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UVR-induced photosynthetic inhibition dominates over DNA damage in marine dinoflagellates exposed to fluctuating solar radiation regimes

E. Walter Helbling [a,b,*], Anita G.J. Buma [c], Willem van de Poll [c], M. Verónica Fernández Zenoff [b,d], Virginia E. Villafañe [a,b]

[a] Estación de Fotobiología Playa Unión, Casilla de Correos N° 15 (9103) Rawson, Chubut, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)
[c] Department of Marine Biology – Centre for Ecological and Evolutionary Studies, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands
[d] Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), Avda. Belgrano y Pje. Caseros (4000) San Miguel de Tucumán, Argentina

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ABSTRACT

The combined effect of solar radiation (UV-B (280-315 nm), UV-A (315-400 nm) and PAR (400-700 nm)) and vertical mixing (i.e., fluctuating radiation regimes) on the marine dinoflagellates *Gymnodinium chlorophorum*, *Heterocapsa triquetra* and *Prorocentrum micans* was investigated during the austral spring in Patagonia, Argentina. Photosynthesis, measured as radiocarbon incorporation, and accumulation of DNA damage, as cyclobutane pyrimidine dimers (CPDs), were investigated under simulated mixed and non-mixed water column conditions using 3 h incubations centered at local noon. Static samples had significant UVR-induced photoinhibition that was higher in *H. triquetra* as compared to the other two species. Increasing mixing speed significantly increased UVR-induced inhibition of carbon fixation in *G. chlorophorum* and *H. triquetra*. No significant UVR effect was observed in *P. micans* under any of the mixing regimes. Most of the loss in carbon fixation in *G. chlorophorum* was due to UV-B while in *H. triquetra* it was due to UV-A. Part of these responses may be associated to the presence of UV-absorbing compounds which were abundant in *P. micans*, and low in *H. triquetra* and in *G. chlorophorum*. However, other variables such as cell size and active repair might have also influenced our results. We did not detect CPD accumulation in any of the species, probably because of the low solar angle that resulted in very low levels of DNA effective UV-B dose. Our results indicate that exposure to solar UVR in the Patagonia area during spring time (even during ozone depletion events) has a clear impact on photosynthesis and much less or negligible on DNA in the three studied species.
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1. Introduction

Wind-induced vertical mixing affects the depth at which phytoplankton cells are found in the water column and thus, the mean irradiance that they receive. Previous studies have shown that vertical mixing can have different effects on aquatic primary production: enhancing (Marra, 1978), decreasing (Kroon et al., 1992) or having no effect (Yoder and Bishop, 1985). In these studies, however, phytoplankton was only exposed to variable Photosynthetically Available Radiation (PAR, 400-700 nm). More recently, the impact of variable (i.e., under mixing conditions) ultraviolet radiation (UVR, 280-400 nm) on phytoplankton primary production was also considered (see review of Neale et al. (2003). For example, studies in Antarctica showed that a shallow upper mixed layer depth (Z_{UML}) enhanced short-term effects of UVR on photosynthesis as compared to conditions of deep mixing (Helbling et al., 1994; Neale et al., 1998a).

* Corresponding author.
E-mail address: whelbling@efpu.org.ar (E.W. Helbling).

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The combined effects of vertical mixing and UVR on carbon fixation were also studied in post-bloom phytoplankton populations from Patagonia showing that the impact of UVR depended on the fraction of the euphotic zone being mixed (i.e., the ratio of the depth of the upper mixed layer (Z_{UML}) to the depth of the euphotic zone, Z_{Eu}) (Barbieri et al., 2002). When the simulated water column had shallow mixing conditions (ratio $Z_{UML}/Z_{Eu}=0.6$) photosynthesis was inhibited by UVR, while an enhancement of photosynthesis in samples receiving UVR was determined under deep mixing (ratio $Z_{UML}/Z_{Eu}=0.91$). Some studies further addressed the effects of UVR under different mixing speeds and variable responses were obtained: On the one hand, modeling showed that fast mixing enhanced photosynthetic inhibition in Antarctic phytoplankton (Neale et al., 1998a). On the other hand, simulated *in situ* mixing studies in tropical waters (Helbling et al., 2003) showed opposite results with increased carbon fixation in samples exposed to UVR under fast mixing conditions. Similarly, fast rotating samples of the red algae *Porphyridium cruentum* were less inhibited by UVR as compared to those under slow mixing or incubated at a fixed depth (Villafañe et al., 2005). Furthermore, it was demonstrated that rapid vertical mixing not only counteracted

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the impact of UVR but also stimulated photosynthesis of phytoplankton from a tropical shallow lagoon (Villafañe et al., 2007).

Studies determining the interactive effect of mixing and UV-B-induced DNA damage on plankton are more scarce, but model predictions showed that the Z_{UML} strongly influences the amount and distribution of DNA damage (Huot et al., 2000) as also found in field studies carried out in different locations (Jeffrey et al., 1996; Boelen et al., 2001; Helbling et al., 2006). Moreover, it was suggested that vertical mixing benefits plankton organisms by transporting cells from the high UV-B levels at the water surface to depths where active repair can takes place (Boelen et al., 2001; Helbling et al., 2006).

Generally speaking, the variables associated with the extent of mixing (e.g. depth and speed) can act synergistically or antagonistically with UVR, depending on the species composition / size structure and on the environmental conditions, thus leading to different responses (Villafañe et al., 2007). The aim of this study is to address the interactive effects of UVR on two different targets, photosynthesis and DNA, under fluctuating solar radiation regimes in three marine dinoflagellate species. The approach was to carry out experiments during the austral spring with selected species, that are commonly found in Patagonian waters, under solar radiation and simulating various mixing conditions.

2. Materials and Methods

2.1. Culture conditions

Experiments were conducted with the marine dinoflagellates *Gymnodinium chlorophorum* Elbrächter & Schnepf (mean diameter, 5 µm), *Heterocapsa triquetra* (Ehrenberg) Stein (mean diameter, 20 µm) and *Prorocentrum micans* (Ehrenberg) (mean diameter, 50 µm). The three species are characteristic of marine Patagonian waters, but while *P. micans* was collected from Golfo Nuevo (Chubut, Argentina) clones of *G. chlorophorum* and *H. triquetra* initially isolated from the North Sea were used in the experiments. The species were maintained in a growth chamber with a 12L:12D photoperiod and maintained semi-continuously in exponential growth in f/2 medium (Guillard and Ryther, 1962) at 18 °C and under 200 µmol photons m^{-2} s^{-1} of PAR illumination for at least one week before being used in experiments.

2.2. Experimental set-up

During September 2004 (early austral spring) experiments were conducted under natural irradiance at the Estación de Fotobiología Playa Unión (EFPU), Chubut, Argentina (43° 18′ S, 65° 02′W) simulating diverse fluctuating radiation regimes. To simulate vertical mixing, the samples (in 50-ml quartz tubes) were attached to horizontal wheels that had on top increasing layers of neutral density screen arranged in circular sectors (i.e., pie-like pieces) that rotated by means of a stepper motor thus varying the irradiance (Helbling et al., 2003). The layer containing the screens had 10 circular sectors of ca 36° going clockwise from none (simulating 100% radiation) to four screens (simulating 6% radiation) and then back to none, thus performing one complete rotation with 10 discrete steps of irradiance (i.e. 100%, 50%, 25% 12.5% and 6% and back to 100%). We used five different speeds of fluctuating irradiance for each species, and the duration of one rotation simulating cells going from the surface (100%) to the bottom of the UML (6%) back to the surface (100%) varied from 12 to 180 min (i.e., from 15 to 1 rotations in 3 hs). We chose the irradiance level at the bottom of the UML as 6% based on previous measurements carried out in the area (Barbieri et al., 2002; Villafañe et al., 2004a).

To implement the radiation treatments, we used three wheels with identical neutral density screens but with different combination of filters / materials that allowed different portions of the solar spectrum to reach the samples: **a**) Full solar radiation (PAR + UVR, 280-700 nm), wheel with uncovered quartz tubes, PAB treatment, **b**):PAR + UV-A (320-700 nm), wheel with tubes covered with UV cut-off filter foil (Montagefolie N°10155099, Folex, 50% transmission at 320 nm), PA treatment; and **c**) PAR only (400-700 nm), wheel with quartz tubes covered with Ultraphan UV Opak Digefra film (50% transmission at 395 nm), P treatment (see Figueroa et al., 1997 for transmission spectra of these materials). The three wheels were placed inside a water bath to control the temperature (18 °C±2 °C) with the tubes just below the surface (<5 mm). It should be noted that this system does not accurately mimic the spectral attenuation in the water column (i.e., the ratio UV-B/UV-A/PAR is higher than normally occurs in the natural environment) but it does provide information on the effects of fluctuating UVR under the worst-case scenario. Simultaneously, we had duplicate “static samples” in quartz tubes that were exposed to fixed solar irradiance under the same conditions as the rotating samples (i.e., in quantity: 100%, 50%, 25% 12.5% to 6% of incident solar radiation, and in quality: PAB, PA and P treatments). In addition, duplicate quartz tubes wrapped in aluminum foil were used as dark controls. The samples (both static and rotating) were then incubated for 3 h (centered on local noon) under solar radiation.

We determined the effects of fluctuating solar radiation on two targets, photosynthesis (carbon incorporation) and DNA. Thus each wheel (and static samples) had duplicate quartz tubes at each irradiance level (total of 10 per wheel) to determine carbon incorporation, and duplicate quartz tubes (total of 10 per wheel) to determine DNA damage. A total of ten experiments (i.e. two for each mixing speed) for each dinoflagellate species were conducted. Additionally, each wheel (and samples under static conditions) had 2-ml quartz biodosimeter tubes per irradiance level to determine UVR-induced DNA damage (i.e., as cyclobutane pyrimidine dimers - CPDs formation).

2.3. Analyses and measurements

2.3.1. Photosynthetic rates

Cultures (3-8 µg chl-a l^{-1}) were transferred during exponential growth to 50-ml quartz tubes and inoculated with 0.1 ml - 5 µCi (0.185 MBq) of labeled sodium bicarbonate (ICN Radiochemicals). The tubes were put in the wheels and, after incubation the samples were filtered onto a Whatman GF/F glass fiber filter (25 mm). The filters were placed in 7-ml scintillation vials, exposed to HCl fumes overnight, dried, and counted using standard liquid scintillation techniques (Holm-Hansen and Helbling, 1995).

2.3.2. Chlorophyll a and UV-absorbing compounds

Chlorophyll-a (chl-a) concentration and the presence of UV-absorbing compounds were determined before exposure by filtering 50 ml of culture onto Whatman GF/F filters (25 mm). The filters were sonicated for 15 min (20 °C) and extracted with 7 ml of absolute methanol in the dark for at least 1 h at 4 °C (Holm-Hansen and Riemann, 1978). After extraction, the samples were centrifuged and the supernatants scanned between 250 and 750 nm using a spectrophotometer (Hewlett Packard model HP-8453E). The peak height at 337 nm was used as an estimate of UV-absorbing compounds concentration (Dunlap et al., 1995). We are aware that UV-absorbing compounds are slightly underestimated by this procedure as it was shown that 20% methanol is the best extraction solvent for these compounds (e.g. Tartarotti and Sommaruga, 2002). However, and since we were limited by the amount of sample, we considered that this procedure was appropriate for the purposes of our investigation. After the spectrophotometric measurements, the same extract was used to determine chl-a concentration with a fluorometer (Turner Designs model TD 700) from the readings before and after acidification with 1 N HCl (Holm-Hansen et al., 1965). The fluorometer is routinely calibrated against absorbance readings using a spectrophotometer.

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2.3.3. DNA damage

After exposure, the samples were filtered onto polycarbonate membrane filters (25 mm, 0.2 µm pore size, Osmonics Inc.) and DNA was extracted immediately following standard techniques (Doyle and Doyle, 1991). The concentration of accumulated CPDs in the extracted and dosimeter DNA was determined using the assay described by Boelen et al. (1999). Briefly, 100 ng of heat denatured DNA was blotted onto nitrocellulose membranes and after blocking and washing steps the membranes were incubated with the primary and secondary antibodies. CPDs were detected using ECL detection reagents (RPN2106 Amersham) in combination with photosensitive films (Kodak-X-AR-5), and quantified using Image Quant software (Molecular Dynamics).

2.3.4. Radiation measurements

Incident solar UV-B (280 - 315 nm), UV-A (315 - 400 nm) and PAR (400 - 700 nm) were continuously measured using a broadband filter radiometer (ELDONET, Real Time Computers, Inc., Germany) that is permanently installed on the roof of the EFPU. In addition, duplicate biodosimeters (i.e. with 10 µg ml^{-1} calf thymus DNA) were incubated in the static and moving systems to determine the DNA effective UV-B dose (Buma et al., 2001) during the experiments. The transmission of the screens on top of the samples was also measured using the ELDONET radiometer.

2.3.5. Calculations

During each experiment both static and mixed samples were exposed to the same incident irradiance (i.e., as they were incubated simultaneously). However, static samples were exposed to five different radiation levels during the whole incubation, whereas the mixed samples were exposed to fluctuating irradiance between 100 to 6%. To compare the two data sets (i.e., static vs. mixed) we calculated the integrated carbon fixation and photosynthetic inhibition in the simulated UML down to the 6% attenuation as follows:

In the mixed condition the experimental set up already integrates the effects of the different irradiance levels (i.e., mean UML irradiance) so, the inhibition in the simulated UML was calculated by comparing the mean carbon fixation (normalized by chl-a) in the different radiation treatments as (Eqs. (1)–(3)):

$$UVR\ inhibition = (P_{PAR} - P_{UVR})/(P_{PAR} * E_{UML}). \quad (1)$$

$$UV\text{-}B\ inhibition = (P_{UV\text{-}A} - P_{UVR})/(P_{PAR} * E_{UV\text{-}B}) \quad (2)$$

$$UV\text{-}A\ inhibition = (P_{PAR} - P_{UV\text{-}A})/(P_{PAR} * E_{UV\text{-}A}) \quad (3)$$

where P_{PAR}, $P_{UV\text{-}A}$, and P_{UVR} represent the carbon fixation in the P, PA, and PAB treatments, respectively, and E_{UML}, $E_{UV\text{-}B}$ and $E_{UV\text{-}A}$ represent the mean PAR, UV-B and UV-A irradiances in the UML, respectively. We normalized the inhibition to irradiance to account for the day-to-day variability, as the experiments were conducted on different days.

For static conditions we used the model of Eilers and Peeters (1988) to fit the data by iteration with a P *vs.* E. curve. The integrated carbon fixed in each radiation treatment was obtained from the area under the curve using as integration limits the carbon fixed (as assimilation numbers) at 6% and 100% irradiance, respectively as:

$$P_{\text{int}} = \int_{6\%}^{100\%} P * dE \quad (4)$$

where P is the assimilation number (in mg C (mg chl-a)$^{-1}$h^{-1}) and E is the irradiance. Then, from these integrated values we calculated the inhibition using the Eqs. (1)–(3), to obtain the integrated inhibition in the simulated UML.

The non-parametric Kruskal Wallis test (Zar, 1999) was used to determine significant differences between the samples in various radiation treatments and mixing speeds, using a 95% confidence limit. We used at least duplicate samples for each radiation / mixing treatment and as each experiment was done twice we considered a minimum n=4 for all analyses.

3. Results

Solar radiation and column ozone concentration data for the area of Playa Unión during September 2004 are shown in Fig. 1. Ozone concentrations (data from http://jwocky.gsfc.nasa.gov) were lower than the mean for the years 1997-2003 during most days (Fig. 1A) with values as low as 249 Dobson Units (D.U.) on September 24. Daily doses of UV-B, UV-A and PAR (Fig. 1B-D) were higher than the mean for the years 1997-2003, except for a few days of heavy cloud cover. There was a clear trend of increasing UV-B towards the end of September (Fig. 1B) with daily doses varying from ~ 5 to ~ 20 kJ m^{-2}. Biodosimeter data (Fig. 1B) also showed a trend of accumulated DNA damage (and DNA weighted dose) in the biodosimeters exposed to full solar radiation. The maximum (clear sky) local noon weighted irradiance for DNA for September 24 (as calculated with the STAR program (Ruggaber et al., 1994)) was 86.4 mW m^{-2}. PAR doses during September 2004 ranged from ~1 to 7 MJ m^{-2} (Fig. 1D). The mean PAR, UV-A and UV-B doses received at the surface of the water bath during the 3 h exposures were 2121 (SD=454), 294 (SD=51), and 6.98 (SD=1.03) kJ m^{-2}, respectively.

3.1. DNA damage

While DNA biodosimeters showed significant ($P<0.05$) CPD accumulation over 3 h on all experimental days (Fig. 1B), no CPD accumulation was found for any of the exposed algal samples during

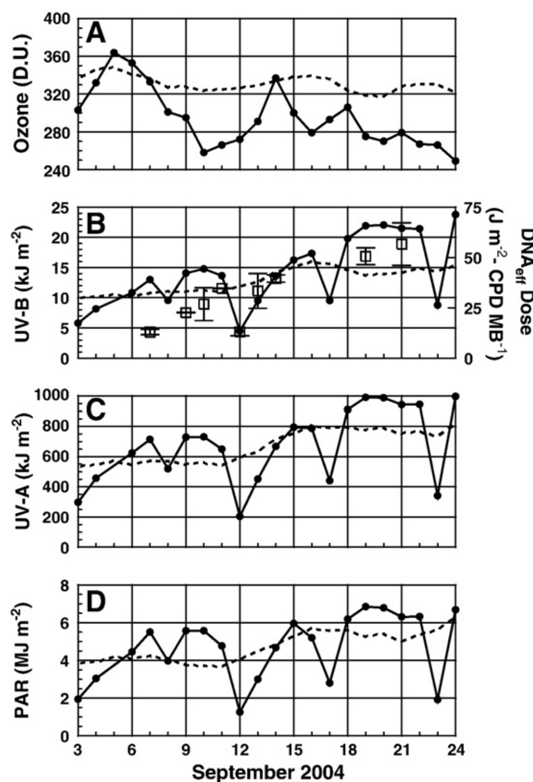

Fig. 1. Radiation conditions and ozone concentrations during September 2004 over the study area. A. Ozone concentrations, in Dobson Units (D.U.); B. UV-B (280-315 nm) daily doses (in kJ m^{-2}) and CPD MB^{-1} in the biodosimeters located in a shade-free area next to the experimental set up; DNA effective doses (open squares, in J m^{-2}) were obtained using the conversion factor of Boelen et al., 1999, and; C. UV-A (315-400 nm) daily doses (in kJ m^{-2}); D. PAR (400-700 nm) daily doses (in MJ m^{-2}). Broken lines represent the 3-day running mean values for the years 1997 to 2003.

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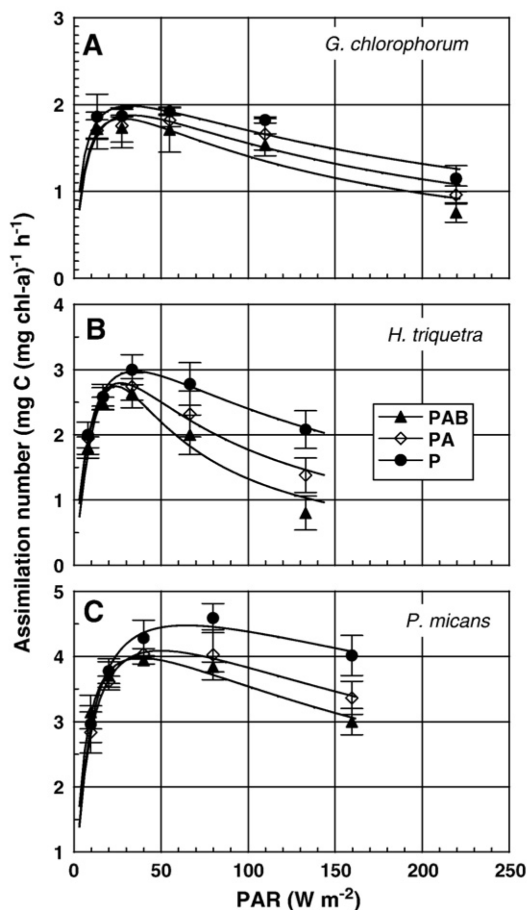

Fig. 2. Carbon fixation per unit chl-a per hour as a function of PAR irradiance received by cells of A. *Gymnodinium chlorophorum*; B. *Heterocapsa triquetra*; and C. *Prorocentrum micans* in static incubations. Symbols represent means ± SD (n = 4), and solid lines the fit using model of Eilers and Peeters (1988).

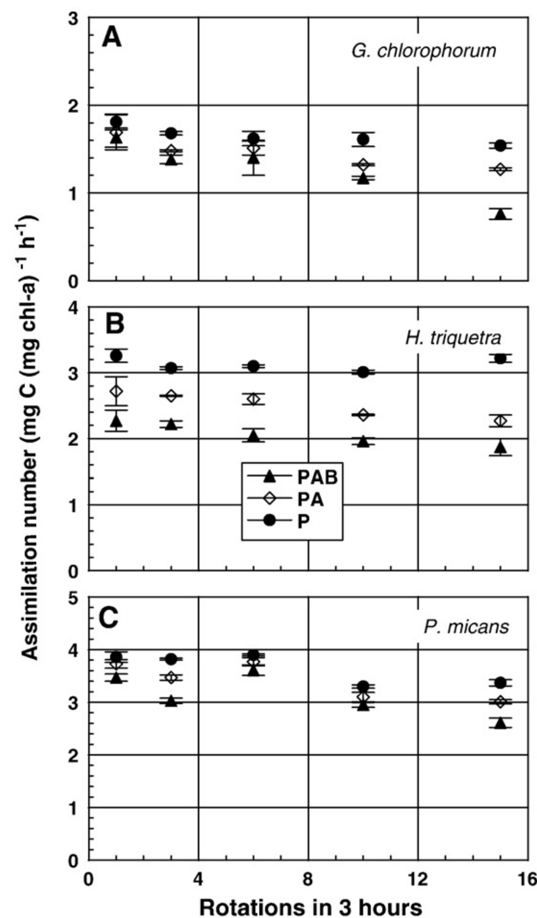

Fig. 3. Carbon fixation per unit chl-a per hour as a function of the number of rotations in 3 h exposure to fluctuating natural irradiance regime for A. *Gymnodinium chlorophorum*; B. *Heterocapsa triquetra*; and C. *Prorocentrum micans*. Symbols represent means ± SD (n = 4).

incubations (data not shown) regardless of the radiation and mixing conditions imposed.

3.2. Photosynthetic rates

There was a significant ($P<0.05$) UVR effect on carbon fixation in the three dinoflagellates when incubated at irradiances >30-40 W m^{-2} under static conditions (Fig. 2). Maximal carbon fixation (P_{max}) under the P treatment were 1.98, 2.97, and 4.47 mg C (mg chl-a)$^{-1}$ h^{-1} for *Gymnodinium chlorophorum* (Fig. 2A), *Heterocapsa triquetra* (Fig. 2B) and *Prorocentrum micans* (Fig. 2C), respectively. The calculated P_{max} values under the PAB treatment were significantly lower ($P<0.05$) than in the P and PA treatments in *H. triquetra* and *P micans*, but not in *G. chlorophorum*. Carbon incorporation of *G. chlorophorum* and *H. triquetra* decreased significantly at higher PAR as compared to P_{max} (49% and 33% at 220 and 140 Wm^{-2}, respectively, Fig. 2). No significant PAR-induced photoinhibition was detected in *P micans* (Fig. 2C) up to 160 Wm^{-2} of PAR.

Assimilation numbers in the mixed conditions varied as a function of speed (expressed as the number of rotations during the incubation period, Fig. 3). In *G. chlorophorum* (Fig. 3A) there was a significant decrease ($P<0.05$) in assimilation numbers with increasing speed in samples under the PAB treatment; a smaller but significant ($P<0.05$) decrease was also found with increasing mixing speed in the P and PA treatments. In *H. triquetra* (Fig. 3B) there were significant differences ($P<0.05$) in assimilation numbers among radiation treatments at all mixing speeds; however, significant ($P<0.05$) decreases with increasing mixing speed were only found in PAB and PA-exposed samples. Finally, in *P. micans* (Fig. 3C) a slight but significant decrease ($P<0.05$) in assimilation numbers in all radiation treatments was found with increasing speed.

The combined effects of UVR and mixing speed on integrated inhibition of photosynthesis are shown in Fig. 4. The integrated inhibition

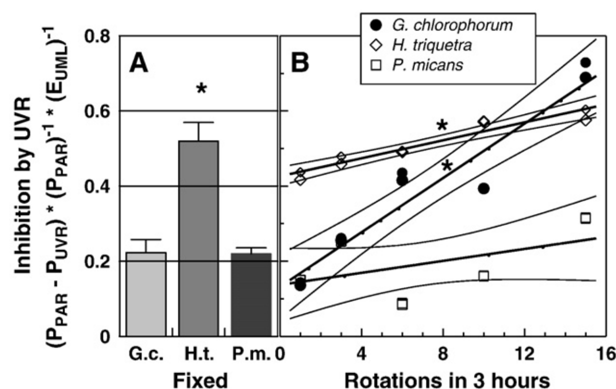

Fig. 4. Inhibition by UVR (relative to samples exposed only to PAR) in *Gymnodinium chlorophorum* (G.c.), *Heterocapsa triquetra* (H.t.) and *Prorocentrum micans* (P.m.). A. Mean integrated inhibition within the simulated UML in static samples; B. Inhibition as a function of mixing speed (expressed as rotations in 3 h incubations). Error bars in A. represent the standard deviation (n = 4); solid lines in B represent the linear regression; the dotted lines are the 95% confidence limits. The asterisk in A. indicates significance difference; the asterisks in B. indicate that the slopes are significant ($P<0.05$).

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Table 1
Integrated carbon fixation per unit chlorophyll per hour (mg C (mg chl-a)$^{-1}$ h^{-1}) in the static condition for *Gymnodinium chlorophorum*, *Heterocapsa triquetra* and *Prorocentrum micans* exposed to different radiation treatments

	Gymnodinium chlorophorum	Heterocapsa triquetra	Prorocentrum micans
PAB	148	123	282
PA	162	146	296
P	178	179	332

The values represent the area under the P *v.* E curves shown in Fig. 2.

per unit energy (units of reciprocal of irradiance) in the static samples, calculated from the integrated carbon fixation per unit chlorophyll per hour (Table 1) is shown in Fig. 4A. The highest inhibition was found in *H. triquetra* - 0.52 (Wm^{-2})$^{-1}$- and it was significantly higher ($P<0.05$) than that determined for *G. chlorophorum* - 0.22 (Wm^{-2})$^{-1}$ and *P. micans* - 0.21 (Wm^{-2})$^{-1}$ (Fig. 4A). In the rotating samples (Fig. 4B) the integrated UVR-induced inhibition of carbon fixation was clearly dependent on mixing speed in *G. chlorophorum*, increasing significantly ($P<0.05$) from 0.13 (Wm^{-2})$^{-1}$ under slow mixing to >0.6 (Wm^{-2})$^{-1}$ under fast mixing. There was also a significant inhibition ($P<0.05$) of carbon fixation in *H. triquetra* (Fig. 4B) with increasing mixing speed from ca. 0.4 to 0.6 (Wm^{-2})$^{-1}$. However, no significant effects of mixing on the integrated UVR-induced photoinhibition were found in *P. micans*. In all species, the integrated inhibition observed in the static samples (Fig. 4A) was higher than that in the mixed ones at the lowest mixing speed (Fig. 4B).

We further examined the relative contribution of the different wavebands - UV-B and UV-A – in inhibiting carbon fixation in relation to mixing speed (Fig. 5). There was a significant increase ($P<0.05$) of integrated UV-B-induced inhibition with increasing mixing speed in *G. chlorophorum* (Fig. 5A). The inhibition increased from ca. 2 (Wm^{-2})$^{-1}$ under the slowest speed of mixing (1 rotation in 3 h) to about 15 (Wm^{-2})$^{-1}$ under the fast mixing conditions (i.e., 15 rotations in 3 h; Fig. 5A). However, the integrated inhibition due to UV-B did not significantly change with increasing mixing speed in *H. triquetra* and *P. micans* (Fig. 5A). On the other hand, all species had a significant increase ($P<0.05$) of integrated UV-A-induced inhibition with increasing mixing speeds (Fig. 5B). Furthermore, *H. triquetra* had a significantly higher integrated inhibition at most of the mixing speeds as compared to the other two species, whereas *P micans* had the lowest one at all mixing speeds (Fig. 5B).

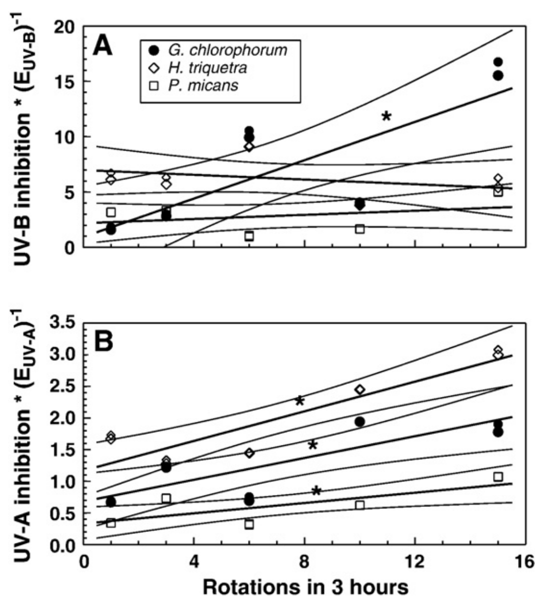

Fig. 5. Inhibition by UV-B (A) and UV-A (B) normalized to the mean irradiance (UV-B and UV-A, respectively) within the simulated UML as a function of mixing speed (rotations in 3 h) in *Gymnodinium chlorophorum*, *Heterocapsa triquetra* and *Prorocentrum micans*. The solid lines represent the linear regression and the dotted lines are the 95% confident limits; the asterisks represent that the slopes are significant ($P<0.05$).

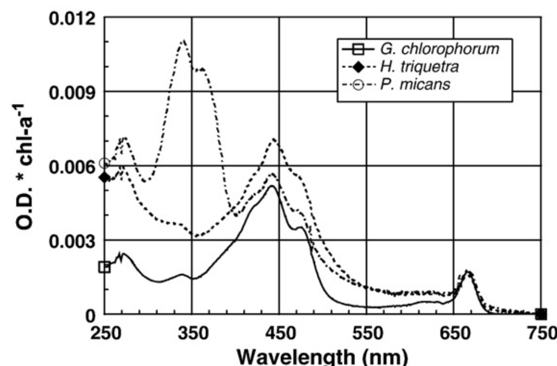

Fig. 6. Spectral absorption characteristics – optical density (O.D) normalized to chl-a as a function of wavelength in *Gymnodinium chlorophorum*, *Heterocapsa triquetra* and *Prorocentrum micans*.

3.3. UV absorbing compounds

The three species had the characteristic peaks of chlorophyll-a (at 440 and 665 nm) and carotenoids (at 470 nm). However, there were species-specific differences in the amount of UV-absorbing compounds detected in the methanol extracts (Fig. 6). For *P. micans*, peaks in the UV region were found at λ_{max}=337 and 358 nm whereas in *G. chlorophorum* and in *H. triquetra* only traces were found. Peak analyses applied to the spectra data at the beginning of experiments indicated significantly more UV-absorbing compounds in *P. micans* extracts ($P<0.05$) as compared to those in the other species.

4. Discussion

Patagonia and coastal areas of the South Atlantic Ocean are characterized by heavy winds during spring (mean daily speeds of 90 km h^{-1}) producing deep mixing of the water column and thus exposing organisms to fluctuating radiation regimes (Villafañe et al., 2004a). Also, at this time of the year the Patagonia area is under the influence of the Antarctic ozone “hole” and hence receiving enhanced UV-B radiation (Helbling et al., 2005). Consequently, and during spring, organisms are subjected to a particular climatology that may affect their performance within the ecosystem. In this study we examined the interactive effects of spring time UVR and fluctuating radiation regimes (i.e., vertical mixing as produced by wind stress) on photosynthesis and DNA of three dinoflagellate species that are normally found in Patagonian coastal waters. This taxonomic group has been chosen as previous studies have shown that they are of ecological importance in Patagonian waters (i.e., as blooming organisms and/or due to their potential toxicity) and because they increase their concentrations during spring-summer in coastal areas (Gayoso, 2001; Villafañe et al., 2004a; Gayoso and Fulco, 2006). In the following paragraphs we will discuss the role of UVR as a stress factor for the photosynthesis process (and not for the DNA molecule) under the experimental and climatological conditions investigated during this study.

During our study, UV-B-induced DNA damage in the form of CPDs was not detected after the 3 h exposures in any of the tested species. Although UV-B was enhanced by ozone depletion, UV-B levels were relatively low during the experimental period (Fig. 1B) as compared to later in the summer. This was also reflected by the low CPD accumulation in the DNA dosimeters (Fig. 1B) which was much lower (about 10 times) than previously found later in the season (Buma et al., 2001). This is expected as there is a relative increase of shorter UV-B wavelengths towards summer, thereby increasing the

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DNA effective dose. However, the lack of CPD accumulation in the dinoflagellates is in contrast with previous results obtained in Patagonia, where significant amounts of them were found (i.e., 300-600 CPDs per megabase in surface waters) in both marine (Buma et al., 2001; Helbling et al., 2001b) and freshwater phytoplankton assemblages (Villafañe et al., 2004b; Klisch et al., 2005). However, these studies were carried out during the summer, when UV-B levels were high (Helbling et al., 2005). Thus, the lack of UV-B induced DNA damage (under both static and mixed conditions) in our study may be due to the relatively low solar radiation at this time of the year (Fig. 1), in spite of ozone depletion, as compared to that in summer due to the low solar zenith angle at that time of the year. In addition, neutral density screen further attenuated the DNA effective irradiance received by the cells (i.e. from 100% to 6%). However, in contrast with the differential spectral attenuation in the water column, the use of neutral density screens resulted in identical reductions for all wavebands. Based on published attenuation coefficients for UV-B and UV-A in the area (Helbling et al., 2005) the calculated ratio UV-B to UV-A ratio at the bottom of a simulated UML (down to 6% irradiance) would be 0.36 of that at the surface. In our experiments the ratio of UV-B to UV-A at the 6% irradiance level was the same as that at the 100% irradiance, thus enhancing UV-B almost 3 times. Consequently, we conclude that DNA damage accumulation played no role in our experiments and that spring time UV-B will probably not cause significant DNA damage accumulation in phytoplankton in the water column. We did not conduct specific experiments to rule out the possibility that the lack of CPDs accumulation resulted from an effective repair that coped with the damage. However, the irradiance levels and biodosimeter data do suggest the relatively low DNA damage.

In contrast, solar UVR clearly affected the photosynthetic rates of *Gymnodinium chlorophorum* and *Heterocapsa triquetra* and in less extent in *Prorocentrum micans.* However, it should be noted that the dinoflagellates were pre-cultivated at relatively high irradiance (200 μmol photons m^{-2} s^{-1}), which has been shown to reduce UVR and high PAR effects (van de Poll et al., 2006). Nevertheless, we observed differences in UVR responses to varying mixing speeds. The mixing speeds used in our experiments varied from 1.2 to 19 cm s^{-1}, which are comparable to previous reported mixing speeds for phytoplankton in various regions (Denman and Gargett, 1983; Neale et al., 2003; Villafañe et al., 2007). We found that UVR-induced photoinhibition in *P. micans* was unaffected by mixing, whereas higher inhibition was found at fast speeds for *G. chlorophorum* and to a lesser extent *H. triquetra* (Figs. 2–5). Enhancement of UVR-induced photoinhibition due to vertical mixing has been previously observed for Southern Ocean phytoplankton (Helbling et al., 1994) whereas opposite results were obtained for tropical assemblages (Helbling et al., 2003; Villafañe et al., 2007) clearly hinting for species-specific responses of organisms. In fact, we simulated mixing for the same portion of the euphotic zone (ca. 60%, down to 14 m) and a similar solar radiation exposure occurred during all the incubations, so it is evident that *P. micans* was the most resistant species while *G. chlorophorum* was the most sensitive. Moreover, it is seen that reciprocity was maintained in *P. micans* but failed in *G. chlorophorum*. This clearly suggests that *G. chlorophorum* was not able to cope with UVR under fast mixing, and that chronic inhibition built up as the cells were more frequently exposed to excess surface irradiances (i.e. fast mixing). It should be also noted that fast mixing could result in less efficient regulatory excess irradiance responses (e.g. regulatory photoinhibition, xanthophyll cycling) that would otherwise (i.e. at lower rotation speeds) be more effective in preventing excess irradiance stress.

The higher resistance of *Prorocentrum micans* as compared to the other two species may be related to the presence of UV-absorbing compounds that were present in high concentrations (Fig. 6). UV-absorbing compounds, generally mycosporine like amino acids (MAAs) have been found to protect dinoflagellates against UVR stress (Neale et al., 1998b) with a negative relationship between the concentration of these compounds and photoinhibition. However, other studies carried out with *P. micans* (Lesser, 1996) indicated that UV-absorbing compounds provided only a partial protection against UV-B effects. We found only traces of UV-absorbing compounds in *Gymnodinium chlorophorum* and *Heterocapsa triquetra*, grown under the same irradiance conditions. It is known that the synthesis of these compounds can be triggered by high irradiances (Sinha et al., 1998). However, previous experiments carried out with *H. triquetra* showed that this species maintained low concentrations of these compounds throughout a 6-days of exposure to solar radiation, whereas *P. micans* maintained them high (Marcoval et al., 2007). One explanation is that UV-absorbing compounds are found in relatively large species, as in small-sized cells the effective concentration would be too high and osmotically disadvantageous (Garcia-Pichel, 1994). Thus the relative high amounts of UV-absorbing compounds in *P. micans* would partially explain its relatively high resistance to UVR under the mixing conditions imposed. On the other hand, the lack of these compounds may also partially explain the relatively high UVR sensitivity of *G. chlorophorum* and *H. triquetra.*

In addition to the differences in the amounts of UV-absorbing compounds, other factors may account for the variability in photosynthesis responses among the three species tested. One of these is the difference in the balance between damage/recovery ability which is also species-specific, as seen in many studies reporting a variety of effects in photosynthetic rates (De Mora et al., 2000; Helbling and Zagarese, 2003). For example, Hiriart-Baer and Smith (2005) reported high recovery rates in freshwater phytoplankton from Lake Erie after 2 h of exposure of damaging radiation. On the other hand, and while full recovery of optimal quantum yield in *Dunaliella salina* was achieved after 30 min exposures to natural solar radiation, recovery was still incomplete after 21 h when a 3 h exposure included UV-B and UV-A (Herrmann et al., 1997). Additionally, cell size has been found to play a significant role when evaluating the impact of UVR on photosynthetic inhibition, with small cells being more resistant as compared to large cells due to a fast acclimation dynamics to changes in radiation conditions (Helbling et al., 2001a). In this sense, our results apparently contrast with previous findings, as in our experiments the smallest species (i.e., *G. chlorophorum*) was the most sensitive. It should be noticed, however, that most of the experimentation conducted in the past to assess the relative importance of cell size in relation to UVR impact, was done under static conditions (i.e., no mixing). Our data show that the sensitivity of *G. chlorophorum* (the smallest species used in our experiments) increased with increasing mixing speed, clearly suggesting an impact on the photosystem (but not on the DNA) with increased frequency of high levels of solar radiation (i.e., due to an increasing number of rotations).

Overall, and given the environmental conditions found during the austral spring in Patagonia, it appears that photosynthesis, and not DNA, is the most important target for UVR in dinoflagellates. While we can not generalize on the effects of these factors to the whole community, our results suggest that a potential increase of solar UVR due to the Antarctic ozone “hole” (i.e. during early spring) will have little impact on DNA as compared to later in the season, as seen in previous studies carried out in this region during summer time (Helbling et al., 2001b; Buma et al., 2001; Villafañe et al., 2004b).

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