



ELSEVIER

## Patterns of DNA damage and photoinhibition in temperate South-Atlantic picophytoplankton exposed to solar ultraviolet radiation

Anita G.J. Buma<sup>a,\*</sup>, E. Walter Helbling<sup>b,c</sup>, M. Karin de Boer<sup>a</sup>, Virginia E. Villafañe<sup>b,c</sup>

<sup>a</sup>Department of Marine Biology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

<sup>b</sup>Estación de Fotobiología Playa Unión, Casilla de Correos N° 153, (9100) Trelew, Chubut, Argentina

<sup>c</sup>Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Casilla de Correos N° 153, (9100) Trelew, Chubut, Argentina

Received 26 December 2000; accepted 7 June 2001

### Abstract

Natural marine phytoplankton assemblages from Bahía Bustamante (Chubut, Argentina, 45°S, 66.5°W), mainly consisting of cells in the picoplankton size range (0.2–2 µm), were exposed to various UVBR (280–315 nm) and UVAR (315–400 nm) regimes in order to follow wavelength-dependent patterns of cyclobutane pyrimidine dimer (CPD) induction and repair. Simultaneously, UVR induced photosynthetic inhibition was studied in radiocarbon incorporation experiments. Biological weighting functions (BWFs) for photoinhibition and for CPD induction, the latter measured in bare calf thymus DNA, differed in the UVAR region: carbon incorporation was reduced markedly due to UVAR, whereas no measurable UVAR effect was found on CPD formation. In contrast, BWFs for inhibition of photosynthesis and CPD accumulation were fairly similar in the UVBR region, especially above 300 nm. Incubation of phytoplankton under full solar radiation caused rapid CPD accumulation over the day, giving maximum damage levels exceeding 500 CPD·MB<sup>-1</sup> at the end of the afternoon. A clear daily pattern of CPD accumulation was found, in keeping with the DNA effective dose measured by a DNA dosimeter. In contrast, UVBR induced photosynthetic inhibition was not dose related and remained nearly constant during the day. Screening of UVBR or UVR did not cause significant CPD removal, indicating that photoreactivation either by PAR or UVAR was of minor importance in these organisms. High CPD levels were found in situ early in the morning, which remained unaffected notwithstanding treatments favoring photorepair. These results imply that a proportion of cells had been killed by UVBR exposure prior to the treatments. Our data suggest that the limited potential for photoreactivation in picophytoplankton assemblages from the southern Atlantic Ocean causes high CPD accumulation as a result of UVBR exposure. © 2001 Elsevier Science B.V. All rights reserved.

**Keywords:** UVR; Phytoplankton; DNA; CPD; Photosynthetic inhibition; Mid-Latitudes

### 1. Introduction

Natural levels of ultraviolet radiation (UVR: 280–400 nm) reduce marine primary productivity in the Antarctic [1–7] as well as in temperate [8,9] and tropical regions [8,10,11]. Therefore, there is increasing awareness that UVR is a strong environmental factor affecting productivity and carbon cycling in most marine systems.

Both UVAR (315–400 nm) and UVBR (280–315 nm) reduce carbon incorporation rates in marine phytoplankton assemblages. It has been shown for Antarctic ice algae that PSII efficiency [12,13] or the RUBISCO pool [14] may be modified by UVR. A reduction in the performance of these targets will decrease the ability of a cell to photosynthes-

ize, thereby hindering the carboxylation process. Another cellular target of UVR is DNA, with UVBR being responsible for the formation of a typical form of DNA damage — cyclobutane pyrimidine dimers (CPDs) — [15–18] such as TT, CC and TC dimers. CPDs may hinder cell cycle progress and replication inhibition, because they obstruct de novo synthesis of cellular components and substances required for growth and cell maintenance. As a consequence, population growth is reduced. However, there are several pathways by which DNA damage can be repaired, one of which is photoreactivation. This type of repair is known to be controlled by wavelengths between 330 and 450 nm [19]. Photoreactivation has been demonstrated to occur in marine viruses [20,21], whereas additional dark repair was found in marine bacteria [22,23].

Any time the damage induction rate exceeds the damage removal rate, damage will accumulate. Over the past years much information has become available on UVBR related

\*Corresponding author. Tel.: +31-50-363-2393; fax: +31-50-363-2251.

E-mail address: a.g.j.buma@biol.rug.nl (A.G.J. Buma).

DNA damage accumulation in marine organisms. The presence of CPDs has been demonstrated in situ in tropical marine bacteria [22–25], marine tropical picophytoplankton [23,27], viruses [27], and fish eggs and larvae [28]. In the Antarctic, CPDs are shown to be induced in situ in ice algae and phytoplankton [6], in several developmental stages of ice fish [29] and in phytoplankton assemblages [30]. Furthermore, noontime CPD levels in the native Patagonian plant *Gunnera magellanica* showed a high correlation with ozone hole related increases in biologically effective UVR [31]. Finally, diel DNA damage patterns showed clear CPD accumulation in surface (sub) tropical planktonic organisms over the day [22,24,27,32,33], indicating that repair mechanisms are not sufficient to undo the damage during UVBR exposure hours.

There are a few studies only that have addressed the impact of solar UVR upon phytoplanktonic species of temperate systems [8,9]. Recently, one study was conducted in the Bahía Bustamante (Chubut, Argentina) area, demonstrating both photosynthetic inhibition and DNA damage in the water column due to UVR [34]. The coastal areas of Patagonia are nutrient rich with high primary productivity rates. Furthermore, these areas are character-

ized by high daily doses of solar radiation during the austral spring and summer and occasional elevated UVBR levels, due to their proximity to the Antarctic polar vortex and the ozone 'hole' [35].

In this paper the dynamics of CPD accumulation and repair in (pico) phytoplankton assemblages from Bahía Bustamante, Chubut, Argentina were studied as a function of wavelength and dose/dose rate. To this end phytoplankton assemblages were exposed to various wavelength bands of the solar spectrum, after which CPD accumulation and wavelength dependent removal were followed. Simultaneously, wavelength effects on photosynthetic inhibition were followed by  $^{14}\text{C}$  spiking experiments.

## 2. Materials and methods

### 2.1. Experimental set-up

Experiments were conducted between January 11 and January 18, 1999 in the Argentinean Sea at Bahía Bustamante, Chubut (45°S, 66.5°W), Argentina (Fig. 1). Surface water samples were collected with a clean bucket,

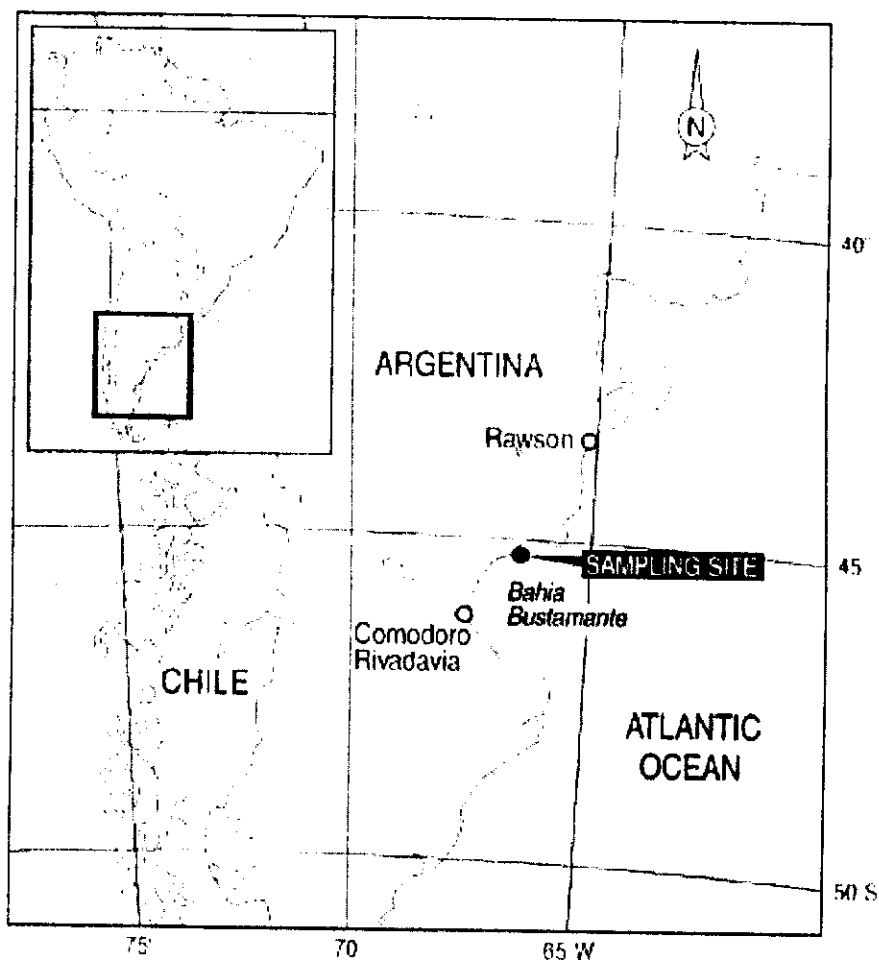


Fig. 1. Map of the Chubut Province, Argentina, indicating the sampling site at Bahía Bustamante. Inset: Relative location of Chubut in South America.

daily between 8 and 9.30 h and transported immediately to shore for incubation purposes. Samples were transferred to 50-ml quartz tubes (for carbon incorporation measurements, see Analyses, Section 2.2) as well as to 10-l polypropylene bags (for DNA damage), which have a high UVR transmission (Fig. 2A). Subsequently, the bags and tubes were exposed to solar radiation in large (about 10 000 l), temperature controlled (running surface seawater — 16–17°C) containers. Bags were placed just below the water surface. Sub-samples were processed for the determination of initial CPD levels, pigment concentration using fluorimetry and phytoplankton composition using classical microscopy. Three types of experiments were conducted:

(a) Quartz tubes were exposed to six different radiation treatments: four sets of tubes were covered with Schott filters (WG 295, WG 305, WG 320, WG 360), one set was covered with Plexiglas UF-3 (cut-off at 400 nm) (Fig. 2B), whereas one set of tubes was without filters thus receiving full solar radiation. The quartz tubes (four in each treat-

ment) were inoculated with labeled radiocarbon (see Analyses, Section 2.2) and incubated for 6–8 h centered around local noon. Simultaneously, small (2 ml) acid cleaned quartz tubes filled with calf thymus DNA in TE buffer ( $10 \mu\text{g l}^{-1}$ ) — DNA dosimeter — [36] were incubated in duplicate under the Schott filters for the entire incubation period. Eight independent experiments were conducted using sharp-cut off filters (Schott) and biological weighting functions (BWF) were calculated using an exposure response curve based on irradiation and a BWF-PI model [37,38]. The spectral dependence of the BWFs in the broadband intervals were extracted using the method of Rundel [39]. An exponential decay function (base 10) was used for each experiment. The exponent of the function was expressed as a third degree polynomial and fitted by iteration (the smaller  $R^2$  obtained was 0.95). There was no significant difference among the calculated BWFs so mean BWFs for the Bahía Bustamante data (photoinhibition, DNA damage in biosimulator DNA) were calculated.

(b) In the second type of experiment, CPD accumulation and repair was studied in several phytoplankton size fractions under various wavelength conditions. For each experiment, seven bags were incubated: two bags were incubated under full solar radiation and harvested around noon or at the end of the afternoon. Similarly, two bags were incubated under UV opaque PMMA (Fig. 2A), for the morning or whole day period. Furthermore, two bags were incubated under full solar radiation during morning hours, after which the bags were covered by either UV opaque PMMA or 3-mm glass plates to remove total UVR or UVBR, respectively (Fig. 2A). This was done to follow afternoon repair of the damage which had been accumulated during the morning period. Finally, the seventh bag was incubated under UVR opaque PMMA during morning hours, after which UVR was admitted during afternoon hours by replacing the PMMA screen by a glass screen. Each bag was accompanied by two DNA dosimeter tubes in order to allow for DNA effective dose assessment during the experiments.

(c) In the third experiment, the daily course of CPD accumulation in two size fractions of phytoplankton was followed under full solar radiation as a function of the dose received. A total of seven bags were placed in the temperature controlled water bath early in the morning, after which they were removed one after the other (at equal PAR doses of  $1 \text{ MJ m}^{-2}$  as estimated by cumulative ELDONET broad band PAR readings (see below) and processed (see Analyses, Section 2.2). In addition, duplicate DNA dosimeter tubes were incubated next to each bag and removed accordingly. Furthermore, carbon incorporation experiments were done to study UVBR inhibition of photosynthesis in the natural phytoplankton population during the day. Two radiation treatments were implemented: quartz tubes were either exposed to full solar radiation or covered with Mylar-D film to screen off

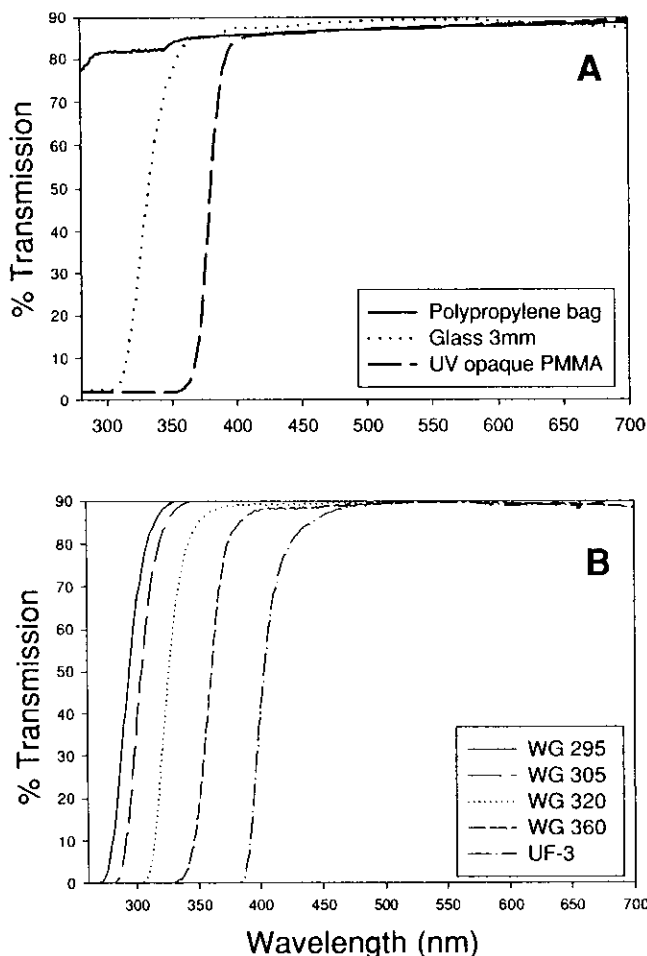


Fig. 2. Transmission characteristics of the materials used for the incubations. (A) Polypropylene bags, glass 3 mm and UV opaque polymethylmethacrylate (PMMA); (B) Schott filters (WG 295, WG 305, WG 320, WG 360) and the UF-3 filter.

UVBR. At equal PAR doses ( $1 \text{ MJ m}^{-2}$ ) four tubes (duplicates for each treatment) were removed during morning and afternoon hours.

During the whole period of experimentation incident solar radiation was recorded continuously using a broad band ELDONET radiometer (Real Time Computers Inc.), measuring UVBR (280–315 nm), UVAR (315–400 nm) and PAR (400–700 nm) at a frequency of one reading per minute. Total ozone column concentration was obtained from satellite data (NASA, Goddard Space Flight Center, <http://jwocky.gsfc.nasa.gov>)

## 2.2. Analyses

For the analysis of DNA damage, samples were harvested and size fractionated over 10-, 2- and 0.2- $\mu\text{m}$  polycarbonate membrane filters (Poretics, 47 mm) and immediately frozen in liquid nitrogen ( $-180^\circ\text{C}$ ) until analysis at the University of Groningen, The Netherlands. The DNA dosimeter samples were harvested and frozen ( $-20^\circ\text{C}$ ) until damage analysis. DNA was extracted from the filters using the same procedure as described in Buma et al. [30] which is a method modified from Doyle and Doyle [40]. To remove the RNA, the extracts were incubated for 1 h with  $75 \mu\text{g ml}^{-1}$  RNase (Boehringer Mannheim) at room temperature. The DNA concentrations of the extracts were 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. [36] employing a primary antibody (H3, Affitech, Oslo, Norway) 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  $\mu\text{m}$ ). The membranes were baked at  $80^\circ\text{C}$  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,  $4^\circ\text{C}$ ). After repeated washing, incubation with the secondary antibody (HRP rabbit-anti-mouse, Dako P0260) was done for 2 h 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 CPD's [36].

For photosynthesis measurements quartz tubes were inoculated with  $5 \mu\text{Ci}$  (0.185 MBq) of labeled sodium bicarbonate [41]. After the incubation period, the samples were filtered onto Whatman GF/F filters (25 mm in diameter), placed in 7-ml scintillation vials and then exposed to HCl fumes overnight. After drying the filters, scintillation cocktail (Wallac Optiphase HiSafe 3) was

added, and the activity measured using a liquid scintillation counter [42].

Pigments were measured by fluorimetry; 100 ml of sample was filtered onto a Whatman GF/F filter (25 mm in diameter) after which the photosynthetic pigments were extracted in absolute methanol during 1 h [43]. Chl-*a* concentration was then calculated from the fluorescence of the extract before and after acidification with 1 N HCl [44], using a Turner Designs fluorimeter (model TD 700), which was calibrated using pure chlorophyll *a* from *Anacystis nidulans* (Sigma C 6144).

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 [45]. In order to further investigate the taxonomic composition of the picophytoplankton community, HPLC analysis of taxon-specific pigments was done on acetone extracts (100%) of GF/F filtered material, after which pigments were analysed following Kraay et al. [46].

## 3. Results

With the exception of January 11, irradiation conditions varied very little during the research period, with clear skies and relatively high daily doses of solar radiation (Fig. 3A, B). January 11 was cloudy, resulting in a 50% reduction of daily doses of UVBR, UVAR and PAR. Daily doses on January 14 were the highest of the study period, with the UVBR dose exceeding  $40 \text{ kJ m}^{-2}$  (Fig. 3A). January 12 and 18 gave intermediate daily doses of the three major wavelength bands. No direct relationship between ozone levels and UVBR dose was found (Fig. 3A). The mean daily doses during the study period were 35.6, 1707, and  $13\,317 \text{ kJ m}^{-2}$ , for UVBR, UVAR, and PAR, respectively.

Over 90% of the DNA was found in the 0.2–2- $\mu\text{m}$  fraction (data not shown) indicating that the phytoplankton was dominated by cells in the picoplankton size range. Microscopic observations supported this finding: the plankton was dominated by very small cells, but in a few cases also diatoms were found (January 11 and January 18) with the main species being *Pseudonitzschia* spp., *Skeletonema costatum*, and *Licmophora* sp. Occasional pigment fingerprinting applying HPLC revealed high levels of chlorophyll-*b*, hinting to the presence of very small green eukaryotes or prochlorophytes in the 0.2–2- $\mu\text{m}$  size range. On January 11 and January 18 elevated levels of fucoxanthin were measured, confirming the presence of diatoms on these days (results not shown).

The mean biological weighting function for photosynthetic inhibition (Fig. 4A) showed a steep decline in sensitivity with increasing wavelength from the UVBR

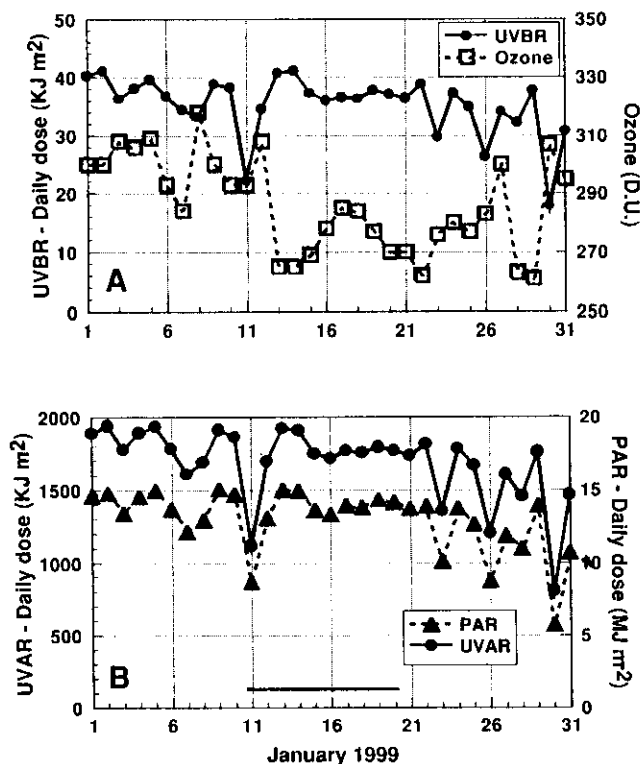


Fig. 3. Irradiation conditions during January 1999 at the study area. (A) Daily doses of UVBR ( $\text{kJ m}^{-2}$ ), and total ozone column concentrations (Dobson units). (B) Daily doses of PAR ( $\text{MJ m}^{-2}$ ) and UVAR ( $\text{kJ m}^{-2}$ ). The black line represents the research period (January 11 to January 18).

into the UVAR region of the spectrum, although inhibition in the UVAR region was also detectable. A comparison with a BWF for a temperate diatom (*Phaeodactylum* sp.) [37] indicated that the BWF for the Bahía Bustamante plankton had a slightly higher response in the UVBR region, but a much lower sensitivity in the UVAR region. The BWF for bare DNA (Fig. 4B) displayed a very strong response in the UVBR region especially when compared with the BWFs derived from the data of Quate et al. [47] and Setlow [48]. In contrast, the BWF for bare DNA showed a relatively good agreement with the Bahía Bustamante phytoplankton response for carbon incorporation (Fig. 4B), especially when considering the wavelengths above 300 nm.

A rapid accumulation of DNA damage was found when phytoplankton was incubated under full solar radiation during the day (Fig. 5A–C). Damage accumulation was the highest on January 14 (Fig. 5C), the clearest day during our experimentation, whereas on a cloudy day (January 11, Fig. 5A, B), accumulation occurred at a lower rate. Furthermore, maximum afternoon CPD levels were lower in the  $>10\text{-}\mu\text{m}$  fraction (Fig. 5A) than in the  $0.2\text{--}2\text{-}\mu\text{m}$  fraction (Fig. 5B). Early morning CPD levels were very variable, but the  $0.2\text{--}2\text{-}\mu\text{m}$  fraction typically contained higher initial CPD levels ( $120.7 \pm 93.1$ ) as compared with the  $10\text{-}\mu\text{m}$  fraction ( $49.5 \pm 33.3$ ). Repair

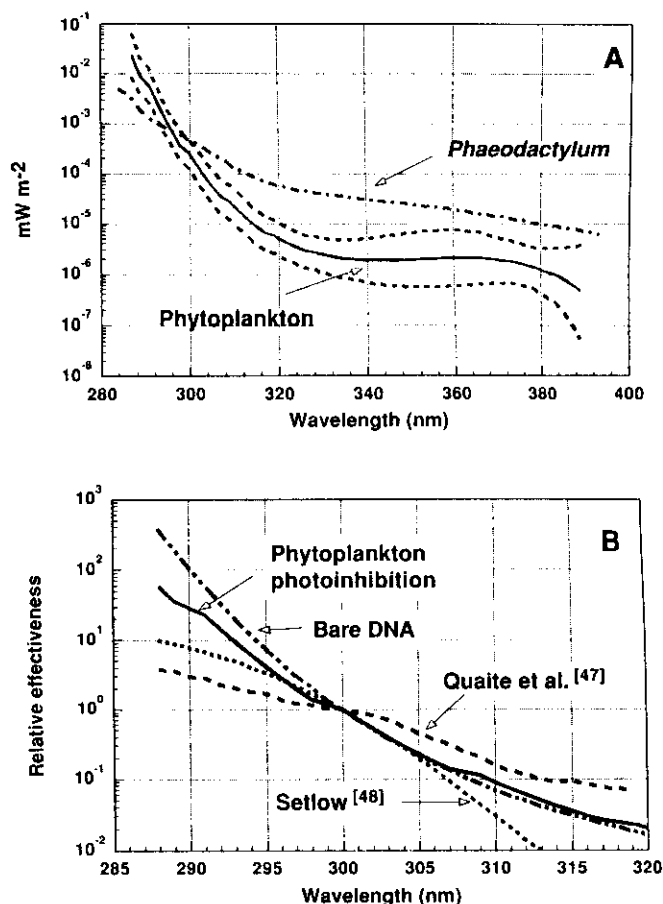


Fig. 4. Mean biological weighting functions (BWFs). (A) Photosynthetic inhibition in phytoplankton from Bahía Bustamante (Phytoplankton); (B) CPD accumulation in bare DNA exposed to sunlight in the Bahía Bustamante area. The BWFs for photoinhibition of photosynthesis in the temperate diatom *Phaeodactylum* sp. [37] and DNA damage according to Setlow [48] and Quate et al. [47] are included for comparison. The dotted lines in (A) are the 95% confidence limits. All BWFs in (B), including the BWF for photosynthetic inhibition from panel (A), have been normalized to 1 at 300 nm.

was studied by incubating samples under various cut-off filters. First, incubation of samples under PAR only, or under PAR (morning) and PAR+UVAR (afternoon), did not result in any removal of initial, early morning CPD levels during the day. Second, samples that had been exposed to full solar radiation during the morning did not show repair of the accumulated damage during afternoon hours: no significant removal of CPDs was seen, either under PAR+UVAR, or under PAR alone, although the variability in the data may have hindered the observation of minor repair, if present.

The daily course of photosynthetic rates and DNA damage accumulation were followed on January 18 (Fig. 6). Carbon incorporation clearly increased during the day in both radiation treatments showing a small, but noticeable, depression in photosynthetic rates at local noon, when the highest irradiation levels were found (Fig. 6A). The inhibition due to UVBR was around 7%, remaining

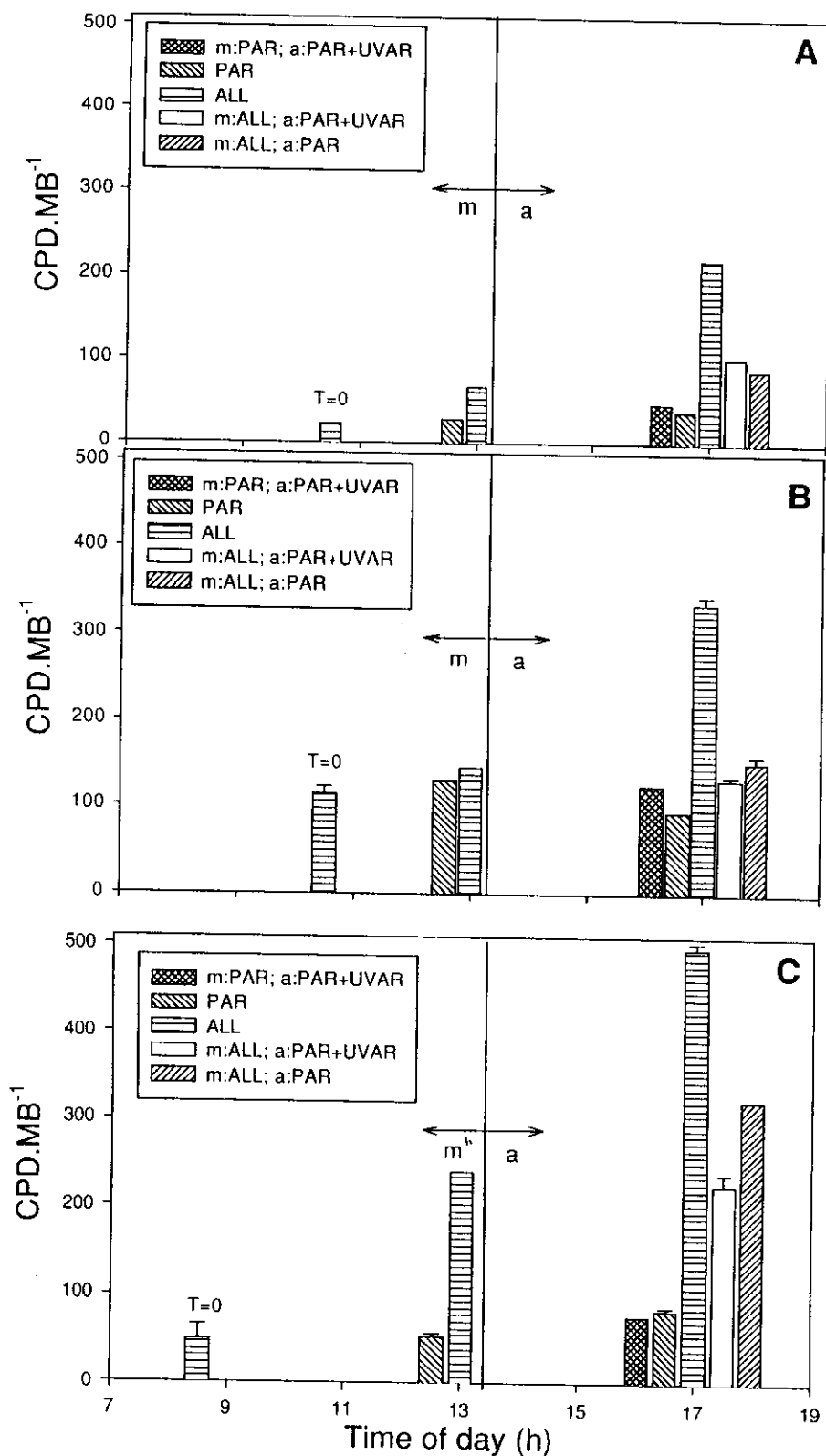


Fig. 5. Accumulation and repair of DNA damage in phytoplankton incubated under various irradiation treatments. (A) January 11, >10  $\mu\text{m}$  fraction; (B) January 11, 0.2–2  $\mu\text{m}$  fraction; (C) January 14, 0.2–2  $\mu\text{m}$  fraction.  $T=0$ : initial CPD levels (pre-treatment). Bars represent various wavelength treatments; m: exposure regimes during morning hours; a: exposure regimes during afternoon hours. Cut-off screens were placed above the bags right after the 'noon' samplings. The lines on top of the bars are standard deviations of the mean (pseudoreplicates).

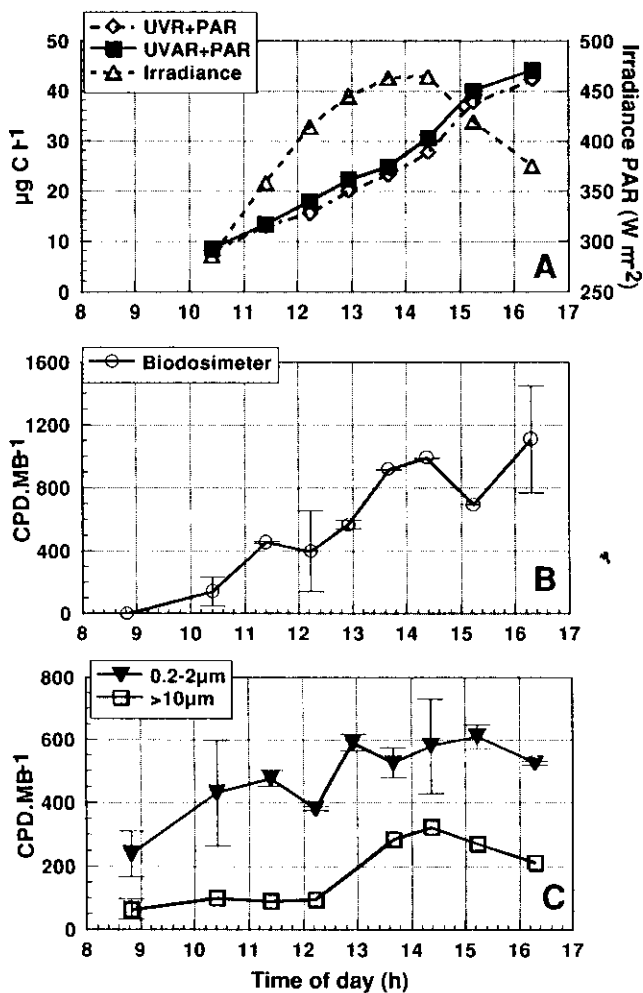


Fig. 6. Daily course of UVR photosynthetic inhibition and CPD accumulation for phytoplankton from Bahía Bustamante on January 18. (A) Effects of total UVR and (UVR+PAR) on photosynthetic rates (carbon incorporation experiments). PAR irradiation during the day is also indicated. (B) DNA effective dose, as measured with the DNA dosimeter; (C) CPD accumulation patterns in two phytoplankton size fractions; open squares:  $>10\mu\text{m}$ ; closed triangles:  $0.2\text{--}2\mu\text{m}$  fraction. Vertical lines indicate standard deviations of the mean (pseudoreplicates for the plankton samples, replicates for the biosimeter samples).

virtually constant during the day (Fig. 6A). This was in sharp contrast with the daily CPD pattern as a steady increase was found during the day in the  $0.2\text{--}2\mu\text{m}$  fraction, roughly following the DNA effective dose, as measured with the DNA dosimeter (Fig. 6B, C). As mentioned before, CPDs were already high at the beginning of the experiment (i.e. at 8.30 h) particularly in the smallest fraction. Both size fractions accumulated CPDs during the day, especially during UVBR peak hours (13.00–14.00 h). Repair appeared to occur in the  $>10\mu\text{m}$  fraction at the end of the afternoon (Fig. 6C).

Compilation of all phytoplankton incubations under full solar radiation conditions did not reveal a clear linear dose response relationship between the DNA effective dose and accumulated CPDs in the plankton during morning and

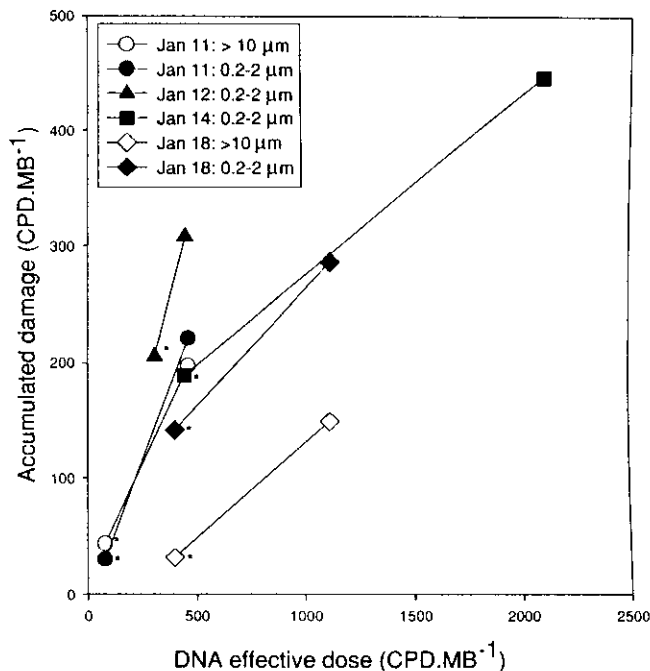


Fig. 7. Dose response relationship of CPD accumulation (accumulated minus initial damage) in two phytoplankton size fractions as a function of the DNA effective dose, measured with the DNA dosimeter (all experiments). The asterisks indicate the morning exposures.

afternoon periods (Fig. 7). The relationship would suggest non-linearity, because at higher DNA effective doses ( $>500\text{CPD.MB}^{-1}$ ) accumulation rates became less rapid, possibly related with minor repair at higher DNA effective doses. However, the scatter in the data was too large to verify this. It is also striking that the  $>10\mu\text{m}$  fraction of the January 18th experiments showed similar levels of accumulated damage, but at much higher DNA effective doses as compared with the other incubations.

#### 4. Discussion

This study shows that CPD accumulation and photosynthetic inhibition both determine the UVR response in phytoplankton assemblages from Bahía Bustamante Argentina, as found before [34]. It also shows that photosynthetic inhibition and CPD accumulation follow different patterns, as previously found for this area [34] and for a tropical region, Lake Titicaca (Bolivia) [32]. UVAR and UVBR inhibit photosynthesis, but CPD accumulation was only related to UVBR exposure (Fig. 6C), which is consistent with the CPD depth profile reported by Helbling et al. [34]. The absence of significant photorepair (Fig. 5) contributes to explain the observed rapid accumulation of CPDs in all fractions. Overall, the high CPD levels found in this study demonstrate that the picophytoplankton assemblages from this area (Bahía Bustamante) are very vulnerable to UVBR induced DNA damage.

The mean BWF for photosynthetic inhibition established for the Bahía Bustamante phytoplankton indicates that this plankton assemblage is slightly more vulnerable to UVBR, but significantly less sensitive to wavelengths  $>300$  nm, than the diatom *Phaeodactylum* sp. [37]. One way to explain this discrepancy is by considering differences in cell size. The plankton in the Bahía Bustamante area was dominated by small cells in the picoplankton size range (0.2–2  $\mu\text{m}$ ), whereas *Phaeodactylum* is a large diatom ( $>20$   $\mu\text{m}$ ). From a number of studies it has become evident that small cells ( $<20$   $\mu\text{m}$  in diameter) are generally more resistant than large cells when looking at photosynthetic inhibition [10,49]. This suggestion is supported here and in the earlier field study, carried out in Bahía Bustamante [34]. In contrast, small cells seem more vulnerable to UVBR stress via CPD formation and accumulation as compared with larger cells, as suggested earlier by Karentz et al. [15]. In support of this, in situ studies have recently demonstrated, for Antarctic phytoplankton assemblages, that small cells contained much more CPDs as compared with larger cells such as diatoms [30].

It is tempting to relate the UVBR effects on photosynthetic inhibition with CPD accumulation, because the BWFs for photosynthetic inhibition and CPD accumulation in the UVBR range showed a strong resemblance, especially above 300 nm (Fig. 4B). One mechanism to explain this would be that DNA damage may inhibit de novo synthesis of proteins, including enzymes such as RUBISCO, required for adequate functioning of the photosynthetic process. However, as shown in the incubation experiment on January 18 (Fig. 6A, B) patterns of UVBR photo-inhibition and CPD accumulation were far from similar, indicating that these two targets (i.e. photosystems, DNA) are affected in an independent way. Also, the BWF for bare DNA was found to differ greatly from all other BWFs including the photoinhibition BWF for Bustamante plankton and the action spectrum of Setlow [48]. The large difference with Setlow's action spectrum ( $<300$  nm) remains unexplained, especially because in an earlier study a high correlation between Setlow weighted UVBR and dosimeter readings were found [36]. The deviation from the BWF for photoinhibition on the other hand could be due to an increasing importance of DNA damage at the shortest UVBR wavelengths (Fig. 4B).

Clearly, CPDs accumulated rapidly when samples were exposed to full solar radiation, even on cloudy days, such as January 11. The  $>10$ - $\mu\text{m}$  fraction also accumulated damage on this cloudy day, although reaching much lower levels at the end of the afternoon than the picophytoplankton fraction, in keeping with the results of a recent study in Antarctic waters [30]. The absolute CPD levels found in this study were very high and consistent with those reported in an earlier study describing in situ CPD levels [34]. Also, absolute CPD levels exceeded levels found in other organisms and areas, applying the same antibody

(H3), such as marine tropical phytoplankton assemblages [26,33] or Antarctic phytoplankton assemblages [30]. Finally they are one order of magnitude higher as compared with high altitude freshwater phytoplankton communities from the Argentinean Andes region (Helbling et al., unpublished results) or Lake Titicaca (Bolivia) [32]. Clearly, the assemblages studied here are very vulnerable for DNA damage induction.

In general, no repair was found during the incubation experiments; damage accumulated during morning hours was not removed during afternoon hours, when samples were exposed to UVAR+PAR or PAR alone. This is in accordance with similar experiments conducted in the Antarctic (Buma et al., unpublished results) where photorepair was minor or absent. The dose response relationship established by pooling all the data (Fig. 7) and the late afternoon decrease in CPDs in the  $>10$ - $\mu\text{m}$  fraction (Fig. 6) imply small but significant repair at higher UVBR doses. In a previous paper [34], repair was found in situ in samples incubated at 3 and 6 m in the water column, where UVBR levels were low, and UVAR and PAR could favor photorepair. Nevertheless, even when photorepair occurs, it will play a rather limited role; photorepair does not prevent the rapid build up of damage during the day, roughly following the DNA effective UVBR dose (Fig. 6B).

Interestingly, the initial damage found early in the morning was not removed by PAR or (PAR+UVAR) treatments (Fig. 5). Pre-treatment CPD levels were very high in this study and higher than those found in other studies in marine tropical picoplankton [22–24], in the Antarctic [30], or in the plankton from Lake Titicaca, Bolivia [32]. In general, residual DNA damage hints to a prolonged history of previous UVBR exposures in the water column, combined with a low repair capacity of cells. In addition, cells may have been damaged to an extent beyond their capability to perform repair. As a result of this they will lose viability, and eventually disappear by lysis, as suggested by Boelen et al. [23]. Also, as shown under laboratory conditions, UVBR exposure causes loss of viability in marine diatoms [16,50]. Indeed, the lack of repair seen in the experiments hints to the presence of non-viable, CPD containing cells, further supported by the rather low assimilation numbers for photosynthesis.

An important consideration with respect to the high UVR vulnerability as demonstrated in this study is the nutrient status of the cells. There are several lines of evidence supporting the assumption that the assemblages were in a post-bloom situation. As discussed before [34], assimilation numbers during the research period were low [ $1 \text{ mg C (mg chl)}^{-1} \text{ h}^{-1}$ ], as compared with the situation in spring [ $5 \text{ mg C (mg chl)}^{-1} \text{ h}^{-1}$ ] (Villafañe, unpublished results). Furthermore, only minor increases in chlorophyll *a* biomass were found during the incubation experiments, irrespective of UVR exposure. Also no changes in CPD levels were found in the repair experiments, suggesting

that damage was not repaired, nor was it diluted by de novo DNA synthesis and cell division by undamaged cells. Therefore, although only supported by indirect evidence, it is likely that the phytoplankton had exhausted the available nutrients at an earlier stage, thereby exhibiting low growth rates during the research period. If this is the case, the observed high UVBR vulnerability, low repair capacity and high initial CPD levels may have been controlled by unfavorable nutrient conditions, in addition to the factors already mentioned above. It is likely that nutrient limitation will affect UVR responses negatively [51], for instance because the depressed ability to generate energy and/or precursors prevent adequate functioning of CPD repair pathways. Future (field) experiments are needed to resolve to what extent the high UVR vulnerability in these picophytoplankton assemblages is related with nutrient conditions.

## 5. Conclusions

This study demonstrates that picophytoplankton assemblages from Bahía Bustamante are very sensitive to UVBR, especially in terms of CPD accumulation. This sensitivity for CPD accumulation may firstly be explained by the small mean cell size of the organisms. Secondly, nutrient limitation may have been involved. Thirdly, if photorepair occurs during the day, the rates are too low to prevent rapid CPD build-up, causing very high CPD levels in the late afternoon. Simultaneously, the phytoplankton assemblage is also affected by UVAR, because photosynthesis is inhibited. This inhibition is less pronounced in comparison with other organisms and assemblages in situ. In conclusion, UVR stress is brought about by effects on both photosystem as well as on DNA, the latter being the most vulnerable in the assemblages from Bahía Bustamante.

## Acknowledgements

We thank M. José, C. Soriano, R. Cariaga and M. Hernández for their help during field work; L. Sala carried out  $^{14}\text{C}$  analysis and L. Taibo helped with computer drawings. We also thank Soriano S.A. and Fundación Playa Unión for logistic support. The comments of two anonymous reviewers and Carlos Ballaré helped to improve our paper. This work was funded by the Dutch Council for Scientific Research (NWO/NAAP), Agencia Nacional de Promoción Científica y Tecnológica — ANPCyT/BID (Project PICT97 07-00000-02206); and The Third World Academy of Sciences — TWAS (Project 98-036 RG/BIO/LA). This is Contribution No. 29 of Estación de Fotobiología Playa Unión.

## References

- [1] R.C. Smith, B.B. Prézelin, K.S. Baker, R.R. Bidigare, N.P. Boucher, T.L. Coley, D. Karentz, S. MacIntyre, H.A. Matlick, D. Menzies, M. Ondrusek, Z. Wan, K.J. Waters, Ozone depletion: Ultraviolet radiation and phytoplankton biology in Antarctic waters, *Science* 255 (1992) 952–959.
- [2] E.W. Helbling, V.E. Villafañe, O. Holm-Hansen, Effects of ultraviolet radiation on antarctic marine phytoplankton photosynthesis with particular attention to the influence of mixing, in: C.S. Weiler, P. Penhale (Eds.), *Ultraviolet Radiation in Antarctica: Measurements and Biological Effects*, Antarctic Res. Ser. Vol. 62, Am. Geophys. Union, Washington DC, 1994, pp. 207–227.
- [3] P.J. Neale, M.P. Lesser, J.J. Cullen, Effects of ultraviolet radiation on the photosynthesis of phytoplankton in the vicinity of McMurdo Station, Antarctica, in: C.S. Weiler, P.A. Penhale (Eds.), *Ultraviolet Radiation in Antarctica: Measurements and Biological Effects*, Antarctic Res. Ser. Vol. 62, Am. Geophys. Union, Washington DC, 1994, pp. 125–142.
- [4] B.B. Prézelin, N.P. Boucher, R.C. Smith, Marine primary production under the influence of the Antarctic ozone hole: Icecolors '90, in: C.S. Weiler, P.A. Penhale (Eds.), *Ultraviolet Radiation in Antarctica: Measurements and Biological Effects*, Antarctic Res. Ser. Vol. 62, Am. Geophys. Union, Washington DC, 1994, pp. 159–186.
- [5] N.P. Boucher, B.B. Prézelin, Spectral modeling of UV inhibition of in situ Antarctic primary production using a field derived biological weighting function, *Photochem. Photobiol.* 64 (1996) 407–418.
- [6] B.B. Prézelin, M.A. Moline, H.A. Matlick, Icecolors '93: spectral UV radiation effects on Antarctic frazil ice algae, *Ant. Res. Ser.* 73 (1998) 45–83.
- [7] A. McMinn, C. Ashworth, K. Ryan, Growth and productivity of Antarctic sea ice algae under PAR and UV irradiances, *Bot. Mar.* 42 (1999) 401–407.
- [8] M. Behrenfeld, J. Hardy, H. Gucinski, A. Hanneman, H. Lee II, A. Wones, Effects of ultraviolet-B radiation on primary production along latitudinal transects in the South Pacific Ocean, *Mar. Environm. Res.* 35 (1993) 349–363.
- [9] E.W. Helbling, S. Avaria, J. Letelier, V. Montecino, B. Ramírez, M. Ramos, W. Rojas, V.E. Villafañe, Respuesta del fitoplancton marino a la radiación ultravioleta en latitudes medias (33°S), *Rev. Biol. Mar. Valparaíso* 28 (1993) 219–237.
- [10] E.W. Helbling, V.E. Villafañe, M. Ferrario, O. Holm-Hansen, Impact of natural ultraviolet radiation on rates of photosynthesis and on specific marine phytoplankton species, *Mar. Ecol. Prog. Ser.* 80 (1992) 89–100.
- [11] V.E. Villafañe, M. Andrade, V. Lairana, F. Zaratti, E.W. Helbling, Inhibition of phytoplankton photosynthesis by solar ultraviolet radiation: Studies in Lake Titicaca, Bolivia, *Freshwat. Biol.* 42 (1999) 215–224.
- [12] B.M.A. Kroon, O. Schofield, B.B. Prézelin, Icecolors '93: UV-B radiation specifically decreases photosystem II (PsII) quantum yield in a field community of Antarctic ice algae exposed to natural daylight, *EOS* 75 (1994) 200.
- [13] O. Schofield, B.M.A. Kroon, B.B. Prézelin, Impact of ultraviolet-B radiation on photosystem II activity and its relationship to the inhibition of carbon fixation rates for Antarctic ice algae communities, *J. Phycol.* 31 (1995) 703–715.
- [14] M.P. Lesser, P.J. Neale, J.J. Cullen, Acclimation of Antarctic phytoplankton to ultraviolet radiation: ultraviolet-absorbing compounds and carbon fixation, *Mol. Mar. Biol. Biotechnol.* 5 (1996) 314–325.
- [15] D. Karentz, J.E. Cleaver, D.L. Mitchell, Cell survival characteristics and molecular responses of Antarctic phytoplankton to ultraviolet-B radiation, *J. Phycol.* 27 (1991) 326–341.
- [16] D. Karentz, Ultraviolet tolerance mechanisms in Antarctic marine organisms, in: C.S. Weiler, P.A. Penhale (Eds.), *Ultraviolet Radiation in Antarctica: Measurements and Biological Effects*, Antarctic

- Res. Ser. Vol. 62, Am. Geophys. Union, Washington DC, 1994, pp. 93–110.
- [17] A.G.J. Buma, E.J. van Hannen, M.J.W. Veldhuis, L. Roza, W.W.C. Gieskes, Monitoring UV-B induced DNA damage in individual diatom cells by immunofluorescent thymine dimer detection, *J. Phycol.* 31 (1995) 314–321.
- [18] A.G.J. Buma, T. van Oyen, W. van de Poll, M.J.W. Veldhuis, W.W.C. Gieskes, The high sensitivity of the marine prymnesiophyte *Emiliania huxleyi* to ultraviolet-B radiation, *J. Phycol.* 36 (2000) 296–303.
- [19] A. Sancar, G.B. Sancar, DNA repair enzymes, *Ann. Rev. Biochem.* 57 (1988) 29–67.
- [20] M.G. Weinbauer, S.W. Wilhelm, C.A. Suttle, D.R. Garza, Photo-reativation compensates for UV damage and restores infectivity to natural marine virus communities, *Appl. Environ. Microbiol.* 63 (1997) 2200–2205.
- [21] S.W. Wilhelm, M.G. Weinbauer, C.A. Suttle, R.J. Pledger, D.L. Mitchell, Measurement of DNA damage and photoreactivation imply that most viruses in marine surface waters are infective, *Aquat. Microb. Ecol.* 14 (1998) 215–222.
- [22] W.H. Jeffrey, R.J. Pledger, P. Aas, S. Hager, R.B. Coffin, R. Von Haven, D.L. Mitchell, Diel and depth profiles of DNA photodamage in bacterioplankton exposed to ambient solar ultraviolet radiation, *Mar. Ecol. Progr. Ser.* 137 (1996) 283–291.
- [23] P. Boelen, M.J.W. Veldhuis, A.G.J. Buma, Accumulation and repair of UVBR mediated DNA damage in marine tropical picoplankton subjected to mixed and simulated non-mixed conditions, *Aquat. Microb. Ecol.* (2001) in press.
- [24] W.H. Jeffrey, P. Aas, M.M. Lyons, R.B. Coffin, R.J. Pledger, D.L. Mitchell, Ambient solar radiation-induced photodamage in marine bacterioplankton, *Photochem. Photobiol.* 64 (1996) 419–427.
- [25] P.M. Visser, E. Snelder, A.J. Kop, P. Boelen, A.G.J. Buma, F.C. van Duyl, Effects of UV radiation on DNA photodamage and production in bacterioplankton in the coastal Caribbean Sea, *Aquat. Microb. Ecol.* 20 (1999) 49–58.
- [26] P. Boelen, M.K. de Boer, G.W. Kraay, M.J.W. Veldhuis, A.G.J. Buma, UVBR induced DNA damage in natural marine picoplankton assemblages in the tropical Atlantic ocean, *Mar. Ecol. Progr. Ser.* 193 (2000) 1–9.
- [27] M.G. Weinbauer, S.W. Wilhelm, C.A. Suttle, R.J. Pledger, D.L. Mitchell, Sunlight-induced DNA damage and resistance in natural viral communities, *Aquat. Microb. Ecol.* 17 (1999) 111–120.
- [28] R.D. Vetter, A. Kurtzman, T. Mori, Diel cycles of DNA damage and repair in eggs and larvae of Northern anchovy, *Engraulis mordax*, exposed to solar ultraviolet radiation, *Photochem. Photobiol.* 69 (1999) 27–33.
- [29] K.D. Malloy, M.A. Holman, D. Mitchell, H. William Dettrich III, Solar UV-B induced DNA damage and photoenzymatic DNA repair in Antarctic zooplankton, *Proc. Natl. Acad. Sci. USA* 94 (1997) 1258–1263.
- [30] A.G.J. Buma, M.K. de Boer, P. Boelen, Depth distributions of DNA damage in Antarctic marine phyto- and bacterioplankton exposed to summertime ultraviolet radiation, *J. Phycol.* 37 (2001) 200–208.
- [31] M.C. Rousseaux, C.L. Ballaré, C.V. Giordano, A.L. Scopel, A.M. Zima, M. Szwarcberg-Bracchitta, P.S. Searles, M.M. Caldwell, S.B. Díaz, Ozone depletion and UVB radiation: Impact on plant DNA damage in southern South America, *Proc. Natl. Acad. Sci. USA* 96 (1999) 15310–15315.
- [32] E.W. Helbling, V.E. Villafañe, A.G.J. Buma, M. Andrade, F. Zaratti, DNA damage and photosynthetic inhibition induced by solar UVR in tropical phytoplankton (Lake Titicaca, Bolivia), *Eur. J. Phycol.* 36 (2001) 157–166.
- [33] P. Boelen, A.F. Post, M.J.W. Veldhuis, A.G.J. Buma, Diel patterns of UVBR induced DNA damage in picoplankton size fractions from the Gulf of Aqaba, Red Sea, *Mar. Ecol. Progr. Ser.* (2001) submitted.
- [34] E.W. Helbling, A.G.J. Buma, M.K. De Boer, V.E. Villafañe, In situ impact of solar ultraviolet radiation on photosynthesis and DNA in temperate marine phytoplankton, *Mar. Ecol. Progr. Ser.* 211 (2001) 43–49.
- [35] V.L. Orce, E.W. Helbling, Latitudinal UVR–PAR measurements in Argentina: Extent of the “Ozone hole”, *Global Plan. Change* 15 (1997) 113–121.
- [36] P. Boelen, I. Obernosterer, A.A. Vink, A.G.J. Buma, Attenuation of biologically effective UV radiation in tropical Atlantic waters measured with a biochemical DNA dosimeter, *Photochem. Photobiol.* 69 (1999) 34–40.
- [37] J.J. Cullen, P.J. Neale, M.P. Lesser, Biological weighting function for the inhibition of phytoplankton photosynthesis by ultraviolet radiation, *Science* 258 (1992) 645–650.
- [38] P.J. Neale, D.J. Kieber, Assessing biological and chemical effects of UV in the marine environment: Spectral weighting functions, in: R.E. Hester, R.M. Harrison (Eds.), *Causes and Environmental Implications of Increased U.V.-B. Radiation*, Issues in Environmental Science and Technology, Vol. No. 14, The Royal Society of Chemistry, Cambridge, UK, 2000, pp. 61–83.
- [39] R.D. Rundel, Action spectra and estimation of biologically effective UV radiation, *Physiol. Plant.* 58 (1983) 360–366.
- [40] J.J. Doyle, J.L. Doyle, Isolation of plant DNA from fresh tissue, *Focus* 12 (1991) 13–15.
- [41] E. Steeman Nielsen, The use of radiocarbon ( $^{14}\text{C}$ ) for measuring organic production in the sea, *J. Cons. Int. Explor. Mer* 18 (1952) 117–140.
- [42] O. Holm-Hansen, E.W. Helbling, Técnicas para la medición de la productividad primaria en el fitoplancton, in: K. Alveal, M.E. Ferrario, E.C. Oliveira, E. Sar (Eds.), *Manual de Métodos Ficológicos*, Universidad de Concepción, Concepción, Chile, 1995, pp. 329–350.
- [43] O. Holm-Hansen, B. Riemann, Chlorophyll a determination: Improvements in methodology, *Oikos* 30 (1978) 438–447.
- [44] O. Holm-Hansen, C.J. Lorenzen, R.W. Holmes, J.D.H. Strickland, Fluorometric determination of chlorophyll, *J. Cons. Int. Explor. Mer* 30 (1965) 3–15.
- [45] V.E. Villafañe, F.M.H. Reid, Métodos de microscopía para la cuantificación del fitoplancton, in: K. Alveal, M.E. Ferrario, E.C. Oliveira, E. Sar (Eds.), *Manual De Métodos Ficológicos*, Universidad de Concepción, Concepción, Chile, 1995, pp. 169–185.
- [46] G.W. Kraay, M. Zapata, M.J.W. Veldhuis, Separation of chlorophylls c1, c2 and c3 of marine phytoplankton by reversed-phase-C18-high-performance liquid chromatography, *J. Phycol.* 28 (1992) 708–712.
- [47] F.E.B. Quate, B.M. Sutherland, J.C. Sutherland, Action spectrum for DNA damage in alfalfa lowers predicted impact of ozone depletion, *Nature* 358 (1992) 576–578.
- [48] R.B. Setlow, The wavelengths in sunlight effective in producing skin cancer: A theoretical analysis, *Proc. Natl. Acad. Sci. USA* 71 (1974) 3363–3366.
- [49] I. Laurion, W.F. Vincent, Cell size versus taxonomic composition as determinants of UV-sensitivity in natural phytoplankton communities, *Limnol. Oceanogr.* 43 (1998) 1774–1779.
- [50] A.G.J. Buma, H.J. Zemmeling, K. Sjollem, W.W.C. Gieskes, UVB radiation modifies protein and photosynthetic pigment content, volume and ultrastructure of marine diatoms, *Mar. Ecol. Progr. Ser.* 142 (1996) 47–54.
- [51] P.J. Neale, Modeling the effects of ultraviolet radiation on estuarine phytoplankton production: Impact of variations in exposure and sensitivity to inhibition, *J. Photochem. Photobiol. B: Biol.* 62 (2001) 1–8 (this issue).