exerting a selective pressure on MPB particularly in sandy sediments, whereas an increased UV-B exposure due to ozone depletion would not severely affect the type of MPB community studied. However, given that UV-B,

at present level, is a selective force in MPB communities of sandy sediments, there is no *a priori* reason to assume that the communities should respond to a less degree to an increase in UV-B levels. Moreover, early successional growth phases of sediment communities are indeed, like periphytic communities, susceptible to moderately enhanced (15%) UV-B levels (Wulff et al. 2000). Epipelic communities on sediments of oligotrophic lakes have also shown a significant response to ambient UV-B (Vinebrook & Leavitt 1999).

Are UV-B effects on microphytobenthos habitat - specific?

The benthic habitats in the above-cited experiments differ in several aspects, such as the type of substratum, water movement and nutrient status. Also the community properties, such as the level of productivity and composition of the food webs (e.g., importance of grazers) vary greatly. Are UVR responses then habitat - specific? The answer appears to be yes, although similarities in responses also exist. When MPB on both hard and soft substrata (and phytoplankton) were studied simultaneously in alpine oligotrophic lakes in Canada (Vinebrook & Leavitt 1996, 1999) it was found that attached periphyton was affected by ambient UV-B, while epipelon of the sediment and phytoplankton remained unaffected. On the other hand, Vinebrook & Leavitt (1998) found that ambient UVR had no effect at all on epilithon, while a significant stimulating effect was found for epipelon. This contradicting result was explained by the fact that established epilithon, and not early successional stages, were studied in the latter experiment. An indication of the importance of the substratum type was also found for sediment MPB in a microtidal brackish - water area in Sweden. More variables were affected by UV-B in the sandy than in the muddy sediment (Sundbäck et al. 1996, 1997, Odmark et al. 1998). This suggests that muddy sediments may function as a refuge for MPB (shallow photic zone, dominance of motile diatoms), while in the sandy sediment, UVR penetrates deeper and the MPB is dominated by small - sized attached species.

Among community properties, grazing pressure is obviously an important controlling factor for the susceptibility of the MPB to UV-B. Heavily grazed periphyton communities become thin and are thus more sensitive to UV-B (McNamara & Hill 2000). On the other hand, if the grazers are more sensitive to UV-B than the algae, the algal growth will benefit from UV-B (Bothwell et al. 1994, Sommaruga 2003).

On a larger geographical scale, climate, latitude / elevation, and general nutrient status of the ecosystem may explain differences in the UV-B responses of MPB. As discussed before, early colonization stages of MPB are more susceptible to UV-B than already established, thick communities. Thus, UV-B can be expected to be a more important controlling factor in for example a cold climate where colonization events are more frequent due to ice and scouring (Vinebrook & Leavitt 1996). Similarly, freshwater periphyton in mid - latitudes (DeNicola & Hoagland 1996, Hill et al. 1997) may be more resistant to current UV-B stress than periphyton communities of higher latitudes (Bothwell et al. 1993, Vinebrook & Leavitt 1999). The combined effects of climate warming and increased input of dissolved organic matter (DOM) have been suggested to moderate the effects of UVR - increase in alpine oligotrophic lakes (Vinebrook & Leavitt 1999). However, climate change in combination with acidification may also increase the exposure of organisms to UV-B, particularly in clear, shallow lakes and streams (Schindler et al. 1996).

EFFECTS OF UVR ON MARINE MACROPHYTE PHOTOSYNTHESIS

Studies on comparative primary productivity of marine macrophytes under different scenarios of UV climate are rather scarce; moreover, there is a diffuse picture of their photoadaptive strategies. Taking into account the distinct origin and the morpho - functional divergences of macroalgal species, a common adaptive strategy is unlikely. Thus, a number of responses can be determined among species. However, a general pattern is observed: under natural radiation levels they show daily photoinhibition – a decrease in the photosynthetic rates / yield (Osmond 1994, Häder et al. 1996b), at least at high zenith angles (Häder et al. 1996a, b, 1997a, b). In most cases, high PAR irradiances at noon cause a decrease in photosynthetic rates (Hanelt 1992, Häder & Figueroa 1997), but UVR also contributes largely to this process (Wood 1989, Larkum & Wood 1993, Dring et al. 1996b, Figueroa et al. 1997b, Hanelt et al. 1997a).

In intertidal algae in particular, the highest photoinhibition values (mainly due to PAR) are found when low tide coincides with local noon (Hanelt 1996, Jiménez et al. 1998). Even algae harvested from rock pools, where they are normally exposed to extreme solar irradiances, show signs of photoinhibition after prolonged periods of exposure (Figueroa et al. 1997b, Häder & Figueroa 1997). Under these conditions, increases in temperature and partial dissecation of algal thallus also contribute to the observed photoinhibition (Hanelt 1992, Figueroa & Gómez 2001). Deep - water algae and those adapted to shaded environments are inhibited even faster when exposed to direct solar radiation (Häder & Figueroa 1997).

Recovery of photosynthesis - measured as an increase in fluorescence quantum yield - starts when irradiance begins to decrease, but remains still at saturating levels. Recovery is species - specific and occurs faster in sun - adapted algae than in algae growing at deep or shaded locations and then transferred to the surface. In the eulittoral red algae *Porphyra leucosticta* (Figueroa et al. 1997b), *Asparagopsis armata* and *Felmanophycus rayssae* (Jiménez et al. 1998) from southern Spain, recovery of photosynthesis occurs immediately after a decrease of only 10 - 20% of solar radiation. However, the brown algae *Padina boryana* recovers with a 30% irradiance decline, whereas in *Sargassum polycystum*, a reduction of 70% in the incident radiation is required (Hanelt et al. 1994b). In their review on red macroalgae, Figueroa & Gómez (2001) reported photoinhibition of ~ 30 - 80% at noon, but most of the species showed full recovery in the afternoon. In contrast, only partial recovery was observed in red algae from the North Sea (Dring et al. 1996b) or from Patagonian waters (Helbling et al., unpub. data). The recovery of macroalgae after UVR exposure (as compared to the PAR control) is highly variable, with little recovery found in *Macrocystis pyrifera* (Clendennen et al. 1996) and in *Gelidium sesquipedale* (Gómez & Figueroa 1998), and high recovery with beneficial effects of UV-B in the brown alga *Dictyota dichotoma* (Flores-Moya et al. 1999) and in the marine angiosperm *Posidonia oceanica* (Figueroa et al. 2002). These specific responses provide important information, as the recovery kinetics gives insights into the photoadaptive strategies of macroalgae and their light - stress tolerance capacity. Thus, those algae capable of dynamic (reversible) photoinhibition under high solar radiation levels and with a rapid recovery capacity will have competitive advantages as compared to those without any efficient photoprotection mechanism.

The ability for dynamic photoinhibition during exposure to high radiation, as well as the general degree of photosynthetic adaptation of individual species to different light regimes influences the upper depth distribution of algal zonation (Henley et al. 1991, Franklin et al. 1996, Hanelt

1996, 1998). In fact, several taxa and life history stages of inter- and subtidal polar algae show a strong correlation between their depth distribution and their capacity to cope with high radiation stress (Hanelt et al. 1997a, Bischof et al. 1998a, b, Hanelt 1998). Thus, species growing in the upper subtidal zone show in general more tolerance to high solar radiation levels, especially to UVR, than algae from deeper waters (Drew 1974, Larkum & Wood 1993, Dring et al. 1996a, b, Hanelt et al. 1997a, b).

Relatively few studies have been conducted on a long-term basis to determine the effects of UVR on photosynthesis and growth of macroalgae (Santas et al. 1998a, Altamirano et al. 2000). In experiments carried out with *Ulva* sp., UV-B caused a decrease of both growth rates and photosynthesis during the first week of exposure to solar radiation, but UV-A stimulated growth as compared to the PAR treatment (Altamirano et al. 2000). However, after two weeks of exposure, no differences were observed between treatments, a fact that hints to the action of acclimation mechanisms, which protect algae against UV stress (see below and Banaszak 2003)

CARBON AND NITROGEN ALLOCATION

There is evidence that UVR, especially UV-B, affects carbon allocation in aquatic autotrophic organisms. This has important consequences for food web dynamics, as these changes will affect growth and consequently, the availability of food for other trophic levels, such as bacteria and heterotrophic microorganisms (Sommaruga 2003, Zepp 2003). Changes in lipid, protein, polysaccharide, and fatty acid levels due to UVR have been determined in some phytoplanktonic and MPB organisms (Döhler & Bierman 1994, Goes et al. 1994, 1997, Norsker & Støttrup 1994, Wang & Chai 1994, Arts & Rai 1997, Odmark et al. 1998, Skerratt et al. 1998). These studies have especially highlighted the variations in responses, according to the specific sensitivity of the organisms. For example, Buma et al. (1996), working with three marine diatoms, found a significant increase in cell protein content when cells were exposed to low UV-B doses, whereas the opposite occurred at higher doses. Veen et al. (1997), working with a chlorophyte, demonstrated an increase in cell protein levels when cells were exposed to UVR. Skerrat et al. (1998) exposed the diatom *Odontella* to UV-B radiation and found a reduction in lipid content whereas an increase in *Chaetoceros* was found. Goes et al. (1994, 1997), working with diverse phytoplanktonic species, found changes in the rates and sizes of storage and structural carbohydrates and polyunsaturated fatty acids when exposed to UV-B. Moreover, Döhler (1997a, b) found UVR – induced changes in pool sizes of diverse amino acids of several Antarctic and temperate marine phytoplankton species with UV-A causing, in general, an increase in their levels, whereas UV-B produced the opposite effect. These results agree with those obtained by Goes et al. (1995), who also showed that UV-B caused changes in amino acid concentrations within the cell. Finally, studies performed to determine UVR effects on the ATP content of Antarctic phytoplankton, showed a reduction in this component when cells were exposed to UVR (Vosjan et al. 1990), but Döhler & Biermann (1994), working with a marine diatom did not find any effect.

Studies performed with MPB communities in sediments have also demonstrated changes in carbon allocation as a result of UVR exposure. The most frequently observed change was a larger relative allocation to proteins at UV-B exposure (Sundbäck et al. 1997, Wulff et al. 1999, 2000). This can be interpreted as a larger proportion of fixed carbon spent on growth when carbon fixation decreases, as microalgal cells tend to retain synthesis of proteins rather

than storage products under adverse environmental conditions (Marañon et al. 1995). Other UV-B effects on carbon allocation were related to polar lipids, which were lower under enhanced UV-B (Odmark et al. 1997). In macroalgae it was also found that UV-B radiation affects carbon and nitrogen allocation, although very few studies have been done in this regard. For example, Altamirano et al. (2000) found that more than 78% of the seasonal changes in the internal content of carbon and nitrogen in the green macroalga *Ulva rigida* were explained by seasonal changes of UV-B.

MECHANISMS TO REDUCE THE EFFECTS OF UVR ON PHOTOSYNTHESIS

Adaptation to UVR assumes the existence of mechanisms that protect the organism or reduce the deleterious effects. According to Roy (2000) four basic mechanisms allow an organism to cope with a stressful situation, i.e., UVR exposure (1) avoidance, (2) reducing the stress by a physiological behavioral mechanism – e.g., through the synthesis of UV - absorbing compounds, (3) repairing the damage produced and, (4) acclimating to the stress allowed enough time. More details on these mechanisms can be found in Helbling & Zagarese (2003).

Avoidance mechanisms seem to be a common strategy against exposure to high levels of UVR. For microalgae living in soft substrata, such as motile cyanobacteria and diatoms (Bebout & Garcia-Pichel 1995, Quesada & Vincent 1997, Underwood et al. 1999), this involves downward vertical migration. Bebout & Garcia-Pichel (1995) showed that by migrating down to 300 µm depth, cyanobacteria could reduce their UV exposure to 10% of that at the sediment surface. For benthic diatoms, the observed downward movement of *Gyrosigma balticum* at high light levels was first suggested to be related to PAR rather than UV-B (Sundbäck et al. 1996, 1997). However, a subsequent fluorescence study indicated that the migration could in fact be a direct response to UV-B (Underwood et al. 1999). Avoidance can also be achieved by means of circadian rhythms that allow an organism to swim down at noon to depths where radiation intensities are low, as occur in some dinoflagellates (Tilzer 1973). However, it should be observed that UVR can alter the motility and phototaxis of some autotrophic organisms, such as several microalgal species (Häder et al. 1995). Moreover, in other organisms, loss of flagella has also been reported (Hessen et al. 1995). Thus, in some sensitive organisms, avoidance mechanisms can be severely altered by UVR exposure.

Another strategy to minimize the effects of UVR is through the presence of UV – screening compounds. The most studied compounds are those collectively named mycosporine like amino acids (MAAs), which are found in many marine and freshwater autotrophic and heterotrophic organisms (Karentz et al. 1991b, Dunlap & Shick 1998, Banaszak 2003). Evidence of their protective role upon physiological mechanisms remains still unclear, and in some cases it seems that they just provide partial protection, as in some cyanobacteria (Garcia-Pichel et al. 1993). In other cases, though, MAAs have been proved to be an effective protection mechanism (Helbling et al. 1996a, Neale et al. 1998a) so that photosynthesis in phytoplanktonic cells with higher amounts of MAAs was less inhibited. In benthic diatoms, however, the production of such protective substances does not appear to be a major strategy. Although MAAs have been detected in MPB of shallow - water subtidal sediments, the concentrations are low and show no significant increase at UV exposure (ambient or increased) (Sundbäck et al. 1996, 1997, Odmark et al. 1998, Wulff et al. 1999), which agrees with the findings of

Peletier et al. (1996). Jeffrey et al. (1999) tested 152 algal species and found that diatoms generally had low concentrations of UV - screening compounds as compared with other algal groups. Moreover, Helbling et al. (1996a) found that pennate diatoms (which usually dominate benthic diatom communities) contained less MAAs than centric diatoms. Other compounds may also have a protective role, functioning as UV - screening agents (Banaszak 2003). For example, scytonemin is a UV - absorbing extracellular substance found in the sheath of cyanobacterial filaments (Garcia-Pichel et al. 1992). In addition, high concentrations of carotenoids as a result of UVR exposure have been observed in diatom mats (Underwood 1999), some cyanobacteria and chlorophytes (Goes et al. 1994), suggesting an UV-protecting function of these pigments.

MAAs have also been reported in green, red and brown macroalgae from tropical, temperate and polar regions (Tsujino et al. 1980, Wood 1989, Karentz et al. 1991b, Karentz 1994, Karsten et al. 1998a, b). The concentration of MAAs in macroalgae has been found to be related to depth zonation and UV exposure (Karentz et al. 1991b). Their accumulation seems to be higher under high than under low daily irradiance values (i.e. different latitudes), and moreover, generally higher in intertidal than that in subtidal algae (Karsten et al. 1998b, Karsten & Wiencke 1999, Hoyer et al. 2001). In addition, this accumulation seems to be a wavelength-dependent process (Karsten et al. 1998a, Franklin et al. 1999, 2001, Karsten & Wiencke 1999), and an UV-B-mediated increment of these compounds has been shown in a variety of algae (DeNicola & Hoagland 1996, Molina & Montecino 1996). In *Chondrus crispus*, both UV-A and UV-B stimulated a strong accumulation of shinorine, whereas the content of palythinol and palythine was mainly stimulated by PAR, indicating a MAA - specific induction triggered by these wavelengths (Karsten et al. 1998a). In *Palmaria palmata*, on the other hand, and when exposed only to PAR, a 6 - fold increase in the porphyra - 334 concentration was observed; the treatment receiving PAR+UV-A gave similar results plus to an accumulation of shinorine; under full solar radiation, accumulation of porphyra - 334, shinorine and palythine was observed (Karsten & Wiencke 1999). In addition, in *Chondrus crispus*, pre - exposure to blue light followed by growth under natural UV-A led to a 7-fold increase in the synthesis of shinorine as compared with growth without the blue light pre-treatment (Franklin et al. 2001). So, it has been hypothesized that there are two photoreceptors for MAAs synthesis in *C. crispus*, one for blue light and one for UV-A, which act synergistically (Franklin et al. 2001). In macroalgae, other types of potentially protective compounds are also found, such as phlorotanins in brown algae (Pavia et al. 1997) and coumarins in the green alga *Dasycladus* (Gómez et al 1998, Pérez-Rodríguez et al. 1998).

In addition, and while UVR – mediated DNA damage occurs in aquatic autotrophic organisms (Buma et al. 1996, 1997, 2001b, Mitchell & Karentz 1993, van de Poll et al. 2001), repair mechanisms of the DNA molecule (Buma et al. 2003) are also present (Mitchell & Karentz 1993). However, the presence of one or other mechanism (i.e., photoreactivation, nucleotide excision repair or recombination repair) is clearly dependant on the species under study and the radiation conditions at which the cells are exposed (Buma et al. 2003).

Finally, acclimation mechanisms to cope with high UVR intensities are important in several aquatic organisms. These usually occur on a long–term basis, when organisms have been exposed for enough time to the stress factor (UVR). One of these acclimation mechanisms is the previously mentioned synthesis of MAAs, as found in some natural populations and cultures of phytoplankton (Villafañe et al. 1995b, Helbling et al. 1996a, Zudaire & Roy 2001).

However, the synthesis of UV - absorbing compounds is not a general response, and several species do not show an increase of MAAs content even after several weeks of exposition to UVR (Behrenfeld et al. 1992, Villafañe et al. 2000). Acclimation can also occur through a change in the community composition (Villafañe et al. 1995b), so that those species more adapted to a particular light regime will dominate. For example, a natural Antarctic phytoplankton population dominated by flagellates (80% in terms of carbon biomass), changed to a diatom dominated population when receiving UVR + PAR; whereas in those samples receiving only PAR, small flagellates still dominated. However, diverse responses are observed in different sites. For example, Mousseau et al. (2000) in their study conducted with an estuarine community also observed changes in diversity when samples were exposed to different radiation treatments. A shift from a diatom – dominated community to small flagellates occurred more rapidly in the treatment receiving enhanced UV-B as compared to those receiving natural UV-B levels. Clearly, responses are strongly species – specific and depend on radiation levels and quality to which organisms are exposed.

OTHER PHOTOSYNTHESIS - RELATED EFFECTS

There are a number of UVR effects that are closely related to the photosynthetic performance of aquatic primary producers. These effects are due to the couplings between radiation – especially UVR - and a number of morphological and biochemical factors within the cell (Hessen et al. 1997). Thus, for example, radiation – induced changes in nutrient uptake, synthesis and allocation of metabolic compounds, motility / orientation, and cell morphology will result in variations in photosynthetic rates. In the following paragraphs we will outline some of these effects - nutrient incorporation and enzyme activities related to carbon and nitrogen metabolism, accumulation or damage on pigments. Specific effects, such as DNA damage, which may induce a reduction in growth rates (Buma et al. 1995) and hence affecting overall primary productivity, are addressed in Buma et al. (2003).

Nutrient incorporation / assimilation and enzyme activities

Growth of aquatic autotrophic organisms is dependent not only on carbon assimilation, but also on the incorporation and assimilation of nitrogen, phosphate, sulfur and several micronutrients (Lobban & Harrison 1997). In general, it is considered that UVR – especially UV-B - is an inhibitor of uptake processes (especially nitrogenous), whereas UV-A stimulates or exerts no significant effects on the uptake of these ions (Döhler 1997a, b). In particular, studies carried out with phytoplanktonic organisms have demonstrated that nitrate and ammonium uptakes are affected by UVR (Döhler 1987, 1992, Döhler & Buchmann 1995). Furthermore, Döhler (1992, 1995, 1997a, b), working with several Antarctic and North Sea phytoplanktonic species has pointed out the diversity of responses among the organisms tested. Thus, samples dominated by the prymnesiophyte *Pheaocystis pouchetii* were very sensitive to UV-B doses (in terms of ^{15}N-ammonium uptake), and so were those containing *Ceratium* sp., *Coscinodiscus* sp. and *Noctiluca* sp. ^{15}N- nitrate uptake was not or only slightly affected by UV-B irradiances (Döhler 1992). On the other hand, in experiments conducted with North Sea natural phytoplankton populations, Döhler & Hagmeier (1997) found that UV-A radiation stimulated ^{15}N – ammonium uptake. Fewer studies have addressed the effects of UVR on P - uptake of phytoplanktonic cells. Hessen et al. (1995), working with the chlorophyte *Chlamydomonas reinhardtii*, found a stimulation

under low UV-B doses (< 3.6 kJoules m^{-2} at 312 nm), but higher inhibition when UV-B doses were higher. In addition, studies on UV-B effects carried out in both sandy and muddy sediments have suggested that the nutrient availability may be an important factor for the susceptibility of MPB communities to UV-B exposure (Odmark et al. 1998). Wulff et al. (2000) designed an experiment to test this hypothesis, and showed that the availability of nutrients indeed can act to mitigate the effects of UV-B on a microbenthic community on a sandy substratum.

Some studies have also addressed the UVR effects on nutrient incorporation in marine macroalgae (Franklin & Forster 1997, Häder & Figueroa 1997). In particular, these studies have focused on UVR effects upon carbon anhydrase (CA) and nitrate reductase (NR) activities (Döhler et al. 1995, Flores-Moya et al. 1998, Gómez et al. 1998, Viñegla et al. 2000a, b, Figueroa & Viñegla 2001). These are important enzymes involved in the incorporation of carbon and nitrogen within the cell (Turpin 1991), thus any stress factor that affects them will ultimately influence photosynthesis. Studies carried out with algae collected from southern Spain (Flores-Moya et al. 1998, Gómez et al. 1998, Viñegla et al. 2000a, Figueroa & Viñegla 2001) found daily variations (i.e., circadian rhythms) in NR and CA activities. In *Dasycladus vermicularis* it was found that these variations were antagonistic during the onset of solar radiation, although these changes only partially matched those of photosynthesis (Gómez et al. 1998), suggesting that these processes are affected differentially by UVR. In long-term studies, it has been shown that UV-A radiation stimulated NR activity, and UV-B decreased both nitrate uptake and NR activities (Viñegla 2000, Viñegla et al. 2000b). On the other hand, UV-B radiation seems to stimulate CA activity in eulittoral algae but not in subtidal (Figueroa 1998, Viñegla et al. 2000b). In addition, experiments were conducted to determine the effects of UVR on the activity of Calvin cycle enzymes, such as ribulose-1,5-biphosphate carboxilase / oxygenase (Rubisco) and glyceraldehyde -3 –phosphate dehydrogenase (G3PDH), and in Arctic macroalgae it was found that the photosynthetic activity decreased due to the negative effects of UVR upon these enzymes (Bischof et al. 2000).

Pigments

Several researchers have reported the decrease of photosynthetic pigments due to exposure to UVR (Helbling et al. 1993, Gerber & Häder 1995, Maske & Latasa 1997). This reduction can be due to a combination of factors, such as the inhibition of *de novo* synthesis and the natural turnover of pigments, or directly to photobleaching (Maske & Latasa 1997). Bleaching can occur not only by UVR, but also due to exposure to high PAR intensities (Maske & Latasa 1997); it is species – specific and also depends on the spectral characteristics of the radiation treatments imposed to the cells. Helbling et al. (1993), working with several marine phytoplankton cultures, found that *Nannochloris oculata* (Eustigmatophyceae) had a decrease in chl *a* content of 30, 60 and 80% under PAR only, PAR + UV-A, and PAR + UVR, respectively, after being exposed for 4.5 h to solar radiation. The prymnesiophyte *Isochrysis galbana*, on the other hand, did not experience significant changes in chl *a* content (for the same radiation treatments) even after 7 h of exposure. Other experiments have also demonstrated the differential sensitivity to UVR of various pigments (Quesada et al. 1995), with the phycobiliproteins being especially sensitive to these wavelengths (Sinha et al. 1995). Absolute amounts of photosynthetic pigments, commonly used as an estimator of growth in

autotrophic organisms, seem to be also affected by UVR. During a long–term experiment, Helbling et al. (1992b) simulated ozone depletion events by moving Antarctic phytoplankton towards the Equator, so that the samples were exposed not only to increased levels of UVR, but also to natural changes in the relative proportions of UV-B and UV-A. They found a decrease in the growth rate of Antarctic phytoplankton exposed to UVR as compared to that exposed to the PAR – only treatment. However, growth rates were not significantly different when the samples were incubated under UVR levels similar to those found at their sampling site in the Antarctic. Data on long-term experiments conducted in both polar areas (Helbling & Villafañe 2002) showed that even though photosynthesis was initially affected by UVR (day 1), growth rates, evaluated either as carbon fixation or chl *a* content did not show any significant differences. In general, studies have demonstrated that different growth responses due to UVR exposure occur not only among taxa (Jokiel & York 1984, Villafañe et al. 2000), but also within the same genus. For example, in the chlorophyte *Dunaliella salina* growth rate was not affected by UVR, whereas in *D. tertiolecta* it was significantly reduced after 3 days of exposure (Villafañe et al. 2000).

The differential sensitivity of pigments to UVR has also been studied for MPB organisms. Phycobilins of cyanobacterial mats appear to be more sensitive than chlorophylls and carotenoids, the latter often increasing at UV-B exposure (Garcia-Pichel & Castenholz 1994, Quesada et al. 1995, Quesada & Vincent 1997). However, in experiments conducted on intact sediment communities dominated by diatoms, no changes in pigment composition (expressed as ratios to chl *a*) were observed (Sundbäck et al. 1996, 1997, Odmark et al. 1998, Wulff et al. 1999, 2000), with one exception: higher carotenoid content was observed at enhanced UV-B levels in a *Gyrosigma* mat, probably reflecting a UV-B - protecting strategy (Underwood et al. 1999).

In marine macroalgae, various responses were also found when addressing the effects of UVR upon various pigments. Exposure of *Porphyra umbilicalis* to artificial UVR levels decreased chl *a* and phycocyanin concentrations by 65 and 67%, respectively, whereas carotenoids and phycoerythrin decreased by as much as 75 and 82%, respectively (Aguilera et al. 1999). Furthermore, and under ambient levels, UVR not only decreased the concentration of chl *a* and biliproteins in the red alga *Porphyra leucosticta*, but the pattern of daily variation was also affected (Figueroa et al. 1997b). The damage of photosynthetic pigments by UVR in *P. leucosticta* was suggested to be the cause of a decrease in photosynthetic rates. However, in *Macrocystis pyrifera*, it was found that the main light - harvesting complex of this alga, the fucoxanthin - chlorophyll protein, was the specific site for UV damage (Wood 1989). Finally, in *Ulva rigida* (Altamirano et al. 1999) and *Dasycladus vermicularis* (Pérez-Rodríguez et al. 1998), the content of chlorophyll and carotenoids was significantly higher in the presence of UV-B than that in the control (PAR - only), suggesting the presence of an efficient protective – pigment mechanism.

Cell morphology and size

When evaluating the photosynthetic responses to UVR of diverse organisms, some studies have revealed the importance of cell size (Karentz et al. 1991a, Helbling et al. 1992b, 2001a, b, Laurion & Vincent 1998). In phytoplanktonic organisms, it was found that, although there is certainly variability in responses, small cells – with a relatively high surface to

volume ratio – are more resistant to photosynthesis inhibition but more vulnerable to DNA damage (Buma et al. 2001b, Helbling et al. 2001a, b). On the other hand, and provided that microplanktonic cells (20-200 µm) do not have high concentrations of UV - absorbing compounds, they are more vulnerable to UVR (in terms of photosynthesis). This has been demonstrated in a comparative study carried out in the Andean lakes (Helbling et al. 2001b), where it was found that larger phytoplanktonic cells had a higher kinetics of inhibition and hence were more affected by UVR than smaller cells. For MPB organisms, on the other hand, there are contradicting findings as to whether UV-B - related changes in species composition are related to cell size or are due to taxon - specific sensitivity (Garcia-Pichel 1994, Peletier et al. 1996, Helbling et al. 2001b). As seen for planktonic algae, increasing size may occur both on an individual species level, as cell division is hampered (Karentz et al. 1991a, Behrenfeld et al. 1992), and on community level, as species with larger cell-size could be favoured (Garcia-Pichel 1994, Wängberg et al. 1996). For MPB there is some indication for the latter, but not for the former. Bothwell et al. (1993) found that large, stalked diatom species increased their dominance at UV-B exposure during periphyton succession, and Vinebrook & Leavitt (1996) found that the growth of the small - sized diatom *Achanthes minutissima* was suppressed under UV-B exposure.

Besides size, the morphology also seems to influence the response of algae to solar radiation. This has been shown particularly for macroalgae. A comparison between the red algae *Porphyra leucosticta* and *Rissoella verruculosa*, which have comparable zonation patterns at intertidal sites, shows the different photo - protective strategies of these algae (Figueroa et al. 1997b, Flores-Moya et al. 1998). This is probably related to different absorption properties because of the thallus thickness and pigment composition. *P. leucosticta* has a thin thallus consisting of one cell layer in which light transmits rapidly and homogenously towards the harvesting complexes, whereas in *R. verruculosa*, which has a more complex structure, some scattering of photons through the multilayered thallus (self-shading) may take place. This was evident when the algae were exposed to full solar radiation, and in *Porphyra* UVR accounted for about 30% of the total photoinhibition, whereas no effects were observed in *Rissoella*. In addition, some studies hint about the importance of different life stages, which are closely related with size. Although studies have focused on the macrothallus or adult stages, it is expected that UVR stress would be more evident in the microscopic life stages (single - and few - celled), mainly due to their structural simplicity. These studies, in addition, bring about important consequences for algal zonation. For example, depth distribution patterns of large kelps have been frequently thought to reflect the light requirements of establishment stages (spores, embryos, etc). Paradoxically, most studies performed to address the relationship between the physiological performance under different light environments have been done with the large sporophytes, whereas spore adaptation has been largely overlooked (Huovinen et al. 2000). The question of whether early developmental stages of macroalgae, particularly spores, are more susceptible to UVR than larger life history phases, has been less addressed (Dring et al. 1996a). If this is so, it is reasonable to think that the physiological adaptation of spore stages (such as the ability to acclimate to different light climates) will have consequences for the whole population dynamics (Reed 1990).

UVR EFFECTS ON AQUATIC PHOTOSYNTHESIS: CONCLUSIONS AND FUTURE RESEARCH

UV can reduce photosynthetic rates of both micro- and macroalgae by **direct effects** on the photosynthetic apparatus through (1) pigment photobleaching in the photosynthetic antenna, (2) reduction of proteins in photosystem II, (3) decrease of enzyme activity in the Calvin cycle, and (4) inhibition of carbonic anhydrase activity, as well as via **indirect effects,** such as DNA damage. Few studies have analyzed the effects of UVR on photosynthetic responses other than carbon assimilation, for example nitrogen or phosphorus assimilation. Thus, analyzing the effects of UVR on such integrated metabolic processes should become an experimental effort of high priority.

A fact that complicates the study of UVR effects on photosynthetic organisms is that ozone depletion is occurring parallel to other global environmental changes, such as the increase in CO_2 and temperature, as well as the increasing eutrophication and acidification of natural waters. In order to predict the effects of increased UV-B radiation on aquatic ecosystems it is necessary to take into account the changes in other environmental factors. The increase in UV-B levels does affect algal physiology and ecology, including biogeochemical cycles in the coastal zone and enhanced radiation may have a significant global - scale climatic impact (Kelly 1986, Williamson & Zagarese 2003, Zepp 2003). Changes in productivity or diversity of aquatic primary producers due to elevated UV-B levels are likely to bring about alterations on several trophic levels of coastal marine food webs. Therefore, changes in community structure and ecosystem function can be expected.

Even *ambient* UVR levels can have a significant effect on benthic and water-column algal communities. For example, a general feature of MPB response is that ambient UV-B levels can exert a selective pressure on *early successional* stages on both hard and soft substrata. However, systems appear to vary greatly in susceptibility, depending on climate, the availability of refuges and nutrients, as well as the level of productivity and structure of the food webs. The local ecological implication of the initial selective pressure during early colonization will thus depend on the general importance of colonization events in relation to UV-B exposure. In the case of macroalgae, UV-B may also function as a selective pressure at the time of early colonization and recruitment. Consequently, macroalgal zonation can be determined by the different resistance against UV-B radiation of spore germination and growth of young plants (Huovinen et al. 2000).

On a longer time scale, algal resistance, shielding properties of the habitat, and trophic cascades may counteract UV-B effects on the primary producer level. For example, increasingly more studies on already established benthic microalgal and phytoplankton communities suggest a UV-B - effect minor to that first expected from short-term experiments. Thus, it is still very difficult to draw predictive conclusions about a general, long-term effect of UV-B on aquatic primary producers. This appears to apply particularly to systems where primary production depends mainly on benthic microalgae. Despite the fact that these communities consist of small organisms, with a rapid turnover, we perhaps still need to address system-level responses to UV-B on even longer time scales (years), like in experiments conducted on terrestrial communities (Björn et al. 1999). However, even results from these terrestrial field experiments show that observed effects are not unambiguous, particularly as effects can be largely modified by other environmental factors (temperature and nutrient availability), which may even reverse the initial UVR effects.

We are now at the stage where we can conclude that UVR affects all types of aquatic primary

producers, although the long-term response at the community level may be highly variable and modified by both environmental and biological factors. Future experimental approaches must include the interaction between different environmental factors in the scenario of ozone depletion. Only then can we expand our knowledge on the effects of increased UV-B, not only at organism level, but also at the community and ecosystem level, which is crucial for understanding the consequences for aquatic biodiversity and productivity.

Chapter 3

Annual patterns of ultraviolet radiation effects on temperate marine phytoplankton off Patagonia, Argentina

Villafañe VE, Barbieri ES, Helbling EW

ABSTRACT

We carried out experiments to evaluate the effects of solar ultraviolet radiation (UVR; 280-400 nm) upon primary production of different natural phytoplankton assemblages (i.e. characteristic of a seasonal cycle) from Patagonia (Argentina) from January 2001 to January 2002. The short-term impact of UVR (i.e. measured as radiocarbon incorporation) was assessed by exposing samples to solar radiation under six radiation treatments: uncovered quartz tubes and tubes covered with different cut-off Schott filters (WG295, WG305, WG320, WG360), and Plexiglas UF-3 (cut-off at 400 nm), so that samples received radiation at five different intervals within the UVR in addition to photosynthetically active radiation (PAR), and only PAR, respectively. Phytoplankton composition and abundance allowed us to differentiate pre-bloom, bloom, and post-bloom periods, with pre- and post-bloom samples characterized by small cells (e.g., flagellates < 10 µm) whereas the bloom was dominated by large diatoms (~50µm). Absolute values of photosynthesis inhibition were lower during the bloom, but biological weighting functions (i.e., inhibition per unit energy), indicated that this assemblage was more sensitive to UVR (especially in the UV-B region, 280-320 nm) than those of pre- and post-bloom periods. UV-A radiation (320-400 nm) accounted for most of the reduction in carbon incorporation (> 60%), especially during the pre- and post-bloom periods. Most of the observed variability was inter-seasonal although small intra-seasonal fluctuations were also observed. Our results indicate that the taxonomic composition and cellular size are especially important when addressing UVR effects upon these assemblages. However, other factors such as mixing can also contribute to the variability in responses to UVR.

INTRODUCTION

Over the past two decades, we have learned much about the effects of ultraviolet radiation (UVR; 280-400 nm) upon phytoplankton (Vernet 2000) especially in relation to the photosynthesis process (Villafañe et al. 2003 and references therein). The large amount of literature available at present, however, reports a wide variability in responses, ranging from strong inhibition, generally due to UV-A (315-400 nm) (Holm-Hansen et al. 1993b, Villafañe et al. 1999), to little or no inhibition when samples are exposed to the very energetic UV-B wavelengths (280-315 nm) (Helbling et al. 1992b), to photosynthesis stimulation under relatively low UVR levels (Nilawati et al. 1997, Barbieri et al. 2002). The reasons behind this variability in responses to UVR are, on one hand, associated with the fact that sensitivity and acclimation capacity to UVR are species – specific (Roy 2000, Vernet 2000). On the other hand, environmental changes such as those occurring in the UVR climate (i.e. seasonal or produced by ozone depletion events) (Blumthaler & Webb 2003), as well as in other abiotic factors, i.e. nutrient availability or temperature (Lesser 1996, Litchman et al. 2002) may account for much of the observed variability in UVR responses of phytoplankton.

Literature on the temporal variability of UVR effects upon natural phytoplankton is rather scarce, as it involves routine sampling throughout the plankton succession when organisms are exposed to different UVR scenarios. However, this information on differential responses to UVR of natural phytoplankton communities is essential to fully understand its impact upon primary productivity from a specific area. In particular, these data are especially important for our study region on the Patagonia coast, where a high standing stock of commercial fish and invertebrate species, e.g. hake and shrimp is sustained (Caille et al. 1997). Here we evaluate short-term responses of different temperate phytoplankton communities –those found during the yearly annual succession – when exposed to natural UVR levels. The study site in Patagonia (Argentina) has been chosen because of its large seasonal variability in chemical and physical characteristics (Helbling et al. 1992a), as well as in phytoplankton taxonomic composition and concentration (Villafañe et al. 1991, Barbieri et al. 2002). Moreover, the study area also is exposed to a variable UVR climate not only due to changes in solar zenith angles, but also because of changes in ozone concentrations (Orce & Hebling 1997, Villafañe et al. 2001), thus making it possible to evaluate and analyze the wide seasonal variations of UVR effects upon natural phytoplankton communities. In addition, our study provides new knowledge about the effects of solar UVR upon natural phytoplankton communities from the South Atlantic Ocean, where photobiological research in this regard has recently started (Buma et al. 2001b, Helbling et al. 2001a, Barbieri et al. 2002).

METHOD

This study was conducted at Bahía Engaño, Chubut, Argentina (43º S, 65º W) (Fig. 1), for the period January 2001 to January 2002. The study site is located in close proximity to the Chubut River estuary, where descriptive studies about the geomorphology (Perillo et al. 1989) and biological and chemical characteristics (Villafañe et al. 1991, Helbling et al. 1992a) have been conducted. To evaluate the annual patterns of UVR effects upon phytoplankton photosynthesis, routine sampling for experimentation was carried out every 7-20 days at a coastal station denoted EGI (Barbieri et al. 2002), throughout the year. Surface water samples were collected using an acid-cleaned (1N HCl) polycarbonate bottle and immediately taken to Estación de Fotobiología Playa Unión (EFPU; 10 min away from the sampling site), where experiments were carried out as described below.
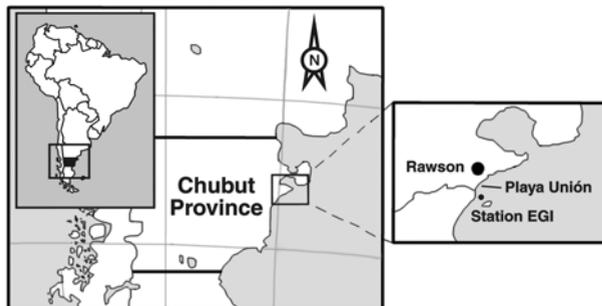

Figure 1: Map showing the study area and the relative position of the Chubut Province in South America.

To determine the effects of solar UVR upon phytoplankton photosynthetic rates, samples were placed in 50 ml quartz tubes and inoculated with 5 μCi (0.185 MBq) of labeled sodium bicarbonate (Steeman Nielsen 1952). The tubes were then placed in a black aluminum frame and exposed to solar radiation under six different radiation treatments (duplicate samples for each treatment): Uncovered quartz tubes and tubes covered with different cut-off Schott filters (WG295, WG305, WG320, WG360), and Plexiglas UF-3 (cut-off at 400 nm), so that samples received radiation at five different intervals within the UVR in addition to photosynthetically active radiation (PAR), and only PAR, respectively (the transmission characteristics of filters and materials are reported in Villafañe et al. (2003)). The whole set-up with the samples was placed in a water bath, with running water as temperature control (< 2 °C change), incubated for $4 – 6$ h (with the incubation centered on local noon) and then filtered onto Whatman GF/F filters (25 mm in diameter). The filters containing the samples were exposed to HCl fumes overnight, dried and counted using a liquid scintillation counter; carbon incorporation was then determined from c.p.m. values (Holm-Hansen & Helbling 1995).

The relative inhibition due to UVR was calculated as follows:

$$Inh = (P_{PAR} − P_{UVx}) / P_{PAR}$$

where P represents the amount of carbon fixed in the PAR-only treatment whereas P_{UVx} represents the carbon fixation in any of the five UVR treatments. Photosynthetic inhibition was expressed indistinctly as stated above or as % (after multiplying the inhibition value by 100). The total relative inhibition due to UVR and UV-A was calculated from the data in the uncovered quartz tubes and the tubes covered with the WG320 filter, respectively; then, the inhibition due to UV-B was calculated as the difference between these data. A non-parametric Kruskal Wallis test was used to test for significant differences between the samples at various radiation treatments, using a 95 % confidence limit.

To determine the wavelength dependence of photosynthesis inhibition, biological weighting functions (BWFs) were calculated using an exposure-response curve based on the irradiance - BWF-PI model (Neale & Kieber 2000). The photosynthetic inhibition for each wavelength interval (i.e. carbon uptake in each UVR treatment as compared to the PAR-only control) over the incubation period was expressed as a function of the average irradiance in the considered interval. The irradiance between each filter interval was determined with the STAR software (Ruggaber et al. 1994) and with data from the ELDONET sensor. The spectral dependence of the BWF in the broadband intervals was extracted using the method of Rundel (1983). An exponential decay function (base 10) was used to fit the data in each experiment, and the exponent of the function was expressed as a third-degree polynomial function; the best fit was obtained by iteration ($r^2 > 0.95$). At least six different and independent experiments were carried out during each phytoplankton condition (i.e. pre-bloom, bloom and post-bloom) to determine the mean BWFs.

Chlorophyll (chl *a*) analyses were done by filtering 100 ml of sample onto a Whatman GF/F filter (25 mm in diameter) and the photosynthetic pigments extracted in 7 ml of absolute methanol during at least 1 h (Holm-Hansen & Riemann 1978). The chl *a* concentration was calculated from the fluorescence of the extract (Holm-Hansen et al. 1965) using a Turner Designs fluorometer (model TD 700). Chl *a* analyses in the pico-nanoplankton fraction was performed as described before, but pre-filtering the sample with a Nitex® mesh (20 μm pore size).

In addition, samples for identification and enumeration of phytoplankton were also taken and placed in 125 ml brown bottles and fixed with buffered formalin (final concentration of 0.4% in the sample); after settling 10-25 ml of sample, they were analyzed with an inverted microscope (Leica model DM IL) following the technique described in Villafañe & Reid (1995).

Incident solar radiation was measured continuously using a broad band ELDONET radiometer (Real Time Computers Inc.) that measures UV-B (280-315 nm), UV-A (315-400 nm) and PAR (400-700 nm) with a frequency of one reading per minute. In addition, continuous monitoring of other atmospheric parameters (i.e. temperature, humidity, wind speed and direction, barometric pressure and rain) was carried out from July 2001 using a meteorological station (Oregon Scientific model WMR-918).

RESULTS

The annual pattern of atmospheric parameters – i.e. incident solar radiation, surface temperature and wind speed, over the study area is shown in Fig. 2.

Incident solar radiation had a high day-to-day variability due to changes in cloud cover (Fig. 2A). Daily doses of PAR varied between 14 MJ m^{-2} and 1 MJ m^{-2} for summer and winter,
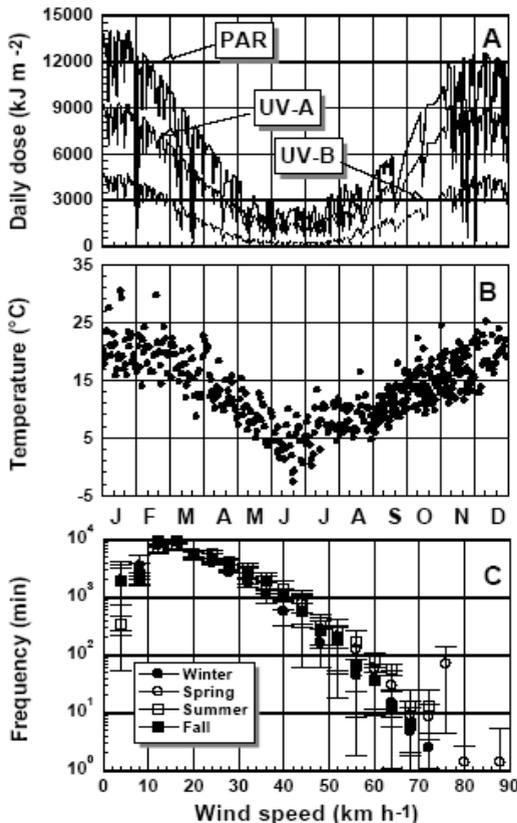

Figure 2: Annual cycle of atmospheric conditions in the study area. **A**) Daily doses of solar radiation (in kJ m^{-2}) for three wavebands: UV-B (280-315 nm), UV-A (315-400 nm) and PAR (400-700 nm). Note that UV-B and UV-A values were multiplied by a factor of 100 and 5, respectively; **B**) Mean daily temperature (°C); **C**) Mean seasonal frequency of wind speed (min). Data from **B**) and **C**) correspond to the period 2001 – 2002.

respectively (Fig. 2A). Daily doses of UVR had a similar pattern, with high values during summer and low ones during winter, with UV-A ranging from ~ 2000 to 150 KJ m^{-2}, whereas UV-B varied from ~ 45 to 5 KJ m^{-2} (Fig. 2A). Mean daily surface temperature presented a similar trend, with values as high as 31 °C during January-February and low during winter time, i.e. minimum values of -3 °C in June (Fig. 2B). Wind speed had a high variability and the maximum frequency was in the interval of 12 -16 km h^{-1} for all seasons. The windy season (i.e. with the highest wind speeds) was determined mainly during spring and to a lesser extent in summer, with values as high as 88 km h^{-1} (Fig. 2C). During this period, predominant winds were from the west.

Phytoplankton abundance, as estimated by chl a concentrations, also varied seasonally (Fig. 3A), with high values (~ 100 mg chl a m^{-3}) during winter (except during July), and low values during summer (< 5 mg chl a m^{-3}), with the exception of a small peak of ~20 mg chl a m^{-3} in mid-February. Samples collected during winter (i.e. the bloom period) were dominated by microplanktonic cells (> 20 µm), whereas samples with low chl a concentration (i.e. the pre- and post-bloom periods) were dominated by pico-nanoplanktonic cells (< 20 µm). Floristic analysis (Fig. 3B) revealed a general pattern of diatom - dominated microplanktonic populations, reaching values as high as 4 x 10^3 cells ml^{-1} during the winter bloom, with the diatom *Odontella aurita* being the dominant species. During the pre-bloom, when dinoflagellates presented their highest numbers for the entire study period (maximum of ~ 60 cells ml^{-1}), samples were always dominated by unidentified monads and flagellates (total cell numbers of ~0.5-3 x 10^3 cells ml^{-1}) (Fig. 3B). The taxonomic composition of the dinoflagellate community during the pre-bloom was characterized mostly by the presence of armored species (e.g. *Prorocentrum micans*, *Alexandrium tamarense*, *Protoperidinium* sp.) as well as diverse cysts (e.g. of the toxic *A. tamarense*). After the winter bloom, the samples were generally dominated by unidentified monads and flagellates (Fig. 3B), although small diatoms of the genus *Thalassiosira* were relatively abundant during spring and early summer.

High variability throughout the year of daily carbon fixation rates also characterized the study area (Fig. 4A). The highest carbon fixation value (i.e. 1.6 g C m^{-3} day^{-1}) was determined during the bloom period, whereas low values (i.e. < 0.1 g C m^{-3} day^{-1}) were obtained during late spring and early summer. Inhibition of photosynthesis by UVR was determined in all experiments, being maximal (i.e. 60 %) during late spring (Fig. 4A). The relative contribution of UV-A and UV-B to the total photosynthetic inhibition varied throughout the year, especially during the pre-bloom period (Fig. 4B). In most cases though, UV-A was responsible for most of the observed inhibition, except for two samples at the end of the experimental period, when UV-B – induced inhibition was > 60% (Fig. 4B).
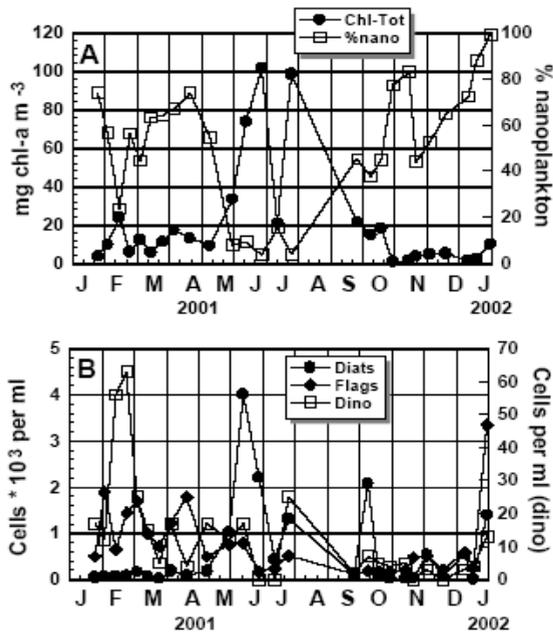
Figure 3: Phytoplankton biomass (estimated by chl a concentration) and composition throughout the study period. **A**) Total chl a concentration (in mg chl a m^{-3}) and percentage of chl a in the nanoplankton fraction (<20 µm); **B**) Phytoplankton cell concentration (in cells ml^{-1}) for diatoms, flagellates and dinoflagellates; note the different scale (y-axis) and units for dinoflagellate concentration.
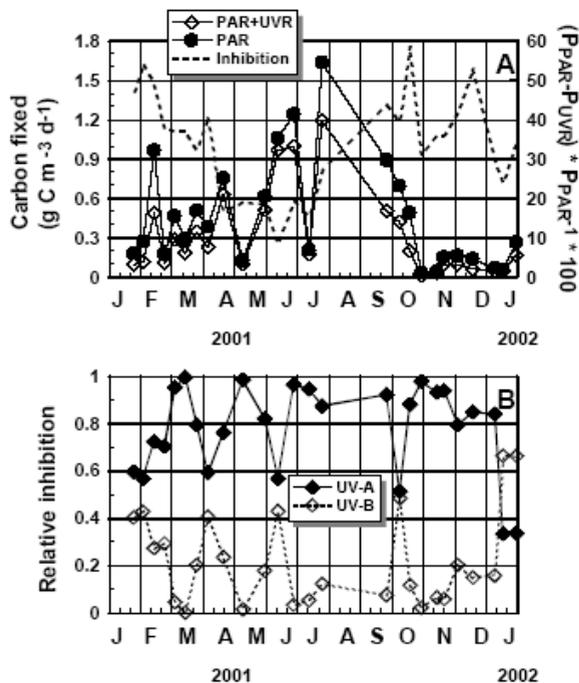

Figure 4: **A**) Daily carbon fixation (in g C m^{-3} day^{-1}; solid lines) and percentage of inhibition by UVR (broken lines) throughout the study period for samples exposed to PAR and PAR+UVR; **B**) Relative contribution of UV-A ( ) and UV-B ( ) to the total inhibition of primary production.

A mean BWF was calculated for the pre-bloom, bloom, and post-bloom periods (Fig. 5).
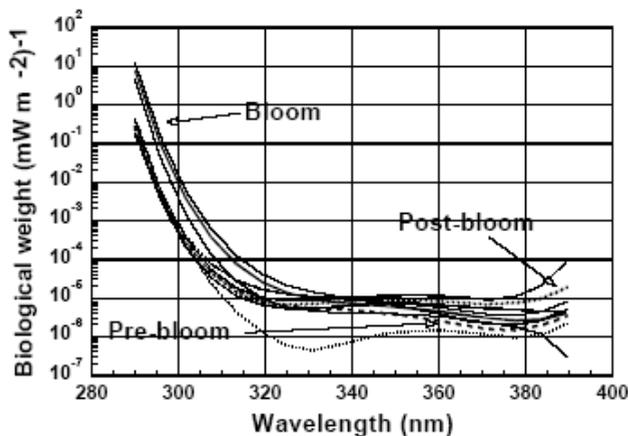

Figure 5: Mean biological weighting functions for pre-bloom, bloom and post-bloom samples. The thin lines indicate one standard deviation.

There were significant differences (see Method for statistical analyses) in the UV-B sensitivity, per unit energy received by the cells, of the phytoplankton samples from the three periods. The biological weights [(mW m^{-2})$^{-1}$] for wavelengths < 320 nm were significantly higher (p < 0.05) in bloom samples, suggesting a higher sensitivity of this assemblage as compared to those of the pre- and post-bloom. The biological weights for wavelength in the UV-A, however, were not significantly different (p > 0.05) between the three conditions (i.e. pre-bloom, bloom, and post-bloom), suggesting a similar response to these wavelengths throughout the year.

DISCUSSION

Many studies have demonstrated the role of solar radiation, especially UVR, in affecting phytoplankton photosynthesis (Villafañe et al. 2003) and hence the overall production of aquatic ecosystems. Most of them, however, have determined these effects within seasons and / or during relatively short periods of time (i.e., during few days or weeks), but only very few have considered the responses of variable communities (i.e. such as those occurring during the plankton succession) to natural radiation levels. In this paper we present data on the effects of solar UVR upon natural phytoplankton communities from Patagonia (i.e. south Atlantic Ocean) when exposed to maximum solar radiation levels as if they were at the surface of the water column (i.e. the worst-case scenario). This information constitutes a single database to: (i) assess the effects of UVR on primary production throughout the year (i.e. with samples exposed to their natural radiation levels); (ii) compare responses of samples that had different light history and acclimation capacity; and (iii) predict and further model the impact of solar UVR upon higher trophic levels of the local aquatic food web.

Previous studies assessing the impact of UVR upon natural phytoplankton assemblages of Chesapeake Bay (Banaszak & Neale 2001) have determined no significant inter-seasonal differences in the responses to UVR. However, a significant intra-seasonal variability in

sensitivity was observed when species were exposed to similar artificial UVR conditions, probably as a result of changes in species composition, light, temperature and nutrient availability. Contrary to these findings, here we report a significant inter-seasonal variability in UVR effects, although small fluctuations are also found in the intra-seasonal scale. The 'seasons' are defined here in relation to three main periods of the phytoplankton seasonal cycle (pre-bloom, bloom and post bloom), each one characterized by different taxonomic composition and abundance (Fig. 3) as well as by specific UVR responses (Figs. 4, 5). In the following paragraphs, we will discuss the main causes of such variability and analyze the overall impact of natural UVR upon primary productivity of this ecosystem of the Patagonia area.

We have determined a general trend of low photosynthetic inhibition values when irradiance levels were low, i.e. during the winter period (Figs. 2A, 4A). However, when considering the absolute carbon fixation and inhibition values (as above) together with the energy received during the experimentation (e.g. BWFs), it is seen that winter bloom cells are indeed more sensitive to UVR than the pre- and post bloom assemblages, especially in the UV-B region (Fig. 5). Thus, the total reduction of carbon incorporation of bloom cells was low just because solar radiation levels during winter were very low (Fig. 2B). On the other hand, pre- and post bloom assemblages (Fig. 3A) presented generally higher inhibition values (Fig. 4A) because of the high radiation levels (Fig. 2A), but the cells were more resistant to UVR than those characterizing the bloom (Fig. 5).

In view of the marked differences between the three main assemblages of the seasonal cycle, we analyzed whether the observed responses to UVR were related to cell size, or to the taxonomic composition of the community. It is seen that bloom assemblages were dominated by microplanktonic cells, whereas pre- and post bloom they were dominated by small cells (Fig. 3A). In fact, the cellular size dependence of UVR-induced inhibition has been the focus of several photobiological studies (Laurion & Vincent 1998, Helbling et al. 2001b). These studies have shown that although there is variability in responses, when addressing photosynthetic inhibition, small cells are more resistant to UVR than large cells, perhaps because they have fast acclimation kinetics due to their high surface to volume ratio. One should aware though that pico- nanoplanktonic cells are generally more vulnerable to DNA damage than to photosynthetic inhibition, as determined in studies carried out by Buma et al. (2001b) and Helbling et al. (2001a) in a nearby area of our study site. Thus, different targets for UVR damage are found in phytoplanktonic cells.

However, when addressing the size dependence of UVR responses, it should be noted that microplankton (especially centric diatoms) are more commonly synthesizers of UV-absorbing compounds such as mycosporine like amino acids (MAAs) (Helbling et al. 1996a); small cells, on the other hand, usually do not contain such compounds due to the energy cost that their synthesis would imply (Garcia Pichel 1994). The protective role of MAAs has been determined in studies carried out with both phytoplankton (Neale et al. 1998a) and zooplankton (Helbling et al. 2002). In our study, though, we did not determine significant amounts of these compounds in any of the communities sampled (data not shown), but we are aware that the methodology used in this study to determine their concentration (i.e. spectrophotometric) is not as sensitive as HPLC techniques. Future studies should fully address the importance of this and other alternative mechanisms (e.g. DNA repair or dynamic rather than chronic inhibition of photosynthesis) that allow phytoplankton species of the Patagonia region to minimize UVR effects.

Another interesting aspect of the UVR effects refers to the relative contribution of UV-A and UV-B to the total photosynthetic inhibition. In our phytoplankton assemblages, the contribution of UV-A was generally higher than that of UV-B (Fig. 4B) as also seen in many other aquatic environments, ranging from polar (e.g. Holm-Hansen et al. 1993a, b) to tropical areas (e.g. Villafañe et al. 1999). 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. In spite of this, we have found in our study some periods in which the relative inhibition due to UV-B and UV-A was rather similar, or even with UV-B surpassing that of UV-A (Fig. 4B). In this case, especially during the pre-bloom, the increase of the relative UV-B–induced inhibition (Fig. 4B) was associated with the dominance of small nanoplanktonic flagellates (Fig. 3B). During the bloom and post-bloom, on the other hand, the increase in UV-B–induced inhibition of photosynthesis was associated with a relative increase of flagellates which occurred together with the large diatoms (Fig. 3B). This obviously hints at a taxonomic dependence on UVR effects where flagellates account for much of the observed UV-B inhibition. In fact, studies have demonstrated the relatively high sensitivity of these small cells to UVR (Villafañe et al. 1995b). We do not know, however, what the reasons are for such "pulses" of variable relative abundance of pico-nanoplanktonic cells, but changes in nutrients input, as normally occur in the area (Helbling et al. 1992a), might account for part of this variability.

Finally, other factors could account for much of the variability in UVR responses of phytoplankton of this region. For example Barbieri et al. (2002), who carried out experimental work in our study area, have shown the importance of mixing in affecting the overall responses to UVR of different phytoplankton communities. These mixing experiments have shown that bloom assemblages use UV-A energy for photosynthesis when PAR levels are low, suggesting a dark acclimation and hence explaining the fact that small amounts of UV-B energy such as those observed during winter and early spring (Fig. 2A), had a negative impact on phytoplankton photosynthesis (Fig. 4B). Deep mixing in the study area, resulting from strong winds during spring and summer (Fig. 2C) precludes large cells from blooming during these periods, resulting in an increase of flagellates that can better utilize solar energy under such conditions (Helbling et al. 1994). Instead, low irradiance levels and shallow mixing (Fig. 2) characterizing the winter period favor the development of large phytoplankton cells, as also seen in the past in the study area (Villafañe et al. 1991, Barbieri et al. 2002) as well as in other coastal areas of Argentina (Gayoso 1999).

In view of our findings, we conclude that several factors account for the overall responses of phytoplankton assemblages to solar UVR and they can be summarized as follows: (i) The irradiance levels at which cells are exposed as well as their previous light history; (ii) their cell size structure, which regulates the effectiveness of solar energy utilization; and (iii) the taxonomic composition of the communities. In addition, other factors such as variations in mixing conditions can interact with solar radiation, affecting not only the underwater radiation field, but also the occurrence of taxonomic groups in the water column. Finally, and although it is difficult to predict the overall impact of UVR upon the productivity of the area, our data suggest that it would be much lower than in other regions of Patagonia, such as the sub- Antarctic (i.e. Beagle Channel) or the Andean lakes (Villafañe et al. 2001) as the bloom occurs during winter, and only its decline period (i.e. September-October) would potentially receive enhanced levels of solar UV-B radiation.

Chapter 4

Photosynthesis versus irradiance (P vs. E) characteristics of coastal phytoplankton assemblages of Patagonia (Argentina): Temporal variability and solar ultraviolet radiation 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 two sites - Bahía Nueva and Bahía Camarones (Chubut, Argentina) and exposed to solar radiation for 4-6 h under three quality radiation treatments (i.e., PAR + UVR, 280-700 nm; PAR + UV-A, 320-700 nm, and PAR only, 400-700 nm), and under 6-8 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 (~ 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 reduced significantly 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.

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) 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 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}), ß (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) (Kirk 1994, Sakshaug et al. 1997). These parameters are dependant 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 dependant on factors such as the irradiance / doses 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 / conditions. Thus, rigorous studies considering the radiation climate as well as the taxonomic structure of natural communities have to be done 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 assess this objective, we collected phytoplankton samples at different times of the year (i.e., during the post- and pre-bloom seasons) in two 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 in the Patagonia coast (Charpy & Charpy - Rubaud 1980, Buma et al. 2001b, Helbling et al. 2001a, Villafañe et al. 2004a) and, in particular, research about the UVR effects on phytoplankton of the area has started relatively recently (Buma et al. 2001a, 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.

MATERIALS AND METHODS

Study area

Nine experiments were done 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 three experiments at Bahía Camarones (44.9° S, 65.6° W) (Fig. 1), a bay with open waters to the Atlantic Ocean located at 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 to obtain 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, being spring and summer the windy seasons (Villafañe et al. 2004a). The attenuation coefficient (K_d) for 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

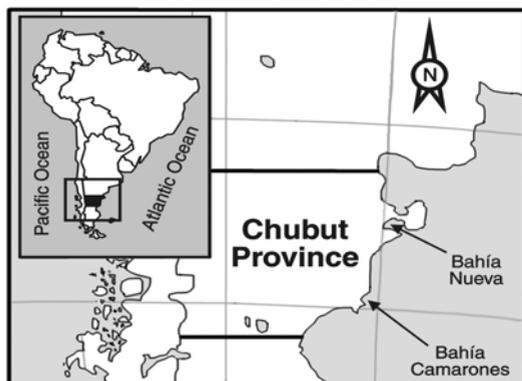8 °C (i.e., June) to 19 °C (i.e., January) (Helbling et al. unpub. data).

Figure 1: Map showing the sampling sites – Bahía Nueva and Bahía Camarones - and the relative position of the Chubut Province in South America.

Experiments and determinations

Surface water samples were collected at 500-1000 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 done during 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 below) under three quality radiation treatments, and under 6-8 levels of ambient irradiance (quantity radiation treatments). The radiation quality treatments were the following: (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. (1997b). The quantity (i.e., irradiance) treatments were obtained by covering the tubes with none and 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., total of 36 or 48 tubes) was then put in a water bath with running water as temperature control (i.e., in Bahía Nueva experiments) or at shore with 1-2 cm of seawater covering the tubes (i.e., in Bahía Camarones experiments), and exposed to natural radiation during 4-6 h, being the incubations centered on local noon. It should be noted though, that other studies have used different incubation periods, ranging from minutes to hours (e.g., Neale et al. 2001). Macedo et al. (2002) found that daily primary production would be understimated when based on 2-4 h incubations and if the P vs. E curves presented a dynamic time-dependent behavior. In our case, we have chosen an incubation time long enough so that any repair mechanism would be at steady state. At the beginning of experiments, samples were taken to determine chlorophyll-a (chl a) concentration, UV-absorbing compounds and floristic composition (see below). In addition, different atmospheric parameters (see below) 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).

Chlorophyll a (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 1-3 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. 1996a).

Floristic analysis. Water samples were fixed with buffered formalin (final concentration in the sample = 0.4 % of formaldehyde). The 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 with 200x for microplankton (> 20 µm) and with 400x 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 Inc.) that measures UV-B (280-315 nm), UV-A (315-400 nm) and PAR (400-700 nm) with a frequency of one reading per minute. 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 a, b, and c parameters as follows:

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

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 (Julian day 305) to June 30, 2003 (Julian 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 Julian day 50. Maximum daily doses were measured during the period December to January, reaching values ~ 12 MJ m^{-2}, 1.8 MJ m^{-2} and 50 KJ m^{-2} for PAR, UV-A and UV-B, respectively (Figs. 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 (Figs. 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°C - 35°C (no data were collected during Julian days 50 to 70).
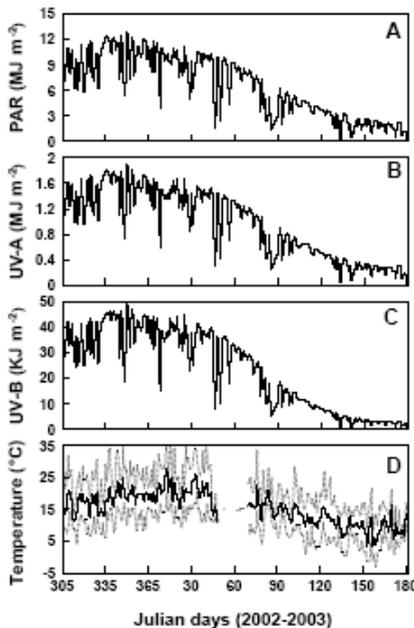

Figure 2: Atmospheric conditions for the period November 1, 2002 (Julian day 305) to June 30, 2003 (Julian day 180). **A**) Daily doses of PAR, 400–700 nm (in MJ m^{-2}); **B**) Daily doses of UV-A, 315-400 nm (in MJ m^{-2}); **C**) Daily doses of UV-B, 280-315 nm (in kJ m^{-2}); **D**) Mean (solid line), maximum and minimum (broken lines) daily temperature (in °C). No temperature data were collected during Julian 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 (Figs. 3A, E) no

photoinhibition was determined, whereas in others (Fig. 3G) it was very important or   evident at irradiances $> 300\,\mu mol\ m^{-2}\ s^{-1}$ (e.g., Figs. 3H, I) or $> 600\,\mu mol\ m^{-2}\ s^{-1}$ (e.g., Figs. 3 B-D). The impact of UVR on P vs. E was important in pre-bloom samples (i.e., fall), causing an additional decrease in CO_2 fixation at high irradiances (Figs. 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 Figs. 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 Figs. 3A-D).
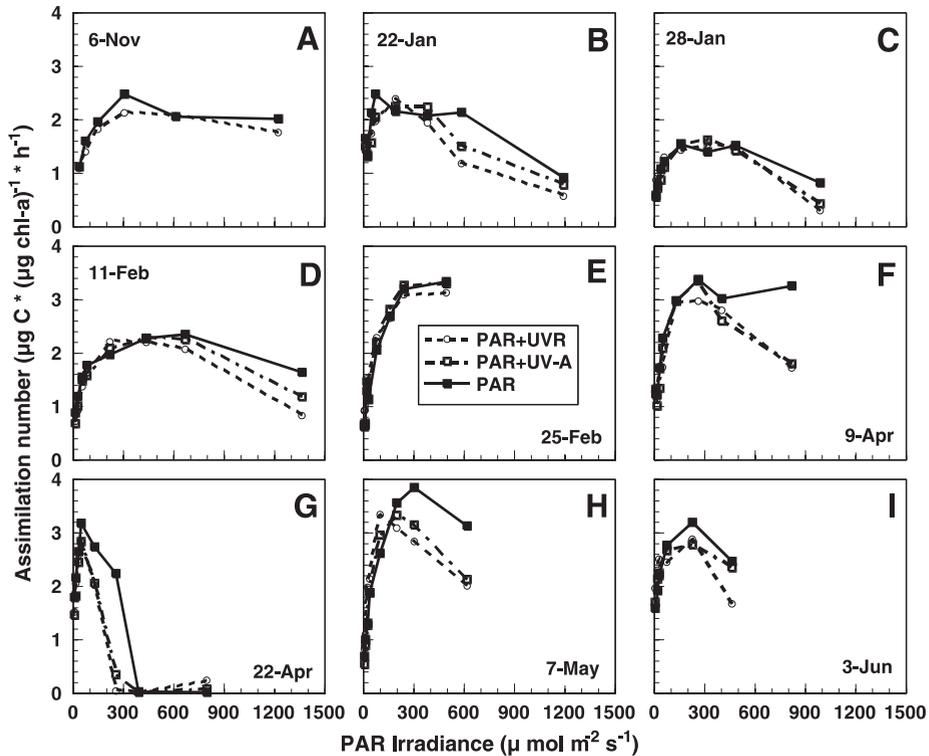

Figure 3: Phytoplankton assimilation numbers (μg C (μg chl-a)$^{-1}$ h^{-1}) as a function of the mean PAR irradiance (in $\mu mol\ m^{-2}\ s^{-1}$) to which samples from Bahía Nueva were exposed. White circles: Samples exposed to PAR + UV-A + UV-B; White squares: Samples exposed to PAR + UV-A; Black squares: Samples exposed to PAR only. The 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.

There was a significant impact of UVR on P_{max} in samples collected during the pre-, but not during the post-bloom (Fig. 4A and Table). This negative effect was mostly due to UV-A, but on same samples (e.g., 9-April), exposure to UV-A resulted in a significantly higher

P_{max} (Fig. 4A). There was not a clear trend in the light saturation parameter E_k according to the sampling period (Fig. 4B). E_k varied between high values (i.e., > 300 µmol m^{-2} s^{-1}) 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 was determined in late April - ~ 50 µmol m^{-2} s^{-1}. 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 previous week 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 two variables, with a decrease of E_k with increasing mean wind speed for all radiation treatments (i.e., PAB, PA, and P).
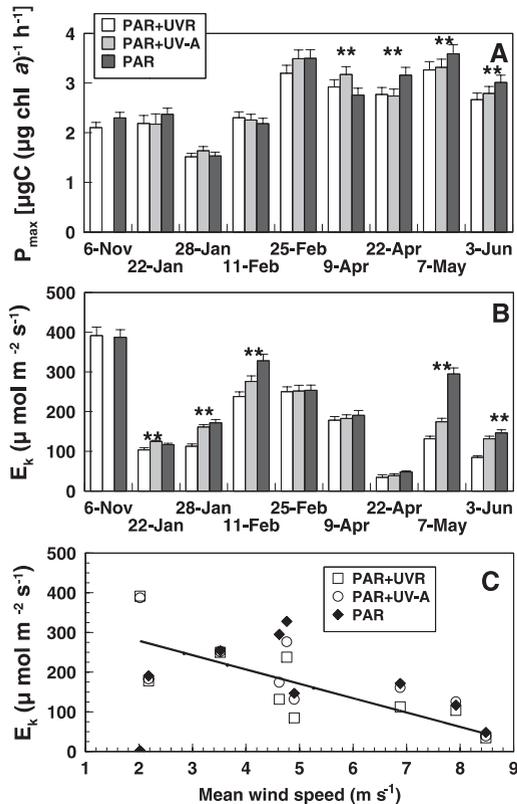

Figure 4: Mean photosynthetic parameters for the nine experiments carried out with waters collected from Bahía Nueva. A) Mean P_{max} (in µg C (µg chl-a)$^{-1}$ h^{-1}); B) Mean E_k (in µmol m^{-2} s^{-1}). White bars: Samples exposed to PAR + UVR; Gray bars: Samples PAR + UV-A; Black bars: Samples exposed to PAR only. The asterisks on top of the bars indicate significant differences ($p < 0.05$). C) Relationship between E_k (in µmol m^{-2} s^{-1}) for the three radiation treatments (i.e., PAR+UVR, PAR+UV-A and PAR only) performed in each experiment and the mean wind speed (in m s^{-1}) of the previous week of experimentation. The line represents the best fit ($R^2 = -0.65$, $p < 0.001$).

Bahía Nueva				
	P_{max}		E_k	
	UV-A	UV-B	UV-A	UV-B
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				
	P_{max}		E_k	
	UV-A	UV-B	UV-A	UV-B
3-Feb	-7.01	-3.31	32.77 *	11.16 *
2-Jun	-1.79	11.27	2.72	24.48 *

Table: 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). The asterisks indicate the degree of significance ($p < 0.05$) (data not available for November 6 and 23 experiments).

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).
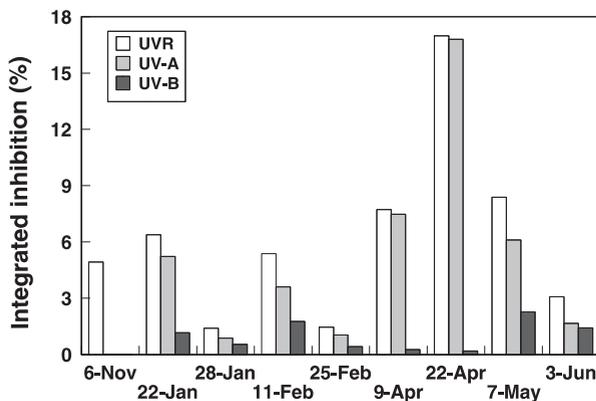

Figure 5: Integrated photosynthetic inhibition (in %) in the euphotic zone due to UVR (white bars), UV-A (gray bars) and UV-B (black bars) during the nine experiments carried out with Bahía Nueva waters.

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 seen 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-Apr); in other experiments though (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) 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}.
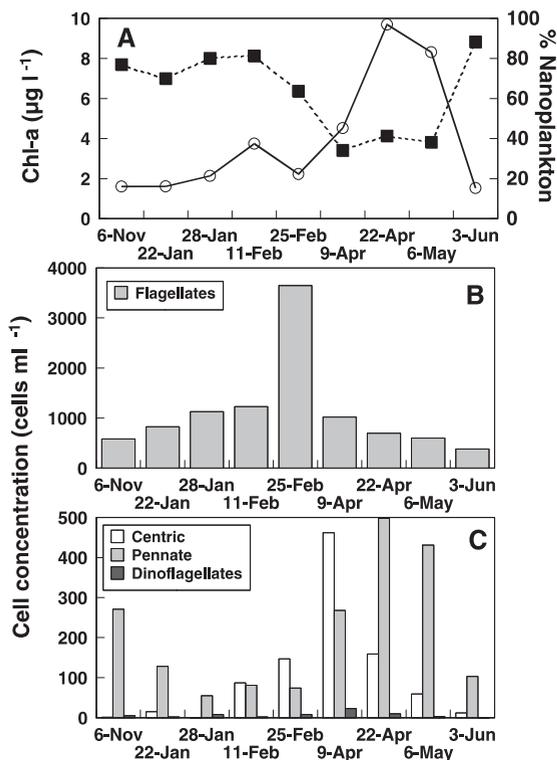
Figure 6: Biological characteristics of samples collected at Bahía Nueva. **A**) Total chl *a* concentration (in µg l⁻¹) (○), and percentage of chl *a* in the nanoplankton fraction (<20 µm) (■); **B**) Unidentified monads / flagellates concentration (in cells ml⁻¹) and, **C**) Centric diatoms (white squares), pennate diatoms (gray squares) and dinoflagellates (black squares) concentration (in cells ml⁻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 - 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 summer and increasing again towards fall (their highest concentration was ~500 cells ml⁻¹ during late April). Centric diatoms reached a maximum concentration of ~ 470 cells ml⁻¹during early April. During late April and May, the most important diatom species was the pennate *Nitzschia longissima* whereas during early April the centrics *Skeletonema costatum* and *Chaetoceros* spp. were abundant, although *N. longissima* also contributed for 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.

Fig. 7 shows the P vs. E curves, photosynthetic parameters and taxonomic composition in the three experiments carried out with samples collected at Bahía Camarones.
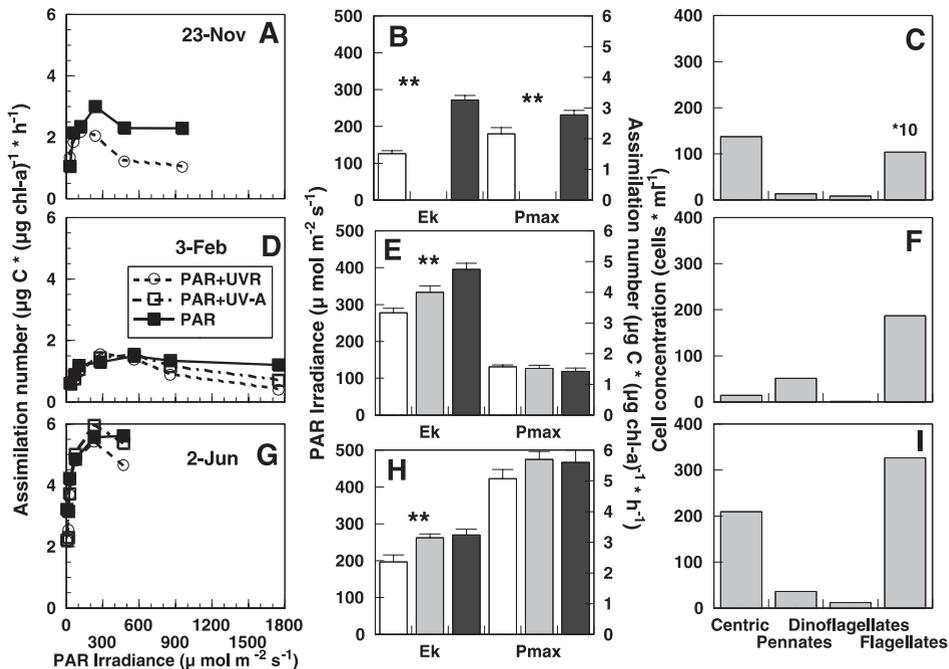

Figure 7: P vs E curves, photosynthetic parameters and species composition of samples collected at Bahía Camarones.   **A**), **D**) and **G**) Phytoplankton assimilation numbers (in µg C (µg chl-a)$^{-1}$ h^{-1}) as a function of the mean PAR irradiance (in µmol m^{-2} s^{-1}) to which samples were exposed. The experiments were carried out on November 23, 2002 (**A**), February 3, 2003 (**D**) and June 2, 2003 (**G**). Open circles: Samples exposed to PAR + UVR; Open squares: Samples exposed to PAR + UV-A; Filled squares: Samples exposed to PAR only. **B**), **E**) and **H**) Mean photosynthetic parameters – E_k (in µmol m^{-2} s^{-1}) and P_{max} (in µg C (µg chl-a)$^{-1}$ h^{-1}) for the experiments carried out on November 23, 2002 (**B**); February 3, 2003 (**E**) and June 2, 2003 (**H**). Open bars: Samples exposed to PAR + UVR; Gray bars: Samples PAR + UV-A; Black bars: Samples exposed to PAR only. **C**), **F**) and **I**) Phytoplankton concentration (in cells ml^{-1}) discriminated in centric and pennate diatoms, dinoflagellates and unidentified monads / flagellates for the experiments carried out on November 23, 2002 (**C**) (note the scale used for quantification of monads / flagellates), February 3, 2003 (**F**) and June 2, 2003 (**I**).

P vs. E curves (Figs. 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}) (Figs. 7G, H), whereas the lowest values were determined during February (i.e., < 1.5 µg C (µg chl *a*)$^{-1}$ h^{-1}) (Figs. 7D, E); the experiments carried out with samples collected in November displayed intermediate values (Figs. 7A, B). E_k values in these three experiments varied within the range 125 – 400 µmol

m^{-2} s^{-1}. The impact of UVR on photosynthetic parameters was also different: UVR had a significant effect on P$_{max}$ only in late November (Fig. 7B) whereas no significant differences between treatments were found in February and June experiments (Figs. 7F, I). On the other hand, UVR had a significant impact on E$_k$ in the three experiments (Figs. 7B, E, H). In addition, UVR-induced photoinhibition was determined in all experiments (Figs. 7A, D, G). The biological characteristics of these samples were the following: 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 cell ml^{-1}. The assemblages were always dominated by unidentified monads / flagellates (Figs. 7C, F, I) however, the diatom community structure was different in the three experiments, with 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 three 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 %) whereas the integrated photosynthetic inhibition due to UV-B was lower, < 4 % in all experiments.
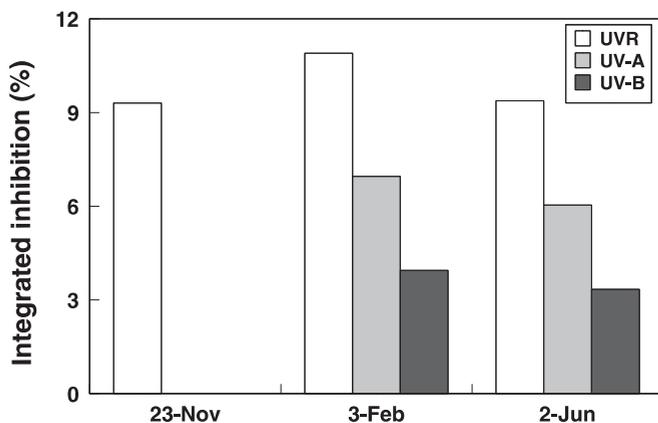
Figure 8: Integrated photosynthetic inhibition (in %) in the euphotic zone due to UVR (white bars), UV-A (gray bars) and UV-B (black bars) during the three experiments carried out with Bahía Camarones waters.

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, and then decreasing 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). Also, and for the area of Golfo Nuevo, previous studies have determined a seasonal cycle in phytoplankton community structure and nutrient concentrations (Gayoso 2001) but we are not aware though, 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, here we focused on the temporal variability of P vs. E relationships of phytoplankton assemblages of two 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 amount of experiments performed but nevertheless, we 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 to variations in radiation levels and temperature (Shaw & Purdie 2001) and to 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 of the Patagonia coast (i.e., Bahía Bustamante, Chubut) with summer (i.e., post-bloom) assemblages having P_{max} values < 1.5 µg C (µg chl a)$^{-1}$ h^{-1} (Helbling et al. 2001a). These P_{max} and those 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 hints for a dependence with 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) appear to be more related to nutrient limitation. Field experiments carried out by us (Chapter 5) have shown that nutrient addition rapidly increased growth rates of summer phytoplankton communities of 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.

In regard to the biological structure of phytoplankton assemblages, our study shows a very good agreement with previous findings for the Bahía Engaño area (also in the Chubut coast), in which three "seasons" are clearly distinguished (Barbieri et al. 2002, Villafañe et al. 2004a): A post-bloom (i.e., spring-summer), a pre-bloom (i.e., fall) season, and a bloom

period during winter; each one characterized by different chl *a* levels and taxonomic composition. Microplankton diatoms increased their concentrations during the pre-bloom, and small cells are especially abundant in the post-bloom period, as also seen here with phytoplankton assemblages of Bahía Nueva (Fig. 6). High chl *a* values towards the cold season seems to be a rather common feature in Patagonia (Gayoso 2001, Barbieri et al. 2002, Villafañe et al. 2004a), suggesting that this phytoplankton dynamics is not local or restricted to a small area, but rather occurring along the coast. One common variable among the different sites seems to be the shallow upper mixed layer (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 previous week of our experiments (Fig. 4C) and we found that with low wind speeds P_{max} increased significantly ($R^2 = -0.67$, $p < 0.05$), as well as the concentration of centric diatoms ($R^2 = -0.72$, $p < 0.01$), which is also in agreement with previous findings, in which we have seen 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 condition, 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 winter time 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 photoacclimation are possible, according to the process 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 have 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 (Figs. 7 B, E, H) and moreover, no apparent relationship between E_k and taxonomic composition (Figs. 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% for each UV-A and UV-B (Helbling & Villafañe 2002). Hence, UVR impact on Patagonian waters would be probably 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. 1992b, 2003).

The different photosynthetic responses to UVR of phytoplankton have been frequently associated to the species – specific sensitivity, with the concomitant differences in their acclimation and repair mechanisms once the effect (or damage) has been produced (Roy 2000, Banaszak 2003, Buma et al. 2003, Villafañe et al. 2003). Such acclimation / repair mechanisms essentially include: (a) Avoidance (through movements away from the radiation source, habitat selection, etc), (b) Screening, either by extra (cuticles, sheaths, etc) and intracellular protective compounds (e.g., MAAs), (c) Repair of both direct and indirect UVR damage (DNA and protein repair, antioxidant enzymes, etc.), and (d) Short- and long-term acclimation (Roy 2000). Particularly, 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 here we did not specifically test differences in acclimation / repair among the assemblages, we evaluated one of them, by measuring in each sample the amount of UV-absorbing compounds (i.e., MAAs). The presence of these compounds is one of the protecting mechanisms that organisms may have against harmful levels of UVR and hence favoring their growth and general performance (Banaszak 2003), including primary productivity (Neale et al. 1998a). 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 photoprotective mechanism was not the most important for phytoplankton (i.e., at least during the study period) to cope with potentially damaging UVR levels. Indeed, other protective mechanisms might be of importance for phytoplankton of this area, and they should be addressed in future studies oriented 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 of the Patagonia area, as seen in studies carried out by Helbling et al. (2001a), Buma et al. (2001b) and Villafañe et al (2004b).

Although our knowledge on UVR-induced effects on phytoplankton has increased a lot, not many 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) and thus, if UVR effects are not taken into account, primary production might be occasionally 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 the radiation treatment under 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), and in the late spring assemblages from Bahía Camarones (Fig. 7B, Table), 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 a previous research (Helbling et al. 1996a) where it was reported the high sensitivity of pennate diatoms to UVR. In regard to the timing of the UVR impact on photosynthetic parameters, it is somehow contradicting that although radiation levels during fall are relatively low (i.e., 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 showing the 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 (providing that cells do not synthesize UV-absorbing compounds) than that in nanoplankton cells. In regard to the light saturation parameter, it was seen that in Bahía Nueva samples, E_k values were significantly reduced by UVR only in some experiments (Fig. 4B), whereas in Bahía Camarones samples, UVR significantly reduced E_k in all of them (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) than in cells coming from shallow UMLs. Previous studies (Helbling et al. 2001b) have also shown that cell size is very important at the time to determine the acclimation of cells to the new irradiance conditions, with nanoplankton acclimating much faster than microplankton cells. Thus, the significant decreases in E_k observed during late fall is probably more related to the species composition rather than to the UML depth.

Our study thus indicates, on 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 relatively high P_{max}; these assemblages, however, might 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 not) of UVR on these parameters in order to have much accurate estimates of CO_2 uptake by phytoplankton.

Chapter 5

Combined effects of solar ultraviolet radiation and nutrient addition upon natural phytoplankton communities off Patagonia (Argentina)

Villafañe VE, Marcoval MA, Helbling EW

ABSTRACT

Experiments to determine the long-term (i.e., 12-14 days) combined effects of solar radiation and nutrient addition were conducted with natural phytoplankton assemblages collected at three sites off the Patagonian coast, Argentina – 42 - 44°S (i.e., Bahía Engaño, Bahía Camarones and Bahía Nueva) during January – March 2003. Samples from each site were put in UVR transparent containers and incubated under three (ambient) radiation treatments: a) Samples exposed to UVR + PAR (280-400 nm) - PAB treatment; b) Samples exposed to UV-A + PAR (320-400 nm) – PA treatment, and, c) Samples exposed to PAR only (400-700 nm) – P treatment. Nutrients (i.e., f/2 concentration) were added to the samples either at the beginning (i.e., N_0 cultures) or after 6-7 days of exposure to solar radiation (i.e., N_x cultures). Growth (i.e., estimated from chl *a* measurements) and floristic composition were monitored every 1-2 days; in addition, primary productivity rates were determined at the beginning and during the exponential phase of N_0 cultures. At the three sites we determined significantly higher growth rates in the N_x than in the N_0 cultures. In addition, we found that photosynthetic inhibition due to UV-A was higher than that produced by UV-B, and that overall inhibition decreased with time suggesting acclimation of cells to the new (i.e., experimental) radiation conditions. At all sites the communities were dominated by small flagellates but differences in the diatom composition were found between experiments, as well as within radiation / nutrient treatments. N_0 cultures of Bahía Engaño were characterized by *Guinardia delicatula*, whereas in the N_x cultures this diatom co-dominated with other species. In Bahía Camarones, and except for N_0_P cultures, where *Asterionellopsis glacialis* was dominant, *Nitzschia longissima* always accounted for an important fraction of the diatom community. In Bahía Nueva *Skeletonema costatum* generally dominated the diatom community, but co-dominated with *Leptocyilindrus* sp. in the treatments N_0 PAB and N_0 PA cultures. Overall, our results indicate that no generalizations can be made in regard to the responses of different phytoplankton assemblages to the combination of solar radiation exposure and nutrient addition. The responses seem to be related to the initial composition of the assemblages, the previous light history and the nutrient status of cells. However, UVR exposure and nutrient addition do seem to account, at least in part, for an important part of the observed phytoplankton biodiversity from Patagonian waters.

INTRODUCTION

Ultraviolet radiation (UVR, 280-400 nm) has been considered the most reactive waveband that might cause negative effects on organisms, hence reducing their performance within their habitat (Caldwell et al. 1995, Häder et al. 1995). In the 80's, and after the discovery of the Antarctic ozone 'hole' (Farman et al. 1985) photobiological studies were devoted to evaluate the impact that solar UVR might have on aquatic organisms, and by the end of that

decade it was thought that enhanced UVR, mainly UV-B (280-315 nm) would cause such deleterious effects on phytoplankton – i.e., significant reduction in primary production and growth rates - that it would result in a collapse of the Antarctic ecosystem (El-Sayed 1988). It is known now however, that although both normal and enhanced levels of UVR do cause significant negative effects on short-term basis (Holm-Hansen 1997, Wängberg & Selmer 1997) there are several mechanisms that act over longer term periods of time (i.e., days, weeks) that allow phytoplankton either to repair the damage produced and / or to acclimate and thus minimize the negative effects caused by the exposure to those short wavelengths (Roy 2000, Banaszack 2003).

Long-term acclimation mechanisms to UVR of phytoplankton cells essentially include, at the individual level, a modification of physiological conditions, such as for example through the synthesis of protective UV-absorbing compounds, e.g., mycosporine-like amino acids (MAAs) (Helbling et al. 1996a, Zudaire & Roy 2001) or carotenoids (Underwood et al. 1999). At the community level, taxonomic alterations (Bothwell et al. 1993, Villafañe et al. 1995a, Wängberg et al. 1996, Cabrera et al. 1997) and changes in growth (Kim & Watanabe 1994, Wängberg et al. 1996) and productivity rates (Lesser 1996, Helbling et al. 1996a) are frequently cited. In addition, other factors (e.g., nutrient status, temperature) can interact on the long run and account for the overall response of phytoplankton when exposed to UVR: For example, Litchmann et al. (2002) demonstrated the increased sensitivity to UVR in nutrient-limited cultures of dinoflagellates, and Lesser (1996) and Lesser et al. (1996) have shown the interaction of UVR effects and temperature on phytoplankton.

In this paper we are evaluating the long-term responses to solar UVR of phytoplankton communities collected at three different coastal sites of the Patagonia region (Chubut, Argentina). We are also considering the contribution of nutrients addition to the overall effects of UVR on these communities. The study sites present very interesting characteristics in regard to their radiation climate (i.e., with relatively high heliophany and with episodic ozone depletion events) (Orce & Helbling 1997), but relatively few studies have evaluated the effects of solar radiation on natural marine phytoplankton communities (Buma et al. 2001a, Helbling et al. 2001a, Villafañe et al. 2001, 2004a, c). In this study, the three sites chosen are relatively close in distance – hence having a rather similar ground radiation climate (Helbling et al. unpub. data) but they present enough differences in their bio-optical as well as in their geo-morphological characteristics that allow interesting comparisons about long-term effects of solar UVR in summer phytoplankton communities of the Patagonia region.

MATERIALS AND METHODS

Sampling sites

Long-term experiments (i.e., 12-14 days) were done with samples collected at three sites of the Chubut coast of Argentina: a) Bahía Engaño (43.3° S, 65° W); b) Bahía Camarones (44.9° S, 65.6° W) and, c) Bahía Nueva (42.7° S, 65° W). The cities of Playa Unión, Camarones and Puerto Madryn are located alongshore Bahía Engaño, Bahía Camarones and Bahía Nueva, respectively (Fig. 1).
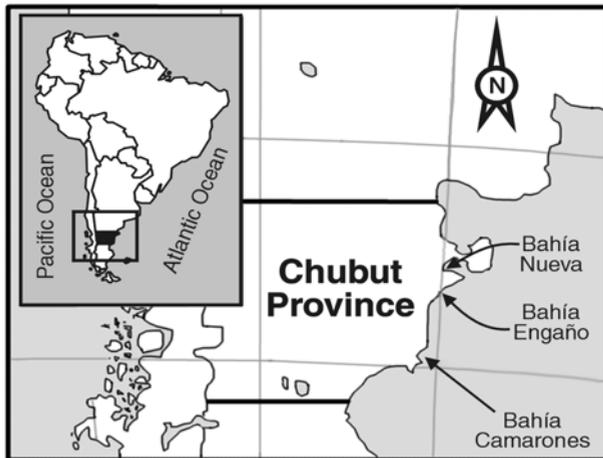
Figure 1: Map of the study area indicating the three sites of sampling (Bahía Engaño, Bahía Camarones and Bahía Nueva). The inset shows the relative position of the Chubut Province within South America.

a) Bahía Engaño: The study area is located at the mouth of the Chubut River, thus receiving nutrients supply through river runoff. The bay is relatively open to Atlantic Ocean waters, and it is characterized by wide sandy beaches alongshore. Several studies have been conducted in the area, oriented to determine its geo-morphological characteristics (Perillo et al. 1989), bloom and nutrient dynamics (Villafañe et al. 1991, Helbling et al. 1992a) as well as UVR responses of natural phytoplankton populations (Barbieri et al. 2002, Villafañe et al. 2004a, c). The long-term experiment with water collected from this site was carried out during the period 17-30 January, 2003.

b) Bahía Camarones: As in Bahía Engaño open waters characterize the area but relatively abrupt cliffs build the coast and rocky shores dominate. Studies assessing the impact of solar UVR upon natural phytoplankton communities have been carried out in the nearby Bahía Bustamante (Buma et al. 2001b, Helbling et al. 2001a). The experiment with water collected from this site was carried out during the period 5-18 February, 2003.

c) Bahía Nueva: This study area is clearly different from the two previously described, as it is located within Golfo Nuevo, which is an enclosed system with relatively little exchange with open waters from the Atlantic Ocean (Rivas & Beier 1990). Phytoplankton studies in this area have particularly focused on monitoring toxic species, such as *Alexandrium tamarense* and *Prorocentrum lima* (Gayoso 2001, Esteves et al. 1992, Gayoso et al. 2002). The experimentation period with water collected in Bahía Nueva was February 25 to March 9, 2003.

Experimentation

Surface water samples were collected 500-1000 m offshore with an acid- cleaned (1 N HCl) polycarbonate carboy and immediately taken to the Estación de Fotobiología Playa Unión (EFPU) where long-term experiments were conducted as following: For each experiment (i.e., with waters from Bahía Engaño, Bahía Camarones and Bahía Nueva, respectively), the samples were put in six 4-liter UV-transparent containers (Plexiglas UVT, GS 2458,

Röhm and Haas, Darmstadt, Germany) and exposed to three radiation treatments: (1) Two samples receiving full radiation (UVR, 280-400 nm, and PAR, 400-700 nm) – uncovered containers - PAB treatment; (2) Two samples receiving UV-A (320-400 nm) and PAR – containers covered with UV cut-off filter foil (Montagefolie, N°10155099, Folex) (50% transmission at 320 nm) - PA treatment; and (3) Two samples receiving only PAR – containers covered with Ultraphan film (UV Opak, Digefra) (50% transmission at 395 nm) - P treatment (the spectra of these materials have been published in Figueroa et al. 1997b). Nutrients were added to the containers (f/2 concentration - Guillard & Rhyter 1962) at different times during the experiments: One container from each radiation treatment received nutrients at the beginning of experimentation (i.e., N_0 cultures), whereas nutrients were added to the other three containers approximately one week after experimentation started (i.e., N_x cultures). The containers where placed in a water-bath with running water as temperature control and exposed to natural radiation for 12-14 days.

At the beginning of each experiment, samples were taken to determine chlorophyll-a (chl a), UV-absorbing compounds (i.e., spectral absorption characteristics) and floristic composition (see below). Sampling was done on daily basis to determine growth rates (i.e., through chl a concentration), whereas every other day sub-samples were taken to determine UV-absorbing compounds and taxonomic composition of the community. In addition, at the beginning (t_0) and during exponential / maximum growth phases (i.e., from the N_0 cultures, and also in N_x cultures in Bahía Engaño samples) sub-samples from each radiation treatment were put in 20 ml quartz tubes to determine photosynthetic rates (see below) under three radiation treatments: (1) Samples receiving full radiation (UVR + PAR) – uncovered tubes; (2) Samples receiving UV-A + PAR – tubes covered with UV cut-off filter foil (as above); and (3) Samples receiving only PAR – tubes covered with Ultraphan film (as above).

Analyses and measurements

The analytical procedure for each determination / measurement was as follows:
Chlorophyll a (chl a). Chl a concentration was measured by filtering a variable amount of water sample onto a Whatman GF/F glass fiber filter (25 mm) and extracting photosynthetic pigments in absolute methanol (Holm-Hansen & Riemann 1978). The fluorescence of the methanolic extract was measured using a Turner Designs fluorometer (model TD700) before and after acidification, and chl a concentration was calculated from these readings (Holm-Hansen et al. 1965). The fluorometer was calibrated using pure chl a from *Anacystis nidulans* (Sigma # C 6144).
UV-absorbing compounds. UV-absorbing compounds were determined by filtering a variable amount of water sample onto a Whatman GF/F glass fiber filter (25 mm) and extracting these compounds in absolute methanol overnight. The estimation of concentration of UV-absorbing compounds (Helbling et al. 1996a) was done by peak analysis of the scans (250-750 nm) obtained using a Hewlett Packard spectrophotometer (model 8453E).
Floristic analysis. Water samples were fixed with buffered formalin (final concentration in the sample = 0.4 % of formaldehyde). The 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 with 200x magnification for microplankton (> 20 µm) and with 400x for nanoplankton cells (< 20 µm). A drop of Rose Bengal was added to the

sample in the settling chamber to better distinguish between cells which were live or dead at the time of collection (Villafañe & Reid 1995).

Photosynthetic rates. Samples were put in 20 ml quartz tubes and inoculated with 2.5 - 5 µCi of labeled sodium bicarbonate - ICN Radiochemicals (Steeman Nielsen 1952). After 4-6 h of incubation, samples were filtered onto a Whatman GF/F glass fiber filter (25 mm). Then the filter was placed in 7 ml scintillation vials, exposed to HCl fumes overnight, dried, and counted using standard liquid scintillation techniques (Holm-Hansen & Helbling 1995).

Radiation measurements and other atmospheric parameters. Incident solar radiation was measured continuously using a broad band ELDONET radiometer (Real Time Computers Inc.) that measures UV-B (280-315 nm), UV-A (315-400 nm) and PAR (400-700 nm) with a frequency of one reading per minute. 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) (no temperature data was obtained during the experiment carried out with waters collected from Bahía Nueva).

Statistics. A non parametric analysis (i.e., Kruskal-Wallis) (Zar 1984) was used to establish differences among treatments; a confidence level of 95% was used in all analyses.

RESULTS

Solar radiation and ambient temperature data during the period January – March 2003 is shown in Fig. 2. There was a day-to-day variability in daily doses due to cloud cover but, in spite of this, there was a clear trend for decreasing values after Julian day 50. Maximum daily doses were measured during January, reaching values of ~ 12 MJ m^{-2}, 1600 KJ m^{-2} and 45 KJ m^{-2} for PAR (Fig. 2A), UV-A (Fig. 2B) and UV-B (Fig. 2C), respectively. Very low values, however, were determined during the experimentation period (i.e., Bahía Camarones experiment) with values of ~ 1 MJ m^{-2}, 300 kJ m^{-2} and 6 kJ m^{-2} for PAR, UV-A and UV-B, respectively. Ambient temperature (Fig. 2D) also had high variability during January - March, with mean values ranging from ~ 11 to 27 °C that fell within the range 5°C - 35°C.

The PAR irradiance conditions during the three experiments is presented in Fig. 3. Maximum PAR irradiance levels were higher during the Bahía Engaño experiment (i.e., ~ 550 W m^{-2}) (Fig. 3A) than during that of Bahía Camarones (i.e., ~ 450 W m^{-2}) (Fig. 3B) and that of Bahía Nueva (i.e., ~ 400 W m^{-2}) (Fig. 3C). In general, maximum PAR values (and also UV-A and UV-B, data not shown) were rather similar within each experiment, with the exception of day #11 in the Bahía Camarones experiment (Fig. 3B), were values as low as 50 W m^{-2} were recorded. During the Bahía Engaño experiment (Fig. 3A) scattered clouds characterized most of the days (except for day #9), whereas experiments with Bahía Camarones and especially with Bahía Nueva waters (Fig. 3B, C, respectively) were done under mostly clear sky conditions.
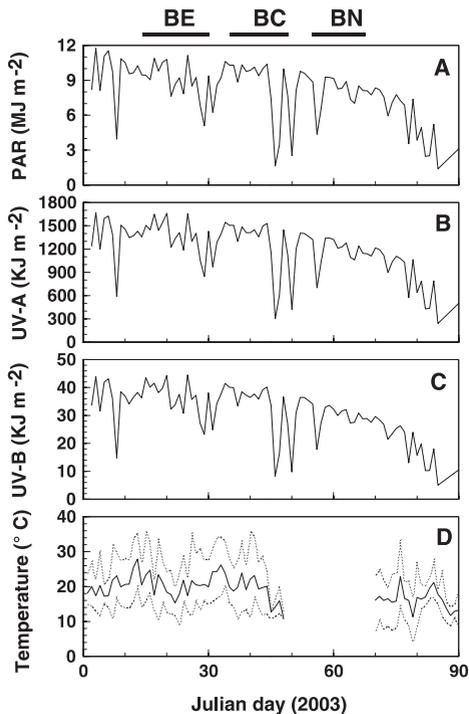
Figure 2: Incident solar radiation and atmospheric temperature during the austral summer (period January – March 2003). Solar radiation is expressed as daily doses for: **A**) PAR (MJ m^{-2}); **B**) UV-A (KJ m^{-2}) and, **C**) UV-B (KJ m^{-2}). (**D**) Mean daily temperature – in °C (solid line) and minimum and maximum values (broken lines). The lines on top **A** indicate the experimentation time at Bahía Engaño (BE), Bahía Camarones (BC) and Bahía Nueva (BN).
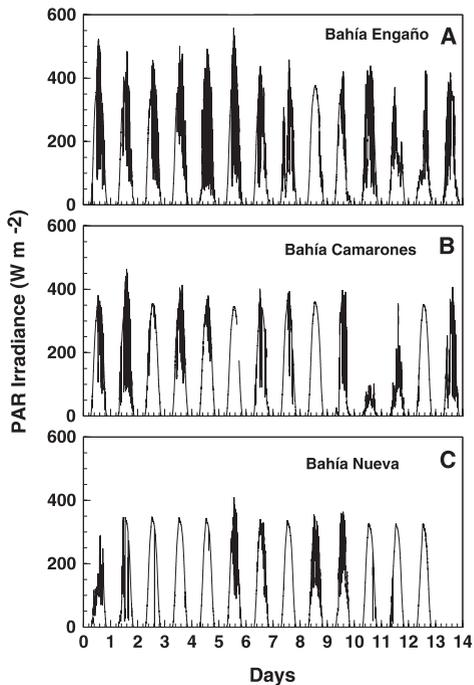
Figure 3: Incident solar radiation (PAR, in W m^{-2}) received by the phytoplankton natural assemblages during the experiments. **A**) Bahía Engaño experiment; **B**) Bahía Camarones experiment and, **C**) Bahía Nueva experiment.

The daily variation in chl *a* concentration for Bahía Engaño, Bahía Camarones and Bahía Nueva waters exposed to different radiation / nutrient treatments is presented in Fig. 4. Chl *a* concentration in the three study sites was either constant or decreased during the first 6-7 days in the N$_x$ samples (open symbols). On the other hand, N$_0$ samples (solid symbols) had an exponential increase in chl *a* concentration from day 1 except for the Bahía Camarones experiment (Fig. 4B) where a short lag phase of one day was noticed. Maximum chl *a* concentration in the three places reached values close to 100 μ chl *a* l^{-1} for N$_0$ samples. With the exception of Bahía Engaño samples, N$_x$ cultures reached lower chl *a* values than N$_0$ samples (although the difference was not significant), and had smaller differences between radiation treatments, with the exception of Bahía Camarones samples.
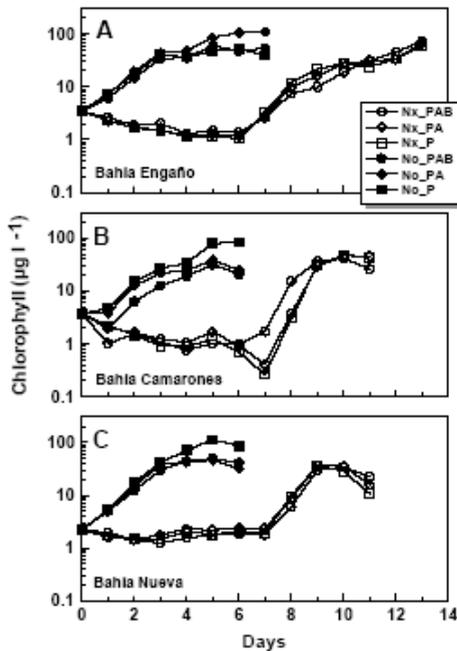
Figure 4: Chlorophyll-a concentration (in $\mu g\ l^{-1}$) during the experiments. Solid symbols indicate the N_0 cultures while open circles indicate the N_x cultures. **A**) Bahía Engaño experiment; **B**) Bahía Camarones experiment and, **C**) Bahía Nueva experiment.

Within the same experiment, growth rates (i.e., μ) were significantly lower in N_0 than in N_x cultures (Table 1).

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_x_PAB	0.95*	1.53*	1.40*
N_x_PA	1.06*	2.13*	1.35*
N_x_P	1.22*	2.38*	1.45*
$p\ (\mu N_0 = \mu N_x)$	0.029	0.002	0.001

The radiation treatments were PAR+UV-A+UV-B (PAB); PAR+UV-A (PA) and PAR only (P). N_0 indicate addition of nutrients at the beginning of the experiment whereas N_x indicate the addition of nutrients after 6-7 days. The asterisks indicate significant differences between N_0 and N_x cultures (i.e., comparing the same radiation treatment).

On the other hand, and within the same nutrient treatment from each experiment, there were not significant differences in growth rates between radiation treatments ($p > 0.05$). When comparing the effect of nutrient addition at the three sites, it is seen that Bahía Camarones samples had the highest change in growth rates, with the lowest μ values in the N_0 (i.e., 0.62 day^{-1} in the PAB treatment) and the highest in the N_x cultures (i.e., 2.38 day^{-1} in the P treatment). On the other hand, samples from Bahía Engaño and Bahía Nueva presented less variation in μ between N_0 and N_x cultures.

Fig. 5 shows the photosynthetic inhibition due to UV-B and UV-A during different stages (i.e., different treatments) of the experiments; the inhibition values were normalized by the mean PAR during the incubation to account for the different radiation conditions during the exposure of samples. In the three experiments photosynthetic inhibition due to UV-A (Fig. 5B) was higher than that produced by UV-B. Within each experiment, there were not significant differences in photosynthetic inhibition due to UV-B between samples incubated at the beginning and later on (i.e., N_0 and N_x cultures) (Fig. 5A). However, in Bahía Engaño and Bahía Camarones experiments (Fig. 5B) photosynthetic inhibition due to UV-A was significantly different when comparing samples incubated at the beginning of the experiment with those collected later on.
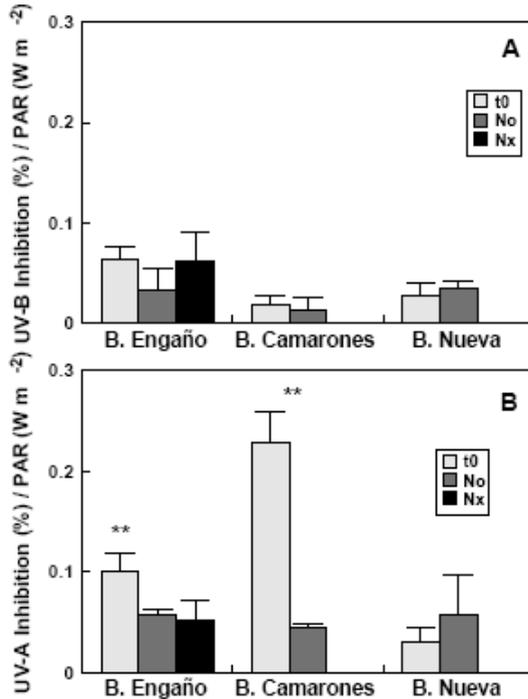

Figure 5: Photosynthetic inhibition due to UV-B and UV-A during the Bahía Engaño, Bahía Camarones and Bahía Nueva experiments when exposed to different nutrient / radiation treatments. T_0 denotes the photosynthetic inhibition (UV-B and UV-A) at the beginning of the experiment, whereas N_0 and N_x are the photosynthetic inhibition in the treatments in which nutrients were added at the beginning or later on in the experiments, respectively. The asterisk on top of the bars indicates significant differences ($p < 0.05$).

The initial taxonomic composition of samples collected at Bahía Engaño, Bahía Camarones and Bahía Nueva waters was different (Fig. 6): Even though at the three sites unidentified monads / flagellates dominated, and that dinoflagellates abundance was negligible (i.e., always accounted for < 1% of total cells throughout experiments), differences in the diatom composition were determined between the study sites, with variable proportion of centrics / pennates (Table 2).

Table 2: Initial composition (cells ml^{-1}) of samples collected at Bahía Engaño, Bahía Camarones and Bahía Nueva.

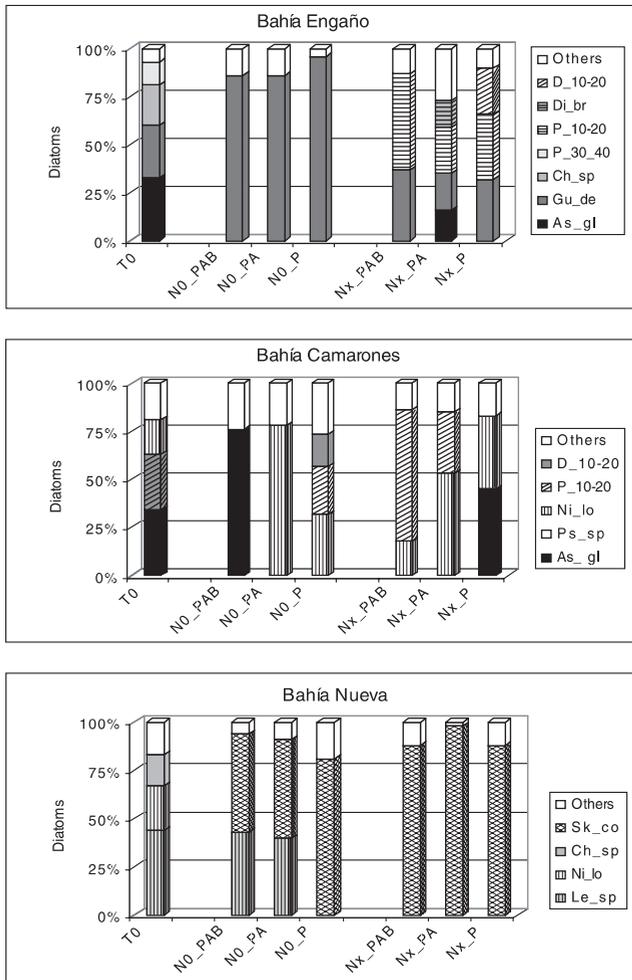| Group	Bahía Engaño	Bahía Camarones	Bahía Nueva
Centric diatoms	47	8	147
Pennate diatoms	45	363	74
Dinoflagellates	14	4	8
Monads / flagellates	1649	2667	3650
Total cells	1775	3042	3879

Figure 6: Relative contribution of diatoms species at the beginning (t$_0$) and during the exponential phase of the N$_0$ and N$_x$ cultures and for the three radiation conditions (i.e., PAB, PA and P, respectively). **A**) Bahía Engaño experiment; **B**) Bahía Camarones experiment and, **C**) Bahía Nueva experiment. D_10-20: discoids 10-20 µm in diameter; Di_br: *Ditylum brightwellii*; P_10-20: pennate 10-20 µm in 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.

The species that accounted for 75% (or more) of the diatom community in Bahía Engaño were pennates 30-40 µm in diameter and *Asterionellopsis glacialis* (Castracane) Round, and the centrics *Guinardia delicatula* (Cleve) Hasle and diverse *Chaetoceros* Ehrenberg species (Fig. 6A). The pennates *A. glacialis*, various *Pseudonitzschia* H. Peragallo in H.

and M. Peragallo species and *Nitzschia longissima* (Brébisson, in Kützing) Ralfs in Pritchard dominated the diatom community in Bahía Camarones (Fig. 6B). On the other hand, in Bahía Nueva the diatoms *Leptocylindrus* Cleve, *N. longissima* and *Chaetoceros* spp. were the most important species at the beginning of the experiment (Fig. 6C). As the experiments progressed, the diatom composition changed, so that in the experiment with waters from Bahía Engaño, *G. delicatula* dominated the N_0 cultures. In the N_x cultures, *G. delicatula* co-dominated together either with small pennate and centric diatoms (10-20 µm in diameter), or with *A. glacialis* and *Ditylum brightwellii* (West) Grunow (i.e., in the PA treatment). In the Bahía Camarones experiment, and except for the samples exposed to full radiation in the N_0 cultures (i.e., where *A. glacialis* was the dominant species), *N. longissima* always accounted for an important fraction of the diatom community. However, it co-dominated together with small diatoms in the other treatments, except for samples exposed only to visible radiation in the N_x cultures (i.e., co-dominance with *A. glacialis*). Finally, in the Bahía Nueva experiment *Skeletonema costatum* (Greville) Cleve accounted for an important proportion of the diatom community and co-dominated with *Leptocyilindrus* sp. in the treatments PAB and PA of the N_0 cultures.

DISCUSSION

Studies have demonstrated that UVR is a very important controlling factor for aquatic communities, producing adverse effects on phytoplankton, e.g., reduction of growth and photosynthetic rates and DNA damage (Häder et al. 1995, Buma et al. 2003, Villafañe et al. 2003) which can affect the overall performance of higher trophic levels within the ecosystem. As a whole, UVR impact on the phytoplankton community depends on the radiation levels at which organisms are exposed, their specific tolerance and their ability to reduce and / or minimize any damage produced (Roy 2000). When assessing UVR effects on phytoplankton, the interaction of UVR with other abiotic factors– e.g., temperature and nutrient status (Lesser 1996, Lesser et al. 1996, Litchman et al. 2002) and the temporal scale of observation (Holm-Hansen 1997, Wängber & Selmer 1997) are important as well. Although extensive research has been carried out to address the short-term effects of UVR on phytoplankton (i.e., with experiments lasting less than one day, see review of Villafañe et al. 2003), the performance of phytoplankton communities over longer temporal scales (i.e., days / weeks - Villafañe et al. 1995b, Helbling et al. 1996a, 2001c, Lesser 1996, Cabrera et al. 1997, Halac et al. 1997, Zudaire & Roy 2001) have been relatively less studied. As a new contribution to the understanding of the UVR effects on the aquatic biota, here we present data on the long-term combined effects of nutrients addition and solar UVR exposure on three summer phytoplankton assemblages from the Patagonia coast off Argentina.

The three sampling sites in the Patagonia coast chosen for our study are relatively close in distance (Fig. 1), but differences in the initial diatom taxonomic composition of the three communities were found, with varied proportions of centrics to pennates (Fig. 6, Table 2). It should be noted that although diatom concentrations were low in the three experiments (Table 2), they contributed for a variable share - and sometimes important - of total carbon biomass (data not shown). These differences in taxonomic structure were rather expected due to the timing of experimentation (i.e., January – March). This timing difference also was reflected in the irradiances / doses levels received by cells, with somewhat large values

during the Bahía Engaño than in the Bahía Camarones and Bahía Nueva experiments (Figs. 2, 3), but probably these variable (ground) radiation levels are not the main factor accounting for differences in the diatom community composition. Instead, phytoplankton diversity differences are more probably related to the geo-morphological characteristics of the three study sites, which are in turn related to water turbidity, underwater radiation field, as well as the physico-chemical environment. Although Bahía Engaño and Bahía Camarones are open waters to the Atlantic Ocean, the former differs from Bahía Camarones because it is located at the mouth of the Chubut River. Bahía Nueva waters on the other hand, encompass a semi-enclosed system with little exchange with open waters. Consequently, there are differences in regard to the radiation field under which cells are exposed, with relatively opaque waters in Bahía Engaño (i.e., K_{PAR} up to 0.9 m^{-1}, Helbling et al. unpub. data) due to heavy sediment load transported by the river (Perillo et al. 1989, Helbling et al. 1992a) as compared to Bahía Camarones ($K_{PAR} = 0.31$ m^{-1}) or Bahía Nueva waters ($K_{PAR} < 0.3$ m^{-1}) (Helbling et al. unpub. data).

On the other hand, the three study sites share climatic characteristics – i.e., they are highly exposed to strong winds during spring / summer seasons (Villafañe et al. 2004a) which results in mixed conditions that seem to favor the development of flagellate - dominated communities as found in the Southern Ocean (Kopczynska 1992, Villafañe et al. 1995a). In fact, time series studies carried out at Bahía Engaño (Barbieri et al. 2002, Villafañe et al. 2004a) have determined pre- and post-bloom communities dominated by unidentified monads / flagellates, and in studies done nearby Bahía Camarones – i.e., Bahía Bustamante (Buma et al. 2001b, Helbling et al. 2001a) and in Bahía Nueva (Gayoso 2001) the authors reported the conspicuous presence of pico - nanoplankton cells during late spring / summer. In addition to strong mixing, other factors might shape the taxonomic structure as found in our study sites. For example, low nutrient concentrations have been found to favor the growth of pico - nanoplankton cells because their high surface-to-volume ratio allows these cells to optimize nutrient utilization (Falkowski 1981). In fact, as shown in our study, nutrient concentration was limiting these post-bloom assemblages, with a relatively long lag phase in N_x cultures and, conversely, a rapid exponential growth when nutrients were added at the beginning of the experiments (i.e., N_0 cultures); moreover, when nutrients were added to the N_x cultures, a fast exponential growth was also observed (Fig. 4).

As phytoplankton assemblages were exposed to the experimental conditions (i.e., different radiation / nutrient treatments), it was determined that growth rates were significantly lower in the N_0 than in the N_x cultures (Fig. 4, Table 1). This response is probably associated to the previous light history of these assemblages (i.e., which were collected during the strong mixing period and thus acclimated to low irradiance levels) so that cells in the N_0 cultures had a high energetic cost in adjusting to the new radiation conditions (i.e., similar of being at the surface). N_x cultures, on the other hand, had enough time to acclimate to the new (and maximum) radiation conditions as imposed in the experiment, so that the "selected" cells took full advantage of nutrient addition and had higher growth rates as compared to the N_0 cultures. In addition, and within the same nutrient treatment, no significant differences in growth rates were found among radiation treatments (Table 1), as also seen in other long-term studies (Villafañe et al. 1995b, Davidson et al. 1996), suggesting that cells acclimated relatively fast and that any adverse effect produced by UVR was not chronic. Measurements of photosynthetic inhibition show that UV-A induced inhibition was higher

that than produced by UV-B (Fig. 5). The fact that UV-A is generally responsible for the bulk of inhibition in diverse freshwater and marine environments of the World – as seen in studies carried out by Bühlmann et al. (1987), Helbling et al. (1992b) and Villafañe et al. (1999) among many others - is because even though UV-B wavelengths are potentially more damaging, the amount of UV-A energy that reaches the Earth´s surface is higher. Nevertheless, we determined in this study that UV-A induced photosynthetic inhibition was significantly higher at t_0 than later on (i.e., N_0 cultures) at Bahía Engaño and Bahía Camarones experiments (Fig. 5B). Again, this seems to be related to the previous light history of cells collected from a mixed environment which are inhibited by UV-A; after a short acclimation period to higher radiation conditions (i.e., 2-4 days) however, photosynthetic inhibition decreased significantly. Particularly, and for the case of Bahía Engaño experiments, we also observed differences in assimilation numbers: N_0 cultures had a mean of 2.0 µg C (µg chl *a*)$^{-1}$ h^{-1}, (SD, 0.3 µg C (µg chl *a*)$^{-1}$ h^{-1}), whereas N_x samples have a mean value of 2.9 µg C (µg chl *a*)$^{-1}$ h^{-1} (SD 0.6 µg C (µg chl *a*)$^{-1}$ h^{-1}) (data not shown). Nevertheless, there were no significant differences in photosynthetic inhibition between these two cultures, either by UV-B (Fig. 5A) or by UV-A (Fig. 5B), suggesting that a period of 4 and 10 days (i.e., for N_0 and N_x cultures, respectively) was enough to acclimate the cells to the new radiation conditions.

Acclimation mechanisms to UVR in phytoplankton can be either physiological or by changes in the taxonomic composition of the community. Long-term physiological acclimation to UVR mainly occurs through the synthesis of photoprotective compounds, especially mycosporine-like aminoacids (MAAs) (Banaszak 2003). However, we did not find significant amounts of these compounds (data not shown) in any of the samples, although we are aware that the technique used by us (i.e., spectrophotometric) is not as sensitive as HPLC analysis. Thus, acclimation mechanisms to UVR (and in our case to nutrient addition) in our phytoplankton communities might probably be related to a selection of more tolerant species, as suggested by Worrest (1983). However, in some cases (i.e., Bahía Nueva or N_0 cultures at Bahía Engaño) this "selection" due to UVR is not clear. Nevertheless, what is clear from our experiments is that the combined response and changes in species dominance are highly dependable on the duration of UVR exposure and nutrient addition. For example, in Bahía Engaño and Bahía Camarones experiments we found that, regardless the treatment, and although there was a change in the relative proportion of cells (as also seen in studies carried out by Halac et al. 1997), 1-4 diatom taxa dominated at the end of each phase of experiments, clearly indicating a selection towards more (radiation / nutrient) adapted cells. The N_0 cultures of Bahía Engaño experiment was dominated by the microplankton diatom *G. delicatula*; however, in the N_x cultures (i.e., already acclimated to the high radiation conditions) smaller cells also contributed to dominance. In the Bahía Camarones experiment though, size does not seem to be determinant, as variable responses were found in the different nutrient / radiation treatments. On the other hand, all Bahía Nueva samples were generally characterized by *S. costatum*, which clearly took advantage of the new nutrient / radiation experimental conditions. In fact, *S. costatum* is a species capable of readily utilize nutrients and radiation to achieve fast growth (Kiefer & Cullen 1990). The effect of different radiation treatments (i.e., within the same nutrient condition) was variable, so that no differences in diatoms dominance were found in the Bahía Nueva experiment (Fig. 6C, see above) and in the N_0 cultures of Bahía Engaño (Fig. 6A). The lack

of differences in taxonomic composition was also determined in long-term experiments conducted in samples exposed and not-exposed to UV-B (Halac et al. 1997, Laurion et al. 1998, Vinebrooke & Leavitt 1999). However, there was a change in diatom dominance in N_x and N_0 cultures of Bahía Engaño and Bahía Camarones, respectively (Figs. 6A & B), which agrees with previous findings (e.g., Villafañe et al. 1995b, Davidson et al. 1996), where radiation seems to play a fundamental role in shaping community structure. One should be aware however, that the experimental conditions imposed to the samples are partially responsible of the type of results obtained by us. For example, when addressing long-term effects of UVR on phytoplankton, Sommaruga (2003) has pointed out the importance of the container's size. This author showed that the effects of UVR in small containers were usually higher than that determined in larger ones, probably because in the former the cells are exposed to "higher" radiation and thus avoidance mechanisms (e.g., via vertical migration or by mixing effects) can not take place.

Overall, our data indicate that it is not possible to generalize the role that both solar radiation and nutrient addition can have even in environments close in proximity as those reported here. Environmental conditions (i.e., light history, nutrient concentration) together with the physiological status of cells are very important to understand the observed responses. In addition, the timing of nutrient enrichment seems to be critical, at least for some assemblages. Thus, for the area studied here, river input (and its control by man activities) might play and important role affecting the responses of natural phytoplankton assemblages.

Chapter 6
In situ impact of solar ultraviolet radiation on photosynthesis and DNA in temperate marine phytoplankton

Helbling EW, Buma AGJ, de BoerMK, Villafañe VE

ABSTRACT

In situ experiments were conducted at various depths in the water column to determine the impact of solar UVR (280-400 nm) upon photosynthesis and DNA of natural phytoplankton assemblages from mid-latitudes of Patagonia (Bahía Bustamante, Chubut, Argentina, 45° S, 66.5° W). The effects of UVR were significant at the surface; however, the impact decreased rapidly with depth: at 3 m there was no measurable DNA damage accumulation whereas at 6 m photosynthetic inhibition was almost zero. UV-A radiation (315-400 nm) was mostly responsible for photosynthetic inhibition, while UV-B radiation (280-315 nm) had a lesser effect on this process. However, UV-B radiation was very effective in damaging the DNA through the formation of cyclobutane pyrimidine dimers (CPDs) in surface waters. The high initial CPD level found in the natural phytoplankton assemblage decreased when samples were incubated at 3 or 6 m, indicating that at these depths repair, dilution or disappearance of damage occurred. Phytoplankton assemblages were dominated by cells less than 2 µm in effective diameter; this cell size category seems to be more resistant to photosynthetic inhibition, but vulnerable to CPD accumulation, as compared with larger eukaryotic phytoplankters (i.e., *Phaeodactylum* sp).

INTRODUCTION

Phytoplanktonic organisms are affected in different ways by ambient levels of solar radiation (Holm-Hansen et al. 1993a, Häder 1997). In particular, the effects of solar ultraviolet radiation (UVR, 280-400 nm) in autotrophic organisms have been addressed due to the discovery of the stratospheric ozone depletion (i.e., the ozone "hole"), as this phenomenon results in an increase of short wavelengths of UV-B radiation (280-315 nm) reaching the Earth's surface (Madronich 1993). One of the most studied effects of solar UVR upon phytoplanktonic organisms is the inhibition of photosynthetic rates, which has been observed in many regions, such as polar areas (Helbling et al. 1992b, Smith et al. 1992, Neale et al. 1998c), temperate (Behrenfeld et al. 1993, Helbling et al. 1993), and tropical environments (Helbling et al. 1992b, Behrenfeld et al. 1993, Villafañe et al. 1999). Another effect of UVR that has been particularly addressed in phytoplanktonic organisms is the damage of genetic material (i.e., DNA) as a consequence of the formation of cyclobutane pyrimidine dimers (CPDs), mainly thymine dimers – T<>T (Karentz et al. 1991a, Buma et al. 1997, Boelen et al. 1999, 2001). Most of the UVR effect studies have been carried out in polar areas (especially Antarctica), as it is thought that organisms of these high latitudes would be especially affected by enhanced levels of solar UV-B radiation during springtime ozone depletion events (Smith et al. 1992, Holm-Hansen et al. 1993b, Arrigo 1994). However, as responses to UVR depend on the particular biological, physical and optical characteristics of the ecosystem under study, it is not possible to safely extrapolate the results obtained in polar areas to other aquatic systems of the planet.

There are not many studies that address the impact of solar UVR upon phytoplanktonic species of temperate systems (Behrenfeld et al. 1993, Helbling et al. 1993). In particular, we are not aware of any studies of the effects of solar UVR on phytoplanktonic species of the temperate region of Patagonia in South America. This is rather surprising, considering the high productivity of the coastal areas of Patagonia (Charpy & Charpy-Roubaud 1980) and the very interesting characteristics that would warrant this type of photobiological studies. The mid - latitude aquatic environments of Patagonia, especially the area including the Atlantic coast, receive high daily doses of solar radiation during the austral Spring – Summer due to a combination of several factors: a) relatively high irradiances and daylight periods of up to 18 h (Orce & Helbling 1997); b) low cloudiness (Lubin & Jensen 1995) and, c) clear skies with very low amount of particles. In addition to these factors, the region is under the influence of periodic events of low column ozone concentrations due to its proximity to the Antarctic polar vortex and the ozone "hole" (Orce & Helbling 1997), thus receiving sporadically higher levels of solar UV-B radiation.

This study evaluates the effects of solar UVR upon photosynthesis and DNA in phytoplanktonic species of the Argentinean Sea – Atlantic Ocean, in the area of Bahía Bustamante, Chubut (Patagonia), which is a nutrient rich - high primary productivity region, and commercially important for the collection of the alga *Gracilaria verrucosa* (Hudson) Papenf. (Rhodophyta). To our knowledge this is the first study that describes *in situ* UVR effects on photosynthetic performance and, simultaneously, DNA damage induction in marine plankton organisms.

MATERIALS AND METHODS

This research work was conducted at Bahía Bustamante, Chubut (45° S, 66.5°W), Argentina (Fig. 1), during January of 1999. Surface water samples were collected early in the morning using acid clean (1N HCl) polycarbonate bottles and incubated *in situ* (0, 3, 6, and 9 m depth) during 6 h centered around local noon (i.e. from 9 am to 3 pm).
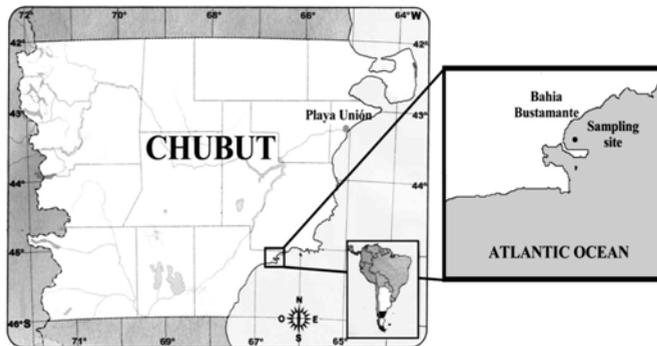
Figure 1: Map of the Chubut Province, Argentina, indicating the sampling and experimentation site at Bahía Bustamante. Inset: Relative location of Chubut in South America.

In order to determine the effects of solar UVR on photosynthetic rates, duplicate samples were placed in 50 ml quartz tubes and inoculated with 5 µCi (0.185 MBq) of labeled sodium bicarbonate (Steeman Nielsen 1952). Three different radiation treatments were implemented at each depth: a) Uncovered quartz tubes [samples receiving both Photosynthetic Available

Radiation (PAR, 400 - 700 nm) and UVR], b) Tubes covered with a Mylar-D film, transmitting UV-A (315 - 400 nm) + PAR and, c) Tubes covered with Plexiglas filter UF-3, so that samples received only PAR. The tubes were then placed in aluminum anodized frames that were connected to a buoy, down to the depths mentioned above. After the incubation period, the samples were filtered onto Wathman GF/F filters (25 mm in diameter), exposed to HCl fumes overnight, dried and counted using a liquid scintillation counter (Holm-Hansen & Helbling 1995).

To evaluate the formation of cyclobutane pyrimidine dimers - CPDs (i.e., DNA damage) and the potential of repairing DNA, water samples containing natural phytoplankton assemblages were placed in 10 l UVR-transparent polypropylene bags hanging next to the aluminum frames. These bags have a very high transparency for all solar wavelengths (Visser et al. 1999). At the start of the experiment (t=0) and after the incubation period, the samples were filtered and fractionated onto 10 µm, 2 µm, and 0.2 µm polycarbonate membrane filters (Poretics, 47 mm) and immediately frozen in liquid nitrogen (-180°C) until analyses, that were carried out at the University of Groningen, The Netherlands. DNA was extracted using a modified method from Doyle & Doyle (1991). Filters were incubated at 60 °C for 30 minutes with 750 µl preheated CTAB isolation buffer [2% (w/v) CTAB (Sigma), 1.4 M NaCl, 0.2% (v/v) β-mercaptoethanol, 20 mM EDTA, 100mM Tris-HCl pH=8.0]. An aliquot of 750 µl CIA [chloroform / isoamylalcohol (24:1)] was added to extract the DNA from cell debris and proteins. After centrifugation (14000 rpm, 10 min.), 0.5 ml of cold isopropanol was added to the upper (water) phase to precipitate the DNA (1h, 4°C). After centrifugation (14000 rpm, 30 min, at 4°C) the supernatant was removed and the pellet was washed with 1 ml of 80% ice-cold ethanol (15 min, -20°C, followed by centrifugation, 30 min. 4°C). Finally the DNA pellet was dried under vacuum and resuspended in TE buffer (1mM Tris-HCl pH=8.0, 0.1 mM EDTA). To remove the RNA, the DNA was incubated for 1 h with 75 µg ml^{-1} RNAse (Boehringer Mannheim) at room temperature. The amount of DNA was determined fluorometrically using Picogreen dsDNA quantitation reagent (dilution 1:400, Molecular Probes) on a 1420 Victor multilabel counter (EG&G Wallac, excitation 485 nm, emission 535 nm).

The amount of CPDs was determined using the method of Boelen et al. (1999) employing a primary antibody directed mainly to thymine dimers. In short, 100 ng of heat denatured DNA samples was blotted onto nitrocellulose membranes (Schleicher and Schuell, Protran 0.1 µm). The membranes were baked at 80°C to immobilize the DNA. After a 30 min blocking step, followed by three washing steps, the membranes were incubated with the primary antibody H3 overnight at 4°C. After repeated washing, incubation with the secondary antibody (HRP rabbit-anti-mouse, Dako P0260) was done for two hours at room temperature. The detection of CPD's was done using ECL detection reagents (RPN2106 Amersham) in combination with photosensitive films (Kodak-X-AR-5). Finally, the films were scanned and the quantification of dimers was done using Image Quant software (version 4.2, Molecular Dynamics). Each blot contained two dilution series of standard DNA with known amounts of CPD's (Boelen et al. 1999, 2001). In order to determine DNA effective radiation and attenuation, a DNA biodosimeter was used as described in Boelen et al. (1999). Duplicate acid-cleaned quartz tubes, containing bare DNA, were placed close to the polypropylene bags and incubated for the duration of the incubation experiment.

Samples were also taken for analysis of chlorophyll-a (chl *a*), floristic composition and absorption characteristics of phytoplankton. The analyses of these samples were done as follows: a) Chl *a* analysis: 100 ml of sample was filtered onto a Wathman GF/F filter (25 mm in diameter) and the photosynthetic pigments extracted in absolute methanol during one hour (Holm-Hansen & Riemann 1978). Chl *a* concentration was then calculated from the fluorescence of the extract (Holm-Hansen et al. 1965), using a Turner Designs fluorometer (model TD 700). b) Samples for identification and enumeration of phytoplankton were placed in 125 ml brown bottles and fixed with buffered formalin (final concentration of 0.4% in the sample); after settling 25-50 ml of sample, they were analyzed with an inverted microscope (Leica DM IL) following the technique described in Villafañe & Reid (1995). Incident solar radiation was measured continuously using a broad band ELDONET radiometer (Real Time Computers Inc.), that measures UV-B (280-315 nm), UV-A (315-400 nm) and PAR (400-700 nm) with a frequency of one reading per minute. Optical characteristics of the water column were determined using a profiling broad band ELDONET radiometer (Real Time Computers Inc.) which measures UV-B, UV-A and PAR, water temperature and water depth; a total of 6 profiles were done with this sensor. This instrument was deployed by hand from a Zodiak at a site next to our array for *in situ* incubations

RESULTS

UV-B radiation decreased with depth in the water column, and the attenuation coefficient (K_{UV-B}) was 0.80 m^{-1} (Fig. 2A), with the 1% radiation level found at 5.8 m. The accumulation of CPDs in bare DNA from the biodosimeter was high at 0 m: 800 CPDs per 10^6 nucleotides. However, DNA effective radiation was so rapidly attenuated that virtually no CPDs could be detected already at 3 m (Fig. 5A). UV-A radiation and PAR penetrated deeper in the water column, with the 1% radiation level for UV-A at 8.7 m, while the irradiance level for PAR at the bottom (i.e., 15 m with high tide) was 10% of the surface irradiance (calculated from K_{PAR}). The attenuation coefficients for UV-A (K_{UV-A}) and PAR (K_{PAR}) were 0.53 m^{-1} and 0.16 m^{-1}, respectively (Figs. 2B, C). Phytoplankton distribution in the water column was rather homogeneous and no stratification was noticeable from the temperature profile (Fig. 3). Although we did not measure salinity, we estimated that there were not significant changes in the density of the upper part of the water column because there were no inputs of freshwater in this system. Chl *a* concentration in the surface sample was 3.1 µg chl-a l^{-1}. Mean surface incident irradiance values during the time of our experimentation were 2.2, 60 and 450 W m^{-2} for UV-B, UV-A and PAR, respectively; the doses during the 6 h incubation period were 47 KJ m^{-2}, 1291 KJ m^{-2} and 9661 KJ m^{-2} for UV-B, UV-A and PAR, respectively.
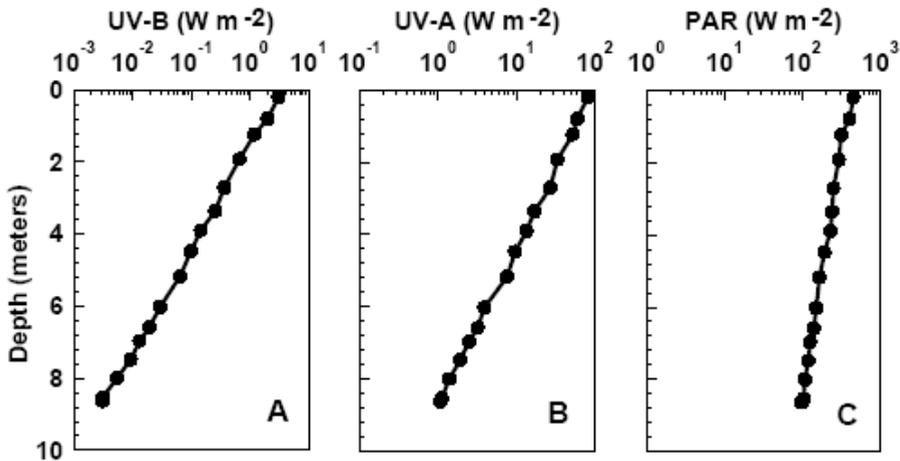
Figure 2: Representative profiles showing the underwater radiation field next to our *in situ* experiments; radiation in W m^{-2}. **A**) UV-B radiation; $K_{UV-B} = 0.80$ m^{-1}; **B**) UV-A radiation; $K_{UV-A} = 0.53$ m^{-1}; and **C**) PAR; $K_{PAR} = 0.16$ m^{-1}. Profiles done on January 13, 1999 (14.00 h local time).
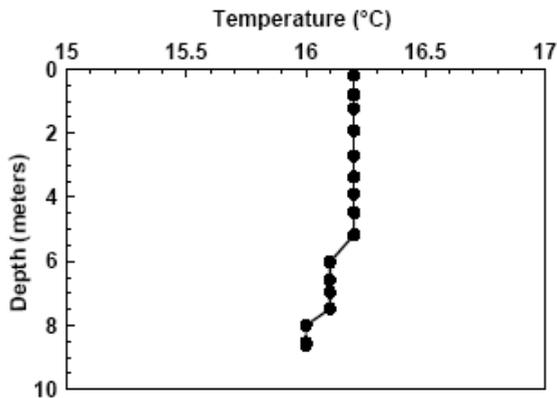

Figure 3: Water temperature profile (in °C) as a function of depth, close to our *in situ* experiments. Data obtained with the temperature sensor on the ELDONET radiometer.

Fig. 4 represents the photosynthetic characteristics of natural phytoplankton assemblages when exposed *in situ* to the three radiation treatments mentioned above. The amount of carbon fixed in surface waters during the 6 h incubation period (Fig. 4A) was about 17.5 µg C l^{-1} in the treatment that received only PAR. The carbon fixation for PAR was rather constant with depth, having a very slight increase at 3-6 m, suggesting little photoinhibition at the surface due to high PAR levels. The amount of carbon fixed by phytoplankton receiving UV-A + PAR and UVR + PAR were 12.6 and 11 µg C l^{-1}, respectively, and no significant differences (p < 0.05) were observed among treatments below 6 m depth (Fig. 4A). Assimilation numbers (Fig. 4B) were rather constant with depth (about 0.9 mg C mg chl a^{-1} h^{-1}) for the treatment that received only PAR, with maximum numbers found at 3 m. Photosynthetic inhibition (Fig. 4C) at the surface was 29 and 7% for UV-A and UV-B, respectively, as

compared with the treatment that received only PAR. This inhibition decreased with depth so that no significant differences ($p < 0.05$) among treatments were found at 6 m. UV-A was responsible for most of the observed inhibition in the upper water column (i.e., upper 3 m).
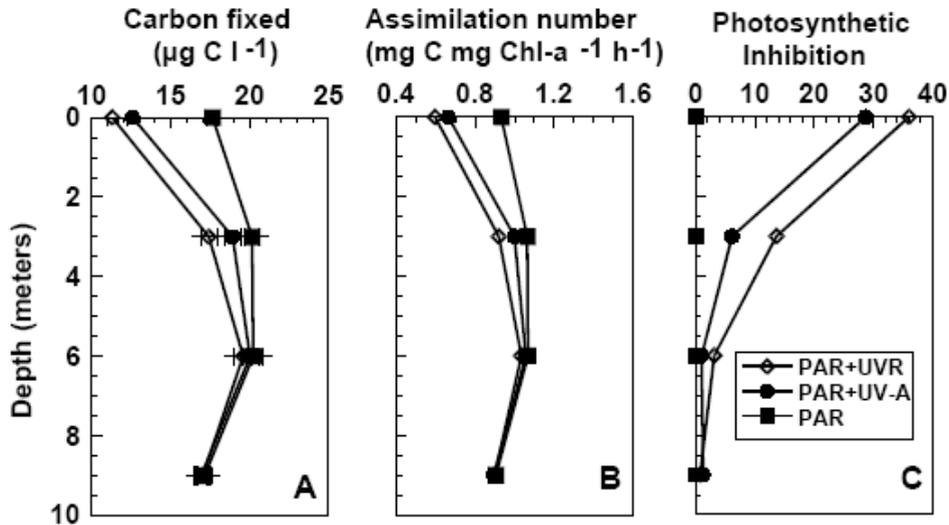

Figure 4: Depth distribution of carbon fixation, assimilation numbers and photosynthetic inhibition for natural phytoplankton populations from Bahía Bustamante exposed to three radiation treatments. **A**) Carbon fixation in $\mu g\,C\,l^{-1}$; **B**) Assimilation numbers in mg C [mg chl a]$^{-1}$ h^{-1}; and **C**) Percentage of photosynthetic inhibition; photosynthetic inhibition in the PAR treatment has been set to zero. The + symbols indicate one standard deviation.

No DNA could be extracted from the 2 and 10 µm fractions, indicating that cells bigger than 2 µm represented a negligible proportion of the plankton biomass in terms of DNA. Microscopic observation of phytoplankton samples also revealed that the phytoplankton crop was mainly composed of picoplanktonic cells (less than 2 µm in diameter), with very few representatives of larger cells such as diatoms (e.g., *Pseudonitzschia* spp., *Skeletonema costatum*, *Licmophora* sp.). The natural phytoplankton assemblage already had a high level of DNA damage at the time the incubation started with a mean of 375 T< >T per 10^6 nucleotides (t = 0, Fig. 5B). The high levels of CPDs in the phytoplankton were consistently observed in all morning samples collected for other experiments (data not presented). At the end of the incubation period, the formation of CPDs increased significantly in surface waters (mean value of 650 T< >T per10^6 nucleotides). In contrast, CPD levels at 3 and 6 m depth diminished to about 250 CPDs per 10^6 nucleotides (Fig. 5B).
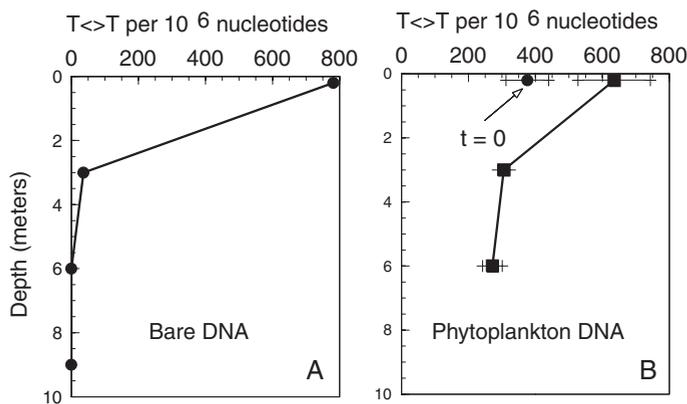
Figure 5: Depth profiles of CPDs formation (T<>T per 10^6 nucleotides). **A)** Bare DNA samples and, **B)** Natural phytoplankton populations. The symbol at t = 0 indicate the amount of CPDs at the start of the experiment. The + symbols indicate one standard deviation.

DISCUSSION

Two of the most important physiological forms of natural UVR stress in phytoplanktonic organisms are photosynthetic inhibition and DNA damage. Many studies have evaluated the role of UVR in inhibiting photosynthesis (Helbling et al. 1992b, Smith et al. 1992, Neale et al. 1998c) and damaging the DNA (Karentz et al. 1991a, Buma et al. 1997, Boelen et al. 2001) in various regions. In this study we consider both targets (i.e., photosynthesis and DNA) at the same time, thus providing a powerful tool to evaluate the overall impact of solar radiation in the very productive waters of the Patagonian coast. Our results show that UVR can simultaneously affect photosynthesis and DNA as observed in surface waters (Figs. 4, 5).

Phytoplankton photosynthesis in our study was more inhibited by UV-A than by UV-B (Fig. 4), with PAR causing relatively minor photoinhibition. The greater inhibition by UV-A, as compared to UV-B, was also observed in other freshwater and marine environments (Bühlmann et al. 1987, Kim & Watanabe 1993, Villafañe et al. 1999). The inhibition of phytoplankton photosynthesis due to solar UVR decreased with depth but it was mostly limited to the upper 3 m of the water column (Fig. 4); below this depth, solar UVR decreased enough (Fig. 2) just to cause a mild effect. In particular, UV-B radiation in our study area (Fig. 2A) had a relatively high attenuation coefficient (i.e., 0.80 m^{-1}), as compared to Antarctic areas with similar particle concentration (Helbling et al. 1994). The high K_{UV-B} in our study most probably reflects the amount of terrigenous material at the coastal station where we conducted the experimentation. However, the attenuation coefficients at Bahía Bustamante were lower than in other coastal areas (Booth & Morrow 1997). When compared with other environments of Patagonia, such as the sub-Antarctic waters of the Beagle Channel, Tierra del Fuego (Helbling et al. 1996c), the total amount of photosynthetic inhibition at the surface was similar at both sites (about 40 %), however, its depth distribution was different (Villafañe et al. 2001). When comparing the overall impact of UVR at these sites, we considered the photosynthetic inhibition as a function of the optical depth (the optical depth of 4.6 is equal to $K_{PAR}*Z_{eu}$). In the Beagle Channel, photosynthetic inhibition in the water column reached down to 3 optical depths (Helbling et al. 1996c, Villafañe et al.

2001), while at Bahía Bustamante it reached down to 1.5 optical depths (Fig. 4C), decreasing, at this latter place, almost exponentially. Thus, the depth integrated loss of carbon fixation (upper 10 m of the water column) due to UV-B (determined when comparing to the PAR treatment that was taken as 'no inhibition'), would be higher for the Beagle Channel assemblages (calculated value of 24%) than for Bahía Bustamante phytoplankton (calculated value of 3%). The integrated inhibition at Bahía Bustamante is closer to the 4.9 % value reported for UV-B inhibition in the Antarctic (Holm-Hansen et al. 1993b).

Initial CPD levels were very high in this study (Fig. 5B) and higher than those found in other areas, for instance in marine tropical picoplankton (Boelen et al. 2001), in the Antarctic (Buma et al. 2001a) or in the plankton from Lake Titicaca, Bolivia (Helbling et al. 2001c). This would hint to a prolonged history of previous UV-B exposures in the water column, combined with a low repair capacity of cells. On the other hand, CPD levels decreased at 3 and 6 m depth, indicating that either repair was taking place, or damage was diluted by *de novo* DNA synthesis in viable cells. Also, CPD formation may result in an increasing proportion of non-viable cells in the plankton assemblage, eventually leading to a loss of cells from the water column through lysis (Boelen et al. 2001). It has to be stressed that the CPD outcomes reflect not only picophytoplankton cells but also heterotrophic bacteria, all contained on the 0.2 µm filter. However, as has been demonstrated recently by Boelen et al. (1999), no significant difference in CPD accumulation could be observed in these two functional picoplankton groups in tropical marine assemblages. Some studies had determined that small cells (i.e., nanoplanktonic, <20 µm in diameter) were generally more resistant that large cells when looking at photosynthetic inhibition (Helbling et al. 1992b, Laurion & Vincent 1998). Simulated *in situ* experimentation data (not shown), conducted in parallel with the *in situ* studies presented in this paper, were used to calculate a BWF for inhibition of phytoplankton photosynthesis. Eight independent experiments were conducted using sharp-cut of filter (Schott); a mean BWFs was calculated using a BWF-PI model (Cullen et al. 1992, Neale & Kieber 2000), and the spectral dependence of the BWF in the broadband intervals was extracted using the method of Rundel (1983). A comparison of Bahía Bustamante BWF with the one for *Phaeodactylum* sp. (Cullen et al. 1992) showed that phytoplankton from Bahía Bustamante were significantly less sensitive to UVR for wavelengths higher than 300 nm. However, in contrast with this, small cells (less than 2 µm) seem to be more sensitive to UVR when looking at CPD formation (Karentz et al. 1991a, Boelen et al. 2001). Also, natural Antarctic picoplankton assemblages displayed significantly higher CPD levels as compared with larger, diatom containing size fractions, as a result of exposure to solar UV-B radiation (Buma et al. 2001a).

Clearly, CPDs were accumulated in the surface, but no accumulation was observed at 3 and 6 m depth, in accordance with the strong attenuation of DNA effective UVR (Fig. 5A). It should be considered that cells move in the upper mixed layer (see Fig. 3 for temperature profile) but in an *in situ* experimentation they are kept at a fixed depth. This means that the surface samples would receive higher irradiance than they would in the upper mixed layer (UML), so an increase in CPDs would be observed. However, the cells incubated at 3 and 6 m, receive less irradiance, thus a decrease in CPDs was noticed (Fig. 5). We have recently shown for plankton organisms from Lake Titicaca (Helbling et al. 2001c), that both UV-A and UV-B inhibit photosynthesis, with UV-A having a stronger effect than UV-B. At the same time CPD accumulation was only related with UV-B exposure, as found in this study

in Patagonia. Moreover, daily patterns of both photosynthetic inhibition and DNA damage accumulation were far from similar. Evidently, DNA damage accumulation and photosynthetic inhibition patterns reveal effects on various, relatively independent cell targets, i.e. nuclear DNA and the photosynthetic apparatus, although interactions between the two processes are imaginable (Helbling et al. 2001c). Assimilations numbers in our coast get up to 5 mg C (mg chl a)$^{-1}$ h^{-1} during the spring bloom, but were lower in the mid-summer post-bloom assemblages (Villafañe, unpub. data). The relatively low assimilation values (about 1 mg C (mg chl a)$^{-1}$ h^{-1}), as compared to the spring bloom, found in this study (Fig. 4), together with the high CPDs values found in the water column, might in fact reveal an interactive process between DNA damage and photosynthetic apparatus.

In conclusion, this study has demonstrated that UVR stress may be brought about by multiple target effects, at least in the surface. The extent to which these targets are affected will be determined by irradiance conditions or species -specific differences in vulnerability, for instance related to cell size.

Chapter 7

Solar UVR – induced DNA damage and inhibition of photosynthesis in phytoplankton from Andean lakes of Argentina

Villafañe VE, Buma AGJ, Boelen P, Helbling EW

ABSTRACT

During January 1999 studies were carried out in temperate lakes of the Andean region of Argentina (41°S, 71°W) to determine the short-term effects of solar ultraviolet radiation (UVR, 280-400 nm) upon natural phytoplankton assemblages. Organisms from one 'clear' (Lake Moreno) and two 'opaque' lakes (Morenito and El Trébol) were exposed to different radiation regimes to assess photosynthesis inhibition and cyclobutane pyrimidine dimers (CPDs) accumulation / repair. UV-B caused significant DNA damage in organisms from 'opaque' lakes, especially those from Lake Morenito. Organisms from the 'clear' Lake Moreno, on the other hand, presented lower CPDs accumulation rates. UV-B had relatively low effects inhibiting photosynthesis in these opaque lakes (2 and 9.5%, for lakes Morenito and El Trébol, respectively) and most of the inhibition was due to UV-A (75 and 71% inhibition for lakes Morenito and El Trébol, respectively). In Lake Moreno, photosynthetic inhibition was 35 and 15% for UV-A and UV-B, respectively. A number of causes seem to account for the different responses observed among phytoplankton assemblages, being one the most important the underwater radiation field and hence the light acclimation history of cells. In addition, factors such as differences in the type and effectiveness of the strategy used by the organisms to cope with solar UVR, as well as differences in the size structure and taxonomic composition of the community are also important at the time to evaluate the overall impact of solar UVR in these lakes.

INTRODUCTION

The effects of solar ultraviolet radiation (UVR, 280-400 nm) upon aquatic photosynthetic organisms have been extensively reported in the literature (see reviews books by De Mora et al. 2000, and Helbling & Zagarese 2003). On one hand, by damaging essential molecules such as proteins and DNA (Buma et al. 1996, Garde & Gustavson 1999), UVR can alter cellular processes such as the uptake of nutrients (Behrenfeld et al. 1995), photosynthesis (Villafañe et al. 2003) or DNA transcription / replication (Setlow et al. 1963), which can finally result in an overall reduction of phytoplankton fitness. On the other hand, UVR wavelengths can be beneficial, as they may enhance photosynthetic rates (Barbieri et al. 2002, Helbling et al. 2003) or they can photodegrade chromophoric dissolved organic matter (CDOM) producing thus the photomineralization of CDOM with the consequently release of inorganic nutrients that are available for photosynthetic organisms (De Lange et al. 2003).

Whether UVR resulting in a negative or advantageous force for phytoplankton will ultimately depend on a combination of factors, among which the penetration of biologically effective radiation in the water column, and hence the amount of UVR received by the cells, plays a determinant role. The penetration of biologically effective radiation is in turn determined by UVR levels reaching the water surface (Madronich 1993) as well as by the optical absorption of different components - the water itself, particulate (both organic and inorganic)

and dissolved matter (Hargreaves 2003). Studies carried out in freshwater environments have shown that CDOM is a good estimator of solar UVR transparency of the water column (Scully & Lean 1994, Morris et al. 1995, Laurion et al. 2000).

Patagonian Andean lakes of Argentina are very different in terms of their biological (Díaz & Pedrozo 1993, 1996, Helbling et al. 2001b) and optical characteristics (Morris et al. 1995; Helbling et al. 2001b; Alonso et al. 2004). This variability in both the community structure and in the underwater radiation field certainly offers a unique opportunity to evaluate UVR effects upon aquatic organisms inhabiting these lakes. In this region, several studies have described the responses to solar UVR of zooplankton organisms (Zagarese et al. 1997a, b, 1998a, b, Alonso et al. 2004), fish larvae (Battini et al. 2000), biological interactions in a mesocosm (Pérez et al. 2003) and photochemical aspects (Zagarese et al. 2001). However, and with the exception of the work of Helbling et al. (2001b) carried out with winter communities exposed to artificial radiation conditions, we are not aware of studies specifically addressing the effects of UVR upon phytoplankton organisms of temperate lakes of Patagonia.

The objective of this study is to determine the effects of solar UVR upon photosynthesis and DNA in phytoplankton from three Andean lakes of Argentina that have marked differences in water transparency. The approach was to determine photosynthetic rates and DNA damage when natural phytoplankton assemblages were exposed to different solar radiation wavebands. It should be noted that while both UV-A (315-400 nm) and UV-B (280-315 nm) can significantly reduce photosynthetic rates, only UV-B causes the formation of cyclobutane pyrimidine dimers (CPDs); UV-A, on the other hand, can induce indirect DNA oxidative damage (Buma et al. 2003). In this work we will estimate DNA damage through the formation of CPDs, which accounts for about 80-90% of photoproducts formed (Buma et al. 2003). It should be considered though, that other UVR-induced photoproducts, such as pyrimidine (6-4) pyrimidone photoproducts ((6-4) PDs) can be as 300 times effecting in blocking DNA polymerase, being therefore more cytotoxic than CPDs (Mitchell & Nairn 1989).

MATERIALS AND METHODS

a) Study site and collection of samples

Experiments were conducted during January 1999 with phytoplankton collected from three Andean lakes of the Patagonia region of Argentina: Moreno, Morenito and El Trébol (41° S, 71° W, 800 m a.s.l.). Lakes Morenito and El Trébol are small lakes with a surface area < 1 km^2 (max. depth ~ 10 m), whereas Lake Moreno is a rather large lake with a surface area of ~ 6 km^2 (mean depth ~ 50 m). Surface water samples were collected daily (early in the morning) using a clean bucket (1N HCl) and transported immediately to the laboratory (aprox. 20 minutes away from the sampling sites) where several experiments were carried out as described below.

b) Experimental

Experiments were done to determine the effects of solar UVR upon phytoplankton photosynthesis rates and DNA under simulated *in situ* conditions in a large pool with running surface water (15 - 17°C) used as temperature control. At the beginning of each experiment sub-samples were processed for the determination of initial cyclobutane pyrimidine dimers

(CPDs) levels, pigment concentration and phytoplankton composition / quantification (see below). Three types of experiments were carried out (all on different dates) to determine UVR effects as follows:

A - *UVR-induced photosynthetic inhibition (all lakes):* Samples were transferred to 50 ml quartz tubes, inoculated with labelled radiocarbon (see below) and incubated for 6 - 8 h around local noon under three radiation treatments (quadruplicates for each treatment): a) Tubes covered with Plexiglas UF-3 (cut-off at 400 nm) so that the samples received only PAR (P treatment); b) Tubes covered with a sharp cut-off Schott filter (WG320) so that the samples received UV-A + PAR (PA treatment); and, c) Tubes without any filter to receive full solar radiation (PAB treatment). The transmission spectra of filters and materials have been published elsewhere (Buma et al. 2001b, Villafañe et al. 2003). Two independent experiments (i.e., different dates) were conducted with waters collected from each lake.

B - *CPDs induction and repair (all lakes):* Samples were dispensed in 10L high -UVR transmission polypropylene bags (the spectral characteristics of these bags have been published in Buma et al. 2001b) to study CPDs accumulation and repair in several microbial size fractions (i.e., 0.2 µm, 2 µm and 10 µm) when exposed to different radiation conditions. The radiation treatments were the following: a) Two bags incubated under full solar radiation and harvested around noon or at the end of the afternoon (PAB treatment); b) Two bags incubated under UVR opaque PMMA that received only PAR (the spectral characteristics of this material have been published in Buma et al. 2001b) for the morning or whole day period – P treatment; c) Two bags incubated under full solar radiation during morning hours, after which the bags were covered by either UVR opaque PMMA or 3 mm glass plates to remove total UVR or UV-B, respectively and (PAB-P and PAB-PA treatments, respectively), d) One bag incubated under UVR opaque PMMA during morning hours, after which UV-A was allowed to pass during afternoon hours by replacing the PMMA screen by a glass screen (P-PA treatment). Each bag had two DNA biodosimeter tubes attached to the side to allow for DNA effective dose assessment during the experiments. The DNA biodosimeters consisted of a small quartz tube filled with a solution of bare DNA - 10 µg/ml calf thymus DNA in TE-buffer (10 mM Tris-HCl; pH=8.0; 1 mM EDTA) (Boelen et al. 1999).

C - *Daily course of CPDs accumulation and photosynthetic inhibition (Lake Moreno only):* Experiments were performed to determine the accumulation of CPDs during the day in the three size fractions (i.e., 0.2-2, 2-10, > 10 µm) from Lake Moreno incubated under full solar radiation. A total of nine bags and eighteen DNA biodosimeter tubes were placed in the temperature-controlled water pool early in the morning. Then, one bag and duplicate biodosimeter tubes were removed one by one at successive PAR doses of 5.5 E/m^2 and processed for CPDs determination. Simultaneously, the daily course of UVR inhibition of photosynthesis was followed in these natural phytoplankton assemblages. For this measurement, three radiation treatments were implemented with eighteen quartz tubes (50 ml) exposed to full solar radiation (i.e., UVR + PAR – PAB treatment), eighteen quartz tubes (50 ml) covered with Mylar-D film (i.e., UV-A + PAR – PA treatment), and eighteen quartz tubes (50 ml) covered with Plexiglas UF-3 (i.e., PAR only – P treatment); the transmission spectra of these materials are published in Helbling et al. (1992b). Two tubes from each treatment were removed, together with a bag and the biodosimeters (i.e., early in the morning, and at equal PAR doses).

c) Analyses and measurements

Photosynthetic rates: Samples for photosynthesis measurements were inoculated with 5 µCi (0.185 MBq) of labelled sodium bicarbonate (Steemann Nielsen 1952). After the incubation period, the samples were filtered onto Whatman GF/F filters (25 mm), placed in 7 ml scintillation vials and exposed to HCl fumes overnight. After drying the filters, scintillation cocktail (Wallac Optiphase HiSafe 3) was added to the vials and the activity measured using a liquid scintillation counter (Holm-Hansen & Helbling 1995).

CPDs formation: Each sample (i.e., in each bag) was size - fractionated by filtration and the filter frozen and stored (-80 °C) until analysis, which was carried out at the University of Groningen (The Netherlands). DNA was extracted from the filters using the procedure described in Buma et al. (2001a). To remove RNA, the extracts were incubated for 1 h with 75 µg / ml RNAse (Boehringer Mannheim) at room temperature. The DNA concentration of the extracts was determined fluorometrically using Picogreen dsDNA quantitation reagent (dilution 1:400, Molecular Probes) on a 1420 Victor multilabel counter (EG&G Wallac, excitation 485 nm, emission 535 nm). The amount of CPDs was determined using the method of Boelen et al. (1999) employing a primary antibody (H3, Affitech, Oslo) directed mainly to thymine dimers. Briefly, 100 ng of heat denatured DNA samples were blotted onto nitrocellulose membranes (Schleicher and Schuell, Protran 0.1 µm) which were then baked at 80°C to immobilize the DNA. After a 30-minutes blocking step, followed by three washing steps, the membranes were incubated with the primary antibody H3 (overnight, 4°C). After repeated washing, incubation with the secondary antibody (HRP rabbit-anti-mouse, Dako P0260) was done for two hours at room temperature. CPDs were detected using ECL detection reagents (RPN2106 Amersham) in combination with photosensitive films (Kodak-X-AR-5). Finally, the films were scanned and the quantification of dimers was done using Image Quant software (version 4.2, Molecular Dynamics). Each blot contained two dilution series of standard DNA with known amounts of CPDs (Boelen et al. 1999). The vulnerability for CPDs induction was assessed by calculating the Mean Damage Ratio (MDR) (Buma et al. 2003) by normalizing CPDs values in microorganisms to the CPDs values obtained in the biodosimeter.

Photosynthetic pigments: Chlorophyll-a (chl-*a*) concentration was determined fluorometrically by filtering 100 ml of sample onto a Whatman GF/F filter (25 mm) after which the photosynthetic pigments were extracted in absolute methanol during 1 h (Holm-Hansen & Riemann 1978). Chl-*a* concentration was then calculated from the fluorescence of the extract before and after acidification with 1N HCl (Holm-Hansen et al. 1965) using a Turner Designs fluorometer (model TD 700).

Cell counts and taxonomic analyses: Samples for identification and enumeration of phytoplankton were placed in 125 ml brown bottles and fixed with buffered formalin (final concentration of 0.4% in the sample); after settling 25-50 ml of sample, cells were analyzed with an inverted microscope (Leica DM IL) following the technique described in Villafañe & Reid (1995). In addition, size distribution and mean cell area of phytoplankton species were determined by attaching a video camera (Philips LDH 0462/00) to the inverted microscope and using image analysis (Wintrack Software, Real Time Computers Inc.). For this measurement, an aliquot of 25 ml of sample was settled overnight; 10-20 fields were analyzed and at least one hundred cells were measured.

Radiation measurements: During the whole experimentation period incident solar radiation was recorded continuously (one reading per minute) with a GUV 511 radiometer (Biospherical Instruments, Inc.) that has four channels in the UVR region of the spectra (305 nm, 320 nm, 340 nm and 380 nm) as well as a broad band PAR channel (400-700 nm). The penetration of solar radiation in the water column was measured at the same dates when experiments B were done (i.e., *CPDs induction and repair*) using an ELDONET broad band filter radiometer (Real Time Computers Inc.) that has sensors for UV-B (280-315 nm), UV-A (315-400 nm) and PAR (400-700 nm) and temperature and depth channels. In addition, DNA biodosimeters were used throughout in simulated *in situ* experiments and incubated *in situ* at different depths in the water column to determine the DNA effective dose (k_{bd-eff}) as described in Buma et al. (2003).

Statistics. A non parametric Kruskal-Wallis analysis (Zar 1984) was used to establish differences among treatments and / or lakes; a confidence level of 95% was used in all analyses.

RESULTS

The water column in the three studied lakes was well mixed, as inferred from the temperature profiles (Fig. 1).
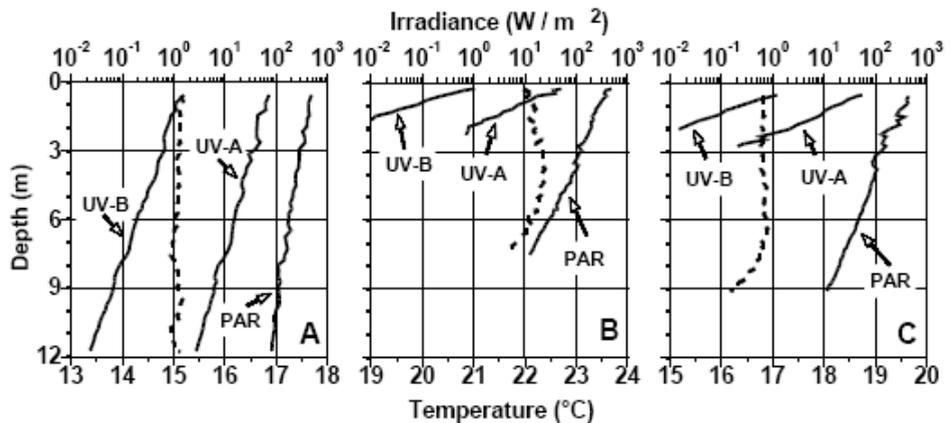
Figure 1: Solar UV-B, UV-A and PAR irradiances and temperature as a function of depth and for the three Andean lakes sampled. **A)** Lake Moreno; **B)** Lake Morenito and, **C)** Lake El Trébol. The broken lines in the figure indicate the underwater temperature (in °C). Solar irradiance for PAR, UV-A and UV-B is expressed in W / m².

Because of their shallowness, mixed conditions were determined down to the bottom in lakes Morenito and El Trébol (Figs. 1B, C); in the deep Lake Moreno, well mixed conditions were found at least in the upper 12 m of the water column (Fig. 1A). The three lakes had differences in temperature, with values of ~22°C in Lake Morenito (Fig. 1B), ~17°C in Lake El Trébol (Fig. 1C), and ~15°C in the large Lake Moreno (Fig. 1A). The underwater optical characteristics of these lakes were also markedly different (Fig. 1, Table 1). Lake Moreno (Fig. 1A) was a clear lake with a relatively deep penetration of solar radiation

(Table 1), and the euphotic zone (1% of surface PAR irradiance) comprised the upper 30 m of the water column. UVR also penetrated relatively deep, and the 1% of surface UV-B and UV-A were found at 12.8 and 16.4 m, respectively. Lakes Morenito (Fig. 1B, Table 1) and El Trébol (Fig. 1C, Table 1) were considered as 'opaque' lakes as solar radiation was attenuated much faster than in Lake Moreno; the euphotic zone in lakes Morenito and El Trébol was measured down to 10 and 11.5 m , respectively. In these two 'opaque' lakes UVR was greatly attenuated and neither UV-B nor UV-A were detected below 3 m (Figs. 1B, C).

Table 1: Attenuation coefficients for UV-B, UV-A and PAR in the three lakes surveyed in this study.

Lake	UV-B (280-315 nm)	UV-A (315-400 nm)	PAR (400-700 nm)
Moreno	0.36 m^{-1}	0.28 m^{-1}	0.15 m^{-1}
Morenito	2.8 m^{-1}	2.18 m^{-1}	0.46 m^{-1}
El Trébol	2.54 m^{-1}	2.39 m^{-1}	0.4 m^{-1}

The biodosimeter profiles from lakes El Trébol and Moreno (Fig. 2) also highlight the differential penetration of UV-B in the water column. CPDs values were high (~2800 CPDs / MB at the sub - surface) and accumulated in the upper 8 m of the water column in the clear Lake Moreno. In Lake El Trébol, on the other hand, CPDs values at surface were lower than in Lake Moreno (~1600 CPDs / MB), and no CPDs accumulation was detected below 0.4 m. The data obtained with the biodosimeters also allowed us to calculate the attenuation of DNA effective doses (k_{bd-eff}), which were 6.24 and 0.74 for lakes El Trébol and Moreno, respectively.
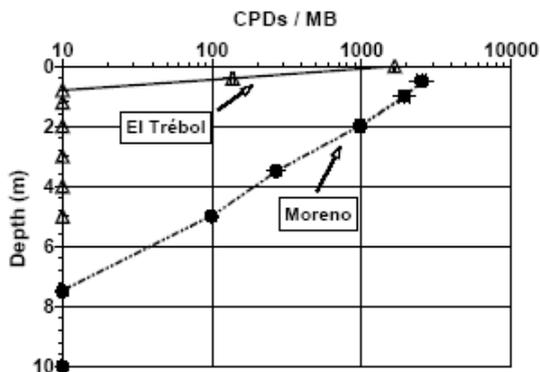
Figure 2: Depth profiles of penetration of biologically effective dose as determined with biodosimeters incubated in lakes El Trébol and Moreno. The (+) symbols indicate the standard deviation.

The lakes also differed in the size structure and composition of the phytoplankton communities. Although during the sampling period phytoplankton communities in the three lakes were characterized by small cells (i.e., < 20 µm in effective diameter), image analyses showed differences in the size distribution of cells (data not shown), with Lake Moreno presenting a slightly higher proportion of large cells as compared to that of lakes El Trébol and Morenito. Microscopical analysis also revealed differences among the lakes in regard to the taxonomic composition: small pennate diatoms characterized Lake El Trébol, whereas unidentified monads / flagellates and chlorophyte colonies dominated in lakes Morenito and Moreno, respectively. Other groups were also present – e.g., large pennate diatoms, dinoflagellates

– but never accounted for a significant proportion of the phytoplankton community. During the sampling period, total cell values were low in the three lakes (< 250 cells / ml) as well as chlorophyll a concentrations (< 1 mg / m³).

A comparison between lakes in regard to CPDs accumulation for the most abundant phytoplankton size group, i.e., the 2-10 µm cell size fraction is shown in Fig. 3.
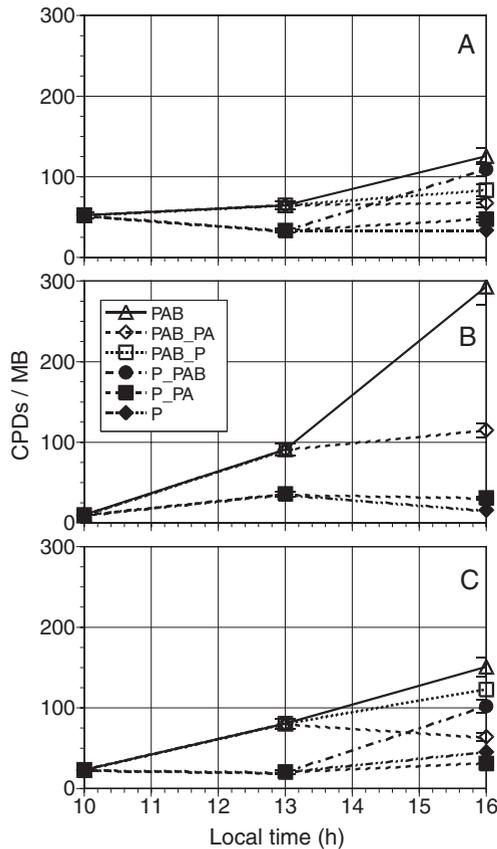

Figure 3: Accumulation of CPDs and repair of DNA damage in phytoplankton (2-10 µm size fraction) incubated under various irradiance treatments. **A)** Lake Moreno; **B)** Lake Morenito and, **C)** Lake El Trébol. Cut-off screens were placed above the samples at 13 h to differentiate DNA damage occurring during morning and afternoon as well as to evaluate photorepair (full explanation in the text). PAB indicates samples exposed to full solar radiation; PA indicates samples exposed to PAR + UV-A and P indicates samples exposed only to PAR. The symbols (⊤) indicate the standard deviation.

Initial CPDs values were 52, 10 and 23 CPDs / MB in lakes Moreno, Morenito and El Trébol, respectively. During the morning, CPDs values increased significantly (P < 0.05) in the two opaque lakes in samples exposed to full solar radiation (i.e., PAB) (Figs. 3B, C) whereas in Lake Moreno CPDs values remained relatively constant (Fig. 3A); CPD values in samples exposed to PAR only also remained constant during the morning. During the afternoon, all samples exposed to full solar radiation significantly accumulated CPDs (P < 0.05) from its noon value, being the damage rate (i.e., damage accumulation during 3 h) highest in Lake Morenito (i.e., final mean values ~ 300 CPDs / MB, Fig. 3B). In Lake El Trébol the damage rate was constant throughout the experiment (Fig. 3C) whereas in Lake Moreno (Fig. 3A) it was significant in the afternoon but not during the morning. As expected, samples exposed in the afternoon to either PAR + UV-A or PAR only did not show significant CPDs accumulation.

The higher CPDs formation during afternoon hours might reflect the impact of higher irradiances received then as compared to those of morning hours (Table 2).

Table 2: Mean solar radiation incident upon the experiments conducted to determine CPDs accumulation and repair (Fig. 3). Morning denotes incubations from 10 to 13h; afternoon denotes incubations carried out from 13 to 16h. PAR irradiances are expressed in μE cm^{-2} s^{-1} and UVR irradiances (i.e., 305, 320, 340 and 380 nm) in μW cm^{-2}.

	Lake Moreno		Lake Morenito		Lake El Trébol	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
PAR	0.174	0.213	0.172	0.215	0.174	0.214
305 nm	4.97	8.44	4.55	7.87	5.42	9.06
320 nm	26.9	36.1	26.2	35.8	27.3	36.9
340 nm	48.5	62.3	47.6	62.4	48.7	62.9
380 nm	63.2	81.1	62.3	81.2	63.3	81.3

Thus, in order to assess this effect, CPDs formation data from biodosimeters is presented in Fig. 4. The CPDs accumulation at the end of the experiments was significantly higher ($P < 0.05$) in the biodosimeter exposed to full solar radiation in Lake Moreno experiments - ~ 5000 CPDs / MB (Fig. 4A) than in those carried out in lakes Morenito and El Trébol (~ 3000 CPDs / MB, Figs. 4B, C). With the exception of Lake El Trébol, there was higher CPDs accumulation in biodosimeter samples collected during the afternoon than in the morning. To evaluate the vulnerability of phytoplankton assemblages of the three lakes in terms of DNA damage, we calculated the mean damage ratio (i.e., MDR) with data from Figs. 3 and 4. Lake Morenito had the highest MDR values ~ mean of 0.17 (SD 0.06), whereas lakes Moreno and El Trébol had MDR mean values of 0.04 (SD 0.02) and 0.06 (SD 0.01), respectively. Photosynthetic rates were rather similar when exposed to full solar radiation (i.e., $P > 0.05$) in the three phytoplankton assemblages (Fig. 5). There was a slight increase in carbon fixation when UV-B was excluded from the samples, but it was significantly higher ($P < 0.05$) only in Lake El Trébol. In the three lakes though, phytoplankton had a significant increase in carbon fixation ($P < 0.05$) when UV-A was additionally filtered out, with the highest values being also observed at Lake El Trébol. The PAR – only treatment also presented significant differences in carbon fixation among the three lakes, being assemblages from Lake Moreno those with the lowest photosynthetic rates (~ 4 μg C l^{-1} h^{-1}) whereas those from El Trébol had the highest values (~ 11 μg C l^{-1} h^{-1}). The mean irradiance received by the cells during the experiments was rather similar - 0.19 μE cm^{-2} s^{-1} for PAR, and 6.49, 30.9, 54.5 and 70.9 μW cm^{-2} nm^{-1} for 305, 320, 340 and 380 nm, respectively.
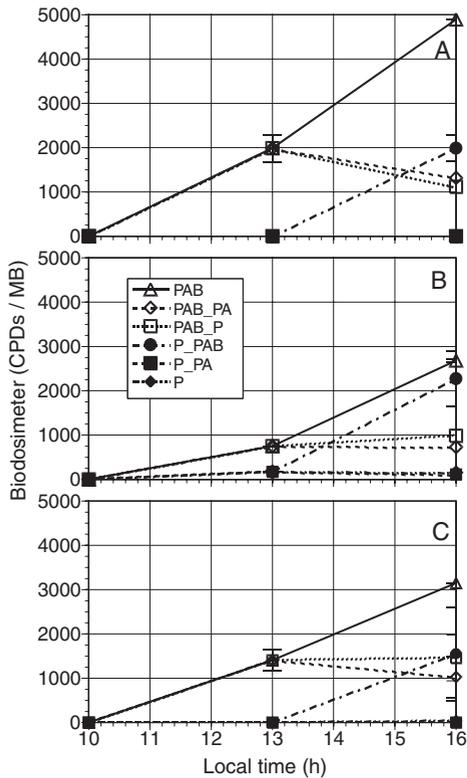
Figure 4: Accumulation of CPDs in biodosimeters incubated under various irradiance treatments (see Fig. 3). **A)** Lake Moreno; **B)** Lake Morenito and, **C)** Lake El Trébol. PAB indicates samples exposed to full solar radiation; PA indicates samples exposed to PAR + UV-A and P indicates samples exposed to only PAR. The symbols (⊤) indicate the standard deviation.
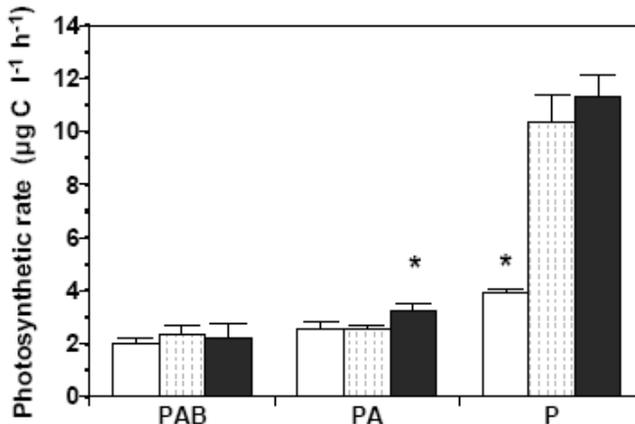
Figure 5: Photosynthetic rates as a function of different quality radiation treatments in phytoplankton assemblages from lakes Moreno (white bars), Morenito (hatched bars) and El Trébol (black bars). The asterisk on top of the bars indicates significant differences (P < 0.05).

Daily inhibition of photosynthesis in parallel to CPDs accumulation was followed in Lake Moreno assemblages (Fig. 6). As seen before, carbon incorporation was significantly higher (P < 0.05) in samples exposed only to PAR as compared to the treatment exposed to full radiation. There was a slight but significant increase in photosynthetic inhibition between these treatments, reaching a difference of 25 µg C / l (Fig. 6A). UV-B was responsible for

more than 65% of the total photosynthetic inhibition (i.e., difference between the values obtained in PAR+UVR and PAR+UV-A treatments divided the total inhibition) during afternoon hours. CPDs accumulation in the 0.2 µm size fraction also increased with time from the initial value of 50 CPDs / MB to ~ 200 CPDs / MB at the end of the experiment (Fig. 6B). In the 2-10 µm and > 10 µm cell size fractions CPDs accumulation was significantly lower (P < 0.05), but also slightly increased throughout the experiment, reaching values of 45 and 23 CPDs / MB (i.e., in the 2-10 µm and > 10 µm cell fractions, respectively). Finally, accumulation of CPDs in the biodosimeter was rather low during morning hours but increased steadily in the afternoon, reaching values of ~ 3000 CPDs / MB at the end of the experiment.
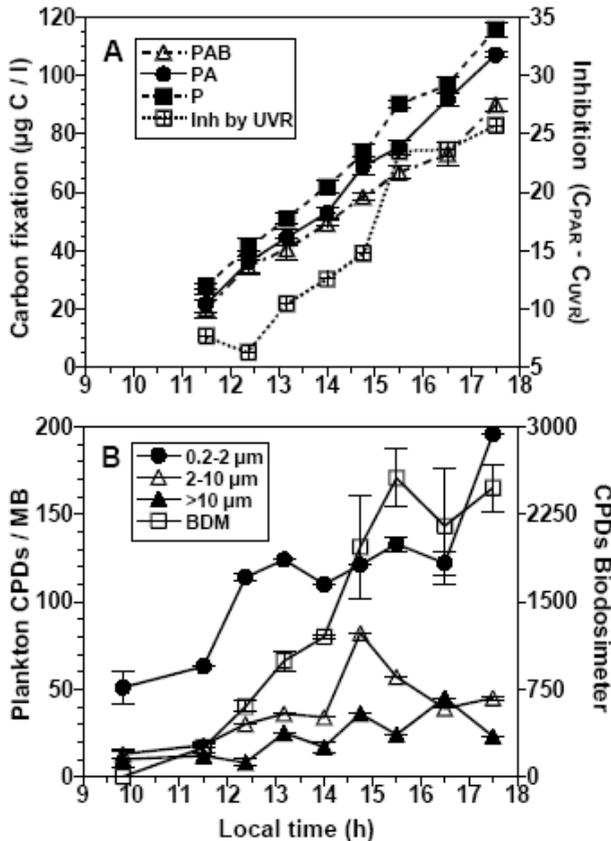
Figure 6: Daily course of UVR photosynthetic inhibition and CPDs accumulation for phytoplankton from Lake Moreno. A) Carbon fixation as a function of different radiation treatments and inhibition due to UVR. B) CPDs accumulation in three different phytoplankton size fractions and DNA effective dose, as measured with the biodosimeter (BDM). The symbols (⊤) indicate the standard deviation.

DISCUSSION

In this study we have shown that natural assemblages from temperate Andean lakes of Patagonia respond in different ways to solar UVR. We particularly focused on two of the most important effects of UVR upon phytoplankton organisms: photosynthetic inhibition (see review by Villafañe et al. 2003) and DNA damage (see review by Buma et al. 2003). So far, many studies have evaluated the role of UVR in inhibiting photosynthesis and damaging

the DNA molecule in various regions of the world, i.e., polar (Helbling et al. 1992b, Smith et al. 1992, Neale et al. 1998b, Buma et al. 2001a, Meador et al 2002), temperate (Helbling et al. 2001a, b, Buma et al. 2001b, Banaszak & Neale 2001), and tropical marine and freshwater environments (Kinzie III et al. 1998, Villafañe et al. 1999, Helbling et al. 2001c, 2003, Boelen et al. 2000, 2001, 2002).

We can summarize the overall effects of solar UVR upon phytoplankton of temperate lakes of Patagonia as follows: In terms of DNA damage (Fig. 3), organisms from Lake Morenito presented the highest CPDs accumulation rates (Fig. 3B) and MDR values, followed by those from Lake El Trébol (Fig 3C). When considering photosynthesis (Fig. 5A) these two lakes also presented high UV-A – induced inhibition (mean of 75 and 71 % for lakes Morenito and El Trébol, respectively) and much lower due to UV-B (2 and 9.5 % for lakes Morenito and El Trébol, respectively). Samples from Lake Moreno, on the other hand, had the lowest CPDs accumulation (Fig. 3A) and MDR values; photosynthetic inhibition was also low (35 and 15% for UV-A and UV-B, respectively) as compared to the other two lakes (Fig. 5). Many causes might account for these differential responses, such as the characteristics of the underwater radiation field, the type and effectiveness of the strategy used by the organisms to cope with solar UVR, and differences in the size structure and taxonomic composition of the community. Optical characteristics in the three lakes were different, and based on underwater radiation measurements (Fig. 1, Table 1) and k_{bd-eff} calculated from the biodosimeters (Fig. 2) we could clearly distinguish two types of environments: One was the 'clear' waters of Lake Moreno, and the other the 'opaque' waters of lakes Morenito and El Trébol. These two types of environments represent two extreme conditions for the area in terms of underwater radiation; however, other studies (e.g., Morris et al. 1995 and Laurion et al. 2000) have determined extreme k_{PAR} values of 5.21 and 0.08 m^{-1} in American lakes and in the tyrolian Alps, respectively. However, the differences in penetration of solar radiation in our study sites are large enough to allow a comparison of the effects of natural radiation upon phytoplankton assemblages exposed and acclimated to two extreme regimes. A major part of the variability of UVR transparency (i.e., k_{UV-B} from 0.36 to 2.8 m^{-1} in lakes Moreno and Morenito, respectively, Fig. 1, Table 1) seems to be related to variations in DOM, especially DOC compounds (e.g., fulvic acids, tannic acids and lignins) as determined in many studies in other parts of the world (Scully & Lean 1994, Morris et al. 1995, Laurion et al. 2000). Although we did not specifically address the variability in DOC concentrations in these lakes, previous studies in the area have determined DOC values ranging from 0.65 to 1.70 g / m^3 in lakes Moreno and El Trébol, respectively (Morris et al. 1995). Our irradiance data as well as the k_{bd-eff} values suggest that the cells in the 'opaque' lakes could be more protected than those in the 'clear' lake. One can argue however, that because of the lower water transparency, cells are exposed to a low mean irradiance and thus 'dark' adapted. This in turn would potentially result in high damage rates if cells are brought to the surface by mixing (Neale et al. 2003). In addition, a recent study (Helbling et al. 2003) has shown that the intensity of mixing (i.e., the turnover speed within the UML) was critical for the acclimation of cells, and phytoplankton photosynthesis could be either enhanced or reduced by solar UVR depending on the mixing rate. In our case, it was seen that phytoplankton from Lake Morenito was the most sensitive to solar radiation, having the highest CPDs accumulation even at lower DNA effective doses (Fig. 7). In the 'clear' Lake Moreno, phytoplankton had the lowest DNA damage even though they received the highest DNA effective doses.
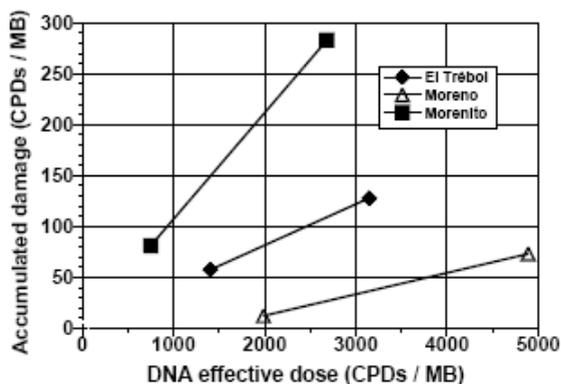
Figure 7: Dose response relationship between CPDs accumulation in the 2-10 µm phytoplankton size fraction as a function of the DNA effective dose for lakes Moreno, Morenito and El Trébol.

It is evident though, that although differences in the underwater radiation field may contribute to different responses from phytoplankton organisms to solar UVR, the optical characteristics would not solely be responsible for the observed responses. The taxonomic composition may account for part of the variability in responses as different assemblages were present in the lakes: small pennate diatoms characterized Lake El Trébol, whereas unidentified monads / flagellates dominated in lakes Morenito and Moreno. In fact, several studies have determined that under similar radiation conditions, a wide range of responses can be observed within different taxonomic groups (Vernet et al. 1994, Helbling et al. 1996a; Sommaruga & Buma 2000) but so far no generalizations can be made in regard to the particular sensitivity to UVR of each taxon. These differences in responses can be attributed not only to intrinsic factors of genetic origin, but also to the presence of photoprotective compounds (i.e., mycosporine like aminoacids – MAAs - or carotenoids) (Vernet et al. 1994, Helbling et al. 1996a) that might allow organisms to improve their overall fitness under UVR stress. For example Neale et al. (1998a) have determined a reduction of UVR – induced photosynthesis inhibition in a dinoflagellate strain, which was attributed to the presence of several MAAs (i.e., mycosporine-glycine, palythine, porphyra-334 and palythene). In our study we did not determine the presence of UV-absorbing compounds in natural phytoplankton assemblages, but the absorption characteristics (data not shown) did not indicate the presence of these compounds; future studies, however, should consider this aspect in greater detail.

We also considered the size structure of the community as potential source for the variability in the responses of phytoplankton to solar UVR. Several studies have demonstrated the size-dependence of UVR effects (Karentz et al. 1991a; Laurion & Vincent 1998; Helbling et al. 2001a, b) with small cells (i.e., high surface to volume ratio) being more resistant when addressing photosynthesis inhibition, but more vulnerable to DNA damage (Helbling et al. 2001b). On the other hand, large cells (providing that they do not have high concentrations of UV-absorbing compounds) are more sensitive to UVR when considering photosynthetic inhibition, but they are more resistant for DNA damage (Karentz et al. 1991a, Helbling et al. 1992b, 1994, Buma et al. 1997, Boelen et al. 2000, Helbling et al. 2001b). In all three lakes the smaller size fraction (0.2 – 2 µm, i.e. heterotrophic bacteria mainly) exhibited more rapid CPDs accumulation as compared with the larger size fractions (i.e. 2-10 µm and

> 10 µm, Fig. 6 and data not shown, for lakes Morenito and El Trébol). This corresponds with studies performed in several regions, where generally higher vulnerability for CPDs induction is found in bacteria as compared with larger, eukaryotic cells (reviewed in Buma et al. 2003). Moreover, a comparison of vulnerability for CPDs induction between these regions clearly showed a very low CPDs induction rate (i.e., normalized to incident DNA effective UV-B) for organisms from Andean lakes as compared with microorganisms from lower altitudes or latitudes. This indicates that physiological and / or ecological acclimation to the prevailing (high irradiance) UV-B regime may occur, but that nevertheless CPDs accumulation cannot be prevented (Buma et al., 2003). Image analyses as well as microscopic observations of our samples indicated that the phytoplankton communities of the three lakes were mainly characterized by small cells (< 20 µm in effective diameter), with a very slight difference in the proportion of large cells (i.e., microplankton) in Lake Moreno. Hence we do not think that these size structures of cells within the communities would account *per se* for the observed differential effects of solar UVR, especially because the differences are found in the 2-10 µm size fraction only (Fig. 3).

Previous studies have revealed the presence of two main mechanisms by which phytoplankton organisms can repair the UVR-induced DNA damage (Sancar & Sancar, 1988, Roy 2000, Banaszak 2003): a) Photoreactivation, which utilizes long UV-A and blue light energy and, b) Nucleotide excision repair, also known as dark repair, because does not require photoreactivating light. Of both mechanisms, photoreactivation seems to be far more common in phytoplankton (Karentz et al. 1991a, Buma et al. 2001a) than dark repair. Our data however, indicate that photoreactivation, if present, was not enough to cope with DNA damage as seen by the continuous increase in CPDs (Fig. 3). Also, there was null or slight decrease in CPDs during the afternoon in the samples were UV-B was filtered out, also suggesting low photoreactivation activity in these lakes. Even though we did not perform experiments to specifically test dark repair, there are some hints that might support the view that dark repair was important for these phytoplankton Andean communities, or at least in the clear Lake Moreno. The cells in our experiments were exposed to the maximum radiation conditions (i.e., surface radiation), but DNA damage in the clear Lake Moreno occurred in the upper 7 m of the water column (Fig. 2), with CPDs formation increasing significantly during the day (Figs 3A, 6B). Early morning CPDs determinations however, were significantly low probably due to dark repair occurring at night. One can not rule out, however, that part of the decrease could be accounted by a potential dilution of the DNA damage either by synthesis of *de novo* DNA or vertical mixing in the water column. In addition to differences in taxonomic characteristics, differences in temperature could account for part of the variability in responses between lakes. For example, studies have revealed the importance of temperature in determining the effectiveness of the photorepair mechanism (Rocco et al. 2002). Here we have found relatively large temperature differences, especially between Lake Morenito (i.e., 22 °C) and the other two lakes (i.e., ~ 15°C), which may result in the higher effectiveness in repair as determined in Lake Morenito.

In conclusion, this study shows that several factors account for the variability in responses of phytoplankton organisms of temperate lakes of the Andes region when exposed to solar radiation. Taxonomic composition, as well as different strategies of protection and repair between organisms from 'opaque' and 'clear' lakes might take place to mitigate UVR – induced damage to acclimate natural assemblages to solar radiation. Our study also highlighted the

importance of DOM in conditioning the underwater radiation field and thus DNA-damage in the phytoplankton assemblages. Even though phytoplankton cells might find 'protection' in 'opaque' waters, this would result in a disadvantage in water bodies exposed to windy conditions such as those in Patagonia, with an overall result of higher DNA damage as compared to 'clear' lakes.

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SUMMARY

Numerous studies carried out since the discovery of the ozone "hole" over the Antarctic continent have motivated photobiological research to determine the potential effects of increased solar ultraviolet radiation – UV-B (280-315 nm) on organisms and ecosystems. Since then, the amount of literature on UVR (280-400 nm) effects has increased a lot especially that concerned with the effects of UVR on photosynthesis of aquatic organisms. The studies have shown that UVR wavelengths – both UV-A (315-400 nm) and UV-B, even at their natural ambient levels, generally cause a reduction in photosynthetic rates, which in turn may reduce primary production of aquatic ecosystems. In fact, extensive research has been done in several aquatic ecosystems of the World, mainly in the Antarctic Ocean, to evaluate the overall impact of solar UVR on phytoplankton organisms. A recent review (Chapter 1) showed what was known about the effects of solar UVR on aquatic systems of the Patagonia area. This work, however, highlighted the fact that although considerable efforts have been put in determining UVR ground levels over the area, as well as the influence of ozone depleted air masses, relatively little research has been done to evaluate the biological effects of these short wavelengths on aquatic organisms.

The Patagonia area, on the southern tip of South America, presents important characteristics which have motivated the research done in this thesis: On one hand, Patagonia is located in close proximity to the Antarctic continent and occasionally receives enhanced UV-B, in addition to normal UVR and PAR. On the other hand, the Argentinean continental shelf area is very productive, as estimated by both remote sensing and scattered descriptive studies, and hence capable of sustaining a high standing stock of consumers, including fish and invertebrate species of commercial interest. Thus, if UVR affects phytoplankton production, it will ultimately affect higher trophic levels of the food web.

On the basis of this background, this thesis has evaluated some of the effects of UVR on aquatic primary productivity of Patagonian environments, for which three areas on the Atlantic coast – Bahía Engaño, Bahía Nueva and Bahía Camarones / Bahía Bustamante, and three lakes from the Andes were chosen as testing sites. UVR effects on phytoplankton primary productivity was assessed by different methodologies / approaches which are described in Chapter 2, including short- and long-term studies, and experiments under both *in situ* and simulated *in situ* conditions. It was determined that UVR does affect phytoplankton communities from Patagonia, as shown in a year-long study conducted at Bahía Engaño designed to evaluate the effects of UVR on primary productivity under variable climatology and species composition (Chapter 3). This study established three general conditions in phytoplankton dynamics throughout the year, with pre-bloom (i.e., late summer-fall), winter-early spring bloom, and post-bloom conditions during late spring-summer. Small-sized cells characterized the pre- and post-bloom communities, which had a relatively high photosynthetic inhibition. During the bloom, which was characterized by microplankton diatoms, photosynthetic inhibition was low. When photosynthetic inhibition was estimated through biological weighting functions this community however, was shown to be more sensitive to UVR. This chapter also highlighted the importance of wind, which conditioned the development of the winter bloom in this area.

Once the seasonal trends of UVR effects were determined (Chapter 3), detailed experimentation was performed to analyze the UVR effects on different parameters and / or processes. Experiments conducted at Bahía Nueva and Bahía Camarones showed that photosynthesis versus irradiance relationships (P vs. E) were variable throughout the study period (Chapter 4). P_{max} displayed a

trend of high values during late summer to late fall (i.e., pre-bloom) and relatively low ones during late spring to early summer (i.e., post-bloom); E_k, on the other hand, did not show such a clear pattern. UVR significantly reduced P_{max} values during the pre-bloom but not during the post-bloom period, and it significantly affected E_k only in some experiments. The temporal variability of P vs. E parameters seems to be greatly influenced by the nutrient status of cells and taxonomic composition, this latter being in turn associated with stratification conditions (e.g., wind speed and duration).

As available literature reports the ability of phytoplankton cells to minimize the overall negative impact of UVR, experiments with waters collected at Bahía Engaño, Bahía Nueva and Bahía Camarones were done to compare the long-term acclimation of natural phytoplankton communities to solar radiation (Chapter 5). These experiments were also designed to evaluate the combined effects of UVR and nutrient addition on phytoplankton growth and species composition. On one hand, it was shown that growth rates were affected by nutrient addition, demonstrating the nutrient-limited conditions of summer (i.e., post-bloom) phytoplankton communities of Patagonian waters. The effects of UVR varied between assemblages, but there was a trend of a shift in the diatom community structure towards the dominance of more resistant species that were, however, different among the three tested assemblages.

Since the DNA molecule is another cellular target that is affected by UVR, studies were performed to jointly assess UVR-induced DNA damage and photosynthesis inhibition (Chapter 6). On one hand, *in situ* studies carried out with marine phytoplankton communities showed that UVR significantly affected both the DNA molecule and photosynthesis at the surface, but these negative effects were rapidly reduced in the water column. This study showed that the impact of cell size on UV vulnerability is strongly determined by the parameter under study: While small cells seem to be very sensitive to DNA damage, large cells are more sensitive to photosynthesis inhibition.

Finally, the study carried out in Andean lakes (Chapter 7) with differential dissolved organic matter (DOM) content demonstrated the variable responses of phytoplankton assemblages to UVR in regard to DNA damage and photosynthesis inhibition. This study reached to the conclusion that differences in the light history of cells are very important so that, in general, assemblages from "opaque" lakes are more sensitive (i.e., particularly in regard to DNA damage) than those from "clear" lakes. This study also highlighted that "shelter" through large amounts of DOM (i.e., implying less penetration of solar radiation) is not in all cases beneficial due to the already high sensitivity of phytoplankton species from "opaque" lakes.

Thus it is concluded from this thesis that natural phytoplankton assemblages from Patagonia display assorted responses to UVR exposure, which depend on a number of factors such as the underwater radiation field, taxonomic composition and size structure, nutrients availability and previous light history. This thesis, far for being exhaustive in regard to the evaluation of the overall UVR impact on phytoplankton from Patagonia waters, opens other key questions related to the importance of winds and depth of the upper mixed layer in conditioning the turnover time of phytoplankton cells in this area, and how this in turn would affect the production of Patagonian waters. Also, as P vs. E parameters are affected by UVR, models and retrieving algorithms for remote sensing should consider this effect (at least for the Patagonia area) to obtain better estimates of carbon fluxes.

SAMENVATTING

De ontdekking van het ozongat boven het Antarctisch continent heeft veel fotobiologisch onderzoek gestimuleerd naar de mogelijke effecten van toenemende UV-B (280-315 nm) straling op organismen en ecosystemen. Sindsdien is de hoeveelheid literatuur over UVR (280 – 400 nm) effecten substantieel toegenomen, in het bijzonder die studies die zich bezighouden met de effecten van UVR op de fotosynthese van aquatische organismen. Deze studies laten zien dat golflengten in het UVR gebied –zowel UV-A (315-400 nm) als UV-B-, zelfs bij natuurlijke niveau's in het algemeen een reduktie veroorzaken in de fotosynthesesnelheid, die op zijn beurt de primaire productie van aquatische ecosystemen zou kunnen verlagen. Bepaalde delen van de wereld maar met name de Antarctische oceaan, zijn zeer intensief bezocht, om het netto effect van natuurlijke UV-straling op fytoplankton organismen te onderzoeken (Hoofdstuk 2). Een recent overzichtsartikel (Hoofdstuk 1) laat zien wat er bekend is over de effecten van natuurlijk UVR op aquatische systemen in Patagonie. Dit hoofdstuk benadrukt echter, dat, ook al zijn er in Patagonie een aantal substantiele inspanningen gepleegd met betrekking tot UVR metingen op zeeniveau, vaak in relatie tot verlaagde ozonconcentraties, er tot nog toe weinig onderzoek was gedaan naar de biologische effecten van deze componenten uit het zonlicht op aquatische organismen.

Patagonie, de zuidelijkste regio van Zuid-Amerika, wordt gekenmerkt door een aantal belangrijke fenomenen die het hier gepresenteerde onderzoek zeer interessant maken. Allereerst ligt Patagonie relatief dicht bij het Antarctische continent waardoor het zo nu en dan wordt blootgesteld aan verhoogde UV-B niveau's, bovenop de natuurlijke UVR en PAR. Ten tweede is berekend uit remote sensing en incidentele beschrijvende onderzoeken dat de Argentijnse continentale shelf zeer productief is en hierdoor in staat is een hoge biomassa van consumenten, inclusief vis en invertebraten te onderhouden, die al dan niet ook commercieel interessant zijn. Met andere woorden, wanneer UVR de primaire productie negatief zou beinvloeden, zou het uiteindelijk effect ook doorwerken op de hogere trofische niveaus van het voedselweb.

Op basis van de beschikbare achtergrondinformatie heeft het hier gepresenteerde promotieonderzoek een evaluatie gemaakt van de effecten van UVR op de aquatische primaire productie van Patagonische systemen, waarvoor drie onderzoekslocaties zijn geselecteerd aan de Atlantische kust – Bahía Engaño, Bahía Nueva and Bahía Camarones / Bahía Bustamante. Ook werden drie meren in het Andes-gebergte onderzocht. UVR effecten op primaire produktie werd onderzocht met verschillende methoden/benaderingen (beschreven in Hoofdstuk 2) inclusief korte- en lange-termijn studies, onder *in situ* en gesimuleerde *in situ* omstandigheden.

Allereerst heeft het onderzoek aangetoond dat UVR de fytoplankton gemeenschappen van Patagnie negatief beinvloedt, zoals dit naar voren kwam tijdens een lange-termijn studie (1 jaar), uitgevoerd in Bahía Engaño. Deze jaar-serie was ontworpen om de verschillende effecten van UVR onder variabele omstandigheden van UVR en soortensamenstelling te ontrafelen (Hoofdstuk 3). Het onderzoek onderscheidde drie algemene condities in fytoplankton dynamiek door het jaar heen, te weten pre-bloei condities (einde zomer-najaar), bloei condities (winter/vroege voorjaar), en tenslotte post-bloei condities tijdens het late voorjaar en de zomer. Kleine cellen karakteriseerden de pre- en post-bloei gemeenschappen, die een relatief hoge fotosynthese-inhibitie tentoonspreidden. Tijdens de bloeiperiode, die werd

gekarakteriseerd door microplanktonische diatomeeën, was de fotoinhibitie laag. Deze gemeenschap was echter gevoeliger voor UVR, wanneer inhibitie werd berekend met behulp van weegfuncties. Het onderzoek benadrukte ook het belang van de wind, die in dit gebied sterk conditionerend werkt op de ontwikkeling van de winterbloei.

Nadat de seizoenstrends waren onderzocht (Hoofdstuk 3), werden gedetailleerde experimenten gedaan om de effecten van UVR op verschillende parameters en processen te onderzoeken. Experimenten uitgevoerd in Bahía Nueva en Bahía Camarones lieten zien dat fotosynthese versus irradiantie relaties (P vs. E) gedurende de onderzoeksperiode zeer variabel waren (Hoofdstuk 4). P_{max} liet een trend zien met hoge waarden in de late zomer tot in de herfst (i.e., pre-bloei), en relatief lage waarden tijdens de laat-voorjaar en vroege zomer perioden (i.e., post-bloei). Echter, E_k liet niet een duidelijke trend zien. UVR blootstelling zorgde voor significante afnames in P_{max} waarden tijdens de pre-bloei periode maar niet tijdens de post-bloei periode, en UVR had alleen incidenteel een significant effect op E_k. De seizoensafhankelijke invloeden op P vs. E parameters lijkt voor een groot deel beinvloed door de nutrienten-status van de cellen alsmede de taxonomische samenstelling van de gemeenschappen, waarvan de laatste weer kan worden geassocieerd met condities van stratificatie (e.g. windsnelheid/duur).

Om de kapaciteit van fytoplankton cellen om negatieve gevolgen van UVR te kunnen minimaliseren te onderzoeken, werden experimenten uitgevoerd met zeewater, verzameld in Bahía Engaño, Bahía Nueva en Bahía Camarones. Het onderzoek was gericht op een vergelijking van lange-termijn aanpassing van verschillende natuurlijke fytoplankton gemeenschappen aan zonlicht (Hoofdstuk 5). Bovendien waren deze experimenten ontworpen om de gecombineerde effecten van UVR en nutrienten-toevoeging op fytoplankton-groei en –samenstelling te kunnen onderzoeken. De resultaten gaven allereerst aan dat de groeisnelheden positief werden beinvloed door nutrienten-additie, waarmee omstandigheden van nutrienten-limitatie (i.e., post-bloei) kon worden aangetoond in fytoplanktongemeenschappen in Patagonia. De effecten van UVR varieerden tussen gemeenschappen, maar in het algemeen was er een trend zichtbaar van een verschuiving in diatomeeen samenstelling ten behoeve van meer resistente soorten die echter verschillend was tussen de drie geteste locaties.

Omdat het DNA molecuul een andere essentiele target is van UVR, werden verschillende onderzoeken uitgevoerd, om gelijktijdig het effect van UVR geinduceerde DNA schade en fotosynthese-inhibitie te onderzoeken (Hoofdstuk 6). De resultaten gaven aan dat bij *in situ* studies met mariene fytoplanktongemeenschappen UVR een significant effect had op zowel het DNA als op de fotosynthese in het oppervlak, maar dat deze negatieve effecten snel uitdoofden met de diepte in de waterkolom. Dit onderzoek toonde aan dat het effect van celgrootte op UV gevoeligheid zeer sterk wordt bepaald door de onderzochte parameter: terwijl kleine cellen zeer gevoelig lijken voor DNA schade, zijn grote cellen juist gevoeliger voor UVR-gerelateerde inhibitie van de fotosynthese.

Tenslotte toonden fytoplanktongemeenschappen uit drie Andes-meertjes met een variabele concentratie opgelost organisch materiaal (DOM) een zeer uiteenlopende UVR-respons, met betrekking tot DNA schade en remming van de fotosynthese. Dit onderzoek leidde tot de conclusie dat verschillen in de licht-historie van de cellen van groot belang zijn, omdat in het algemeen gemeenschappen uit "UV ondoorlatende" meren UVR-gevoeliger waren (met name wat betreft DNA schade) dan die van "UV doorlatende" meren (Hoofdstuk 7). Het

onderzoek toonde met name aan dat de "bescherming" door grote hoeveelheden DOM (als implicatie voor een snelle uitdoving van zonlicht) niet altijd even gunstig is, omdat hierdoor de UVR gevoeligheid van organismen erg wordt versterkt.

De conclusie van het hier gepresenteerde promotieonderzoek is dat natuurlijke fytoplanktongemeenschappen van Patagonie een zeer gevarieerde UVR respons laten zien, die afhangt van een veelheid aan factoren, zoals het onderwater lichtveld, soortensamenstelling en gemiddelde celgrootte, nutrienten-beschikbaarheid een de licht-historie van de organismen. Dit promotieonderzoek is verre van kompleet met betrekking tot het geven van een overall beeld van UVR effekten op fytoplankton uit Patagonie. Het opent nieuwe sleutelvragen gerelateerd aan het belang van wind-mixing en de diepte van de gemengde laag in het conditioneren van de turn-over tijd van fytoplankton cellen en hoe dit de productie van Patagonische wateren zou kunnen beinvloeden. Tenslotte, omdat P versus E parameters duidelijk door UVR werden beinvloed, zullen modellen en het afleiden van algoritmes voor remote sensing (tenminste geldend voor de regio Patagonie) in de toekomst rekening moeten houden met UVR effekten, om hiermee een betere schatting van koolstof-fluxen te kunnen maken.

ACKNOWLEDGMENTS

There are many people that I would like to thank for helping me in many ways to do this work. First of all, I wish to thank Anita Buma and Hein de Baar, who helped me in every single way to present this thesis at RuG. The possibility of working with Anita opened a completely new UV world for me – thanks for the nice chats, ideas as well as for the good times that we spent together in Groningen and in Playa Unión.

For my husband Walter and our son Tomás – I simply do not have words to thank you both. You gave me much love, constant support and help of all kinds throughout this fascinating (and long) road. My gratitude also goes to other members of my family, especially to my parents, which are always at my side…

It was a pleasure to work at the EFPU with Elena Barbieri, Rodrigo Gonçalves and Alejandra Marcoval. We have shared great conversations and asados, and why not? Also work!! After all, we spent a lot of time working with data and fighting with computers. Thanks also for the good times spent together when we were away from home - in Bariloche, Germany and in China. My special thanks go to Niní Albarracín for her never ending work in maintaining our phytoplankton cultures, and to Cristian Barrio for his excellent devices and set ups for experiments. A special gratitude also goes to Leonardo Taibo, who set up our computers and helped out with drawings at the right time. I also want to thank the members of Fundación Playa Unión who always supported my work; especially to Carlos Malbárez, also a dear family friend.

During all this time, I also had the opportunity to work and interact with very nice people who enriched my views and allowed me to participate in joint research activities at their labs or here in Patagonia; much of our fruitful discussions were then converted into papers. My special thanks are due to Franceso Zaratti (Universidad Mayor de San Andrés, Bolivia), Felix L. Figueroa (Universidad de Málaga, Spain), Donat Häder (Friedrich Alexander Universität, Germany), Horacio Zagarese (INTECH, Argentina), Kunshan Gao (Shantou University, People's Republic of China) and M. Eugenia Farías (PROIMI, Argentina). Special thanks are also due to Kristina Sundbäck (Göteborg University, Sweden) and Peter Boelen (Univesity of Groningen) for their valuable contribution to the work presented in this thesis. I would also like to thank Marcos Andrade and Valeria Lairana from Bolivia, Karin de Boer from the Netherlands, Nathalie Korbee and Roberto Abdala from Spain, Hongyan Wu (Nancy) and Wanchun Guan (Spring) from China, and Michael Lebert and Peter Richter from Germany: Field work in Lake Titicaca, Patagonia and Nan'Ao was especially enjoyable thanks to them.

This thesis was carried out with the support of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina) and Agencia Nacional de Promoción Científica y Tecnológica, The Third World Academy of Sciences and Fundación Antorchas.

Thanks to all!!! Muchísimas gracias a todos!!!!