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Biological weighting function for xanthophyll de-epoxidation induced by ultraviolet radiation

Cristina Sobrino[a,1,*], Patrick J. Neale[b], Olimpio Montero[a] and Luis M. Lubián[a]

[a]Instituto de Ciencias Marinas de Andalucía (CSIC), Avda República Saharahui, 2, 11510, Puerto Real (Cádiz), Spain
[b]Smithsonian Environmental Research Center, PO Box 28, Edgewater, MD 21037, USA
[1]Present address: Smithsonian Environmental Research Center, PO Box 28, Edgewater, MD 21037, USA

Correspondence
*Corresponding author,
e-mail: sobrinoc@si.edu

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The light-induced de-epoxidation of xanthophylls is an important photoprotective mechanism in plants and algae. Exposure to ultraviolet radiation (UVR, 280–400 nm) can change the extent of xanthophyll de-epoxidation, but different types of responses have been reported. The de-epoxidation of violaxanthin (V) to zeaxanthin (Z), via the intermediate antheraxanthin, during exposure to UVR and photosynthetically active radiation (PAR, 400–700 nm) was studied in the marine picoplankter *Nannochloropsis gaditana* (Eustigmatophyceae) Lubián. Exposures used a filtered xenon lamp, which gives PAR and UVR similar to natural proportions. Exposure to UVR plus PAR increased de-epoxidation compared with under PAR alone. In addition, de-epoxidation increased with the irradiance and with the inclusion of shorter wavelengths in the spectrum. The spectral dependence of light-induced de-epoxidation under UVR and PAR exposure was well described by a model of epoxidation state (EPS) employing a biological weighting function (BWF). This model fit measured EPS in eight spectral treatments using Schott long pass filters, with six intensities for each filter, with a $R^2 = 0.90$. The model predicts that 56% of violaxanthin is de-epoxidated, of which UVR can induce as much as 24%. The BWF for EPS was similar in shape to the BWF for UVR inhibition of photosynthetic carbon assimilation in *N. gaditana* but with about 22-fold lower effectiveness. These results demonstrate a connection between the presence of de-epoxidated Z and the inhibition under UVR exposures in *N. gaditana*. Nevertheless, they also indicate that de-epoxidation is insufficient to prevent UVR inhibition in this species.

Introduction

Microalgae in the planktonic environment are episodically exposed to high photosynthetically active radiation (PAR) and ultraviolet radiation (UVR), both of which can have detrimental effects (Neale et al. 2003). They have several types of protective mechanisms to counteract these effects. Under high PAR, the absorption of light energy in excess of the capacity of photosynthesis to dissipate energy results in a build-up of the thylakoid ΔpH that is generated by photosynthetic electron transport. This decrease in pH within the thylakoid lumen is a signal of excessive irradiance and induces

Abbreviations – A, antheraxanthin; BWF, biological weighting function; Chl, chlorophyll a; EPS, epoxidation state; NPQ, non-photochemical quenching; PAR, photosynthetically active radiation; ROS, reactive oxygen species; T, transmittance; UV-A, 320–400 nm radiation; UV-B, 280–320 nm radiation; UVR, ultraviolet radiation; V, violaxanthin; Z, zeaxanthin.

the reversible de-epoxidation of specific xanthophylls (Demmig-Adams 1990, Pfündel and Bilger 1994, Yamamoto 1979). In several types of algae (primarily chlorophytes) and higher plants, de-epoxidation of violaxanthin (V) results in zeaxanthin (Z), via the intermediate antheraxanthin (A) (V Cycle). The presence of de-epoxidized xanthophylls is correlated with photoprotective mechanisms, such as the non-photochemical quenching (NPQ) of absorbed excitation energy, which protects PSII and the cell in general, from the production of damaging reactive oxygen species (ROS) such as O_2^-, H_2O_2 and O_2 (Gilmore and Yamamoto 1993, Niyogi et al. 2001). NPQ also prevents the over-reduction of the electron-transport chain and the over-acidification of the lumen (Demmig-Adams 1990, Horton et al. 1994, for review Müller et al. 2001).

In contrast with PAR, solar UVR does not significantly contribute to total excitation energy absorbed by the harvesting antenna and directed to reaction centres (Halldal and Taube 1972). The damaging effects of UVR are instead caused by the direct absorption by a target molecule or by the indirect effect of ROS produced by UVR photochemistry. These mechanisms may affect many possible targets such as nucleic acids, PSII, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) or membrane lipids, among others (for review Vincent and Neale 2000). Damage to these cellular components results in inhibition of photosynthetic carbon assimilation (Day and Neale 2002) with specific effects dependent on wavelength (Cullen and Neale 1997, Neale et al. 1998, Sobrino et al. 2005).

Biological weighting functions (BWFs) describe the wavelength dependence of UVR effects and are usually obtained from polychromatic exposures (for reviews see Cullen and Neale 1997, Neale 2000). There can be significant variation in the BWFs for different types of UVR responses (Flint and Caldwell 2003, Neale 2000). However, there are very few BWFs for UVR effects in algae besides inhibition of photosynthesis (Neale 2000, Villafane et al. 2003). BWFs are key to understanding UVR effects in aquatic environments, where spectral composition is highly variable, and predicting how a biological process responds to the increase in the short wavelength UV-B (280–320 nm radiation) because of the depletion of the ozone layer (Day and Neale 2002). They also allow the comparison between the spectral irradiances used in the different UVR studies (Cullen and Neale 1997, Neale 2000).

The many molecular targets together with the strong dependence on spectral conditions implies that responses to UVR will depend on which process is being measured and what is the spectral composition of exposure. Differences in experimental conditions between studies can sometimes lead to apparently contradictory conclusions. Previous results suggest this may be the case for studies of the effect of UVR on xanthophyll de-epoxidation. It has been reported that UVR can decrease de-epoxidation in algae and higher plants (Bischof et al. 2002, Mewes and Richter 2002, Pfündel et al. 1992), by inhibiting the V-deepoxidase enzyme (Pfündel et al. 1992) as well as increasing the epoxidation activity (Mewes and Richter 2002). Conversely, increased xanthophyll de-epoxidation has been described in cultured and natural samples of phytoplankton exposed to UVR under controlled conditions (Döhler and Haas 1995, Döhler and Hagmeier 1997, Goss et al. 1999, Zudaire and Roy 2001), while no differences were observed between cultures of *Gymnodinium breve* exposed to PAR only and PAR + UVR treatments under natural sunlight (Evens et al. 2001).

This study uses a filtered xenon lamp, which gives PAR and UVR irradiances similar to natural proportions (Neale and Fritz 2001, Neale et al. 1994), to assess the spectral dependence of xanthophyll de-epoxidation to UVR in *Nannochloropsis gaditana* (Eustigmatophyceae). *N. gaditana* is an excellent species for the study of the UVR effect on the xanthophyll cycle, because the violaxanthin is the main carotenoid in this alga and constitutes, together with the chlorophyll a (Chl), the major light-harvesting pigment in the Eustigmatophyceae (Lubián et al. 2000, Owens et al. 1987). *Nannochloropsis* is also a good model organism for the picoplankton (cells with a diameter less than 3 μm), which has been described as one of the dominant contributors to both primary production and biomass in open oceanic waters (Campbell et al. 1994, Li 1994, Van den Hoek et al. 1995).

Complementary experiments to this study have also shown that photosynthetic carbon assimilation is very sensitive to inhibition by UVR exposure in *N. gaditana* especially in comparison with dinoflagellates and diatoms (Sobrino et al. 2005). A photosynthesis irradiance model incorporating a BWF to account for UVR effects (BWF_T/P-I model) was developed that accurately predicted the photosynthetic response of *N. gaditana* to a wide range of UVR and PAR exposures. The BWF for *N. gaditana* inhibition of photosynthesis defines an effective (weighted) irradiance for inhibition (E^*_{inh}). Weighted irradiances below a threshold ($E^*_{inh} = 1$) have no net effect and irradiances above the threshold result in decreased (inhibited) rates of photosynthesis (Sobrino et al. 2005). Here, we use a similar approach to define a BWF and associated model for the effect of UVR on xanthophyll de-epoxidation.

Materials and methods

Culture growth conditions

Cultures of *N. gaditana* Lubián (Eustigmatophyceae), strain 06/0201, were provided by the Culture Collection of Marine Microalgae of the Instituto de Ciencias Marinas de Andalucía (CSIC, Cádiz, Spain). Cultures were grown at 20°C with aeration under continuous illumination with cool-white fluorescent lamps providing 150 µmol photons m^{-2} s^{-1} of PAR (QSL 100 Biospherical Instruments, San Diego, CA). The growth medium was filtered seawater from the Gulf Stream with salinity adjusted to 35‰ and enriched with f/2 nutrients. Experiments were carried out during the fourth day after a standardized inoculation (Initial density = 3×10^6 cell ml^{-1}), in the middle of the exponential growth phase. The Chl concentration in the samples was 1.3 ± 0.1 µg ml^{-1}.

Experimental setup and pigment analysis

Four millilitres of culture dispensed into 3 cm diameter quartz cuvettes were exposed to PAR and UVR during 1 h in a special incubator, the photoinhibitron (Cullen et al. 1992, Neale et al. 1998, with modifications as described in Neale and Fritz 2001). The incubator uses a 2500 W xenon lamp. UVR exposure is accompanied by high PAR, similar to solar irradiance, and the UV-B : UV-A (320–400 nm radiation) ratio brackets solar UVR (Fig. 1) (see also Neale and Fritz 2001). A mirror directs the horizontal lamp beam upward to the bottom of a temperature-controlled aluminium block that holds the quartz cuvettes. Spectral irradiance in each cuvette was configured by filtering lamp irradiance with a Schott WG series long-pass filter (nominal 50% transmittance (T) cutoff at 280, 295, 305, 320, 335, 350 or 370 nm) or a GG series filter (nominal 50% T at 395 nm) which were combined with neutral density screens for a total of 48 treatments of varying spectral composition and irradiance. Neutral density screens used were nylon window screens and metal screens with various pore sizes. Irradiance spectra (1 nm resolution) were measured with a scanning monochromator (SPG 300 Acton Research, Acton, MA) equipped with a photomultiplier tube detector (Neale and Fritz 2001, Neale et al. 1998). The system is calibrated for absolute spectral irradiance with a 1000 W NIST-traceable lamp

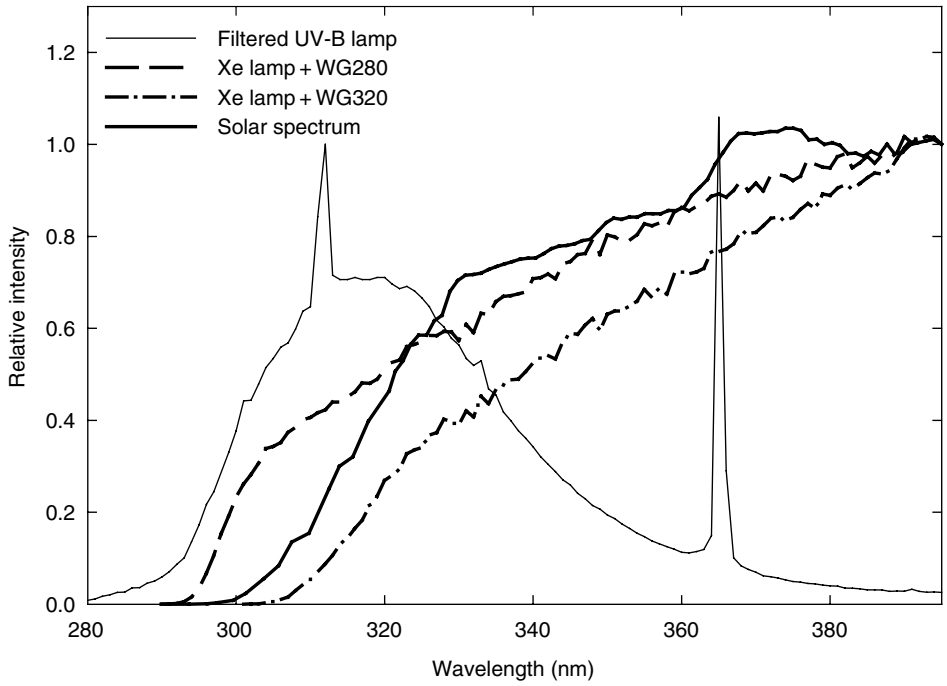

Fig. 1. Comparison of the spectra of solar radiation, fluorescent lamp frequently used in ultraviolet radiation (UVR) studies (FS40 T12-UV-B, Light Sources Inc.,West Haven, CN, USA) and filtered Xenon-Arc lamp. Values are normalized by a maximum value in the UVR region for each light source (313 nm for the UV-B lamp and 395 nm for solar radiation and Xe-lamp). Data from solar spectrum (thick line) come from Neale (2001). The spectrum was smoothed for wavelength >330 nm (10 nm bandwidth) to facilitate comparison with other spectra. For the Xenon-Arc lamp, two example spectra from the present study are given: Xe lamp + WG280, the treatment with the most short-wavelength UVR (long dashes), and Xe lamp + WG320, a treatment with an intermediate level of UVR (short dash and dots). The spectrum for FS40 UV-B lamp was filtered by cellulose acetate (thin line, modified from Cullen and Lesser, 1991).

and by reference to Hg emission lines for wavelength. Two different sets of experiments were performed. In the first set, duplicate culture samples were exposed in the photoinhibitron exclusively to high irradiance of 1500 ± 270 μmol m^{-2} s^{-1} PAR (n = 16), while in the second set, the neutral density screens were used to produce up to six irradiances for each Schott filter, in which PAR irradiance ranged from a minimum of 250 μmol m^{-2} s^{-1} to a maximum of 1900 μmol m^{-2} s^{-1} (45–415 W m^{-2}) (n = 48). Exposure start and end times were offset by 10 min between groups of six samples. Immediately after exposure, the contents of each group of cuvettes were rapidly filtered through six Whatman GF/F glass microfiber filters mounted on a 1225 Sampling Manifold (Millipore, Billerica, MA). Two non-exposed samples, just before (T_0) and after (T_E) the total exposure period, were also analysed in order to monitor possible changes in the culture during the experiment. The filters were immersed in 2 ml methanol and extracted overnight at 4°C. After filtration of the extracted samples, the pigment concentration was determined by high-performance liquid chromatography (HPLC) as described in Lubián and Montero (1998). The experiments were repeated three times with three independent cultures.

Spectral model for variation in epoxidation state

The epoxidation state (EPS) values were calculated as EPS = (V + 0.5A) / (V + A + Z). The EPS values obtained from samples exposed in the photoinhibitron were fitted to a model of EPS response as a function of UVR and PAR. The general equation for the model is described as

$$EPS(E^*) = EPS_{\text{active}}\exp(-E^*) + EPS_{\text{constant}} \quad (1)$$

where $EPS(E^*)$ is the predicted epoxidation state as a function of a given weighted or *effective* irradiance E^* (Cullen and Neale 1997, Neale 2000). EPS_{active} is the change in EPS associated with full de-epoxidation of the pool of cycling V, and EPS_{constant} is the EPS obtained using only violaxanthin that is not involved in the violaxanthin cycle. E^* is a single, non-dimensional measure of PAR and UVR *effectiveness* in inducing de-epoxidation. It is obtained by summing the product of spectral exposure $E(\lambda)$ (mW m^{-2} nm^{-1}) and a co-efficient of effectiveness or weight, $\epsilon(\lambda)$ (reciprocal mW m^{-2}), over a series of narrow wavelength bands ($\Delta\lambda$, 1 nm) for the UVR range (280–400 nm) and the product of PAR irradiance, E_{PAR} (W m^{-2}), with ϵ_{PAR} (reciprocal W m^{-2}), the average weight over the PAR range (400–700 nm) (Cullen and Neale 1997, Neale 2000). Therefore,

$$E^* = (\epsilon_{\text{PAR}}E_{\text{PAR}}) + \sum_{\lambda=280}^{\lambda=400}\epsilon(\lambda)E(\lambda)\Delta(\lambda) \quad (2)$$

The set of weights or weighting coefficients, $\epsilon(\lambda)$ that measure the strength of UVR action at each wavelength constitute the BWF (Cullen and Neale 1997, Neale 2000).

Model parameters giving the best fit to the observed EPS were obtained using two approaches based on two different methods for the estimation of the BWF. In the first approach, $\epsilon(\lambda)$ was assumed to be a first order exponential function of wavelength following an approach originally described by Rundel (1983), in which the parameters (a_i) can be estimated by non-linear regression (see also Buma et al. 2001, Cullen and Neale 1997).

$$\epsilon(\lambda) = \exp[-(a_0 + a_1\lambda)] \quad (3)$$

and therefore (by substitution in equation 2)

$$E^* = (\epsilon_{\text{PAR}}E_{\text{PAR}}) + \sum_{\lambda=280}^{\lambda=400}\{\exp[-(a_0 + a_1\lambda)]\}E(\lambda)\Delta(\lambda) \quad (4)$$

In the second approach, UVR effects on de-epoxidation are assumed to be correlated with UVR inhibition of photosynthesis, the justification for such an assumption will be discussed. According to the previously developed model for UVR inhibition of photosynthesis (Sobrino et al. 2005), weighted irradiance for inhibition (E^*_{inh}) is defined as

$$E^*_{\text{inh}} = (\epsilon_{\text{inhPAR}}E_{\text{PAR}}) + \sum_{\lambda=280}^{\lambda=400}\epsilon_{\text{inh}}(\lambda)E(\lambda)\Delta(\lambda) \quad (5)$$

where ϵ_{inhPAR} and $\epsilon_{\text{inh}}(\lambda)$ are the weighting coefficients for PAR and UVR inhibition of photosynthesis. For the analysis here, the BWF for de-epoxidation [$\epsilon(\lambda)$] was assumed to be proportional to the BWF for the UVR inhibition of photosynthesis [$\epsilon_{\text{inh}}(\lambda)$] i.e. $\epsilon(\lambda) = F\epsilon_{\text{inh}}(\lambda)$, where F is a proportionality factor. Then, E^* is defined as:

$$E^* = (\epsilon_{\text{PAR}}E_{\text{PAR}}) + F\left[\sum_{\lambda=280}^{\lambda=400}\epsilon_{\text{inh}}(\lambda)E(\lambda)\Delta(\lambda)\right] \quad (6)$$

The parameters EPS_{active}, EPS_{constant}, ϵ_{PAR} and, depending on approach, a_0 and a_1 or F were estimated using the SAS statistical analysis program (V.8), nlinfit procedure (Marquardt optimization). The 95% confidence intervals for the BWFs were obtained by

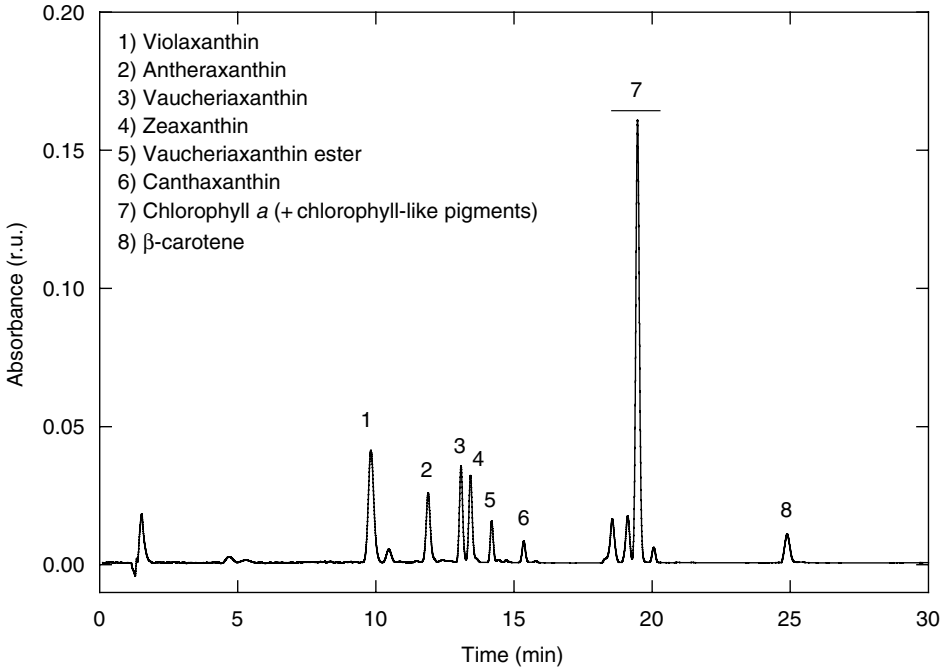

Fig. 2. An example chromatogram of a *Nannochloropsis gaditana* pigment extract obtained by high-performance liquid chromatography analysis. Notice that *N. gaditana* only has chlorophyll a and that violaxanthin is the main carotenoid (Lubián et al., 2000).

propagation of errors from the parameter standard errors and correlations. The procedure required only five iterations to converge to final estimates, and results were unaffected by different initial values.

Results

The protocol utilized for HPLC analysis of pigment extracts allowed for separation of cellular Chl and seven carotenoids, including V, A and Z (Fig. 2). In non-exposed samples (T_0 and T_E values), Chl, V, A and Z did not change significantly over a 1-h period (Fig. 3A, B). In exposed samples, Chl remained similar to the non-exposed samples, but V, A and Z changed by varying amounts depending on spectral treatment (Fig. 3A, B). The decrease in V and A concentrations was concomitant with the increase in Z. Moreover, the conversion of V and A to Z increased with the total quantity of irradiance as well as with the inclusion of shorter wavelengths in the spectral treatment (Fig. 3B, C). Total xanthophylls (V + A + Z) were similar among samples exposed to the different spectral treatments after 1 h in the photoinhibitron, although they increased significantly from a mean of 12.5 ± 1.4 fg cell^{-1} in non-exposed samples to 15.8 ± 3.2 fg cell^{-1} in the exposed samples.

Maximal EPS was found in non-exposed samples with values of 0.91 ± 0.03. In exposed samples, EPS showed values between 0.81 ± 0.04 and 0.44 ± 0.04. EPS decreased both with increasing PAR and as shorter wavelength UVR was included in the exposure (Fig. 4).

When the model (equation 1) was fit to EPS responses to PAR and UVR exposure, the estimated EPS_{active} and $EPS_{constant}$, with SE, were 0.50 ± 0.04 and 0.39 ± 0.05, respectively. These values were, respectively, similar to the variation range from the minimum to maximum EPS (0.47) and minimum EPS observed in the exposed samples (0.44). ϵ_{PAR} showed a value of $4.38 \times 10^{-3} \pm 0.94 \times 10^{-3}$ (W m^{-2})$^{-1}$. Consequently, the fitted model equation can be expressed as

$$EPS(E^*) = 0.50 \exp^{(-E^*)} + 0.39 \qquad (7)$$

where

$$E^* = (0.00438\ E_{PAR}) + \sum_{\lambda=280}^{\lambda=400} \epsilon(\lambda) E(\lambda) \Delta(\lambda) \qquad (8)$$

For the spectral dependence, equivalent fits ($R^2 = 0.90$) were obtained using either a BWF described by a simple exponential function (equation 3) or by scaling the BWF for UVR inhibition of photosynthesis in *N. gaditana* (Sobrino et al. 2005) by a factor *F* (equation 5). Estimated coefficients using the former approach are $a_0 = 9.11 \pm 0.57$ and $a_1 = 0.08 \pm 0.036$ while for the latter $F = 0.045 \pm 0.014$. Therefore, $\epsilon(\lambda)$ and E^* with fitted coefficients using both the approaches are given as

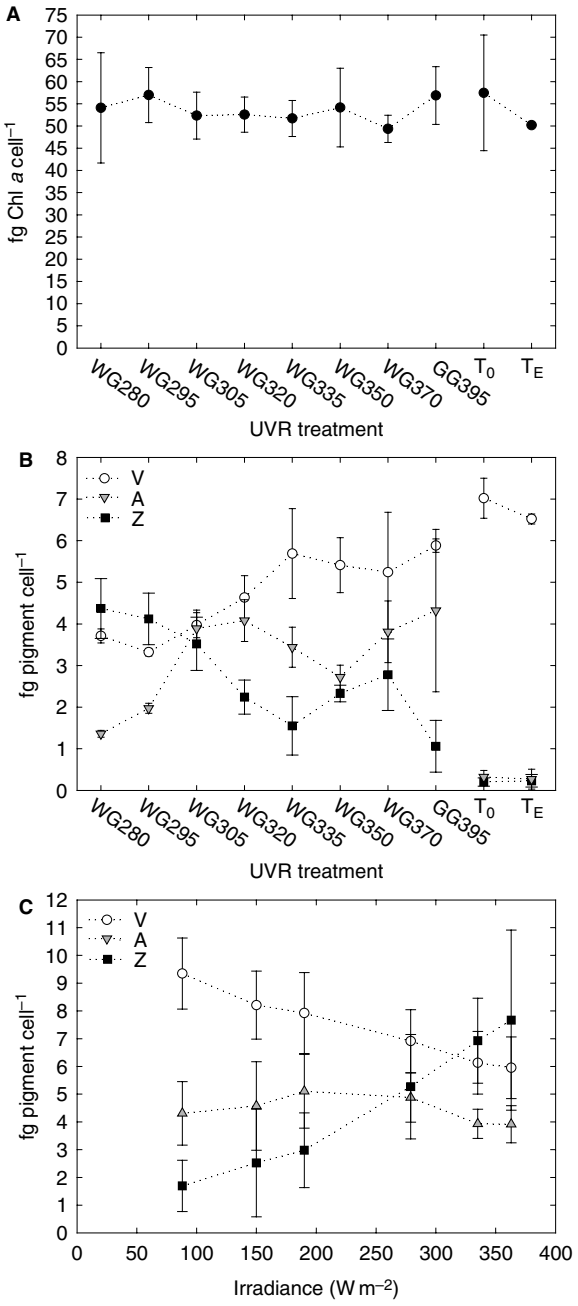

Fig. 3. Chlorophyll a (Chl), violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z) concentrations (fg cell^{-1}) after 1 h ultraviolet radiation (UVR) exposure in the photoinhibitron. T_0 and T_E correspond to the pigment concentrations of non-exposed cultures just before (T_0) and after (T_E) the exposure. (A) Chl concentration and (B) V, A and Z concentrations of samples exposed in the photoinhibitron to eight different UVR spectral treatments with similar PAR intensities (1500 ± 270 μmol m^{-2} s^{-1}). (C) V, A and Z concentrations of samples exposed in the photoinhibitron to six different intensities (PAR, 370–1530 μmol m^{-2} s^{-1}) using a WG320 filter and neutral density filters. The treatments using WG320 were chosen to show an intermediate response to UVR. The results show the average value with standard deviation of three independent experiments.

(1) using Rundel (exponential) method

$$\epsilon(\lambda) = \exp\{-[9.11 + 0.08(\lambda)]\} \quad (9)$$

$$E^* = (0.00438\ E_{PAR}) + \sum_{\lambda=280}^{\lambda=400} \exp\{-[9.11 + 0.08(\lambda)]\} E(\lambda)\Delta(\lambda) \quad (10)$$

(2) scaling the BWF for UVR inhibition of photosynthesis in *N. gaditana* (Sobrino et al. 2005)

$$\epsilon(\lambda) = 0.045\ \epsilon_{inh}(\lambda) \quad (11)$$

$$E^* = (0.00438\ E_{PAR}) + \sum_{\lambda=280}^{\lambda=400} 0.045\epsilon_{inh}(\lambda) E(\lambda)\Delta(\lambda) \quad (12)$$

Both the BWFs (Fig. 5) show that UVR irradiance at shorter wavelengths induces stronger xanthophyll de-epoxidation, similar to the spectral pattern of UVR effectiveness in other BWFs. For the spectral range of 290–340 nm, the slopes (on a log scale) were similar between the BWF fitted by the Rundel (exponential) method and the BWF that is proportional to the BWF for UVR inhibition of photosynthesis in *N. gaditana* [$\epsilon_{inh}(\lambda)$] (Sobrino et al. 2005). The weights estimated using the two approaches were not significantly different over this range, based on 95% confidence interval (Fig. 5). At longer wavelengths, the slope of the $\epsilon_{inh}(\lambda)$-based BWF decreases (Fig. 5). The differences at longer wavelengths are at least partly due to the fact that the BWF for inhibition of photosynthesis in *N. gaditana* was obtained using a more complex statistical analysis (Principal Component Analysis. Cullen et al. 1992, Neale 2000), whereas the BWF fit by the Rundel method is constrained by a single exponential slope. In either approach, the fit will be dominated by the treatments producing the largest responses (those with the highest E^*). The weighted UVR irradiance in the photoinhibitron treatments is largest in the 300–320 nm band (data not shown); hence, treatment exposures extending through this range contributed the most to the fit, and the Rundel BWF confidence interval is the smallest in this wavelength range (Fig. 5). It is notable that for this wavelength range, the slope of the fitted exponential function is similar to that of the BWF for the inhibition of photosynthesis.

The variation in EPS during the 1-h UVR + PAR treatment was exponentially related to weighted exposure over the full range, which confirms the applicability of the model over a wide range of irradiance and spectral composition (Fig. 6).

The comparison between the responses estimated for *N. gaditana* UVR inhibition of photosynthesis and EPS,

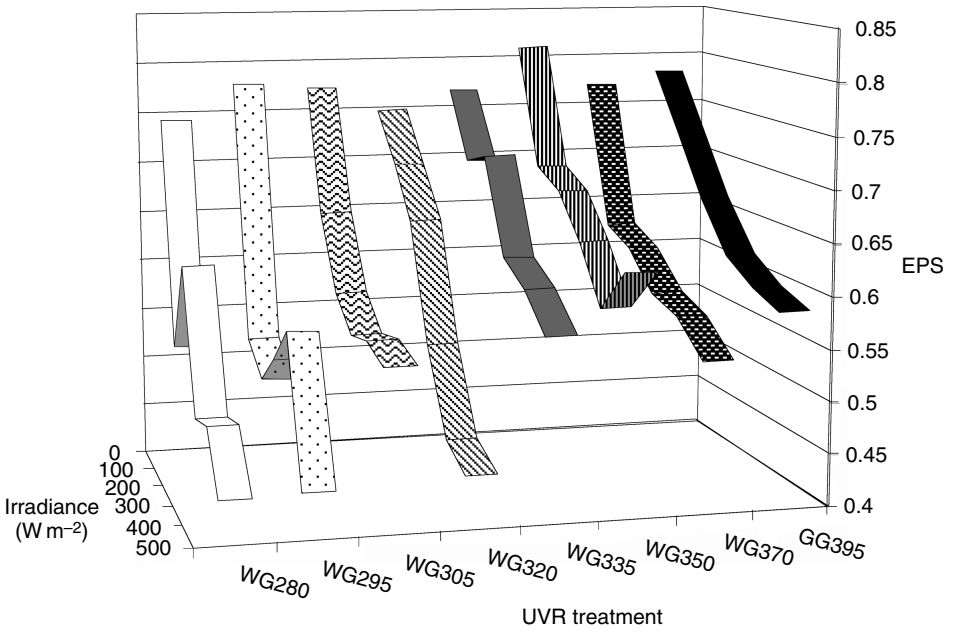

Fig. 4. Epoxidation state [EPS = (V + 0.5 A) / (V + A + Z)] versus unweighted photosynthetically active radiation (PAR) (W m^{-2}) obtained for *Nannochloropsis gaditana* cultures after 1 h exposure to ultraviolet radiation (UVR) and PAR polychromatic irradiances in the photoinhibitron. The departures from a smooth response curve for any given spectral treatment arise from spectral variations in the xenon lamp beam (which are accounted for in the statistical analysis) not from experimental error. This variation is more pronounced for spectral treatments with the most damaging wavelengths (i.e. using WG280 or WG295).

each obtained using the appropriate BWF (Sobrino et al. 2005 and Fig. 5), illustrates the interactions between PAR and UVR exposure in determining EPS (Fig. 7). Inhibition of photosynthesis (continuous line) is strongly dependent on UVR exposure and only slightly affected by PAR (Sobrino et al. 2005, see also Neale 2000, Villafane et al. 2003). Based on the estimated ϵ_{inhPAR} for *N. gaditana* (Sobrino et al. 2005), PAR exposures up to the maximum in the photoinhibitron do not

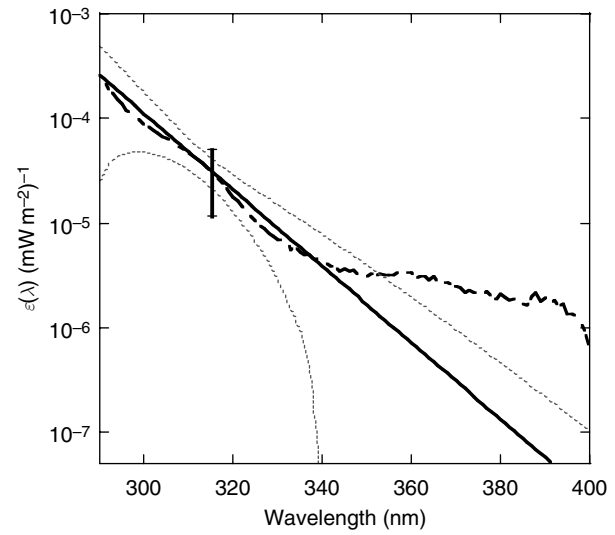

Fig. 5. Biological weighting functions [BWFs, $\epsilon(\lambda)$ (mW $m^{-2})^{-1}$] for decreased epoxidation state (EPS) induced by ultraviolet radiation exposure as estimated by statistical analysis of data from *Nannochloropsis gaditana* cultures. The continuous line represents the BWF for the EPS determined by the Rundel method (Rundel, 1983), with the upper and lower 95% confidence interval indicated by the small dashed lines. The broken line represents the BWF estimated by scaling the BWF for inhibition of photosynthesis in *N. gaditana* ($\epsilon_{inh}(\lambda)$, Sobrino et al. 2005) with the vertical line at 315 nm, indicating the 95% confidence interval for the scaled BWF arising from the variance in the estimate of *F* (equation 5).

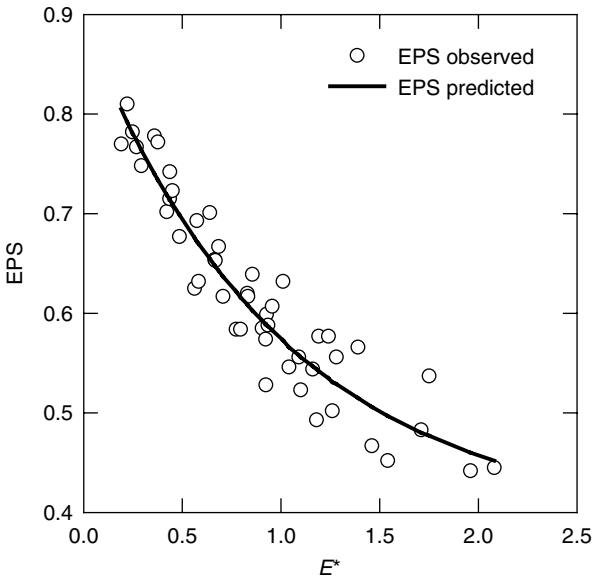

Fig. 6. Exposure-response curve (ERC) for *Nannochloropsis gaditana* epoxidation state (EPS) values. Open circles are observed EPS values obtained during a 1-h incubation in the photoinhibitron and the continuous line shows the predicted EPS values using the scaled biological weighting function for inhibition of photosynthesis in *N. gaditana* (equation 12) (R^2 = 0.90).

significantly inhibit photosynthesis ($E^*_{inh} < 1$). In contrast, EPS is strongly affected by PAR, and UVR effects are secondary. Therefore, there is a range of $EPS(E^*)$ values for any given UVR exposure (E^*_{inh}) depending on the relative level of PAR. The broken curves (dashed and dashed-dotted) show responses for the minimum and maximum E_{PAR} in the photoinhibitron and delimit the range of all predicted EPS for our experiments (cf. Fig. 4): When UVR exposure is low, i.e. weighted intensities smaller than the inhibition threshold ($E^*_{inh} = 1$), $EPS(E^*)$ varies between near maximal values for minimum PAR, to almost 80% de-epoxidation of the active pool for maximum PAR. However, even the maximum PAR exposure does not induce full de-epoxidation of the active pool. The latter is only predicted for the highest UVR exposures, which are typically combined with high PAR exposures in the photoinhibitron to simulate solar spectral balance.

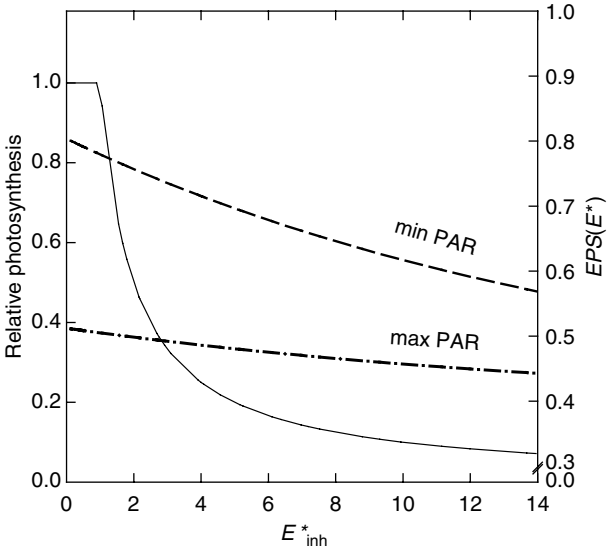

Fig. 7. Comparison between predicted epoxidation state (EPS) and photosynthetic response of *Nannochloropsis gaditana* cultures (Sobrino et al. 2005), exposed to photosynthetically active radiation (PAR) + ultraviolet radiation (UVR). Weighted irradiance (E^*_{inh}) is calculated using the biological weighting function (BWF) for the inhibition of photosynthesis in *N. gaditana* (equation 5) and spans the full range of PAR + UVR treatments in the photoinhibitron for EPS measurements (minimum $E^*_{inh} = 0.12$, maximum $E^*_{inh} = 13.7$). $EPS(E^*)$ was calculated using Equations 11 and 12 over the full range of weighted UVR in combination with the minimum (dashed) and maximum (dotted) PAR used in experimental exposures. The continuous line shows the predicted photosynthetic rates in the photoinhibitron relative to the potential rate in absence of inhibition (P^B/P^B_{pot}). In the BWF_T-PI model $P^B/P^B_{pot} = \min(1, \frac{1}{E^*_{inh}})$, (Sobrino et al. 2005). Note that the threshold of damage is at $E^*_{inh} = 1$, and inhibition of photosynthesis occurs only when $E^*_{inh} > 1$.

Discussion

Photoautotrophic organisms have developed several mechanisms that protect the photosynthetic apparatus from damage because of exposure to UVR and excess PAR such as avoidance, screening, NPQ and repair. Among these, the formation of de-epoxidated xanthophylls, correlated with the development of NPQ (Demmig-Adams 1990, Horton et al. 1994), has been described as an effective protective mechanism under severe PAR (for review Müller et al. 2001) and UVR exposure (Götz et al. 1999). However, the role of UVR in the stimulation of the xanthophyll de-epoxidation is still unclear (Bischof et al. 2002, Döhler and Haas 1995, Döhler and Hagmeier 1997, Goss et al. 1999, Mewes and Richter 2002, Pfündel et al. 1992, Zudaire and Roy 2001).

Our data show that in *N. gaditana* exposed to 48 different spectral treatments, the de-epoxidation of V to Z, through the intermediate A, occurs under PAR but is even greater under PAR + UVR exposure. The exposures were performed using a solar simulator lamp (Xenon lamp) that, after appropriate filtration, gives PAR, UV-A and UV-B similar to natural proportions. Under these conditions, the spectral treatments allowed the activation of PAR and UV-A-related repair mechanisms (Neale 2001, Quesada et al. 1995, Sobrino et al. 2005) and the determination of responses under realistic light exposures. The results agree with those published by Goss and collaborators (1999) in which the exposure of *Phaeodactylum tricornutum* to PAR and UVR produced more conversion of diadinoxanthin to diatoxanthin than PAR alone. They also reported an increase in NPQ and a decrease of oxygen production during the UVR exposure as well as the recovery of the photosynthetic rates after UV light was off. However, an enhancement of epoxidation compared with de-epoxidation can also occur under highly damaging spectral treatments. This hypothesis may account for the contrasting results of Mewes and Richter (2002), in which epoxidation state increased during exposure to UV-B. The authors demonstrated that the enhancement of epoxidation was because of UV-B-damaged thylakoid membranes increasing the permeability to ions. The partial loss of the pH gradient across the thylakoid membrane caused by UV-B radiation increased the lumen pH and, consequently, the epoxidation activity. The relative importance of these damage mechanisms may depend on the spectral balance between UV-B, UV-A and PAR in the experimental treatment and the activation of the repair mechanisms (Neale 2001, Sobrino et al. 2004, Vincent and Neale 2000) (Fig. 1). Factors that affect the resistance to UVR, such as presence of protective compounds, species sensitivity or

nutrient conditions, may also alter the relative response of the xanthophyll cycle (Litchman et al. 2002, Neale et al. 1998). Furthermore, it has been suggested that the physiological differences between species, independently of the UVR effect, may also affect the response of the xanthophyll cycle (Jacob et al. 2001).

The results obtained in our study also show that Z formation, concomitant with the decrease in V and A concentrations, is dependent on the exposure spectrum. As shorter wavelengths are included in the spectral irradiance, the Z cellular concentration increases, and therefore, the EPS decreases. The values showed that maximum de-epoxidative capacity in *N. gaditana* was similar to previously published results obtained under extreme light and temperature conditions (Lubián and Montero 1998). The exposure of the cells to eight different spectral regimes (Schott long-pass filters), with up to six intensities for each filter, allowed the determination of a new exposure-response model and a BWF for the xanthophyll de-epoxidation. In the exposure-response model, EPS was well described by an exponential function of PAR and weighted irradiance, E^*($R^2 = 0.90$). The model results also suggest that in *N. gaditana*, 56% of total V is involved in the V cycle (EPS_{active}) while 44% does not undergo de-epoxidation ($EPS_{constant}$) but takes part in other processes (Siefermann and Yamamoto 1975).

The UVR spectral dependence was described by the BWF and showed that de-epoxidation increases with exposure to shorter, more damaging, wavelengths, with the UV-B having the greatest capacity per unit energy to induce de-epoxidation. The BWF for induction of de-epoxidation obtained by scaling the BWF for UVR inhibition of photosynthesis (carbon assimilation) in *N. gaditana* (Sobrino et al. 2005) resulted in a similar fit ($R^2 = 0.90$) as the BWF obtained using an independent method (Rundel method). The fitted scaling factor (F) suggests a 22-fold lower effectiveness of UVR-induced de-epoxidation compared with UVR inhibition (equation 11). The agreement in the shape of both the BWFs suggests a connection between the energy dissipation process performed by Z and inhibition of photosynthesis under UVR exposures. Specifically, as the latter increases with exposure to shorter wavelengths or higher irradiance, there is a greater effect on EPS, producing more Z and limiting the excitation pressure on the photosynthetic electron-transport chain. Excitation pressure could be increased by UVR inhibition through decreased dissipation of reductant and adenosine 5′-triphosphate (Neale et al. 1993). The latter may occur as UVR damages 'downstream' components of the photosynthetic pathway, for example, through lower activity of Rubisco (Bischof et al. 2002, Lesser et al. 1996, Nogués and Baker 1995, Vu et al. 1982). In any case, the inhibition of photosynthesis by UVR exposure is relatively greater than the increase in de-epoxidation. Although the xanthophyll cycle contributes to the defense of the photosynthetic apparatus from damage from UVR, it is clearly insufficient to prevent such damage altogether.

It has been suggested that the presence of Z limits damage of the PSII reaction centre and also decreases D1 protein degradation avoiding the inactivation of PSII (Götz et al. 1999, Jahns et al. 2000). We do not know if this occurs in *Nannochloropsis* under our experimental conditions, but it is possible that in a similar way, Z accumulation could be a response to a decrease in PSII activity because of direct UVR damage (Renger et al. 1986, Trebst and Depka 1990, Turcsanyi and Vass 2000). In contrast, Pfündel et al. (1992) argued that PSII inhibition by UVR could result in oxidation of electron carriers between photosystems, which reduces V availability, decreasing Z formation (Pfündel et al. 1992, Siefermann and Yamamoto 1975).

The relative response of EPS to PAR + UVR suggests that xanthophyll de-epoxidation is mainly related to protection from excess PAR and is secondarily a response to UVR. Exposure to PAR alone, or PAR with little additional UVR ($E^*_{inh} < 1$), could induce up to 80% of maximum de-epoxidation, while the remaining 20% is predicted to occur only with combined exposure to UVR and PAR (Fig. 7). Nevertheless, UVR induction of de-epoxidation is not conditioned on simultaneous exposure to high PAR. Exposure to full band UVR + PAR at low irradiance is as effective at inducing de-epoxidation as high irradiance of PAR alone. For example, the EPS is as low under a full UVR + PAR (WG280) treatment at 150 W m^{-2} PAR, as it is under a PAR alone (GG395) treatment at 400 W m^{-2} PAR (Fig. 4).

In conclusion, our results confirm the induction of xanthophyll de-epoxidation under solar-simulated UVR and PAR exposures and describe its response and wavelength dependence in *N. gaditana*. However, more experiments using natural sunlight exposures are necessary to confirm the results obtained under artificial conditions and to determine the molecular mechanisms involved. Measurements with natural phytoplankton assemblages are also essential to assess the photoprotective role of xanthophyll cycles as a response to environmental variations in UVR exposure.

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