Revista Chilena de Historia Natural
74:293-311, 2001

Ecophysiological strategies in response to UV-B radiation stress in cultures of temperate microalgae isolated from the Pacific coast of South America

Estrategias ecofisiológicas en respuesta a la radiación ultravioleta-B en cultivos de microalgas templadas aisladas de la costa sudamericana del Pacífico

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ABSTRACT

Marine microalgae exposed to ultraviolet radiation (UV) have complex adaptive responses provided by a series of protection and repair mechanisms. Interspecific differences in UV sensibility could result in differential selection of the more tolerant species, having consequences for the structure of phytoplankton assemblages. The relative importance of protection and photorepair mechanisms of microalgal cells exposed to potential UV-B stress was studied in monocultures with different taxonomic, ecological and size characteristics obtained from the Chilean coast. Differences in photosynthesis and growth rates were predicted, since the ability to effectively acclimate to UV is not universal between microalgal species. The dinoflagellate *Alexandrium catenella* Whedon et Kofoid Balech, the diatom *Phaeodactylum tricornutum* Bohlin, the chrysophyte *Aureococcus* sp. and the cyanobacterium *Spirulina subsalsa* Oersted were acclimated during exponential cell growth under PAR + UV-A radiation (365 nm, 140-240 kJ $m^{-2} d^{-1}$) and thereafter exposed 2 h d^{-1} to high and low UV-B radiation (312 nm, maximum 3.1 kJ $m^{-2} d^{-1}$) at the center of the 16 h light period. Measured parameters were growth rates (μ), in vivo spectral absorption, cellular fluorescence capacity, pigment concentration, photosynthesis and photoreactivation during three cycles in controls and treatment samples. Growth rates diminished less than 35 % in *Phaeodactylum* and *Aureococcus* compared to 80-100 % decrease in *Alexandrium* and *Spirulina*. In these two last species, a significant increase in UV absorbing substances was observed, probably related to the presence of mycosporine-like aminoacids (MAAs) and scytonemin, respectively, and also lower photoreactivation efficiency compared to *Phaeodactylum* and *Aureococcus*. The analysis of photosynthetic performance under different PAR/UV-A ratios for *Alexandrium* and *Phaeodactylum*, could also explain the differences in μ. These results suggest that in time, species with high rates of photorepair might be more tolerant to UV-B than those species, which depend on the synthesis of UV absorbing compounds as their principal protection mechanism.

Key words: photosynthesis, absorbance, photoreactivation, growth, UV response.

RESUMEN

Las microalgas marinas expuestas a radiación ultravioleta (UV) presentan una respuesta adaptativa compleja, dada por una serie de mecanismos protectores y reparadores. Diferencias interespecificas en susceptibilidad al UV podrían resultar en la selección de las especies más tolerantes, alterándose así la estructura de los ensambles fitoplanctónicos. La importancia relativa de los mecanismos de protección y reparación frente a un potencial incremento de UV-B se estudió en monocultivos de cuatro especies aisladas de la costa de Chile que presentan diferencias taxonómicas, ecológicas y de tamaño: *Alexandrium catenella* Whedon et Kofoid Balech (dinoflagelado), *Phaeodactylum tricornutum* Bohlin (diatomea), *Aureococcus* sp. (crisoficea) y *Spirulina subsalsa* Oersted (cianobacteria bentónica). Diferencias en tasas de fotosíntesis y crecimiento (μ) se producirían porque la capacidad efectiva de aclimatación al UV entre microalgas no es universal. Cultivos en fase exponencial fueron primero aclimatados a 16 h $día^{-1}$ de PAR + RUV-A (365 nm, 140-240 kJ $m^{-2} d^{-1}$) y luego los tratamientos suplementados con 2 h d^{-1} alto y bajo RUV-B (313 nm, dosis máxima: 3,1 kJ $m^{-2} d^{-1}$) al centro del período iluminado. Durante tres ciclos de fotoperiodo se estimó tasa de crecimiento (μ), absorción espectral in vivo, capacidad de fluorescencia celular, concentración de pigmentos fotosintéticos, tasa de fotosíntesis y fotorreactivación en cultivos control y tratamientos. Mientras que μ en *Phaeodactylum* y *Aureococcus* disminuyó en menos de un 35 %, *Alexandrium* y *Spirulina* presentaron una disminución entre 80-100 %. En estas últimas dos especies se observó un incremento significativo de compuestos absorbedores de RUV, probablemente debido a la presencia de sustancias tipo micosporinas (MAAs) y scytonemin, así como una menor eficiencia de

fotoreactivación respecto de *Phaeodactylum* y *Aureococcus*. El análisis de las tasas de fotosíntesis de *Phaeodactylum* y *Alexandrium* frente a distintas razones PAR/UV-A, podrían también explicar las diferencias en μ. Estos resultados sugieren que en el tiempo, las especies con altas tasas de fotorreactivación podrían ser mas tolerantes al UV-B que aquellas especies que dependen de la síntesis de compuestos absorbedores de UV como mecanismo principal de protección.

Palabras clave: fotosíntesis, absorbancia, fotoreactivación, crecimiento, respuesta UV.

INTRODUCTION

The deleterious effect of UV-B radiation (limits 280-320 nm, Neale 2000) has been recognised as a potentially significant stress factor for populations of marine phytoplankton world-wide (Vernet 2000). At the population level, the net effect of damaging radiation is a function of the history of radiation, the radiation dose and the sensitivities of the individual species (Cullen & Neale 1993, Karentz 1994, Xiong et al. 1997). On the other hand, tolerance of phytoplankton to UV-A (320-400 nm) and UV-B radiation has received increasing attention in the past one to two decades in view of global increases in UV radiation reaching the surface of the earth as a result of the gradual depletion of stratospheric ozone (Frederick et al. 1994, El-Sayed et al. 1996). Phytoplankton species have evolved the ability to effectively acclimate to UV-B radiation in response to variations in UV levels across latitudes, seasons and depths, which could confer them, at least temporarily, some competitive advantages over species with limited tolerance to this radiation stress.

As the position of phytoplankton cells in the water column is limited by the availability of light for photosynthesis, cells are invariably exposed to the shorter, damaging wavelengths included in sunlight. Nearly all cell components can absorb UV-B radiation, yet most of the UV-B damages result from absorption of the radiation by proteins and nucleic acids. Depressions in primary production are attributed primarily, to direct damage to photosystem II (Iwanzik et al. 1983) and inhibition of the CO_2 fixing enzyme RUBISCO. Inhibition of the synthesis of chlorophyll a (Chl a) and other light harvesting pigments further limits recovery of the photosynthetic apparatus (Strid et al. 1990, Häder & Häder 1991, Fischer & Häder 1992, Molina & Montecino 1996). Damage to DNA from exposure to UV-B radiation results primarily in the production of pyrimidine dimers, which can be cumulative, resulting eventually in mutation or death (Karentz et al. 1991, Mitchell & Karentz 1993).

Our increased understanding of UV-B radiation effects on marine phytoplankton has revealed a complex and diverse array of mechanisms involved in protection and damage repair (Vernet 2000). It is possible that some of these mechanisms have a phylogenetic basis or represent pre-adapted tolerance (Reynolds 1997), whereas others have evolved in response to local conditions. Many algal cells are capable of synthesising UV-absorbing compounds that act as UV screens, thus preventing or reducing UV damage (Carreto et al. 1989, Karentz et al. 1991, Roy 2000), including Mycosporine-like amino acids (MAAs), which are ubiquitous among marine algae (Nakamura et al. 1982). Nevertheless, active synthesis of MAAs in response to UV stress is not universal (Hannach & Sigleo 1998). The extracellular pigment scytonemin is found in several cyanobacterias especially in benthic forms (García-Pichel & Castenholz 1991, Quesada et al. 1995). Scytonemin has a broad band absorbance in the UV-A range, (maximum at 365-375 nm) and may be effective at low UV doses (Retamal 1999). Also, repair mechanisms include renewal of damaged proteins by *de novo* synthesis (Mate et al. 1998) and light-dependent (photorreactivation) or independent DNA repair (Karentz et al. 1991). It has been suggested that the increased UV-B resistance of low latitude species is associated with highly efficient DNA repair mechanisms (Karentz 1994, Karentz & Spero 1995).

Tolerance to UV-B radiation stress is based on the efficiency of repair and protection mechanisms. The net effect reflects a balance between damage, repair and protection costs, with consequences for the survival, growth and reproductive success of the species under UV stress (Vincent & Neale 2000).

Since UV-B radiation dose decrease with latitude, differential tolerance can be expected among algae from different latitudes. In the Antarctica, phytoplankton exposed to a sudden UV-B radiation increase suffered significant growth inhibition (Jokiel & York 1984, Davidson et al. 1994, Helbling et al. 1994); whereas marked resistance and acclimation to UV-B radiation have been found at low latitudes (Helbling et al. 1992, Hazzard et al. 1997). The greater inhibition of growth at high latitudes may also be explained by the temperature dependency of biosynthetic repair (Roos & Vincent 1998). Thus, although the current increase in UV-B radiation in the southern tip of South America is considered modest compared to more equatorial latitudes (Frederick et al. 1994), it may be sufficient to elicit changes in the species composition of local phytoplankton assemblages. It is of particular importance to

identify which mechanisms could be involved in the development of harmful algae blooms in Southern Chile (Muñoz & Avaria 1997), based on the various ecophysiological strategies that allow species to exist in environments where chronic exposure to potentially damaging levels of UV radiation is unavoidable. Here we compare the physiological responses to UV-B radiation in four species of phytoplankton isolated along the coast of Chile. The species selected for this study are phylogenetically diverse and differ widely in habitat and cell size.

MATERIAL AND METHODS

Cultures and experimental conditions

Stock cultures of the dinoflagellate *Alexandrium catenella* Whedon et Kofoid Balech (ACC01, stocks from Universidad de Chile), the diatom *Phaeodactylum tricornutum* Bohlin (MLB292, stocks from Universidad de Valparaíso), and the chrysophyte *Aureococcus* sp. (MLB192, stocks from Universidad de Valparaíso) were maintained for months in 250 ml Erlenmeyer flasks in f/2 (Guillard 1975) at 15 ± 2 °C and 90 µmol photons $m^{-2} s^{-1}$ PAR with a 16:8 h L:D photoperiod. Stocks of the cyanobacterium *Spirulina subsalsa* Oersted (MLB193) were grown in Provasoli (Collantes & Melo 1995) at 18.5 °C, 40 µmol photons $m^{-2} s^{-1}$ and 8:16 h L:D in a small culture chamber. All cultures were periodically refreshened in order to maintain the cells under continuous growth. *A. catenella* was originally isolated from the Aysén region (45° 32' S, 73° 34' W) and *P. tricornutum*, *Aureococcus* and *S. subsalsa* from the intertidal zone at Montemar (32° 58' S, 71° 35' W).

A bigger culture chamber was equipped with a combination of fluorescent lamps providing PAR (Philips TLD 18W/33), UV-A radiation (365 nm, Philips TLD 18W/08), and UV-B radiation (313 ± 12 nm, Q-Pannel). Figure 1A shows the weighted emission of UV lamps. PAR was measured with a Licor quantum meter and a LI-250 cosine sensor. UV-A and UV-B radiation were measured with a VLX-3W interference filter radiometer (Cole Parmer, France). All measurements were done in air with the sensor covered by bag material and the appropriate cut-off filter. Because flat sensors were used, total flux was estimated from the sum of upward and downward measurements at specific locations within the growth chamber.

Inoculates from the stock were diluted to exponential growth densities (determined previously) and transferred in 100 ml UV-B-transparent polyethylene bags (Whirl-Pak NASCO, U.S.A.) to the big culture chamber for acclimation to PAR and UV-A radiation prior to UV-B exposure. Cells were considered acclimated when the in vivo chlorophyll a (Chl a) fluorescence per cell did not change significantly with time (3 to 4 days). Cultures were then assigned to three experimental conditions: high UV-B, low UV-B, or control (without UV-B). All bags received PAR + UV-A radiation on a 16:8 h L:D photoperiod. UV-B exposed bags received a high or a low dose of UV-B radiation, provided daily for 2 h at the center of the 16 h light period. High and low UV-B represent unweighted doses at 3.0-3.1 and 0.7-1.4 kJ $m^{-2} d^{-1}$ respectively, obtained by varying the distance to the emission source (Table 1). Whereas control bags were wrapped in polyester film to eliminate wavelengths < 320 nm, UV-B exposed bags were maintained in cellulose acetate film envelops to cut wavelengths < 280 nm (Fig. 1B). All cut-off filters attenuated PAR by 10 %. Meanwhile, three consecutive experimental runs (4-5 d each) were performed under similar

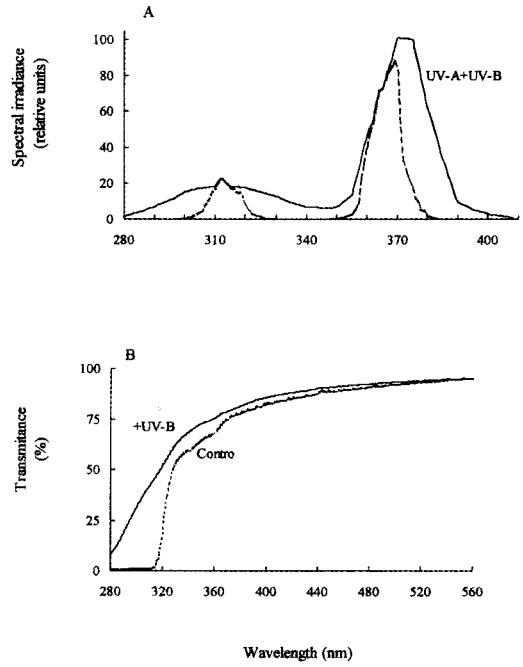

Fig. 1: (A) Radiation spectra of the UV-B (313 ± 12 nm) and UV-A (365 ± 12 nm) lamps in relative units and weighted emission of both lamps and (B) transmittance of cut-off filters used for the UV-B treatments (cellulose acetate film) and control samples (polyester film).

(A) Espectros de radiación de lámparas de UV-B (313 ± 12 nm) y UV-A (365 ± 12 nm) en unidades relativas y emisiones ponderadas de ambas lámparas y (B) transmitancia de filtros bloqueadores usados en los tratamientos con UV-B (lámina de acetato de celulosa) y en las muestras controles (lámina de poliester).

TABLE 1

Photosynthetic available radiation (PAR), UV-B and UV-A values for the different treatments and species arranged according to nominal size from 14 to 22 x 10^3 μm^3. PAR and UV-A were provided during 16 h and low and high UV-B radiation for 2 h daily, these are unweighted doses at 0.7-1.4 kJ m^{-2} d^{-1} and 3.0-3.1, respectively, obtained by varying the distance to the emission source that produced changes in the UV-A and UV-B proportions in relation to PAR. The different parameters that were measured are assigned for each taxon. Values represent mean ± standar error

Valores de radiación fotosintéticamente activa (PAR), UV-B y UV-A para los diferentes tratamientos y especies ordenados de acuerdo al tamaño nominal, desde 14 hasta 22 x 10^3 μm^3. Las radiaciones PAR y UV-A fueron entregadas durante 16 h y baja y alta radiación UV-B por 2 h diariamente, las que representan dosis no ponderadas de 0,7-1,4 y 3,0-3,1 kJ m^{-2} d^{-1}, respectivamente, obtenidas por variar la distancia a la fuente de emisión, lo que produjo cambios en las proporciones de UV-A y UV-B en relación a PAR. Los diferentes parámetros medidos son asignados a cada taxón. Los valores representan la media ± error estándar

Taxa	Nominal size (μm^3)	Treatment	PAR (μmol m^{-2} s^{-1})	UV-A (kJ m^{-2} d^{-1})	UV-B (kJ m^{-2} d^{-1})	UV-A/PAR (%)	UV-B/PAR (%)	Parameters
Alexandrium catenella	22 438	Control	119 ± 2.9	202 ± 10	0	12.1	0	a, b, c, d, e
		Low UV-B	111 ± 2.8	157 ± 23	0.8 ± 0.1	10.6	0.6	
		High UV-B	116 ± 3.0	214 ± 31	3.1 ± 0.2	14.9	2.2	
Phaeodactylum tricornutum	180	Control	110 ± 2.9	202 ± 30	0	14.7	0	a, b, c, d, e
		Low UV-B	100 ± 2.8	157 ± 10	0.9 ± 0.1	12.5	0.7	
		High UV-B	102 ± 2.9	201 ± 22	3.1 ± 0.2	15.7	2.4	
Spirulina subsalsa	137	Control	35 ± 1.3	107 ± 9	0	48.6	0	a, b, c
		Low UV-B	35 ± 1.4	141 ± 10	1.4 ± 0.1	64.4	6.4	
		High UV-B	35 ± 2.0	167 ± 12	3.1 ± 0.2	76.2	14.1	
Aureococcus sp.	14	Control	119 ± 2	156 ± 17	0	8.7	0	a, b, c
		Low UV-B	121 ± 5	242 ± 6	0.7 ± 0.02	13.7	0.4	
		High UV-B	127 ± 4	242 ± 12	3.0 ± 0.2	13.8	1.7	

[a] Growth rate [b] Chlorophyll a $cell^{-1}$
[c] Specific in vivo absorbance [d] Cellular fluorescence capacity (CFC) [e] Photosynthesis (P-E curves)

conditions of irradiance for *A. catenella*; one or two experimental runs (2-3 d) were performed for each of the other species. All experiments were run in triplicate (3 bags per treatment combination).

Photoreactivation

In a separate experiment, photoreactivation efficiency was estimated from cell viability following exposure to UV-B radiation. Photoreactivation experiments were performed on samples removed from the PAR + UV-A acclimated cultures described above prior to their UV-B exposure. Two series of 20 ml samples (n = 3) were exposed to a range of UV-B doses (0-7.5 kJ m^{-2}) in the absence of PAR. Immediately after this treatment, one series of cultures was incubated for three days under full range PAR (400-700 nm) to allow for photorepair of UV-B damage. For the other series under PAR, lacking the blue range of the spectrum (450-700 nm), a cut-off yellow filter was used to block photorepair wavelengths. Cell numbers were determined using the methods described for growth rate measurements and used to calculate rates of cell survival. The percent of photoreactivation was estimated from the difference between the survival rates of the series of cells allowed photorepair by subtraction of the respective areas under the curves (Retamal 1999). The photoreactivation efficiency at each UV-B dose was estimated as the survival rate of cells not allowed to photorepair relative to the survival rate of cells allowed to photorepair. Curves were compared through the Peto-Peto test (Pike & Thomson 1986).

Photosynthesis versus irradiance

Photosynthesis versus irradiance curves were determined for UV-B exposed (high UV-B, low UV-B) and control (UV-B excluded) cultures of

A. catenella and *P. tricornutum*, both before and after 3 and 2 days of UV-B cycles, respectively. Photosynthetic rates were measured by $NaH^{14}CO_3$ uptake in a thermoregulated photosynthetron incubator at 12 irradiance levels ranging from 10 to 600 µmol photons m^{-2} s^{-1}. Aliquots of 1 ml were incubated for 1 h with 1 µCi ^{14}C ml^{-1}. The samples were then fixed with formaldehyde, acidified by addition of 250 µl 6N HCl and shaked for 1 h to remove excess $^{14}CO_2$ (Montecino et al. 1996). Total activity was measured with phenethylamine. Data of P-E were fit to the model of Jassby & Platt (1976):

$$PP = P_{max} \times (\exp(\alpha \times E/P_{max}) - \exp(-\alpha \times E/P_{max}))/(\exp(\alpha \times E/P_{max}) + \exp(-\alpha \times E/P_{max})),$$

where PP is the photosynthetic rate, E is PAR, P_{max} is the light–saturated photosynthetic rate, and a is the light limited photosynthetic rate per unit of PAR. Photosynthetic rates were normalised to cell-concentration and the photosynthetic parameters were compared using the nonparametric Mann-Whitney U test (Zar 1984).

Specific in vivo absorption (a), chlorophyll a (Chl a) and cellular fluorescence capacity (CFC)*

In vivo absorbance spectra (280-700 nm) were recorded on 5-15 ml samples concentrated onto 25 mm GF/F glass fiber filters using a Shimadzu single beam UV-1203 spectrophotometer attached to a computer. Sample filters were extracted in hot methanol, scanned again, and the second scan subtracted from the first to obtain absorption due to pigmented substances only (Kishino et al. 1985, Bricaud & Stramski 1990). A filter saturated with culture medium was used as a blank. Spectral absorption coefficients ($a_p(\lambda)$, m^{-1}) were obtained from the expression:

$$a_p(\lambda) = 2.3\ [OD(\lambda) - OD(750)] / ß\ (V_f/A),$$

(Cleveland & Weidemann 1993)

where $OD(\lambda)$ is the wavelength-specific optical density, OD(750) is the optical density at 750 nm (a correction for residual scattering), ß is a dimensionless pathlength amplification factor, V_f is volume filtered (m^3), A is the clearance area of the filter (m^2), and 2.3 converts from log base 10 to log base e. Specific absorption coefficients ($a^*_p(\lambda)$, m^2 mg^{-1}) were obtained as $a_p(\lambda)$/Chl a (Stuart et al. 1998). In order to quantify the total absorption due to UV-absorbing substances, $a^*_p(\lambda)$ was integrated at 2 nm intervals across the range 310-340 nm. Total absorption of UV-B exposed samples was expressed relative to the control and analysed by using the Mann -Whitney U test, Kruskal-Wallis test and the Tukey a posteriori test at P = 0.05 (Zar 1984).

Chlorophyll a was determined spectrophotometrically using the equations of Jeffrey & Humphrey (1975) in 10-15 ml samples concentrated onto 25 mm glass fiber filters (MFS), cold extracted in 90 % acetone for 24 h and clarified by low centrifugation.

For CFC measurements, samples (4 ml, n = 3) were dark adapted for 30 min and transferred in dim light to a Turner 111 fluorometer. Fluorescence was measured over the first 5 s of exposure to the excitation beam (F_b). Thereafter an aqueous solution of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) was injected to the samples to a final concentration of 3 x 10^{-5}M and fluorescence was maximised after 30 s, and this plateau value was recorded as F_a. CFC was obtained according to

$$CFC = F_a\text{-}F_b/F_a \quad \text{(Vincent et al. 1984)}$$

Measurements in UV-B exposed samples were expressed relative to the control. The effect of UV-B radiation on in vivo absorbance, Chl a and CFC was evaluated by the non parametric Kruskal-Wallis test (Zar 1984).

Ribulose-1,5-biphosphate carboxylase/oxygenase (RUBISCO) polypeptides

The RUBISCO pool was estimated in *A. catenella* and *P. tricornutum* by centrifuging 30-60 ml samples followed by ELISA characterisation (Orellana & Perry 1992).

Growth rates (µ)

Three replicates for each treatment were incubated during 14 days for *A. catenella* and counted every 72 h from 1 ml aliquots fixed with formaldehyde (5 %) in a Sedgwick-Rafter counting chamber. *Pheodactylum tricornutum* and *Aureococcus* sp. cultures (n = 3) were counted daily for 7–8 days in a Fuchs-Rosenthal chamber (Martínez et al. 2000). Samples were manipulated under sterile conditions to minimize bacterial growth. Cell densities of *S. subsalsa* (n = 3) were estimated daily for 10 days from the optical density at 665 nm using a Unicam UV/VIS spectrophotometer (Retamal 1999). Cell-specific growth rates (µ) were determined by linear regression of the natural logarithm of cell abundance versus time through the next expression:

$$\mu = (\ln_{Cf} - \ln_{Ci}) / (t_f - t_i)\ (d^{-1})$$

where C_f is cell concentration at final time (t_f) and C_i is cell concentration at initial time (t_i) during the growth exponential phase (Kain 1987).

RESULTS

Tolerance to UV-B radiation varied between taxa, and the different responses of the measured parameters are described below for each of them.

UV absorption

The *in vivo* absorption normalised by Chl a (a_p*) and integrated between 310 and 340 nm (specific integrated absorption, $\int_{310}^{340} ap^*$) increased both under High and Low UV-B radiation in different degrees according to each species. This increase relative to the control samples, expressed as ${}_{st}a_p$* ($\int_{310}^{340} ap^*$ treatment / $\int_{310}^{340} ap^*$control) can be attrib-

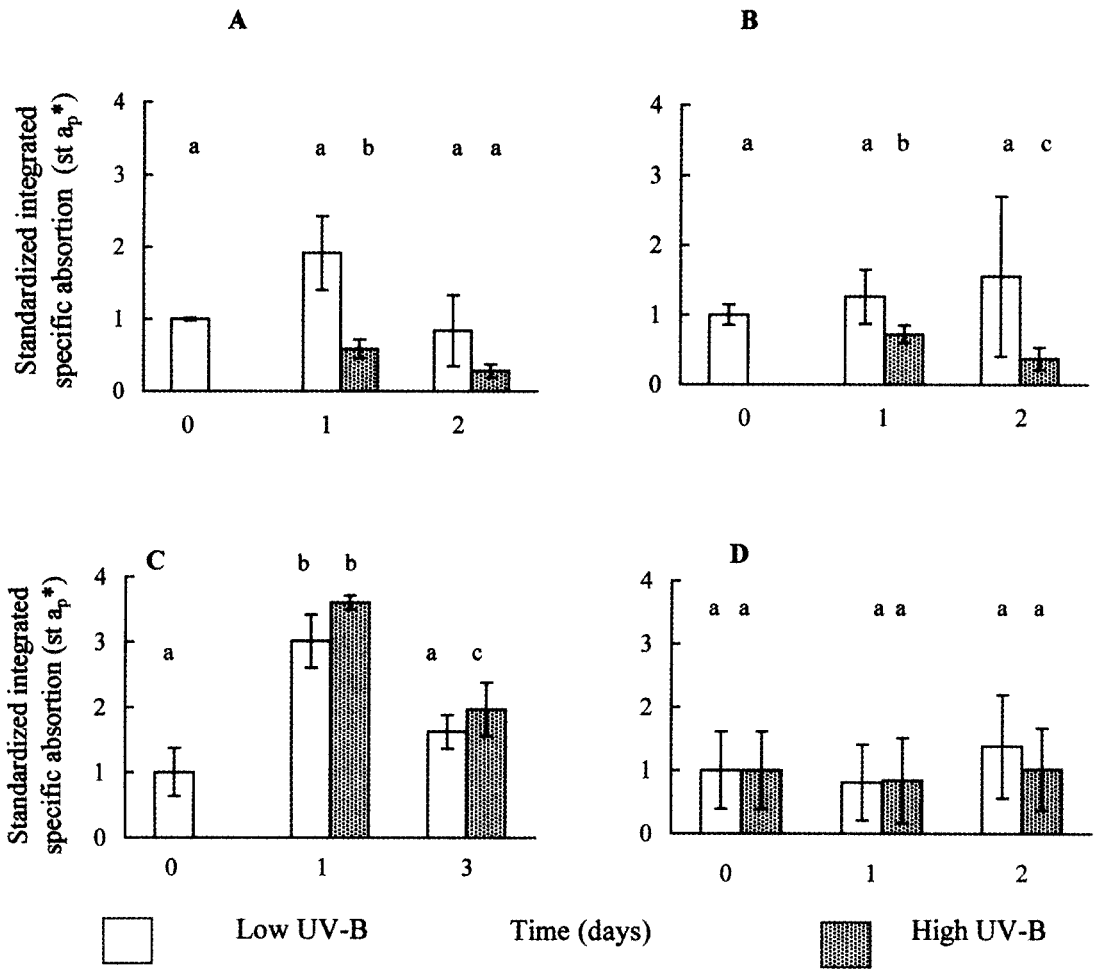

Fig. 2: Integrated specific absorption ($\int a_p$*) and standardized by each control (${}_{st}a_p$* = $\int a_p$* treatment / $\int a_p$*control), estimated from in vivo spectra between 310-340 nm, at different times (in days), after exposures cycles of 2 h day^{-1} to high and low UV-B in consecutive experiments with (A) *Phaeodactylum tricornutum* (not diluted) (B) *Phaeodactylum tricornutum* (diluted) (C) *Alexandrium catenella* and (D) *Aureococcus* sp. Subscripts a, b and c on top of each bar, denote significant differences between treatments and time (Tukey groups).

Absorción específica integrada ($\int a_p$*) y estandarizada por cada control (${}_{st}a_p$* = $\int a_p$* tratamiento / $\int a_p$*control), estimada de espectros *in vivo* entre 310-340 nm, en diferentes tiempos (en días), después de ciclos de exposición de 2 h día^{-1} a alta y baja UV-B en experimentos sucesivos en (A) *Phaeodactylum tricornutum* (no diluido) (B) *Phaeodactylum tricornutum* (diluido), (C) *Alexandrium catenella* y (D) *Aureococcus* sp. Los subíndices a, b y c en la parte superior de cada barra indican diferencias significativas entre tratamientos y tiempo (grupos Tukey).

uted to Mycosporine-like aminoacids (MAAs). These changes in ${}_{st}a_p^*$ were significant for *P. tricornutum* with differences according to the cell density of the samples (Fig. 2A and 2B). Comparing between high and low UV-B treatments, a significant ${}_{st}a_p^*$ increase ($P = 0.006$) of 70 % occurred at low UV-B radiation, and only after the first day of exposure (Tukey test, $P = 0.005$) in the samples that were not diluted. Therefore, another experiment was run with cultures that were maintained optically thin, by diluting 1:1 with fresh medium. In these refreshened samples, significant differences in ${}_{st}a_p^*$ were found between treatments at both times. At low UV-B the ${}_{st}a_p^*$ increase was 57 % after one day and 76 % after the second day of exposure when compared with the high UV-B treatment ($U = 9.0$, $P < 0.05$, Fig. 2B). The highest increase in ${}_{st}a_p^*$ was shown by *A. catenella* already after one day of exposure at both low and ligh UV-B (67 and 72 % respectively) when compared to time zero ($P < 0.05$) with no differences between these two treatments (Fig. 2C). After three days of exposure the difference in ${}_{st}a_p^*$ decreased by 50 % with high UV-B intensity ($P < 0.05$), nevertheless it was 50 % higher compared to time zero (Fig. 2C). While in *Aureococcus* sp. changes in ${}_{st}a_p^*$ were not significant in time or between high and Low UV-B treatments (Fig. 2D), in *S. subsalsa* after the second day of exposure ${}_{st}a_p^*$ increased both under UV-B (low intensity) and when exposed to UV-A. This increase reached 74 % between 310-340 nm, and 83 % between 365-375 nm after 4 days of exposure when compared to time zero ($P < 0.05$) (Fig. 3A and 3B).

Cellular photosynthetic capacity (CFC)

Results analysed as CFC standardized values (${}_{st}CFC = CFC_{treatment}/CFC_{control}$), showed that in *P. tricornutum* after one day exposure to UV-B under non diluted and diluted conditions ${}_{st}CFC$ values always decreased, and between treatments this decrease was significantly greater ($P < 0.05$) with high UV-B (Fig. 4A and 4B). In the samples that were not diluted, the ${}_{st}CFC$ decreased significantly ($P = 0.001$) and remained depressed until day three under low and high UV-B exposure. With high UV-B the decrease was greater and reached 90 % after the third day of exposure compared with the low UV-B treatment (Fig. 4A). In the daily medium diluted samples of *P. tricornutum,* the ${}_{st}CFC$ significantly decreased with high UV-B, and contrary to the behaviour under low UV-B between days one and three (Fig. 4B) showed no recovery after the third day of exposure ($P < 0.05$).

A. catenella showed a variable response in its photochemical efficiency (${}_{st}CFC$) in replicated experiments (codes 13a and 15). Fig. 4C shows that under low UV-B no effect was found com-

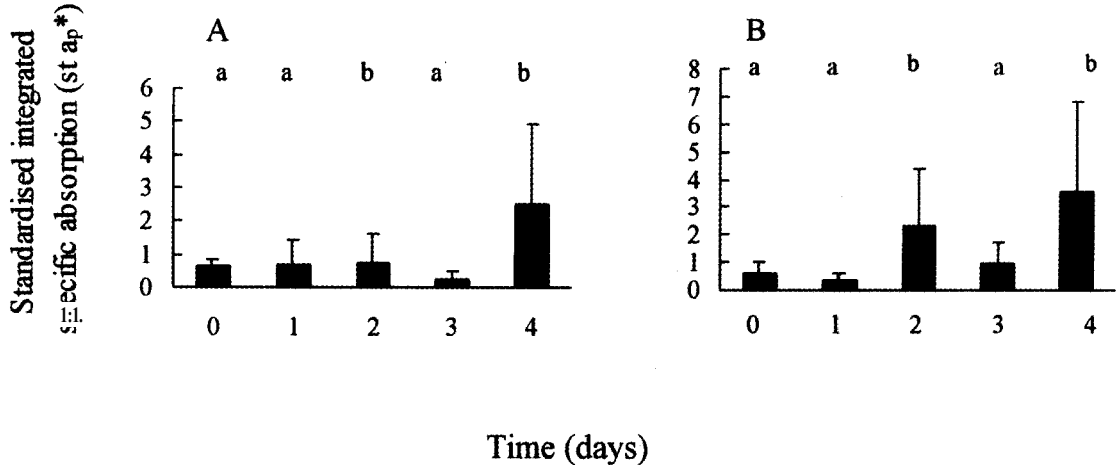

Fig. 3: Integrated specific absorption ($\int a_p^*$) and standardized by each control (${}_{st}a_p^* = \int a_p^*$ treatment / $\int a_p^*$control) estimated from in vivo spectra at different times (in days) after exposures cycles of 2 h day^{-1} to low UV-B in (A) *Spirulina subsalsa* $\int a_p^*$ between 310-340 nm, and (B) *Spirulina subsalsa* $\int a_p^*$ between 365-375 nm. Subscripts a and b on top of each bar, denote significant differences in time (Tukey groups).

Absorción específica integrada ($\int a_p^*$), estandarizada por cada control (${}_{st}a_p^* = \int a_p^*$ tratamiento / $\int a_p^*$control) y estimada de espectros *in vivo* en diferentes tiempos (en días), despues de la exposición a baja UV-B en ciclos de 2 h día^{-1} en (A) *Spirulina subsalsa* $\int a_p^*$ entre 310-340 nm y (B) *Spirulina subsalsa* $\int a_p^*$ entre 365-375 nm. Los subíndices a, b y c en la parte superior de cada barra indican diferencias significativas en el tiempo (grupos Tukey).

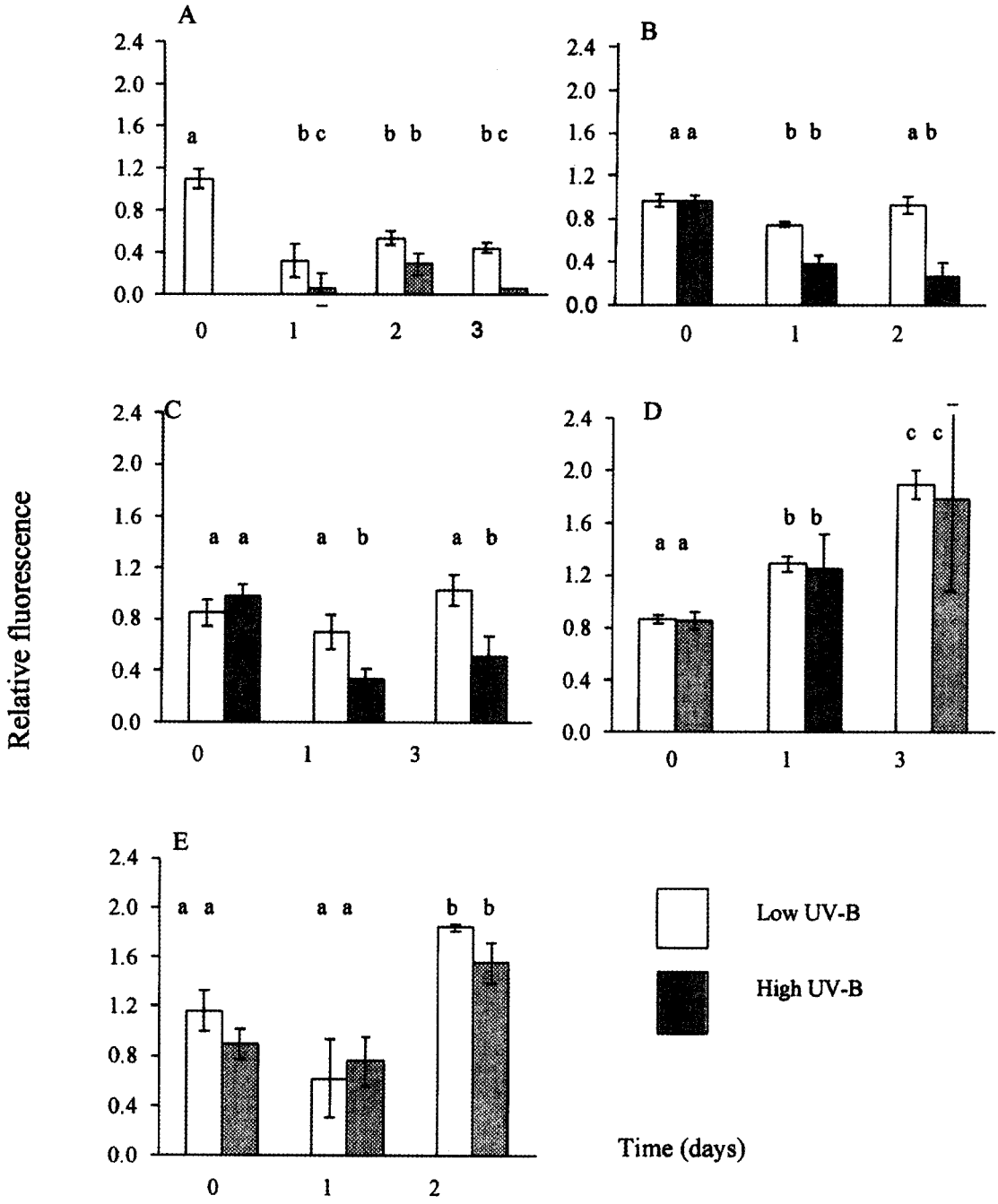

Fig. 4: Relative cellular photosynthetic capacity (CFC treatment / CFC control) at different times (in days) after exposures cycles of 2 h to high and low UV-B in (A) *Phaeodactylum tricornutum* (not diluted), (B) *Phaeodactylum tricornutum* (diluted), (C) *Alexandrium catenella,* experiment 1, (D) *Alexandrium catenella,* experiment 2, and (E) *Aureococcus* sp.

Capacidad fotosintética celular relativa (CFC tratamiento / CFC control) en diferentes tiempos (en días) después de ciclos de exposición de 2 h a alto y bajo UV-B en (A) *Phaeodactylum tricornutum* (no diluido), (B) *Phaeodactylum tricornutum* (diluido) (C) *Alexandrium catenella,* experimento número 1, (D) *Alexandrium catenella,* experimento número 2 y (E) *Aureococcus* sp.

pared to high UV-B, where after one day of exposure a significant 50 % of irreversible decrease occurred (Mann-Whitney test, $U = 9.0$, $P < 0.05$). However, in the second experiment a significant increase in $_{st}$CFC independent of UV-B quantities was observed, which was higher with more time of exposure to UV-B (Fig. 4D). After the first day the increase was 33 % and after the third day of exposure the increase was 54 % higher than at time zero ($P < 0.05$, Fig. 4D) ($_{st}$CFC values higher than 1.0 may be attributed to the effect of UV-A on control samples, see below and in Discussion). Also in *Aureococcus* sp. the $_{st}$CFC increased after the second day of exposure to low and high UV-B respectively (Kruskal-Wallis test, $P < 0.05$), when compared to time zero (Fig. 4E).

Chlorophyll a

A different response was obtained between species according to UV-B cycles and with no significant Chl a differences between UV-B dose (Table 2A). Changes in the concentration of this photosynthetic pigment per cell, showed a significant decrease in *A. catenella* and *S. subsalsa* only on the third day under high UV-B (Tukey, $P = 0.01$). In *P. tricornutum* the Chl a concentration changed according to cell concentration of the non refreshened samples, and in *Aureococcus* sp. no differences were found between time zero and day three (Table 2B). *A. catenella* with a time zero value of 18.7 ± 1.4 pgChl a cell^{-1} in the first experiment, showed a 12.8 % decrease and start-

TABLE 2

(A) Results of the statistical analysis of chlorophyll a concentration variability in *A. catenella*, *P. tricornutum*, *Aureococcus* sp. and *S. subsalsa*. MANOVA according to the different taxa, time (UV-B cycles in days) and dose (high and low UV-B)

(A) Resultados del análisis estadístico de la variabilidad de la concentración de clorofila a en *A. catenella*, *P. tricornutum*, *Aureococcus* sp. and *S. subsalsa*. ANDEVA multivariado de acuerdo a los diferentes taxa, tiempo (ciclos de UV-B en días) y dosis (alto y bajo UV-B)

Source of variation	Sum of squares	Degrees of freedom	Mean square	F-value	P-value
Taxa	501.64	2	250.82	1398.45	<0.001
Time	1.526	2	0.763	4.25	0.017
UV-B dose	0.070	2	0.035	0.19	0.823
Taxa x time	3.249	4	0.812	4.53	0.002
Taxa x UV-B dose	0.058	4	0.015	0.081	0.988
Time x UV-B dose	0.741	4	0.185	1.032	0.395
Taxa x time x UV-B dose	1.112	8	0.139	0.775	0.625
Error	15.604	87	0.179		

(B) Concentration of chlorophyll a (pgChl a per cell) in *A. catenella*, *P. tricornutum*, *Aureococcus* sp. and *S. subsalsa*, arranged according to cell size, in the controls (Time = 0) and according to Table 2A. The mean pgChl a cell^{-1} values ± standard error (n = 6) for all treatments during three UV-B cycles are shown. The range of the number of cells ml^{-1} in control samples is given below name of each species' name. Superscripts a and b indicate differences through time (Tukey test, $P < 0.05$)

Concentración de clorofila a (pgCl a por célula) en *A. catenella*, *P. tricornutum*, *Aureococcus* sp. y *S. subsalsa*, en los controles (Tiempo = 0) y de acuerdo con la Tabla 2A. Se presentan los valores promedio de pgCl a cel^{-1} ± error estándar (n = 6) de todos los tratamientos durante tres ciclos de UV-B. También se indica el rango de células ml^{-1} en las muestras control bajo el nombre de cada especie. Los superíndices a y b indican diferencias en el tiempo (prueba de Tukey, $P < 0,05$)

UV-B cycles (d^{-1})	*A. catenella*		*P. tricornutum*		*Aureococcus* sp.	*S. subsalsa*
	2.64-5.45	2.30-5.1	772-2047	336–918	4770 – 14900	485000-556000
0	18.7 ± 1.4^a	28.6 ± 3.6^a	0.66 ± 0.14^a	0.27 ± 0.04^a	0.047 ± 0.001^a	0.017 ± 0.002^a
1	20.7 ± 0.2^a	29.1 ± 0.3^a	0.28 ± 0.01^b	0.26 ± 0.03^a	0.072 ± 0.001^b	0.018 ± 0.005^a
2	-	-	0.27 ± 0.01^b	0.33 ± 0.02^a	0.057 ± 0.007^a	0.010 ± 0.001^a
3	16.3 ± 1.6^b	19.7 ± 1.3^b	-	-	0.051 ± 0.007^a	0.009 ± 0.005^b

ing with 28.6 ± 3.6 pgChl a cell^{-1} in the second experiment the decrease reached 31 %. In *P. tricornutum* Chl a diminished significantly after one UV-B cycle (from 0.66 ± 0.14 to 0.28 ± 0.01 pgChl a cell^{-1}) in the not diluted cultures. This response more than an UV effect relates to PAR availability (selfshading), considering that in the diluted cultures Chl a concentrations (~0.29 pgChl a cell^{-1}) did not change significantly in time. *Aureococcus* sp. showed values ≅ 0.05 pgChl a cell^{-1}, and in *S. subsalsa* Chl a decrease from 0.017 ± 0.002 pgChl a cell^{-1} to 0.009 ± 0.005 pgChl a cell^{-1} (Table 2B).

P versus E and photosynthetic parameters in A. catenella *and* P. tricornutum

As a function of PAR irradiance, photosynthetic performance (P-E curves) measured through autotrophic carbon fixation (P) after *A. catenella and P. tricornutum* samples were exposed to UV radiation, was different between these two species and different experiments (Fig. 5). P results were normalized to cell counts because low light photosynthetic efficiency (a) values could not be compared between these two species when normalized to Chl a. This occurred as a result of the different proportions of UVA/PAR, or the accumulated amount of UVA received during the 16 hours light period in the control samples that were placed nearest to the UV-B and UVA lamps. Consequently the amount of UVA received by the control treatment was 1.3 times higher than the treatments under high and low UV-B. This affected differentially *A. catenella* (Fig. 5C, 5D, 5E and 5F) compared with *P. tricornutum* (Fig. 5A and 5B). The same applies to the results in the photosynthetic parameters a and maximum carbon fixation (P_{max}, cell^{-1}), the CFC response (Fig. 4D) and also in the RUBISCO pool, that were not significantly lower than the controls (data not shown). A similar experiment (code N°15) showed the same CFC decrease in the controls, generating the observed relative increase in the UV-B treatments with a photosynthetic efficiency that was also affected (data not shown).

In *P. tricornutum,* the analysis of the response in a and in the light-saturated rate of photosynthesis (P_{max} cell^{-1}) in thicker growing cultures (Fig. 5A), indicates no statistically significant differences between treatments, compared to those obtained when selfshading was avoided in the culture samples through daily dilution with fresh medium (Fig. 5B). The comparison of both photosynthetic parameters P_{max} cell^{-1} and a between exposure cycles T1 and Tf in *A. catenella*, show a significant decrease for all treatments (Table 3A). Between experiments, these parameters in *P. tricornutum* under different cell concentration (non diluted and diluted) were also significantly different (Table 3B).

Photoreactivation

In *A. catenella, P. tricornutum, Aureococcus sp.* and *S. subsalsa* the highest survival percentage of the cells grown under full PAR (control) was statistically different from the cells incubated in the absence of blue light (400-450 nm) ($P < 0.05$). The clearest response was shown by *P. tricornutum*, based on the highest difference (> 48 %) between both curves (Fig. 6A) compared to 23 % in *A. catenella* (Fig. 6B), 24 % in *S. subsalsa* (Fig. 6D) and 12 % higher survival in *Aureococcus* sp. (Fig. 6C). In order to further quantify the differences between species, the results are also compared through the relationship between UV-B dose versus photoreactivation efficiency (survival percentage of the control sample/ survival percentage of the treatment sample). The highest photoreactivation efficiency with increased UV-B dose was shown by *P. tricornutum*, evidenced by the smallest slope (-2.82, $R^2 = 0.88$). For *S. subsalsa* the slope was –3.78 ($R^2 = 0.73$), for *A. catenella* -5.97 ($R^2 = 0.99$) and for *Aureococcus* sp. -7.89 ($R^2 = 0.99$). By statistical comparison of these curves, the photoreactivation efficiency was the same between *A. catenella* and *S. subsalsa*.

Growth rates under different UV-B dose

Under control conditions (PAR + RUV-A) microalgae exhibited differences in μ-values according to taxonomic differences (Table 4). Meanwhile, *P. tricornutum* and *Aureococcus* sp. showed the highest μ-values (0.65-0.88 d^{-1} and 0.71 d^{-1} respectively), *A. catenella* and *S. subsalsa* presented the lowest μ-values ranging from 0.25 to 0.08 d^{-1}. This same pattern could be observed among taxa faced with increased UV-B doses, while μ-values in *A. catenella* and *S. subsalsa* decreased respectively to 0.03 and 0.02 d^{-1}or even went negative, *Aureococcus* sp. and *P. tricornutum* under high UV-B decreased their growth rates to 0.40–0.55 d^{-1}. According to this differential response, *Aureococcus* sp. and *P. tricornutum* exhibited an μ inhibition < 50 % where *P. tricornutum* presented the lowest effect (Mann-Whitney U test, $P < 0.05$), opposite to the highest sensitivity in *S. subsalsa* and *A. catenella* (Fig. 7).

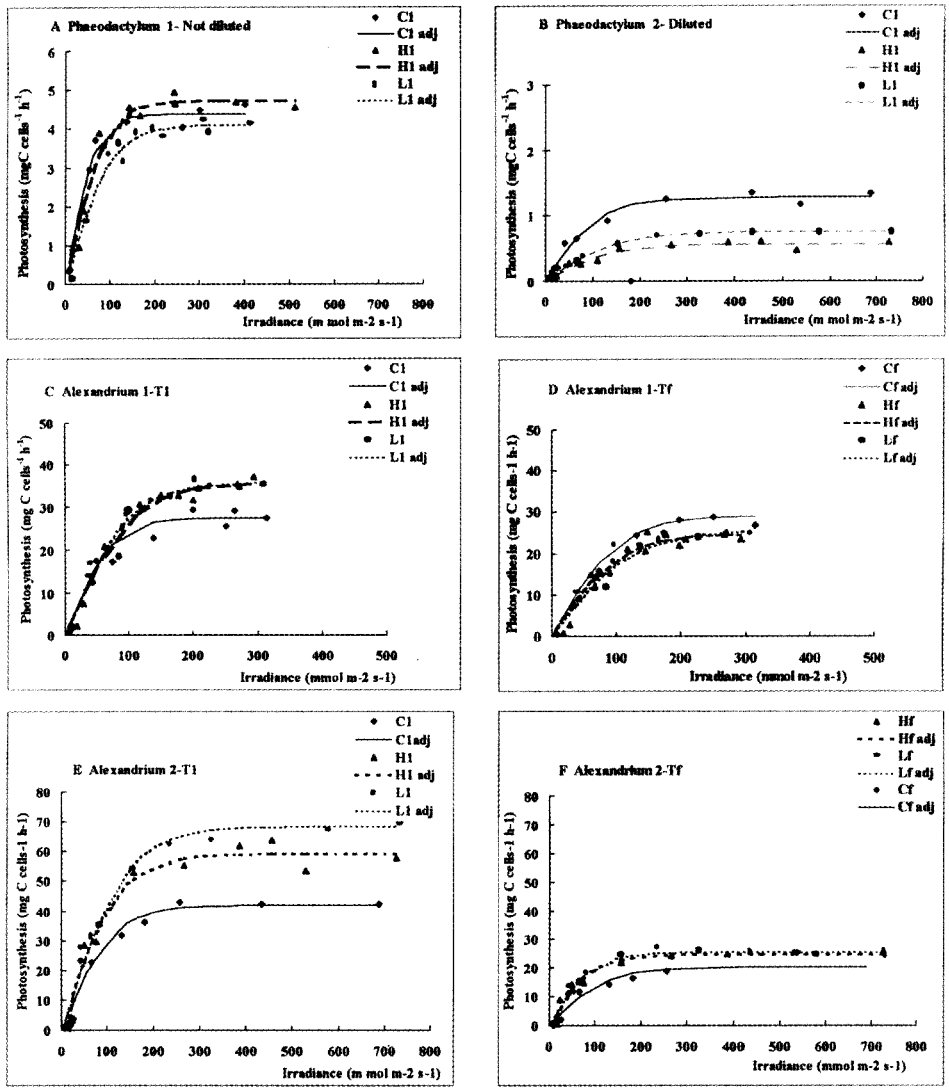

Fig. 5: Experiments of photosynthesis (normalized by cell counts, mgC cells^{-1} h^{-1}) versus light, in *Phaeodactylum tricornutum* and in *Alexandrium catenella*. (A) Non-daily diluted samples of *Phaeodactylum tricornutum* that were just previously exposed during 2 h to high and low UV-B and samples without UV-B (controls); (B) daily diluted samples of *Phaeodactylum tricornutum* that were just previously exposed during 2 h to high and low UV-B and samples without UV-B (controls); (C) *Alexandrium catenella* experiment number 1 (at T1) with samples that were just previously exposed during 2 h to high and low UV-B and samples without UV-B (controls); (D) *Alexandrium catenella* experiment number 1 (at Tf) after 3 days of 2 h daily cycles of high and low UV-B and samples without UV-B (controls); (E) *Alexandrium catenella* experiment number 2 (at T1) with samples that were just previously exposed during 2 h to high and low UV-B and samples without UV-B (controls); (F) *Alexandrium catenella* experiment number 2 (at Tf) after 3 days of 2 hours cycles of high and low UV-B and samples without UV-B (controls).

Experimentos de fotosíntesis (normalizados por recuentos celulares, mgC cels^{-1} h^{-1}) versus luz en *Phaeodactylum tricornutum* y *Alexandrium catenella*. (A) Muestras de *Phaeodactylum tricornutum* no diluidas diariamente y previamente expuestas a alta y baja UV-B durante 2 h y muestras sin UV-B (controles); (B) muestras de *Phaeodactylum tricornutum* diariamente diluidas y previamente expuestas a alta y baja UV-B durante 2 h y muestras sin UV-B (controles); (C) experimento número 1 en *Alexandrium catenella* (en T1) con muestras previamente expuestas a alta y baja UV-B durante 2 h y muestras sin UV-B (controles); (D) experimento número 1 en *Alexandrium catenella* (en Tf) después de 3 días de ciclos de 2 h diarias con alta y baja UV-B y muestras sin UV-B (controles); (E) experimento número 2 en *Alexandrium catenella* (en T1) con muestras previamente expuestas a alta y baja UV-B durante 2 h y muestras sin UV-B (controles); (F) experimento número 2 en *Alexandrium catenella* (en Tf) después de 3 días de ciclos de 2 h diarias con alta y baja UV-B y muestras sin UV-B (controles).

TABLE 3

(A) Comparison of the photosynthetic parameter α (mgC cell^{-1} h^{-1} µmol m^{-2} s^{-1}) and maximum P_{cell} (mgC cell^{-1} h^{-1}) between day one (T_1) and day three (T_{final}) of UV-B radiation cycles in *Alexandrium catenella*. Values represents mean ± standard error

Comparación del parámetro fotosintético α (mgC cell^{-1} h^{-1} µmol m^{-2} s^{-1}) y máxima P_{cell} (mgC cell^{-1} h^{-1}) entre los días con un ciclo (T_1) y tres (T_{final}) ciclos de UV-B en *Alexandrium catenella*. Valores corresponden a promedio ± error estándar

Parameter	$T_{(1)}$	$T_{(final)}$	P-value
α	0.438 ± 0.042	0.223 ± 0.036	0.05
P_{cells}	55.39 ± 7.76	23.54 ± 1.68	0.05

(B) Comparison of the photosynthetic parameter α (mgC cell^{-1} h^{-1} µmol m^{-2} s^{-1}) and maximum P_{cells} (mgC cell^{-1}h^{-1}) between experiments with *Phaeodactylum tricornutum* cultures after two cycles of UV-B radiation under high (selfshading) and low cell density (not selfshading) conditions. Values represents mean ± standard error

Comparación del parámetro fotosintético α (mgC cell^{-1} h^{-1} µmol m^{-2} s^{-1}) y máxima P_{cell} (mgC cell^{-1} h^{-1}) entre experimentos con cultivos de *Phaeodactylum tricornutum* después de dos ciclos de radiación bajo condiciones de alta (autosombreadas) y baja densidad celular (no autosombreadas). Valores corresponden a promedio ± error estandar

Parameter	Non-selfshading	Selfshading	P-value
α	0.006 ± 0.002	0.053 ± 0.008	0.05
P_{cells}	0.82 ± 0.21	4.40 ± 0.18	0.05

DISCUSSION

In this study differential interespecific sensitivity to UV-B stress, was found between the four microalgal strains isolated from the Pacific coast of South America, as reflected in photosynthetic performance (Fig. 4 and 5) and growth rates (Fig. 7). This was related to the ability of the cells to acclimate, using different strategies, mainly through photoreactivation (Fig. 6) and synthesis of UV absorbing compounds (Fig. 2 and 3). Great variation in microalgae sensitivity (Xiong et al. 1996) is due to the presence of different mechanisms that deal with the direct effects (Siebeck 1981, Mitchell & Karentz 1993, Vincent & Roy 1993, Davidson et al. 1994), of which the increase in the cellular concentration of screening agents has received broad experimental support (Carreto et al. 1989, Lesser et al. 1996, Jeffrey et al. 1999). When exposed to UV, photosynthesis of the dinoflagellates *Heterocapsa* and *Prorocentrum* was less affected than *Phaeodactylum* (Ekelund 1994) similarly to what was found here between *P. tricornutum* and the dinoflagellate *A. catenella*. Nevertheless, the increase in screening agents in *A. catenella* as observed in *Prorocentrum micans* (Lesser 1996), did not provide complete protection against UV effect, especially in relation to the effect of UV-A. In our experiments, slightly different proportions of UVA/PAR affected differentially the control samples of *A. catenella* (Fig. 5C, 5D, 5E and 5F) compared with *P. tricornutum* (Fig. 5A and 5B). This occurred because in the experimental design we prioritized optimal distribution of treatment replicates in the big culture chamber in relation to PAR, situating the control samples nearest to the UV-B and UVA lamps. The absence of response in *Aureococcus* sp. in terms of an increase in UV absorbing compounds or photoreactivation capacity is noteworthy, because its growth diminished less than 50 %. The question remains if this chrysophyte is screening UV through the cell wall constituents or using other efficient repair mechanisms. Under enhanced UV-B, chrysophytes were reported to replace diatoms in a mesocosmos experiment (Mostajir et al. 1999).

The UV-B effects on the physiology and ecology of marine phytoplankton (Vernet 2000) have been categorised in four basic responses: avoidance, stress reduction, damage repair and acclimation. Moreover, photoprotection and repair processes dealing with PS II damage are known to be dependent on previous light history and photosynthetic physiology of microalgae (Roy 2000). Striking differences emerge between the susceptibility of cells to UV radiation on PS II photochemistry and inhibition of PS II. Damage of the

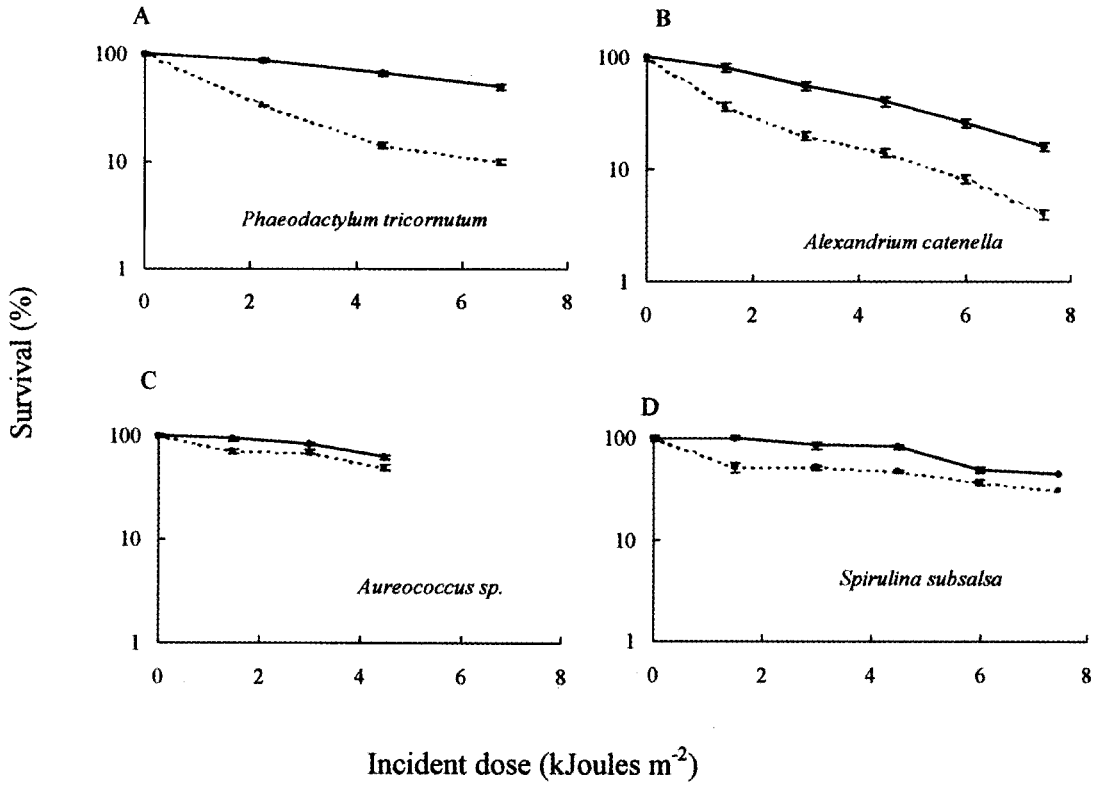

Fig. 6: Comparison of the photorepair capacity through the percentage of survival measured as the area between treatments after 3 days, from samples exposed at different UV-B doses (kJ m^{-2}), starting with incident UV-B = 0. Control samples incubated under PAR (400-700 nm) correspond to the continuous line and treatments incubated without blue light (450-700 nm) correspond to the dashed lines in (A) *Phaeodactylum tricornutum*, (B) *Alexandrium catenella*, (C) *Aureococcus* sp. and (D) *Spirulina subsalsa*.

Comparación de la capacidad de fotorreparación a través del porcentaje de sobrevivencia medido como el área entre tratamientos después de 3 días de exposición de muestras a diferentes dosis de UV-B (kJ m^{-2}), comenzando con UV-B = 0. Las muestras controles, incubadas bajo PAR (400-700 nm), corresponden a las líneas continuas y los tratamientos, incubados sin luz azul (450-700 nm), corresponden a las líneas punteadas, en (A) *Phaeodactylum tricornutum* (B) *Alexandrium catenella* (C) *Aureococcus* sp. y (D) *Spirulina subsalsa*.

photosynthetic reaction centre is rapidly reversible, and in natural phytoplankton communities photodamage to PS II appears to be completely repaired overnight (Vassiliev et al. 1994). From studies in microalgae thylakoid membranes and PS II polypeptides, it has become clear that D1 degradation is not the immediate cause of photoinhibition but a consequence of inactivation of PS II primary photochemistry following exposure to high levels of PAR (Long et al. 1994). Furthermore, gene induction is also a defence response against UV stress (Mate et al. 1998). CFC as a crude measurement of open RC II traps (Vincent et al. 1984) showed that *A. catenella* was the species that recovered even under high UV-B conditions. Also their Chl a concentration per cell did not change significantly between UV-B treatments (Table 2A), and was in fact more dependent on PAR or cell density (Table 2B). The same behaviour was observed in *Aureococcus* sp.; nevertheless, in *P. tricornutum* CFC decreased under both high and low UV-B in the diluted and non diluted samples (Fig. 4A and 4B). Chl a concentration per cell changed around the limits of 0.3-0.5 pgChl a $cell^{-1}$ reported for this species. *P. tricornutum* presented a slightly lower growth rate of 0.9 d^{-1} versus 1.2 d^{-1} (Geider et al. 1985) with the highest photoreactivation capacity. The cost of the protection mechanisms to the plants are not known (Rozema et al. 1997). Nevertheless it has to be taken into account that in small cells there is not much cytoplasm to accumulate MAAs (García-Pichel 1994) and investment in the synthesis of UV-B absorbing secondary metabolites is expensive. Photorepair is a nearly universal, blue light-dependent, and primary mechanism of

DNA repair. Therefore, especially in small phytoplankton species, light correction of UV damage should be an important factor in cell survival (Karentz et al. 1991), although (Davidson et al. 1994) found no consistency in relation to size. According to our results, under high and low UV-B, photosynthesis damage was prevented in a short time scale (one day) in the larger cells through the synthesis of UV absorbing compounds. Nevertheless, chronic UV-B exposure (3 days) decreased *A. catenella*'s photosynthetic capacity and at a threshold of 2.9 kJ m^{-2} d^{-1} of UV-B radiation, there was no investment in growth and μ reached 83 % inhibition (Martínez et al. 2000). This would support the idea of avoidance responses, such as vertical migration in relation to the radiation source, which could be the case for this species in the natural environment. Inhibition of growth was also found despite MAAs and scytonemin synthesis in *S. subsalsa* (Fig. 3A and 3B), inhabiting tidal pools and growing at low PAR intensities of 30-40 μmoles m^{-2} s^{-1}. The more efficient use of low light produces a smaller dependence on irradiance of the division rate than for the carbon uptake (Rivkin & Putt 1987), concluded that benthic algae are saturated at very low PAR intensities in relation to photosynthesis and cell division. In relation to growth, short term variations in irradiance and cellular metabolism, photosynthesis and nutrient uptake is a matter of debate. Wängberg et al. (1996) described the effects of UV-B on some of these processes and suggested that biomass and composition of marine phytoplankton was modified by UV radiation.

Aside from UV absorbing compounds and increase in photoprotective pigments (carotenoids) found in *S. subsalsa* (Retamal 1999), the arrangement of the filaments (trichomes) can influence survival, because less damage was found in the

TABLE 4

Growth rates (± standard deviation, $days^{-1}$) for the four taxa arranged according to nominal size in different experiments under control conditions (PAR + UV-A), and low and high UV-B treatments

Tasas de crecimiento (± desviación estándar, $días^{-1}$) de cuatro taxa ordenados de acuerdo al tamaño nominal en diferentes experimentos bajo condiciones de radiación control (PAR + UV-A) y tratamientos de baja y alta UV-B

Species	Experiment code	Treatment	Growth rate ($days^{-1}$)
Alexandrium catenella	9	Control	0.22 ± 0.02
		Low UV-B	0.25 ± 0.04
		High UV-B	0.03 ± 0.02
	13a	Control	0.16 ± 0.02
		Low UV-B	0.07 ± 0.01
		High UV-B	0.03 ± 0.03
	15	Control	0.25 ± 0.06
		Low UV-B	0.29 ± 0.02
		High UV-B	0.19 ± 0.03
Phaeodactylum tricornutum	13b	Control	0.65 ± 0.03
		Low UV-B	0.63 ± 0.03
		High UV-B	0.55 ± 0.05
	9b	Control	0.88 ± 0.03
		Low UV-B	0.63 ± 0.04
		High UV-B	0.41 ± 0.01
Spirulina subsalsa	L 1	Control	0.22 ± 0.19
		Low UV-B	0.10 ± 0.08
		High UV-B	-0.01 ± 0.01
	L 2	Control	0.08 ± 0.06
		Low UV-B	0.02 ± 0.02
Aureococcus sp.	14	Control	0.71 ± 0.01
		Low UV-B	0.55 ± 0.04
		High UV-B	0.40 ± 0.02

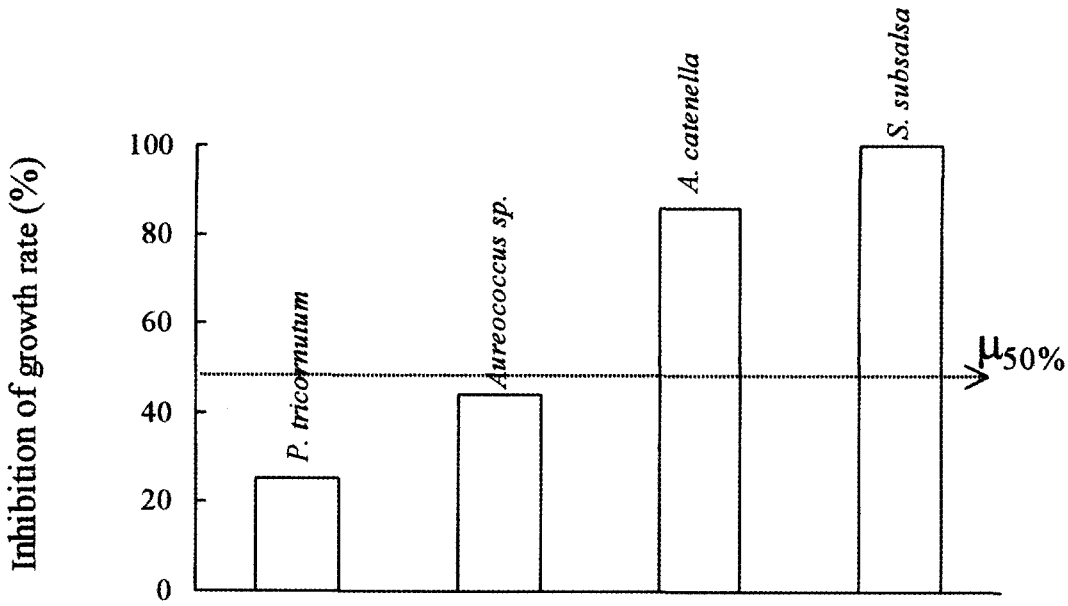

Fig. 7: Comparison of growth rate (μ) inhibition, expressed as percentage of μ decrease [($\mu_{treatment}$-$\mu_{control}$/ $\mu_{control}$) x100] of all species, considering those experiments that received similar doses of UV-B as shown in Table 1.

Comparación de la inhibición de la tasa de crecimiento (μ), expresada como el porcentaje de disminución de μ [($\mu_{tratamiento}$-$\mu_{control}$/$\mu_{control}$) x100] de todas las especies, considerando solamente los experimentos con dosis similares de UV-B mostradas en la Tabla 1.

larger trichomes (> 160 mm) probably related to an increased selfshading effect. Responses also varied with exposure times and in this benthic species a significant response was obtained after 2-4 cycles of UV-B (Fig. 3), suggesting adaptation to chronic UV environments. In Antarctic phytoplankton, selfshading can provide additional protection and Lesser et al. (1996) found a decrease of 22 % in light saturated rates of photosynthesis, using cut-off filters at 375 nm, in cultures maintained at low levels of Chl a (30 μgl).

In the present study, the size of the samples was predetermined (concentration and volume) to allow for the different measurements. Therefore, in our case Chl a concentrations were up to 10 times higher, and consequently the effect of UV could have also been diminished by selfshading as it was the case in *P. tricornutum* (Fig. 5A). Photoinhibition can also be prevented experimentally by acclimation with PAR (Neale et al. 1994). In the long term a decrease in sensitivity means an increase in protection/resistance of phytoplankton (Villafañe et al. 1995). Short term inhibition and in the long term a steady state acclimation and recovery of RUBISCO was found to ameliorate the inhibition of carbon fixation (Hazzard et al. 1997).

Our results from monocultures would confirm that an increase of the UV component of radiation in surface waters, could modulate the structure and function of phytoplankton assemblages as observed in mixed cultures, different phytoplankton groups (Helbling et al. 1994) and in the depth-differential inhibition of Antarctic phytoplankton photosynthesis (Neale et al. 1998). A decrease in primary production with UV exposure was less at lower latitudes (Helbling et al. 1993). Nevertheless, shifts could also be expected according to nutrient concentrations (Behrenfeld et al. 1995), because more UV-B tolerant species can have advantages during nutrient competition (Behrenfeld et al. 1992). Other shifts can be biologically controlled, like the change from autotrophic to heterotrophic conditions reported for the brown tide forming species *Aureococcus anophagefferens* (Bricelj & Lonsdale 1997). At the community level, natural plankton assemblages exposed to UV-B showed an increase in the abundance of bacteria, flagellates and small phytoplankton, shifting from a herbivorous food web to a microbial food web (Mostajir et al. 1999).

Experimental UV-B doses were lower compared with field measurements obtained with the same instrument at 50° S in the spring of 1997 that

ranged from 8.6-30.2 kJ m^{-2} d^{-1}. Ecological significant penetration of UV-B depends not only on phytoplankton biomass but also on the abundance of dissolved organic compounds (Kirk 1994, Goes et al. 1995). Therefore, in relation to the amount of UV-B radiation reaching the earth's surface and the extent of the Ozone Hole (Smith et al. 1992, Häder 1996, Orce & Helbling 1997, Rozema et al. 1997), the underwater light field needs also to be taken into account to predict changes in phytoplankton abundance.

Our study has extended the knowledge of microalgal response specificity and the realization of the possibility that cell acclimation can lead to a reduced effect of UV on production rates, from tropical and subtropical organisms to include organisms from lower latitudes. Species having a superior net growth performance under UV enhancement will be selected, given that species redundancy will discriminate in favour of those with superior preadapted tolerance (Reynolds 1997). In summary this study has shown that at low UV-B doses, efficient mechanisms are operating to prevent UV-B damage. The different strategies between taxa (Fig. 8) can be associated with cell size and shape or different taxonomic groups (Laurion & Vincent 1998). Extrapolations of laboratory culture results have to be taken with extreme caution because the small differences in wavelengths and in the proportions of radiation in the experimental design, plays a major role in the time course of effects. Nevertheless, species with high rates of photorepair could have some competitive advantages over less tolerant UV-B specie, since species with lower rates of photorepair will have to expend energy for repair and acclimation mechanisms which would otherwise be used for growth. These advantages should insure that inhibition at the level of phytoplankton assemblages is hardly conceivable in the long term.

ACKNOWLEDGEMENTS

This work was funded by FONDECYT grant 1960875, concurrent lately to the Program FONDAP-HUMBOLDT. At Universidad de Chile, Faculty of Sciences we also acknowledge Dr. Raúl Morales for providing the UV sensor, for laboratory facilities Dr. Fernando Zambrano with the spectrophotometer and Dr. Tito Ureta for the Scintillation counter. We are deeply thankful to Gloria Collantes, Miriam Seguel, Dr. Benjamin Suarez and Dr. Nestor Lagos for providing the cultures and also Verónica Muñoz for helping during the experiments and analysis of samples. Finally, we acknowledge Walter Helbling and Alejandro Buschmann for the invitation to the Minisimposium of Photobiology at the 1999 Phycology Congress.

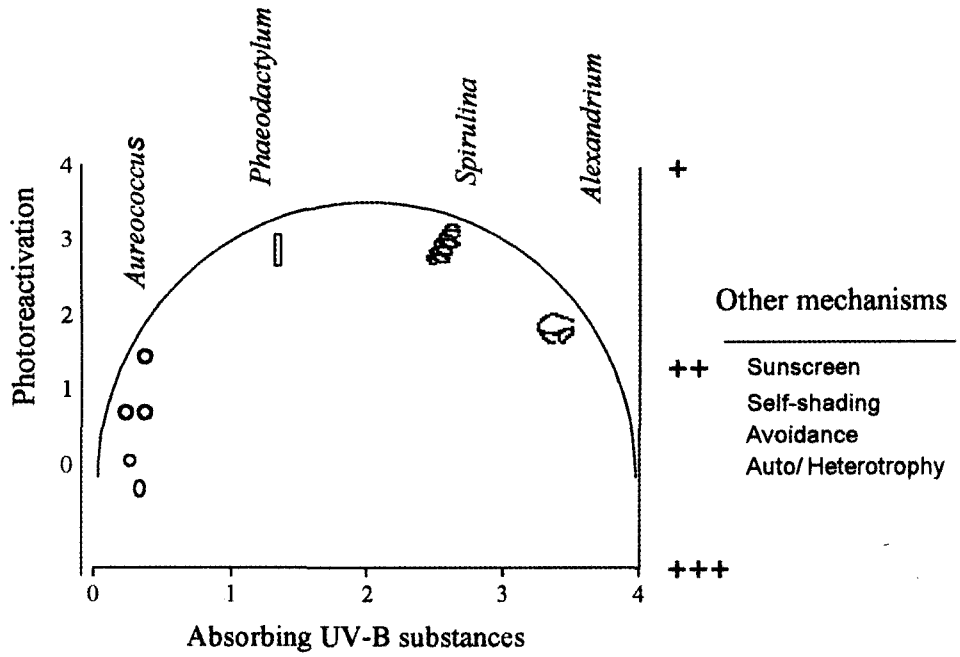

Fig. 8: Predictive diagram for the relationships between photoreactivation, absorbing UV-B substances, and other protection mechanisms for the four microalgae. Scales are in arbitrary units.

Diagrama predictivo de las relaciones entre fotorreparación, sustancias absorbedoras de UV-B y otros mecanismos de protección para las cuatro microalgas. Las escalas están en unidades arbitrarias.

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Associate Editor: P. Ojeda
Received May 12, 2000; accepted April 17, 2001