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Hernando, Marcelo; Malanga, Gabriela; Puntarulo, Susana; Ferreyra, Gustavo

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Research Article

Non-enzymatic antioxidant photoprotection against potential UVBR-induced damage in an Antarctic diatom (*Thalassiosira* sp.)

Marcelo Hernando[1], Gabriela Malanga[2], Susana Puntarulo[2] & Gustavo Ferreyra[3,4]
[1]Facultad de Medicina, Universidad de Morón, Cabildo 134 (1708), Buenos Aires
[2]Physical Chemistry-PRALIB, School of Pharmacy and Biochemistry
University of Buenos Aires, Junín 956, CP 1113, Buenos Aires, Argentina
[3]Institut des Sciences de la Mer (ISMER)
310 allée des Ursulines, Rimouski (Qc.), G5L 3A1, Canadá
[4]Instituto Antártico Argentino, Cerrito 1248, C1010AAZ Buenos Aires, Argentina

ABSTRACT. In January 1999, unialgal cultures of the diatom *Thalassiosira* sp., solate from natural phytoplankton assemblages from Potter Cove, Antarctica, were exposed to solar ultraviolet radiation (UVR, 280-400 nm) in order to study the long-term acclimation of this species. Ultraviolet radiation B (UVBR, 280-315 nm) inhibited the growth rate during the first and second days of exposure. No UVBR inhibition was observed on the third day. The initial content of α-tocopherol (13 pmol $(10^4 \text{ cell})^{-1}$) showed a marked decrease during the exponential growth phase (4 pmol (10^4 cell^{-1}) by day 3). The initial content of β-carotene (3 pmol $(10^4 \text{ cell})^{-1}$) did not show significant differences over time in cells exposed to UVBR. Two mycosporine-like amino acids (MAAs) were identified: porphyra-334 and shinorine. Cellular concentrations of MAAs increased significantly on days 2 and 3, and exposure of the algae to UVBR significantly enhanced this value. The relative importance of MAAs concentration was significant ($P < 0.05$) in relation to the α-tocopherol content. A positive correlation was shown between cellular MAAs concentration and growth rate. Our results suggest that photoprotection against UV-induced damage is characterized by short-term consumption of α-tocopherol and longer-term synthesis of MAAs. The UVBR damage/repair ratio during long-term exposure involves the combined action of several endogenous factors within the cell, with MAAs synthesis being the most effective factor related to photoprotection.

Keywords: β-carotene, α-tocopherol, growth rate, diatoms, MAAs, photoprotection, Antarctica.

Fotoprotección antioxidante no-enzimática contra el potencial daño inducido por UVBR en una diatomea antártica (*Thalassiosira* sp.)

RESUMEN. Durante enero 1999, cultivos unialgales de una diatomea *Thalassiosira* sp., aislada de poblaciones fitoplanctónicas naturales de Caleta Carlos Potter, Antártida, fueron expuestos a radiación solar ultravioleta (UVR, 280-400 nm) con el objetivo de estudiar su aclimatación a largo plazo. La radiación ultravioleta B (UVBR, 280-315 nm) inhibió la tasa de crecimiento durante el primer y segundo día de exposición. No se observó inhibición por UVBR durante el tercer día. El contenido inicial de α-tocopherol (13 pmol $(10^4 \text{ cel})^{-1}$) mostró una marcada disminución durante la fase de crecimiento exponencial (4 pmol $(10^4 \text{ cel})^{-1}$ al día 3). El contenido inicial de β-caroteno (3 pmol $(10^4 \text{ cel})^{-1}$) no mostró diferencias significativas entre días en células expuestas a UVBR. Se identificaron dos aminoácidos parecidos a micosporina (MAAs), porfira-334 y shinorina. Las concentraciones celulares de MAAs mostraron aumentos significativos en los días 2 y 3, que resultaron mayores cuando las algas fueron expuestas a UVBR. La importancia relativa de la concentración de MAAs fue significativa ($P < 0.05$) en relación al contenido de α-tocopherol. Se encontró una relación positiva entre la concentración celular de MAAs y la tasa de crecimiento. Estos resultados sugieren que la fotoprotección contra el daño inducido por UV está caracterizado por un consumo a corto plazo de α-tocopherol y una síntesis de MAAs a largo plazo. El balance UVBR daño/reparación a largo plazo, compromete la acción combinada de varios factores celulares endógenos, siendo la síntesis de MAAs el factor más efectivo relacionado con la fotoprotección.

Palabras clave: β-caroteno, α-tocoferol, tasa de crecimiento, diatomeas, MAAs, fotoprotección, Antártida.

Corresponding author: Marcelo Hernando (mphernando09@hotmail.com)

INTRODUCTION

Increased levels of UVBR reach the Earth's surface, particularly in Polar region, due to the anthropogenic depletion of the stratospheric ozone layer (Frederick & Lubin, 1988; Madronich, 1993). The identification of repair and protective mechanisms that may allow Antarctic phytoplankton species to survive and reproduce under natural conditions of ultraviolet radiation (UVR, 280-400 nm) and particularly under increased ultraviolet-B radiation (UVBR, 280-315 nm) flux scenarios (Lubin *et al*., 1989) is a critical issue. UVR can cause significant damage to a variety of cell targets. However, growth and biomass accumulation will result from the complex interactions between the harmful direct and indirect effects of UVR, and a series of counteracting repair mechanisms (Lesser *et al.*, 1994; Bischof *et al.*, 2000).

One of the main difficulties of evaluating the potential ecological impact of increased UVBR on natural phytoplankton is that UVR sensitivity is species specific (Hannach & Sigleo, 1998; Hernando & San Román, 1999; Hernando *et al*., 2006). Prymnesiophytes and dinoflagellates are often considered more UVR tolerant groups (Neale *et al*., 1998a). Diatoms, which in polar region often dominate algal blooms mainly in spring, show a wide range of responses to UVR exposure. Furthermore, centric diatoms seem to be more UVR-resistant than pennate diatoms, after a suitable acclimation period (Helbling *et al*., 1996). Acclimation rates vary with species (Hannach & Sigleo, 1998), but also with environmental conditions, such as the light history of the cells (Neale *et al.*, 1998b), temperature (Roos & Vincent, 1998) and nutrient availability (Döhler, 1997). These factors may affect the rate of synthesis of photoprotective compounds as well as the responses of other UVR protective strategies (Roy, 2000).

UVR has been shown to be very effective in inducing lipid peroxidation of biological membranes (Takeuchi *et al.*, 1995; Hideg & Vass, 1996), polyunsaturated fatty acids (Yamashoji *et al.*, 1979) and phospholipid liposomes (Pelle *et al.*, 1990). Moreover, UVBR can destroy the natural lipid soluble antioxidants and promote the formation of lipid peroxidation products (Salmon *et al.*, 1990; Malanga & Puntarulo 1995; Estevez *et al.*, 2001). On the other hand, several biological effects of UVBR involve endogenous photosensitization and the formation of reactive oxygen species (Martin & Burch, 1990). There are a variety of sensitizers within cells which absorb UVBR. Interaction between excited sensitizers and triplet oxygen, produces active oxygen stressor intermediates consisting of singlet oxygen, super-oxide radical, hydroxyl radical and hydrogen peroxide (Ichiki *et al.*, 1994). One of the possible mechanisms that could counteract the damage caused by UVBR induced oxidative stress is the synthesis of both enzymatic and non-enzymatic antioxidants (Davidson, 1998; Niyogi, 1999). The non-enzymatic antioxidants are generally small molecules, such as ascorbate and glutathione acting in the aqueous phase, whereas the lipophilic antioxidants (such as α-tocopherol and β-carotene) are active in the membrane environment. The hydrophilic ascorbate and the lipophilic tocopherol might be effective in modulating tissue injury by predominantly reacting chemically with free radicals (e.g., alkoxyl and peroxyl radicals) whereas the predominant photoreaction of the lipophilic ß-carotene might be the scavenging of excited species such as singlet oxygen and triplet states. Thus, the antioxidant action of these compounds *in vivo* is different qualitatively and quantitatively and depends on the type of photo-oxidative stress imposed (Fuchs, 1998).

The negative effects of UVR are also minimized through the presence of screening compounds. The most studied of these compounds are mycosporine-like amino acids (MAAs), which have been found in many marine and freshwater algae (Karentz *et al.*, 1991; Banaszak, 2003). MAAs have been shown to be an effective protection mechanism (Neale *et al*., 1998a), and their synthesis was reported for both, natural populations and cultures of phytoplankton (Helbling *et al.*, 1996, Zudaire & Roy, 2001; Hernando *et al*., 2002; Callone *et al*., 2006). However, the synthesis of MAAs is not a general response, and several species do not show an increase in the content of these compounds even after several weeks of exposure to increased UVR (Villafañe *et al.*, 2000).

The aim of this work was to study the antioxidant and photoprotective responses of the Antarctic diatom (*Thalassiosira* sp.) to UVBR, after exposure to surface solar radiation under culture conditions. The relative contribution to the total protection capacity of MAAs, α-tocopherol and β-carotene was analyzed.

MATERIALS AND METHODS

Experimental design

The experiments were carried out in 1999 at Potter Cove (South Shetland Islands, Antarctica, 62°14'S, 58°38'W) (Fig. 1) during the summer (January). Surface water samples were collected with a five-liter Niskin bottle and maintained in the laboratory at 2°C. *Thalassiosira* sp. single cells were isolated with micropipettes using an inverted microscope, and

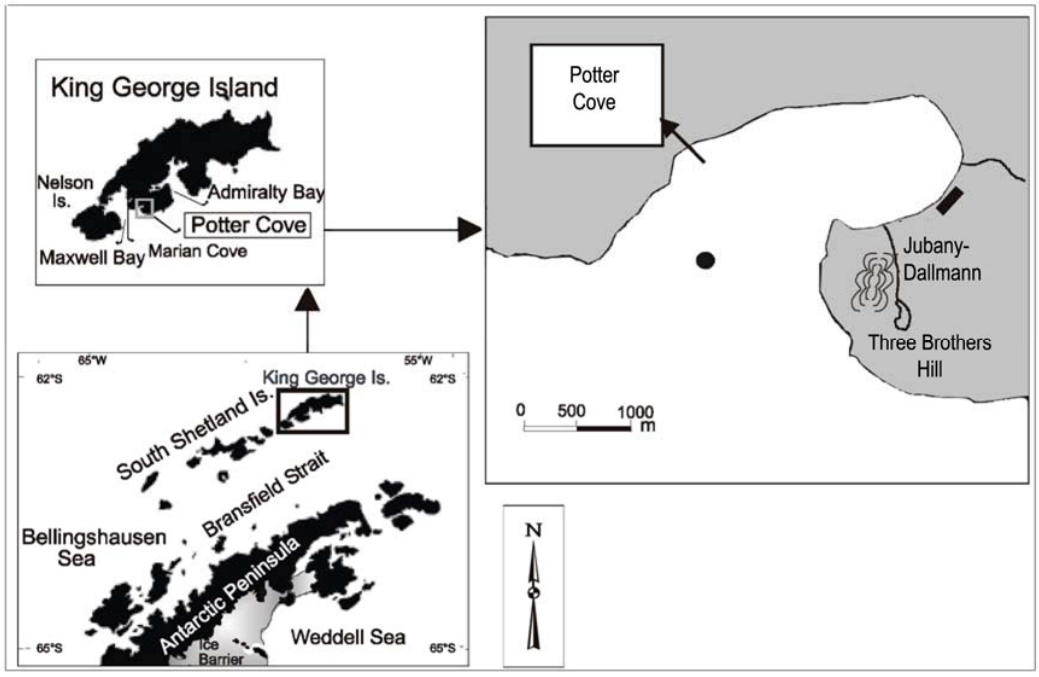

Figure 1. Map showing the location of Potter Cove (62º14´S, 58º38´W) in relation to King George Island and the Antarctic Peninsula. The filled circle indicates the sampling site. Taken from Schloss (1997).

Figura 1. Mapa mostrando la ubicación de la Caleta Potter (62º14'S, 58º38'W), Isla Rey Jorge y la Península Antártica. El círculo negro indica el sitio de muestreo. Tomado de Schloss (1997).

inoculated into 200 mL flasks with 0.7 µm filtered seawater plus sterile F/2 culture medium (10 mL L^{-1} of seawater) (Guillard, 1975). Cultures were grown at 210 µmol m^{-2} s^{-1} Photosynthetically Available Radiation (PAR, 400-700 nm) and exposed to ´cool-white´ fluorescent tubes on 15/9 h light/dark periods at 3ºC. Prior to the experiments and once exponential growth was reached, aliquots of the mono-specific culture were inoculated into a series of 1000 mL vessels covered with a Mylar foil (DuPont, which has 50% transmission at 316 nm), allowing the samples to receive only ultraviolet A radiation (UVAR, 315-400 nm) and PAR. The algae were pre-adapted to solar radiation in an outdoor water bath with flowing seawater maintaining a temperature between 1 to 2ºC for 10 days, from December 26, 1998 to January 4, 1999. After this period, the cultures were transferred to six 1000 mL quartz vessels and exposed to two natural irradiance treatments: without UVB (PAR+UVAR) and with UVB (PAR+UVAR +UVBR). For the without UVB treatment, the bottles were covered with Mylar foil (Hernando & San Román, 1999). Three replicate samples were used for each treatment. The algae were exposed to solar radiation in an outdoors water bath with flowing seawater to maintain the temperature between 1-2ºC. The initial concentrations of cells were on the same order of magnitude as those reported previously (Zudaire & Roy, 2001; Leu *et al*., 2006; Marcoval *et al*., 2007). The sterile culture medium was added to the different treatments at time 0 (t_0) of the experiments and after 3 days of exposure. Sub-samples of 80 mL were taken daily at 09:00 AM to determine chlorophyll-*a* (Chl-*a*) concentrations, MAAs, cell number and the content of α-tocopherol and β-carotene.

Determination of Chl-*a* and cell counting

For Chl-*a* analyses, 20 mL water were filtered in to Whatman fiberglass filters (GF/F, 25 mm) followed by extraction of the pigments in absolute methanol (Holm-Hansen & Riemann, 1978). Sub-samples for cell counts were kept in dark bottles and fixed with formalin previously neutralized with sodium borate (final concentration 0.4% w/v). Cells were enumerated with a microscope using a Sedgwick-Rafter counting slide according to Villafañe & Reid (1995).

Irradiance measurements

Incident solar radiation was monitored continuously during the experiment using a spectroradiometer (model GUV 510, Biospherical Instruments, Inc.),

which records irradiances at four wavelengths in the ultraviolet region (305, 320, 340 and 380 nm), and PAR. Data were recorded every minute at a site located close to the experimental setup. The equation from Orce & Helbling (1997) was used for calculating UVBR and UVAR doses expressed in kJ m^{-2}.

Growth measurements

Cell instantaneous growth rate was determined according to the following equation,

$$\mu = \ln (N_1/N_{n-1}) /t_1$$

where μ is a specific rate constant (day^{-1}), N_1 is the cell concentration at the time of the measurement, N_{n-1} is the cell concentration at the previous time period, and t_1 is the time (days) between both measurements.

Lipid soluble antioxidants

For identification of antioxidants 20 mL samples were taken daily and filtered onto Whatman GF/F filters and frozen (-20°C) until the time of analysis. The content of α-tocopherol and β-carotene in the cell homogenates was quantified by reverse-phase HPLC with electrochemical detection using a Bioanalytical Systems LC-4C amperometric detector with a glassy carbon working electrode at an applied oxidation potential of 0.6 V (Desai, 1984). Extraction from the samples was performed with 1 mL of methanol and 4 mL of hexane. After centrifugation at 600 g for 10 min, the hexane phase was removed and evaporated to dryness under N_2. Extracts were dissolved in methanol:ethanol (1:1 v/v) and injected for HPLC analysis: d, l- α-tocopherol from synthetic phytol (Sigma) and β-carotene were used as standards.

MAAs measurements

For identification of MAAs, 10-30 mL sub-samples were filtered onto Whatman GF/F filters and frozen (-20°C). Filters were extracted with a Vibra Cell probe sonicator (Sonics & Materials; 1 min, 100 W pulse mode, 0ºC) into 4 mL 100% HPLC grade methanol. The extracts were filtered (Whatman GF/F) and dried using a centrifugal vacuum evaporator (Centrivap, Labconco, Co.). The residue was redissolved in 500 µL water and vortexed for 30 s. After passing through a 100 kDalton ultrafilter (Ultraspin™), samples were analysed by HPLC according to the method of Carreto *et al*. (2002). Briefly, the individual MAAs were separated by reverse phase gradient elution on an Alltima (Alltech) C_{18}, 5 µm column (4.6 mm i.d.×150 mm length) protected by its own Alltech guard column (4.6 mm i.d.×20 mm length) and incubated to 30°C. The gradient included an initial isocratic hold of 8 min with 100% Solvent A (0.2% acetic acid) followed by a 12 min linear gradient from 0 to 40% of Solvent B (methanol: acetonitrile: 0.2% acetic acid, 25:25:50) at a flow rate of 1.0 mL min^{-1}. Individual peaks were identified by their online absorption spectra, retention time and co-chromatography with authentic standards isolated from the red algae *Porphyra* sp.

For quantification of MAAs, three daily replicate sub-samples were extracted for two hours in 7 mL of methanol at 8ºC. After extraction, samples were centrifuged and the supernatant was scanned from 250 to 750 nm with a Shimadzu (model UV-1203) spectrophotometer. For each spectrum, a peak analysis was performed using appropriate software to determine the height of the peak in the UVR region. Considering that both MAAs (Shinorine and Porphyra-334) showed a maximum optical density (OD) at 334 nm (Takano *et al*., 1979; Tsujino *et al*., 1980) this wavelength was selected from quantification of the UV-absorbing compounds. The concentration of MAAs was expressed as the peak height at 334 nm (in mm) per cell concentration, according to Dunlap *et al*. (1995).

Statistical analyses

Repeated measures ANOVA (RMANOVA) were performed (Statistica, version 5.1) to determine the significance of the differences observed for the abundance, cell number, Chl-*a*, growth rate, α-tocopherol and β-carotene in each treatment. Normality was checked using a one-sample Kolmogorov-Smirnov test, whereas the sphericity assumption that concerns variance homogeneity was checked using Mauchley´s test. When such requirements were not satisfied, a standard transformation of the data was applied (1/square root on α-tocopherol and β-carotene data). The days of exposure and the treatment were the factors considered. A Tukey test was additionally done to determine the differences in each factor. When interaction was significant or the assumptions of sphericity were not satisfied, a one factor ANOVA was performed (Scheiner & Gurevitch, 2001). Multiple regression was used to study the relationship between α-tocopherol and MAAs (independent variables) and the growth rate (dependent variable) of *Thalassiosira* sp.

RESULTS

The surface daily doses of UVBR, UVAR and PAR both during the study and the pre-exposure period, showed variation (Orce & Helbling, 1997) (Fig. 2). During the period of exponential growth, the average daily doses from January 4 to 8, 1998 were: 49, 1110,

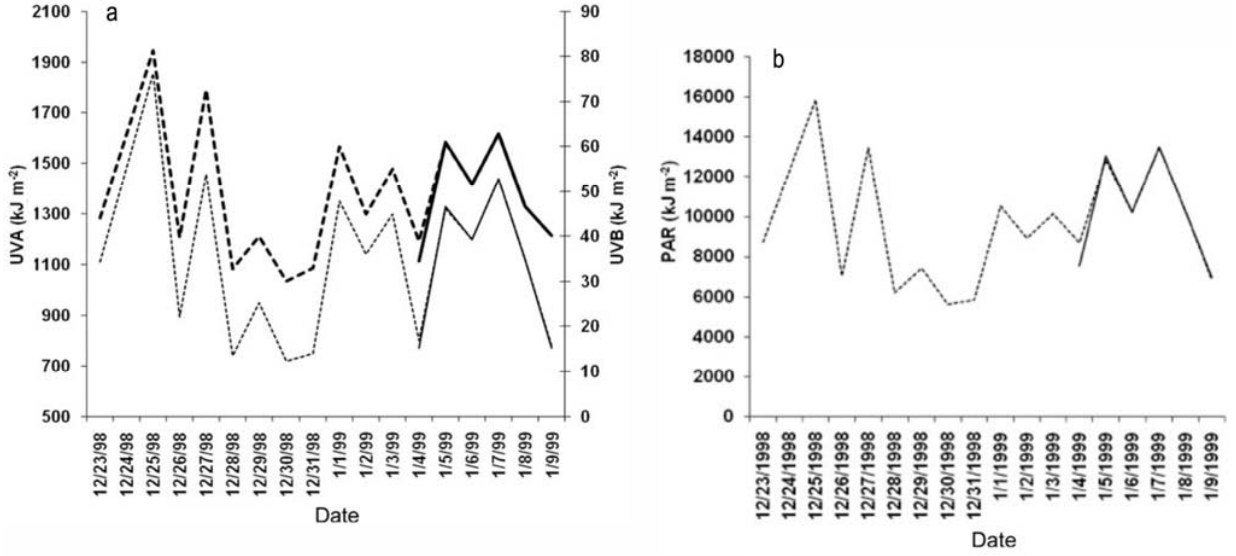

Figure 2. Variations in UVR doses and PAR at Potter Cove from December 23, 1998 to January 9, 1999. a) UVBR (280-315 nm) and UVAR (320-400 nm) in kJ m^{-2}, b) PAR doses (400-700 nm), in kJ m^{-2}. The line (—) shows total UVAR, (--) shows data of UVAR under de experimental conditions (experimental doses at the first day are lower than total doses considering that the experiment started at 09:00 AM), (▬) shows total UVBR and (▪▪▪) shows data of UVBR under de experimental conditions.

Figura 2. Variaciones en la dosis de radiación UV en la Caleta Potter, del 23 de diciembre 1998 al 9 de enero de 1999. a) UVBR (280 a 315 nm) y UVAR (320-400 nm), en kJ m^{-2}, b) dosis PAR (400-700 nm), en kJ m^{-2}. La linea (—) muestra la radiación UVA total, (----) muestra los datos de UVAR en condiciones experimentales (las dosis experimentales durante el dia inicial son menores a las dosis totales ya que el experimento se inicia a las 09:00 AM), (▬) muestra la radiación UVB total y (▪▪▪) muestra los datos bajo condiciones experimentales.

and 10300 kJ m^{-2} for UVBR, UVAR, and PAR, respectively. The ozone concentration showed an average value of 316 ± 10 D.U. The data variability was primarily due to changes in cloud cover.

After the second day of acclimation to exposure to radiation without UVB, the cell number of the culture was significantly increased, and this increase continued up to day 5 (Fig. 3a). In contrast, biomass accumulation in the UVB treatment started after a third-day and continued until day 5. The UVB treatment showed significant differences with the without UVB treatment during days 2-4. Cell numbers in the without UVB treatments were 45 and 27% higher than in the UVB treatment by day 2 and 4, respectively (Fig. 3a). Chl-*a* content in the cultures showed the same profile (Fig. 3a inset).

During the first two days of exposure, growth rates of the cells subjected to UVB exposure were significantly inhibited as compared to the cells without UVB treatment (Fig. 3b). At day 3, the growth rate in cells exposed to UVB treatment was significantly higher than those grown without UVB exposure. No significant differences in growth were observed between treatments from day 4 to the end of the study.

The content of α-tocopherol showed a significant decrease as compared to the beginning of the experiment over the studied period, both after exposure to UVB radiation and without UVB treatment. However, the values were significantly lower than those recorded after exposure to radiation without UVB at day 5 (Fig. 4a). When UVBR was excluded, the content of α-tocopherol reached maximum values of approximately 8 pmol $(10^4 \text{ cell})^{-1}$ (Fig. 4a). No significant differences were observed between days in the content of β-carotene on algae exposed to UVBR or to radiation without UVB (Fig. 4b). The content of β-carotene reached a maximum value of approximately 4 pmol $(10^4 \text{ cells})^{-1}$ (Fig. 4b).

The chromatogram of the cell extract of the diatom *Thalassiosira* sp. showed a simple MAAs profile (Fig. 5). Although diatom species generally contain low concentrations of MAAs, some species are reported to lack MAAs (Riegger & Robinson, 1997; Jeffrey *et al.*, 1999; Laurion *et al.*, 2003). In coincidence with previous reports (Riegger & Robinson, 1997; Hernando *et al.*, 2002) *Thalassiosira* sp. showed high amounts of two MAAs, porphyra-334 and shinorine. The concentration of porphyra-334 exceeded that of

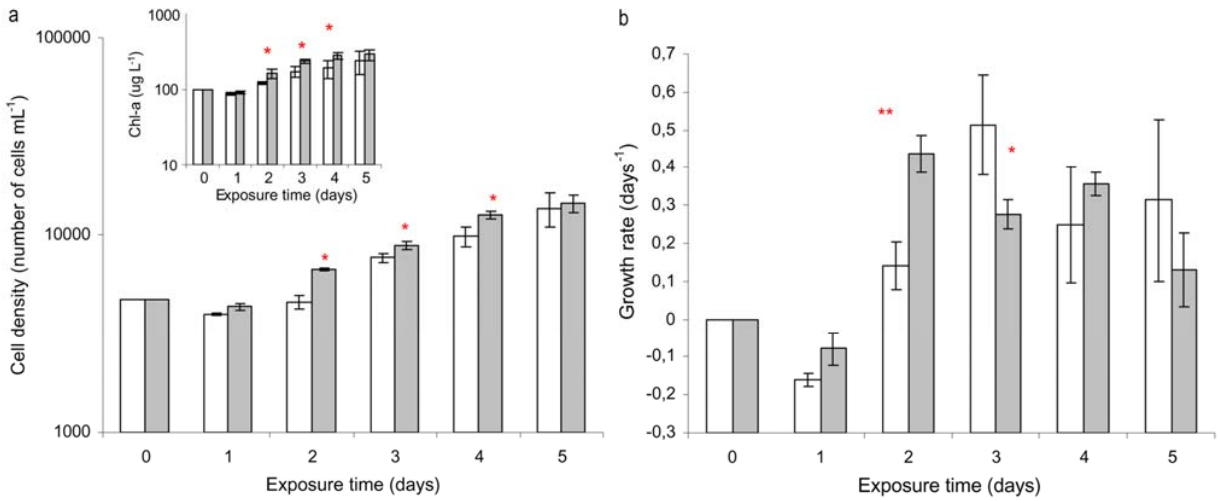

Figure 3. Time course evolution of the culture assessed as: a) cellular density expressed as number of cells mL^{-1}. Inset: time course evolution of the culture assessed as Chl-*a* biomass expressed as ug L^{-1}, b) Exponential growth rate of *Thalassiosira* sp. exposed to natural solar radiation over 5 days. Open bars (□) represent the parameters in algae cultures exposed to the UVB treatment, and dark bars (▒) represent the values in cells exposed to the without UVB treatment. Vertical bars represent the mean ± standard deviation. * Significantly different from growth rate at the same day. Tuckey Test, $P < 0.05$. ** Significantly different from growth rate at the same day. Tuckey Test, $P < 0.01$.

Figura 3. Secuencia temporal del cultivo celular expresado en: a) la densidad celular como número de células mL^{-1}. Inserto: secuencia temporal del cultivo celular evaluado como biomasa por Chl-*a* expresado en ug L^{-1}, b) Tasa de crecimiento exponencial de *Thalassiosira* sp., expuesta a la radiación solar natural durante 5 días. Las barras abiertas (□) representan los parámetros en cultivos de algas expuestas al tratamiento UVB, y las barras oscuras (▒) representan los valores en células expuestas al tratamiento sin UVB. Las líneas verticales representan la media ± desviación estándar. * Significativamente diferente para la tasa de crecimiento en el mismo día. Test de Tuckey, $P < 0{,}01$).** Significativamente diferente para la tasa de crecimiento en el mismo día. Test de Tuckey, $P < 0{,}01$.

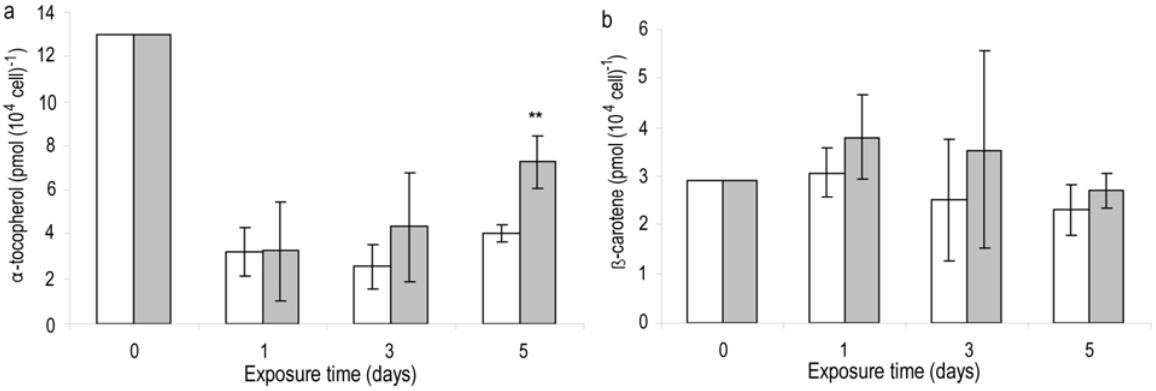

Figure 4. Effect of UVBR on content of lipid soluble antioxidants as a function of exposure time: a) α-tocopherol, b) ß-carotene. Open bars (□) represent the parameters in algae cultures exposed to the UVB treatment, and dark bars (▒) represent the values in algae cells exposed to the without UVB treatment. Vertical bars represent the mean ± standard deviation. ** Significantly different from growth rate at the same day. Tuckey Test, $P < 0.01$.

Figura 4. Efecto de la UVBR sobre el contenido de antioxidantes liposolubles en función del tiempo de exposición: a) α-tocoferol, b) ß-caroteno. Las barras abiertas (□) representan los parámetros en cultivos de algas expuestas al tratamiento UVB, y las barras oscuras (▒) representan los valores en células de algas expuestas al tratamiento sin UVB. Las líneas verticales representan la media ± desviación estándar. ** Significativamente diferente para la tasa de crecimiento en el mismo día. Test de Tuckey, $P < 0{,}01$.

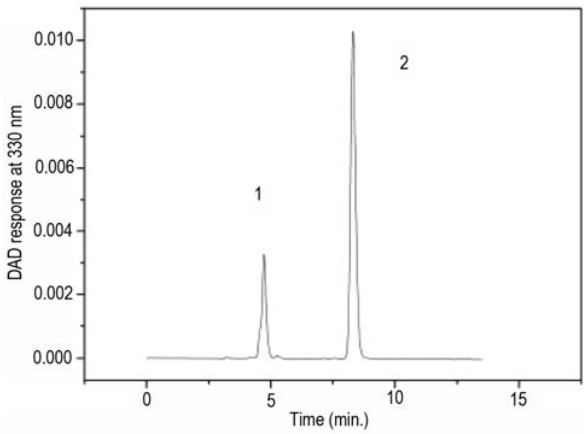

Figure 5. Typical chromatogram of cell extracts of the diatom *Thalassiosira* sp. Peak 1 corresponds to shinorine, and peak 2 to porphyra-334. Pigments identification was performed by diode array detection (DAD) at λ = 334 nm.

Figura 5. Cromatograma típico de extractos celulares de la diatomea *Thalassiosira* sp. Máximo de elución 1 corresponde a shinorina, y el máximo de elución 2 a porphyra-334. La identificación de los pigmentos fue realizada vía detección por arreglo de diodos a λ = 334 nm.

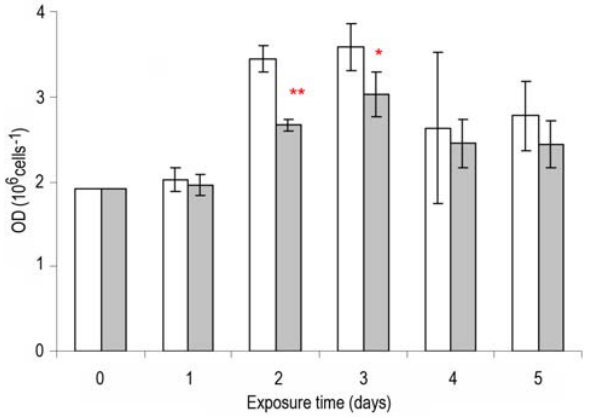

Figure 6. Concentration of MAAs after radiation treatments, normalized to total cell numbers. Open bars (□) represent the parameters in algae cultures exposed to the UVB treatment, and dark bars (▒) represent the values in algae cells exposed to the without UVB treatment. Vertical bars represent the mean ± standard deviation. * Significantly different from growth rate at the same day. Tuckey Test, $P < 0.05$. ** Significantly different from growth rate at the same day. Tuckey Test, $P < 0.01$.

Figura 6. Concentración de MAAs, normalizada por el número de células totales frente los tratamientos de radiación. Las barras abiertas (□) representan los parámetros en cultivos de algas expuestas al tratamiento UVB, y las barras oscuras (▒) representan los valores en células de algas expuestas al tratamiento sin UVB. Las líneas verticales representan la media ± desviación estándar. * Significativamente diferente para la tasa de crecimiento en el mismo día. Test de Tuckey, $P < 0,05$. ** Significativamente diferente para la tasa de crecimiento en el mismo día. Test de Tuckey, $P < 0,01$.

shinorine by approximately 9 times. The cellular concentration of MAAs (estimated spectrophotometrically as OD $(10^6 \text{ cells})^{-1}$) showed a significant increase as compared to control values until day 3. Upon day 2 and 3 the content of MAAs in algae exposed to UVB treatment was higher than in the cells subjected to treatments without UVB ($P < 0.01$) (Fig. 6).

Considering the relationship between α-tocopherol and MAAs and the growth rate, the regression coefficients corresponding to the UVB treatment were markedly different ($P < 0.05$). MAAs presented significant partial correlation coefficients ($P < 0.05$), while no significance was observed for α-tocopherol.

On the other hand, no significant correlations were found between both defense compounds and growth rate for cells exposed to radiation without UVB ($P > 0.05$), but a significant regression for MAA was observed.

DISCUSSION

The results presented here suggest the presence of a combined action against UV-induced damage, characterized by a short-term consumption of α-tocopherol and a long-term synthesis of MAAs. UVR impact on the phytoplankton community depends on the radiation levels to which algae are exposed, their specific tolerance and their ability to reduce the damage (Roy, 2000). There are several mechanisms that act over longer periods of time (i.e., days, weeks) that allow phytoplankton to acclimate, and thus minimize the negative effects caused by the exposure to those short wavelengths (Roy, 2000; Banaszak, 2003). Long-term acclimation mechanisms to UVR of phytoplankton cells essentially include physiological adaptations, such as the synthesis of protective UV-absorbing compounds (Helbling *et al*., 1996; Zudaire & Roy, 2001). Our data on *Thalassiosira* sp. behavior are in agreement with previous field observations made in Potter Cove (Ferreyra *et al.*, 1998), which showed a significant positive correlation between UVBR and MAAs concentration Hernando *et al*. (2002) showed the algae

capacity to synthesize photoprotective compounds in a time scale (hours), which reasonably fits the time scale of changes in irradiance in Antarctica.

The data presented here indicated that MAAs concentration per cell of *Thalassiosira* sp. was significantly higher in the UVB treatment on day 3 (Fig. 6a), when growth inhibition was not observed. Accordingly, Leu *et al.* (2006) showed an increase in the ratio of the photoprotective pigments diadinoxanthin and diatoxanthin/Chl-*a* content during days 0 to 7 in an Arctic diatom *Thalassiosira antarctica* var. *borealis* exposed to UV radiation. In our study, a significant decrease was observed in the MAA concentration by days 4 and 5, however, there was no growth inhibition throughout the time of exposure. The reasons for the strong reduction in MAAs are not clear but may be related to the energetic cost required for the long-term synthesis of these compounds, reducing the energy available for growth and cell division (Zudaire & Roy, 2001; Hernando *et al.*, 2002). MAAs seems to play a significant role in the photoprotection of Antarctic phytoplankton since *Thalassiosira* sp. cultures showed an initial period of growth inhibition and the rate of synthesis of MAAs (principally *Porphyra*-334) was detectable in all the exposure regimes (with UVBR, or without UVBR or all the irradiance spectrum) in the short-term experiment (12 h) and during the 2 initial days of the long-term experiment (Hernando *et al.*, 2002).

The diatom *Thalassiosira* sp. is a commonly occurring marine Antarctic diatom which may occasionally bloom during early summer, thus this species would be able to naturally cope with UVBR generating defense pigments in the time scale of hours-days, which fits reasonably with the time scale of changes in irradiance and vertical mixing. These data support previous field observations made in Potter Cove (Ferreyra *et al*., 1998), which show a significant positive correlation between UVB irradiance and MAAs concentration present in the natural phytoplankton community within the time scale of a day. In our study, the decrease in Chl-*a* content after UVB exposure (Fig. 3a, insert) is consistent with the hypothesis that under UVBR stress conditions, there is an active down regulation of photosynthesis in the cells for achieving protection (Malanga *et al.*, 1997). PSII is one of the main targets likely to be affected by UVR (Melis *et al*., 1992), and several sites might be susceptible to damage (Bumann & Oesterhelt, 1995) in association to the D1 protein, which connects the primary electron donors with the active PSII receptors. Leu *et al.* (2006) reported a decrease in optimum quantum yield of PSII between day 6 and 15 in the Arctic diatom *Thalassiosira antarctica* var. *borealis,* exposed to high UV conditions. The degradation of D1 protein and therefore degradation of chlorophyll would produce nitrogen released that could then be used to synthesize MAAs. Phytoplankton repair UV-induced damage using several mechanisms, many of which involve N_2-requiring enzymes (Roy, 2000). The Biological Weighting Functions (BWFs) predict that increased UVBR due to ozone depletion would cause a more than 1.5-fold greater additional inhibition of N_2-limited compared to nutrient-sufficient estuarine dinoflagellates and *Akashiwo sanguinea* (= *Gymnodinium sanguineum*) and *Gymnodinium* (= *Gyrodinium*) cf. *instriatum* (Litchman *et al*., 2002). The importance of N_2 availability to many mechanisms of defense and repair of UV effects is noteworthy due to the widespread N_2 limitation is in marine waters (Falkowski & Raven, 1997).

UVBR exposure has been demonstrated to stimulate the generation of reactive oxygen species that could be responsible for the effects of UVBR on cell membranes (Predieri *et al.,* 1995). The connection between oxidative damage on the one hand, and antioxidant defense mechanisms on the other, has been described in both, animal and plant cells (Kingston-Smith & Foyer, 2000). Consequently, in our study, lipid soluble antioxidant content was evaluated. No significant differences were observed between days in the content of β-carotene in *Thalassiosira* sp. exposed to radiation without UVB (UVAR+PAR). The content of the β-carotene in the without UVB treatment was lower than the α-tocopherol content tested. Besides, it did not show any differences between days or treatments ($P > 0.05$). However, a significant decrease on the content of α-tocopherol in the UVB exposed cells during the entire experiment was observed. The decrease in α-tocopherol content as compared to the initial day of exposure, suggests an active generation of active oxidant species during the culture growth, leading to the consumption of the antioxidant. This observation could explain the minimum reduction on the growth rate as the result of a large decrease of the α-tocopherol content in *Thalassiosira* sp. cells. In addition, the growth rate was significantly higher during day 3 in algae exposed to UVBR, as compared to time 0 values, suggesting that cells were able to cope with UVBR damage, and that the only significant effect was the delay in the timing of the triggering of the exponential phase of growth. Similar acclimation to UV radiation was reported in *Thalassiosira antarctica* var. *borealis* after 15 days of exposure to

UVBR (Leu *et al*., 2006); as well as in a Chlorophyte from Antarctica (after 14 days) (Lesser *et al*., 2002).

However, the simultaneous action of other defense mechanisms may help the cells cope with UVR damage, like the synthesis of MAAs. Zudaire & Roy (2001) postulated that the long-term response of Antarctic *Thalassiosira weissflogii* to UVR involves both a short-term activation of the xanthophyll cycle followed by a long-term synthesis of MAAs. It cannot be ruled out the contribution of other protecting mechanisms such as an active xanthophyll cycle or self-shading as the cultures grew. In fact, the xanthophylls cycle has been shown to be an effective protective mechanism in the diatom *Thalassiossira weisflogi* (Zudaire & Roy, 2001). Considering the possible effect of self-shading (Garcia-Pichel, 1994), the growth curves had a plateau after the exponential growth. At this point (i.e., end of the exponential growth) self-shading might have a role in protecting the cells as was seen in studies carried out with the cyanobacterium *Arthorspira platensis* (Wu *et al*., 2005). Nevertheless, it has been showed that for diatom concentrations of approximately 10^5 cells mL^{-1}, the self-shading mechanism had an insignificant role (Marcoval *et al*., 2007). The cellular concentration in the study presented here was approximately 8×10^2 cells mL^{-1}, and the microalgae biovolume was close to those values reported by Marcoval *et al*. (2007).

CONCLUSIONS

Climate warming results in a decrease in summer sea ice in the polar regions, including the coastal regions of King George Island (Serreze *et al*., 2007; Schloss *et al*., 2008), which will alter water column stratification and mixed layer depth in Antarctica (Finkel *et al*., 2010). This effect alters the environmental conditions that influence phytoplankton in the coastal regions (Irwin & Finkel, 2008). To improve predictions of recent changes on the water column stratification in the area of the West Antarctic Peninsula, plankton physiologists needs to determine how to quantify the key physiological responses of major bloom forming species. For Potter Cove, Schloss & Ferreyra (2002) suggested that during low wind conditions (wind speed < 4 m s^{-1}) the strong input of freshwater frequently stabilizes the water column, thus increasing the residence time of cells within the first few meters (5-10 m). Increased vertical stratification and reduction of the vertical mixed layer extension in Antarctic coastal regions are anticipated to select species that are less susceptible to photodamage, with higher rates of repair, and/or other strategies to deal with high light stress (Finkel *et al.,* 2010). The results seem to indicate that if phytoplankton remains for a sufficiently long period in a shallow upper mixed layer, the cellular concentrations of MAAs should be high and chlorophyll low. Consequently, these cells will be more photoprotected against UVBR than those present deeper in the water column. Such cells, when exposed to mixing, will receive higher irradiances when transported to the surface and might be severely inhibited by UVR. While the capacity for acclimation seems high, sudden light exposure after a long period of low light appears to affect quality more than quantity, with PAR having high impact on these changes too (Leu *et al*., 2006).

A significant result from this work is that UVBR-induced damage affected the triggering of exponential growth, but neither affects the final biomass accumulation nor the growth rate during the exponential phase. Overall, our results support the idea that the UVBR damage/repair balance involves the combined action of several endogenous cellular factors within the cell, with MAAs synthesis being the most effective factor related to photoprotection in association with an initial consumption of α-tocopherol, for the particular case of the studied species.

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