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Photochemical responses of three marine phytoplankton species exposed to ultraviolet radiation and increased temperature: Role of photoprotective mechanisms

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ABSTRACT

We carried out experiments using long-term (5–7 days) exposure of marine phytoplankton species to solar radiation, in order to assess the joint effects of ultraviolet radiation (UVR) and temperature on the photochemical responses and photoprotective mechanisms. In the experiments, carried out at Atlantic coast of Patagonia (43°18.7′S; 65°2.5′W) in spring-summer 2011, we used three species as model organisms: the dinoflagellate *Prorocentrum micans*, the chlorophyte *Dunaliella salina* and the haptophyte *Isochrysis galbana*. They were exposed under: (1) two radiation quality treatments (by using different filters): P (PAR, >400 nm) and PAB (PAR + UV-A + UV-B, >280 nm); (2) two radiation intensities (100% and 50%) and (3) two experimental temperatures: 18 °C and 23 °C during summer and 15 °C and 20 °C in spring experiments, simulating a 5 °C increase under a scenario of climate change. In addition, short-term (4 h) artificial radiation exposure experiments were implemented to study vertical migration of cells pre- and non-acclimated to solar radiation. We observed species-specific responses: *P. micans* displayed a better photochemical performance and a lower inhibition induced by UVR than *D. salina* and *I. galbana*. In accordance, *P. micans* was the only species that showed a synthesis of UV-absorbing compounds (UVACs) during the experiment. On the other hand, non-photochemical quenching (NPQ) was activated in *D. salina* at noon throughout the exposure, while *I. galbana* did not show a regular NPQ pattern. This mechanism was almost absent in *P. micans*. Regarding vertical migration, *I. galbana* showed the most pronounced displacement to deepest layers since the first two hours of exposure in pre- and non-acclimated cells, while only non-acclimated *D. salina* cells moved to depth at the end of the experiment. Finally, temperature partially counteracted solar radiation inhibition in *D. salina* and *I. galbana*, whereas no effect was observed upon *P. micans*. In particular, significant UVR and temperature interactive effects were found in *I. galbana*, the most UVR sensitive species. The joint effects on UVR and temperature, and the species-specific photoprotective responses will affect the trophodynamics and production of aquatic ecosystems in a way that is difficult to predict; however the specificity of the responses suggests that not all phytoplankton would be equally benefited by temperature increases therefore affecting the balance and interaction among species in the water column.

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1. Introduction

High intensities of solar radiation, which include ultraviolet (UVR, 280–400 nm) and visible (PAR, 400–700 nm) components may negatively affect some species in the surface layers of the water column. The UVR impact on phytoplankton cells comprises short- and long-term effects such as the decrease of photosynthesis and growth rates, and damage to different cellular targets, such as to the DNA molecule, among others [1,2]. However, some phytoplankton species have evolved photoprotective mechanisms that can be selectively activated, depending on the exposure length and/or species-specific features, thus alleviating the impact of UVR in physiological processes such as photosynthesis. For motile species, one of these short-term mechanisms is the downward migration in the water column [3]. Radiation can trigger movement in flagellated species [4,5]. Avoidance of excessive radiation can result from movement in response to high irradiance [6,7] or also by a circadian response to daily light patterns [8]. In this

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way, flagellate cells maintain an appropriate level of light, and limit the loss of cells in the photic zone, if the water is not too turbulent [9]. However, under pronounced stratification, microorganisms are somewhat physically forced to stay close to the water surface, exposing cells to high radiation.

Another short-term mechanism includes the enzymatic conversion of xanthophylls which helps cells to cope with UVR and high levels of PAR [10,11]. The de-epoxidation of diadinoxanthin, violaxanthin and antheraxanthin reduces any potential damage to PSII by enhancing the energy dissipation of excess light, measured as non-photochemical quenching, NPQ [12]. Over long-term (days) periods of exposure to high radiation levels, more permanent, physiological changes can occur, which are considered part of an acclimation process. One of such long-term mechanisms includes the synthesis of photoprotective compounds such as carotenoids, which act as antioxidants, and UV-absorbing compounds, UVACs – mainly mycosporine like amino acids, MAAs. UVACs exhibit absorption peaks within 310–360 nm [13] and are broadly distributed in tropical, temperate and polar environments, although not all the phytoplankton species synthesize them, e.g., they are less common in chlorophytes [14]. The acclimation ability under UVR is species-specific: Helbling et al. [15] found less photosynthetic inhibition and higher MAAs concentration in centric diatoms as compared to pennate species, whereas Hannach and Sigleo [16] demonstrated that MAAs synthesis was higher in dinoflagellates and haptophytes than in diatoms, chlorophytes and prasinophytes species.

This entire suit of individual and biochemical responses is in turn affected by temperature. For example, the dinoflagellates *Lingulodinium polyedrum* and *Ceratium furca* showed differences in swimming speeds at different temperatures: Between 22 and 26 °C, both species migrated at a rate of 0.7–1.0 m h^{-1}, while temperatures below *ca.* 20 °C caused a marked decrease in swimming speed [17]. Moreover, *Gyrodinium dorsum* cells showed significant higher velocity at 32 °C than at 11 °C [18]. The synthesis of protective compounds is also affected by temperature, as it regulates the enzymatic cell machinery. In this regard, Halac et al. [19] demonstrated that the diatoms *Chaetoceros gracilis* and *Thalassiosira weissflogii*, at 23 °C and during short-term exposures to solar radiation showed lower photoinhibition as compared to samples exposed at 18 °C, mainly due to heat dissipation processes (NPQ) mediated by xantophyll pigments, which was more efficient at high temperatures.

So far, long-term studies about the interactive effects of UVR and temperature on phytoplankton photosynthesis and the associated photoprotective mechanisms are rather scarce. Sobrino and Neale [20] demonstrated that photosynthesis in phytoplankton exposed to UVR is highly dependent on temperature. Higher temperatures decreased the sensitivity to UVR due to the temperature dependence of repair mechanisms. However, Lionard et al. [21] did not find any significant effect of temperature or UV-B (280–315 nm) or their interaction, neither on photosynthetic performance nor in diadinoxanthin-based xanthophyll cycle pool size, likely associated to the presence of diatoms, the dominant algal group in the studied communities. This variability in the interactive effects of UVR and temperature on phytoplankton may be partly explained by other factors, such as the optimal temperature range for UVR sensitivity and the capacity of acclimation in each species under different temperatures.

In a context of climate change, it is essential to know the extent of these combined effects of different variables as well as the mechanisms that phytoplankton cells use to cope with their potential impact. Thus the aim of this study was to evaluate the long-term (days) photochemical responses to UVR of three phytoplankton species characteristic of Patagonian waters in terms of (a) effective photochemical efficiency, and (b) three key photoprotective mechanisms – dissipation of excess energy, synthesis of photoprotective compounds, i.e., carotenoids/UVACs, and vertical migration – that might help to mitigate the negative effects of UVR on the photosynthetic process. As temperature increase could interact antagonistically with UVR levels to reduce its negative effects, we also asked whether a potential increase in temperature, such as it may occur in a context of climate change [22], would influence the studied responses. Moreover, we also evaluated the effects of an attenuated irradiance condition such as occurring when cells are in deeper layers in the water column e.g. when the water column was mixed by wind. Because of the utmost importance of Patagonia within a photobiology context, i.e., the region normally receives high levels of UVR and it is periodically under the influence of ozone depletion events [23], this kind of studies are essential to assess the potential responses of local phytoplankton species under a scenario of climate change.

2. Materials and methods

2.1. Culture collection/study site

Prorocentrum micans Ehrenberg (Dinophyceae), *Dunaliella salina* (Dunal) Teodoresco (Chlorophyceae) and *Isochrysis galbana* Parke (Prymnesiophyceae) from the Microalgae Culture Collection at Estación de Fotobiología Playa Unión (EFPU, Argentina) were grown in 1 l Erlenmeyer flasks in f/2 medium [24] with a photoperiod 12L:12D in a chamber (Sanyo model ML 350). Cells were pre-acclimated during two weeks prior to experimentation at the local oceanic mean surface temperature corresponding to the experimental season: 18 °C, experiments with *P. micans* during the period 9–15 February 2011, or 15 °C, experiments with *D. salina* and *I. galbana* during the periods 12–16 and 21–25 November 2011, respectively. During this pre-acclimation period, the cultures received constant PAR – 235 μmol photons m^{-2} s^{-1}, equivalent to the saturation light value (I_k) for coastal Patagonian areas [25]. Nevertheless, in the water column, cells would experience irradiances above and below that level, depending not only on the position of the cells in the water column but also on other factors, such as the depth of the upper mixed layer and the attenuation coefficient in the water column, among others. Light was provided by cool white fluorescent lamps (Philips daylight) and photon flux densities were measured with a spherical micro quantum sensor (Walz GmbH, model US-SQS/WB). Cells were harvested during the exponential growth phase and used in the experiments as described below. Experiments to determine the long-term responses of these three phytoplankton species to solar radiation were performed at EFPU located in the Patagonian coast of Argentina (43°18.7′S; 65°2.5′W).

2.2. Experimentation/sampling protocol

Two types of experiments were carried out to evaluate: (a) Long-term effects on photosynthesis and photoprotective compounds when cells were exposed to solar radiation conditions, and (b) Short-term effects on vertical migration of both, pre-acclimated cells to solar radiation and no-acclimated cells, when exposed to an artificial radiation source.

2.2.1. Solar radiation exposure experiments

Prior to experimentation cells in exponential growth were diluted (6:1) with f/2 medium [24]; this reduced the cell concentration and avoided self-shading effects. Samples (duplicates for each treatment) were incubated under solar radiation during 7 (*P. micans*) or 5 days (*D. salina* and *I. galbana*) in quartz tubes (300 ml) under the following treatments: 1) Radiation quality

(RQ): (a) PAB treatment – full solar radiation, i.e., UVR + PAR (>280 nm) – this was achieved by using the quartz tubes without any filter; (b) P treatment (>400 nm), samples received only PAR as tubes were covered with Ultraphan film (UV Opak, Digefra, Munich, Germany, 50% of transmittance at 395 nm). The transmittance spectra of these materials are published in Figueroa et al. [26]. (2) Radiation intensity (RI): 100% and 50%, tubes uncovered or covered with one layer of neutral density screen, respectively; and 3) Temperature (T): a) control, average of local ocean surface temperature, 18 or 15 °C, as explained before, and b) increased (+5 °C). Samples were put in thermostatic baths (Frío 21, Argentina) with two independent circuits of circulating water that kept temperature at 18 °C (*P. micans*) or 15 °C (*D. salina* and *I. galbana*) (control temperature) and 23 °C (*P. micans*) or 20 °C (*D. salina* and *I. galbana*) (increased temperature). A total of 16 tubes were used in the experiments i.e., duplicates of a 3 × 2 matrix (2 RQ × 2 RI × 2 T). After exposing samples under solar radiation for 11 h (08:00 to 19:00 h, local time), they were taken to the laboratory, in two growth chambers at the corresponding exposure temperatures without light. The following morning, samples were taken outdoors and exposed to solar radiation in their respective thermostatic bath.

The sampling during these experiments was done as follows: Photosynthetic variables (see below) were obtained three times a day: morning (08:00), noon (13:00) and afternoon (19:00). Additionally, every other day, samples were collected for analysis of chlorophyll-*a* (chl-*a*), carotenoids, UVACs and cell concentrations. After sampling, samples volume was reduced to 200 ml, and 100 ml of fresh f/2 medium was added, therefore reaching a final volume of 300 ml. In this way, cultures were maintained at exponential phase which was confirmed by cell counting throughout the experiments.

2.2.2. Artificial radiation exposure experiments

Vertical migration was evaluated by exposing *D. salina* and *I. gabana* cells pre-acclimated to solar radiation (PAR + UVR) i.e., samples coming out from the previously described experiment under solar radiation. For comparison, cultures maintained in an incubator under constant light conditions (235 μmol quanta m^{-2} s^{-1}) on a 12 h L:12 h D photoperiod and at 15 °C, were also exposed to artificial radiation under the same quality radiation and temperature conditions than the pre-acclimated cells. Logistic difficulties precluded us to carry out the vertical migration experiment with *P. micans*. Vertical migration was determined in 4 h exposures under a solar simulator (Hönle System Sol 1200, Hönle, Germany) inside a temperature-controlled chamber (Minicella, Argentina). This exposure period was selected based on previous reports [4,27] which showed that the motility of different flagellated species was negatively affected within 4 h of exposure at high UVR and PAR intensities i.e., similar as those used in our experiments (see below). Cultures were dispensed into four vertically-placed (“column”) glass aquaria (60 cm depth and 15 × 8 cm side) containing 5 l of autoclaved seawater (final cell concentration: ~500–750 cel ml^{-1}). An initial homogeneous vertical distribution was achieved by gently mixing the aquaria before the exposure. The aquaria were placed inside a water bath (50 l) to keep the temperature constant (15 or 20 °C ± 1 °C). Only RQ and temperature, same treatments as described above, were tested in these experiments. Due to space restrictions under the solar simulator, temperature treatments were done at different times, i.e., in replicate experiments, to assure a homogeneous light field for all the exposed samples. Distance from the top of the columns to the solar simulator was adjusted so that the light radiation intensity at the water surface was ~150, 60 and 1.5 W m^{-2} for PAR, UV-A and UV-B, respectively. Vertical distribution was estimated by determining cell concentrations in 2-h intervals at 4 defined depths: 5, 18, 29 and 40 cm, hereafter levels 1, 2, 3 and 4, respectively. The sampling device consisted of 4 syringes with 4 tubes of different lengths, i.e., one per depth.

2.3. Analyses and measurements

2.3.1. Radiation and temperature

Solar radiation was continuously monitored using a broad-band filter radiometer (ELDONET, Real Time Computer, Möhrendorf, Germany) that measures UV-B (280–315 nm), UV-A (315–400 nm) and PAR (400–700 nm) with a frequency of one datum per minute. Irradiance output of the solar simulator lamp was measured with an Ocean Optics spectroradiometer (HR 2000CG-UV-NIR). Temperature inside the water baths was controlled with sensors attached to each temperature channel and adjusted automatically throughout the experimental period, whereas temperature inside the column glass aquaria was measured with digital thermometers.

2.3.2. Fluorescence measurements

The photosystem II effective photochemical efficiency of charge separation (*yield*, Y) was determined by measuring the *in vivo* chl-*a* fluorescence emission using a portable pulse amplitude modulated fluorometer (Water-ED PAM, Walz, Germany). The Y was calculated using the equations of Genty et al. [28] as:

$$Y = \Delta F/F'_m = (F'_m - F_s)/F'_m \quad (1)$$

where F'_m is the instantaneous maximum intensity of chl-*a* fluorescence in an irradiated cell induced by a saturating white light pulse (~5300 μmol photons m^{-2} s^{-1} in 0.8 s) in the presence of a weak actinic light, and F_s the current steady-state fluorescence induced by weak actinic light in light-adapted cells. The non-photochemical quenching (NPQ) of chl-*a* fluorescence is a proxy for the non-radiative dissipation of light energy absorbed in excess, and it is the most important short-term photoprotective mechanism activated by saturating PAR intensities. NPQ was determined daily from F_m and F'_m values. F_m was measured before solar exposure (i.e., in dark-adapted samples), whereas F'_m was measured during the exposure. NPQ was then calculated as:

$$NPQ = (F_m - F'_m)/F'_m \quad (2)$$

There were no significant differences between NPQ values calculated in this way and those obtained directly using the PAM fluorometer therefore, so we used the data provided by the instrument. To avoid data below the instrument detection limit, NPQ values corresponding to $(F_s/F'_m) \leqslant 0.70$ and $(F'_m - F_s) \geqslant 200$ were discarded. Chl-*a* fluorescence measurements were made six times for each sample.

2.3.3. Pigments and UV-absorbing compounds (UVACs)

Chl-*a*, carotenoids and UVACs concentration was estimated by filtering 30–50 ml of sample onto a Whatman GF/C filter (25 mm) and extracting the photosynthetic pigments and UVACs in absolute methanol [29]. A scan between 250 and 750 nm was done using a Hewlett Packard spectrophotometer (model HP 8453E) and chl-*a* and carotenoids concentration were calculated using the equations of Porra [30]. UVACs were estimated from the height of the absorbance peak at 337 nm [31]. Other studies [32] suggested the use of 20% methanol as the best extraction solvent for these compounds; however since we were limited by the volume of samples, we used just one scan for determining all pigments and UVACs. This might have resulted in a slight underestimation of UVACs, but this did not change the general trend during the experiment. Once scanned, chl-*a* was also measured with a fluorometer [33] (Turner Designs, model TD700).

2.3.4. Cell concentration and growth rate determination

Aliquots of samples after exposure to solar (10–20 ml) and artificial (2 ml) radiation were fixed with buffered formalin, final concentration of formaldehyde in the sample: 0.4%, and immediately stored in darkness. Cells were counted with a Fuchs- Rosenthal chamber (Marienfeld, Germany) under a compound microscope (Zeiss model D-7082, Germany). To be considered as a representative sample, at least 100 cells had to be counted. Growth rates were calculated every day before adding culture medium, as follows:

$$\mu = \ln(N_2 - N_1)/t \quad (3)$$

where N_2 and N_1 are cell abundances after and before the experimental period (t), respectively.

2.4. Statistical and data analysis

For the long-term experiments, the following factors were considered: RQ, RI, and temperature, each of them with two levels. The variables were *yield* (Y), NPQ, chl-*a*, carotenoids, UVACs and cell concentration. The short-term experiments considered temperature and radiation quality as factors, and vertical migration, evaluated as cell concentration at different depths throughout time, as the response variable. RQ and RI treatments were done in duplicate and data was reported as the mean and half mean range. One-way and two-way repeated measurements ANOVA test was used to determine differences among radiation quality, and temperature treatments and interactions between them, using a 95% confidence limit and one degree of freedom [34]. A post hoc Tukey analysis was applied when a combination of radiation and temperature treatments was significant. The normality and homoscedasticity of data were confirmed by using a Kolmogorov–Smirnov test and plotting the residuals, respectively.

The decrease of Y (ΔY_{UVR}) induced by UVR, i.e., Y in the PAB treatment relative to that in the P control, over the incubation period was calculated as:

$$\Delta Y_{UVR} = (Y_P - Y_{PAB})/(Y_P)^{*}100 \quad (4)$$

where Y_P and Y_{PAB} are the PSII effective photochemical efficiency of charge separation in the P and PAB treatments, respectively.

3. Results

3.1. Solar radiation conditions

In general, irradiances were quite similar within each experimental period (Fig. 1), with no significant differences determined among days. However, some differences in the mean irradiances among the experiments were found (Table 1). Mean irradiances were higher during *D. salina* experiments than in the ones with *P. micans* and *I. galbana*: PAR intensities were 19% and 26%, while UV-A was a 17% and 25% and UV-B was a 32% and 25% above the levels recorded during the *P. micans* and *I. galbana* experiments, respectively (Table 1).

3.2. Long-term UVR and temperature effects on PSII

3.2.1. Yield variation

Yield (Y) values throughout the long-term solar radiation exposures showed a general daily pattern, decreasing towards noon (Y_{13h}), recovering towards the afternoon (Y_{19h}) and in some species even reaching higher values in the morning of the next day (Y_{8h}) in comparison to the previous afternoon, e.g., *D. salina* and *I. galbana*; Fig. 2. However, the three species displayed differences in the Y decrease at noon and Y recovery at morning, as well as different responses to radiation and temperature treatments. For simplicity, we show in Fig. 2 only the data obtained under the 100% intensity treatment, while Table 2 shows the whole data set. *P. micans* did not show significant differences in the Y between RQ or temperature treatments (Fig. 2A and B). In general, there were not significant differences between PAB and P treatments in *D. salina* but responses under control and increased temperatures were different, with higher Y values in the latter (Fig. 2C and D). *I. galbana* showed significant differences in the afternoon (Y_{19h}) and in the morning (Y_{8h}), being Y values higher under P than in the PAB treatments (Fig. 2E and F). Moreover, *I. galbana* also showed differences between temperature treatments: Y morning values (Y_{8h}) were higher at increased temperature, especially under the PAB treatment (Fig. 2E and F). Table 2 shows the ANOVA tests for significance in the Y among treatments in the morning (Y_{8h}) and at noon (Y_{13h}). Under 100% radiation intensity conditions, and for RQ treatments, significant differences in Y_{8h} values were only found in *I. galbana* ($p \leqslant 0.05$; Table 2) whereas the Y_{13h} was significant different in both *D. salina* and *I. galbana*, ($p \leqslant 0.05$; Table 2). On the other hand, differences between temperature treatments, either in Y_{8h} or $Y_{13h,}$ were significant in *D. salina* and *I. galbana*, especially under PAB conditions for the latter species ($p \leqslant 0.05$, Table 2). Additionally, interaction between UVR and temperature was confirmed for *I. galbana*: Y_{8h} and Y_{13h} values in the PAB treatment were higher at increased in comparison with control temperature ($p \leqslant 0.01$; Table 2). In general, the significant differences observed in Y_{8h} and Y_{13h} between RQ treatments were found only at 100% irradiance condition while temperature treatments were significant different in both irradiance conditions, 100% and 50% of radiation intensity (Table 2).

3.2.2. PSII inhibition induced by UVR

Different responses among species were observed in the UVR-induced PSII inhibition (Fig. 3). Table 3 shows the complete results of the ANOVA tests of UVR inhibition between temperature treatments during the morning and at noon for the whole experiment. UVR-induced inhibition was not significant in *P. micans* under both irradiance conditions (Fig. 3 A-B; Table 3). In contrast, UVR inhibited *D. salina* at noon, especially during the first three days of exposure and then decreasing throughout the experiment; this effect was significantly lower during the afternoon and in the morning (20%, $p \leqslant 0.05$; Fig. 3C and D). Increased temperature had an antagonistic effect, decreasing the UVR-induced inhibition, especially during the days 2 and 3 and under 100% of irradiance ($p \leqslant 0.05$; Fig. 3 C; Table 3). During the first three days a significant lower UVR-induced inhibition was observed at 50% of irradiance ($p \leqslant 0.05$; Fig. 3D) in comparison with the 100% irradiance treatment, and no differences between temperature treatments were observed at those intensities (Fig. 3D; Table 3). Finally, *I. galbana* showed higher UVR-induced PSII inhibition than the other two species, especially during the afternoon (Fig. 3; $p \leqslant 0.05$). This inhibition was more pronounced at noon and during the afternoon and decreased only in the morning (Fig. 3E and F). UVR inhibition was higher under control than under increased temperature only in the 50% of irradiance conditions (Fig. 3F; Table 3). In the two latter species, cells under increased temperature were the least inhibited by UVR.

3.3. Photoprotective mechanisms

3.3.1. Dissipation of excess radiation energy (NPQ)

In general, NPQ reached maximal values at noon and decreased during the afternoon and the morning (Fig. 4A–C). This mechanism in *P. micans* was only evident during the first day, decreasing to values near zero during the rest of the experiment (Fig. 4A) whereas *D. salina* displayed high NPQ values at noon during the last days of exposure ($p \leqslant 0.05$; Fig. 4B). These values were similar

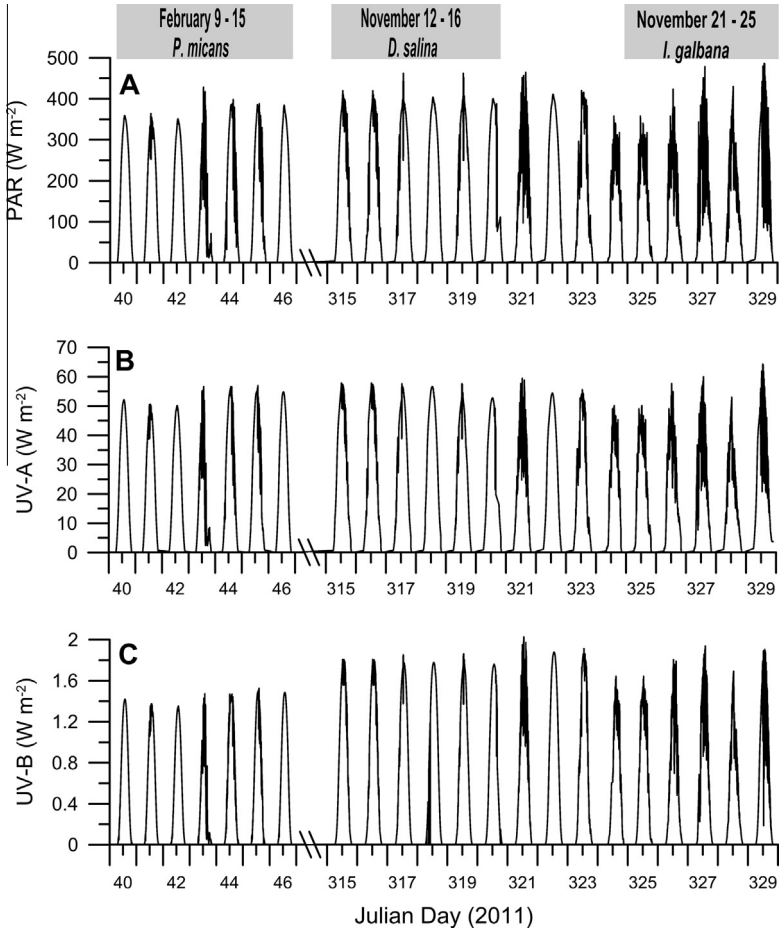

Fig. 1. Solar radiation conditions during exposure of *Prorocentrum micans*, *Dunaliella salina* and *Isochrysis galbana*. Daily irradiance in W m^{-2}: PAR, 400–700 nm (A); UV-A, 315–400 nm (B); UV-B, 280–315 nm (C).

Table 1
Mean irradiances of PAR, UVA and UVB (mean ± SD) during exposure period of *Prorocentrum micans*, *Dunaliella salina* and *Isochrysis galbana* to natural solar radiation.

	Mean solar irradiance (W m^{-2})		
	PAR	UVA	UVB
Prorocentrum micans	174 ± 29	25 ± 4.2	0.56 ± 0.10
Dunaliella salina	215 ± 10	30 ± 1.5	0.8 ± 0.03
Isochrysis galbana	160 ± 50	22 ± 3.0	0.6 ± 0.06

between radiation treatments, but significant differences ($p \leqslant 0.05$) were found among temperature treatments, being higher under control temperature than under increased temperature conditions (Fig. 4B). In general, *I. galbana* displayed low NPQ values during the whole experiment, (Fig. 4C) and did not show significant differences between PAB and *P* treatments under increased temperature conditions ($p \geqslant 0.05$; Fig. 4C); no data are available for PAB under control temperature. Moroever, NPQ values in cells under *P* did not show significant differences between control and increased temperature ($p \geqslant 0.05$; Fig. 4C). Altogether, *D. salina* showed the most pronounced and sustained heat dissipation mechanism throughout the experiment.

3.3.2. Synthesis of UV-absorbing compounds (UVACs) and carotenoids

Only *P. micans* showed a significant increase of UVACs ($p \leqslant 0.001$) during the solar radiation exposure, without significant differences among radiation or temperature treatments (Fig. 4D). However, under 50% of irradiance, UVACs concentration was significantly lower than at full intensities ($p \leqslant 0.001$) during the last two days, especially under *P* and increased temperature conditions, (see Fig. 4D). In contrast, *D. salina* and *I. galbana* maintained UVACs concentration near zero in all experimental conditions (Fig. 4 E-F). In general, carotenoids concentration decreased throughout the experimental period, i.e. ~75% in *P. micans* and *D. salina* towards the end of the experiments (Fig. 4G and H). The latter species showed a significant higher carotenoid concentration during the last day of exposure under PAB and control temperature treatments ($p \leqslant 0.05$; Fig. 4H). However, the carotenoid concentration was maintained relatively low in *I. galbana* cells, with no significant differences between treatments (Fig. 4I).

3.3.3. Vertical migration

There were no significant differences in cell concentration when comparing each depth between temperatures for both radiation treatments, thus only control temperature results are presented for *D. salina* and *I. galbana*. *D. salina* cells pre-acclimated to solar radiation (PAB) during 5 days did not display a clear migration pattern, with no significant differences in cell concentration among depths for both radiation treatments, with the exception of level 1 after 4 h (Fig. 5A–C). On the other hand, *D. salina* cells no- acclimated to solar radiation showed in some cases a displacement to deeper layers, with a higher proportion of cells at levels 3 and 4 (29 and 40 cm depth) than at surface layers ($p \leqslant 0.05$; Fig. 5D–F).

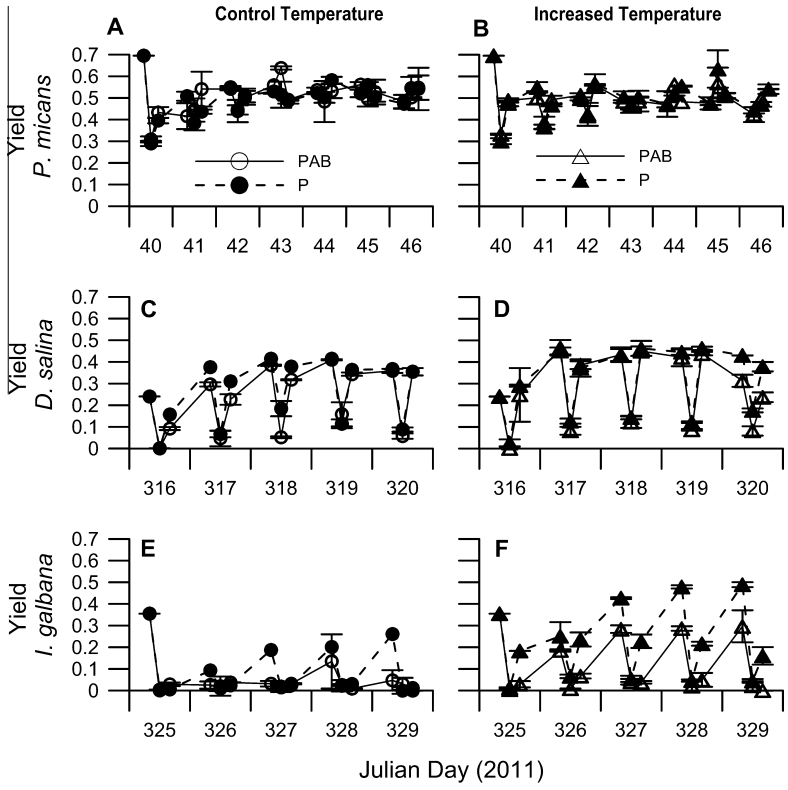

Fig. 2. PSII effective photochemical efficiency (*yield*, Y) in *Prorocentrum micans* (A and B), *Dunaliella salina* (C and D) and *Isochrysis galbana* (E and F) exposed to solar radiation during 5–7 days. Changes in *yield* are shown in cells maintained at control temperature (A, C and E; circles) and increased temperature (B, D and F; triangles) under PAB (PAR + UVR; open symbols) or P (PAR; closed symbols). The lines on top of symbols are the half mean range.

Table 2
Statistical results of two-ways repeated measurements ANOVA to establish differences in morning (Y_{8h}) and noon Y values (Y_{13h}) between radiation treatments, between temperature treatments and combined radiation-temperature during 5–7 days of solar radiation exposure. Radiation quality treatments were: PAB = PAR + UV-A + UV-B, P = PAR; temperature treatments were: control temperature (18 or 15 °C) and increased temperature (+5 °C; 23 or 20 °C). Irradiance conditions were: 100 or 50% of radiation intensity.

Statistical data Long-term solar radiation exposures										
	Differences between radiation quality treatments				Differences between temperature treatments				Differences between radiation quality * temperature treatments	
	Temp	Rad. Int.	Y_{8h}	Y_{13h}	Rad. Qual.	Rad. Int.	Y_{8h}	Y_{13h}	Y_{8h}	Y_{13h}
P. micans		100%	n.s.	n.s.		100%	n.s.	n.s.	100% R; n.s. PAB18 °C = P23 °C	100% R; n.s. PAB18 °C = P23 °C
	18 °C				PAB					
		50%	n.s.	n.s.		50%	n.s.	n.s.	PAB23°C = P18 °C 50% R; n.s.	PAB23°C = P18 °C
		100%	n.s.	n.s.	P	100%	n.s.	n.s.	PAB18°C = P23 °C	50% R; n.s.
	23 °C	50%	n.s.	n.s.		50%	n.s.	n.s.	PAB23°C = P18 °C	PAB18°C = P23 °C PAB23°C = P18 °C
D. salina		100%	n.s.	n.s.		100%	$p < 0.001$	$p < 0.05$	100% R; n.s. PAB18 °C = P23 °C	100% R; n.s. PAB18 °C = P23 °C
	15 °C				PAB					
		50%	n.s.	n.s.		50%	$p < 0.05$	$p < 0.05$	PAB23°C = P18 °C	PAB23°C = P18 °C
		100%	n.s.	$p < 0.05$		100%	$p < 0.01$	$p > 0.05$		
	20 °C				P				50% R; n.s.	50% R; n.s.
		50%	n.s.	n.s.		50%	$p < 0.05$	$p < 0.05$	PAB18°C = P23 °C PAB23°C = P18 °C	PAB18°C = P23 °C PAB23°C = P18 °C
I. galbana		100%	$p < 0.01$	$p < 0.001$		100%	$p < 0.001$	$p > 0.05$	100% R	100%R
	15 °C				PAB				PAB18 °C≠P23 °C $p < 0.001$	PAB18 °C≠P23 °C $p < 0.01$
		50%	$p < 0.01$	n.s.		50%	$p < 0.01$	$p < 0.001$		
		100%	$p < 0.05$	n.s.		100%	n.s.	$p < 0.01$	50%R PAB18 °C≠P23 °C	50%R; n.s. PAB18 °C = P23 °CPAB23
	20 °C				P				$p < 0.01$	°C = P18 °C
		50%	n.s.	n.s.		50%	n.s.	n.s.		

I. galbana cells showed a gradual movement to deep layers in all the studied conditions during the short-term exposure (Fig. 6). After 2 h, pre-acclimated cells under PAB conditions concentrated at level 3 in a significantly higher proportion than at the other depths ($p \leqslant 0.01$; Fig. 6B). Although a major displacement to level 3 was also observed in the P treatment, differences among the cell

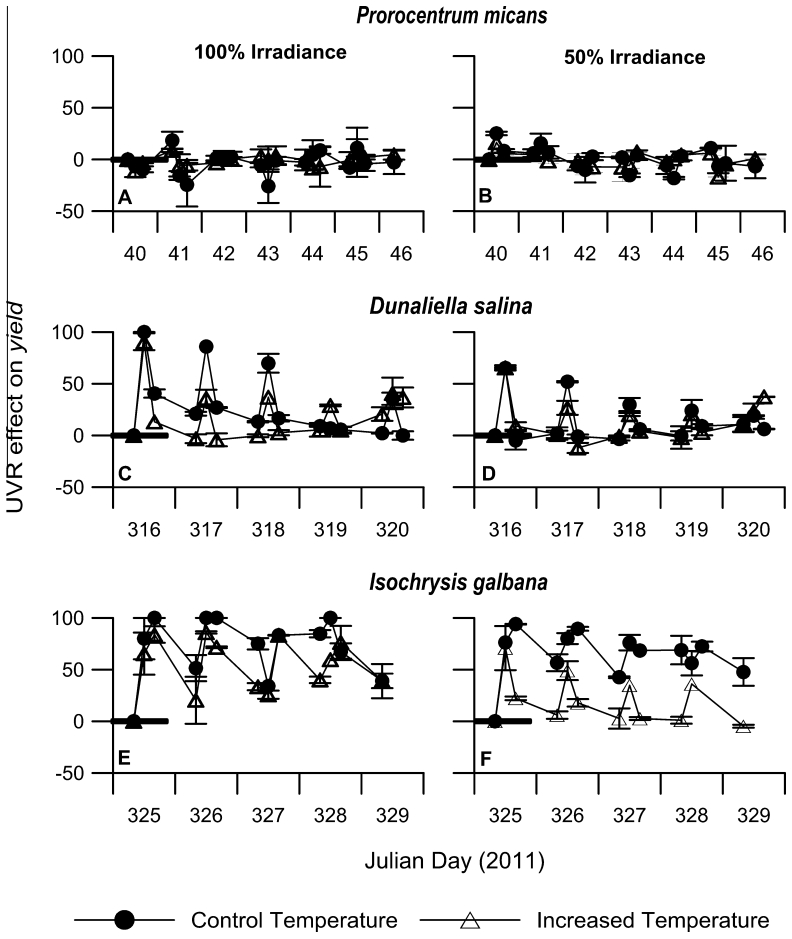

Fig. 3. Percentage of *yield* inhibition induced by UVR in *Prorocentrum micans* (A), *Dunaliella salina* (B) and *Isochrysis galbana* (C) exposed to solar radiation during 5–7 days. UVR inhibition values are shown in cells maintained at control temperature (circles) and increased temperature (triangles) and calculated as $(Y_P–Y_{PAB})/(Y_P) * 100$ where Y_P and Y_{PAB} are the *yield* in the P and PAB treatments, respectively. The lines on top of symbols are the half mean range.

Table 3
Statistical results of one-way repeated measurements ANOVA to establish differences in PSII inhibition induced by UVR during morning (8 h) and noon (13 h) between temperature treatments during 5–7 days of solar radiation exposure. Temperature treatments were: control (18 or 15 °C) and increased levels (+5 °C; 23 or 20 °C); irradiance conditions were: 100% or 50% of radiation intensity.

	Radiation intensity	*F*		Df		Y_{8h}	Y_{13h}
		Y_{8h}	Y_{13h}	Y_{8h}	Y_{13h}		
Differences in UVR effect between temperature treatments							
P. micans	100%	0.58	0.03	2	2	n.s.	n.s.
	50%	1.13	0.77	2	2	n.s.	n.s.
D. salina	100%	1.10	6.07	2	2	n.s.	$p < 0.05$
	50%	4.13	7.25	2	2	n.s.	$p < 0.05$
I. galbana	100%	2.86	2.64	2	2	n.s.	n.s.
	50%	8.77	31.1	2	2	$p < 0.05$	$p < 0.05$

proportions were not significant among levels 1, 2 and 3 (Fig. 6B). At the end of the experiment, the proportion of cells under the PAB or P treatments, were significantly higher at level 3 ($p \leqslant 0.01$; Fig. 6C). Similarly, and after 2 h, *I. galbana* cells no-acclimated to solar radiation and exposed to artificial PAB were distributed in a higher percentage at levels 2 and 3 ($p \leqslant 0.01$; Fig. 6E) while those under the P treatment did not show significant differences in their distribution among levels (Fig. 6E). At the end of exposure the highest proportion of cells, under either the PAB or P treatment, were found at levels 3 and/or 4 ($p \leqslant 0.01$; Fig. 6F).

3.4. Growth rates

The growth of the three species, estimated using cell abundance throughout the experiment, is presented in Table 4. There were significantly higher ($p \leqslant 0.05$) growth rates of *D. salina* in the P treatment as compared to PAB under both temperature treatments. Moreover, increased temperature benefited growth since rates were higher than in control. On the other hand, significant differences in growth rates between temperature treatments were observed in *P. micans* and *I. galbana* under P ($p \leqslant 0.05$), being higher at increased temperature.

4. Discussion

The results of our study presented here contribute to understand the impact of temperature and UVR upon different phytoplankton species when exposed to solar radiation levels that normally occur in the Patagonia area and simulating an increase of temperature due to climate change as predicted by the year 2100 i.e., a scenario of increase in coastal surface temperature [22]. The first evident finding of our study is related to the species-specific nature of photochemical responses and photoprotective mechanisms. Moreover, UVR-induced PSII inhibition was different among species and that was probably due to differences in their photoprotective mechanisms.

Each species showed a particular response during the exposure: Whereas *P. micans* displayed a slight difference in the daily

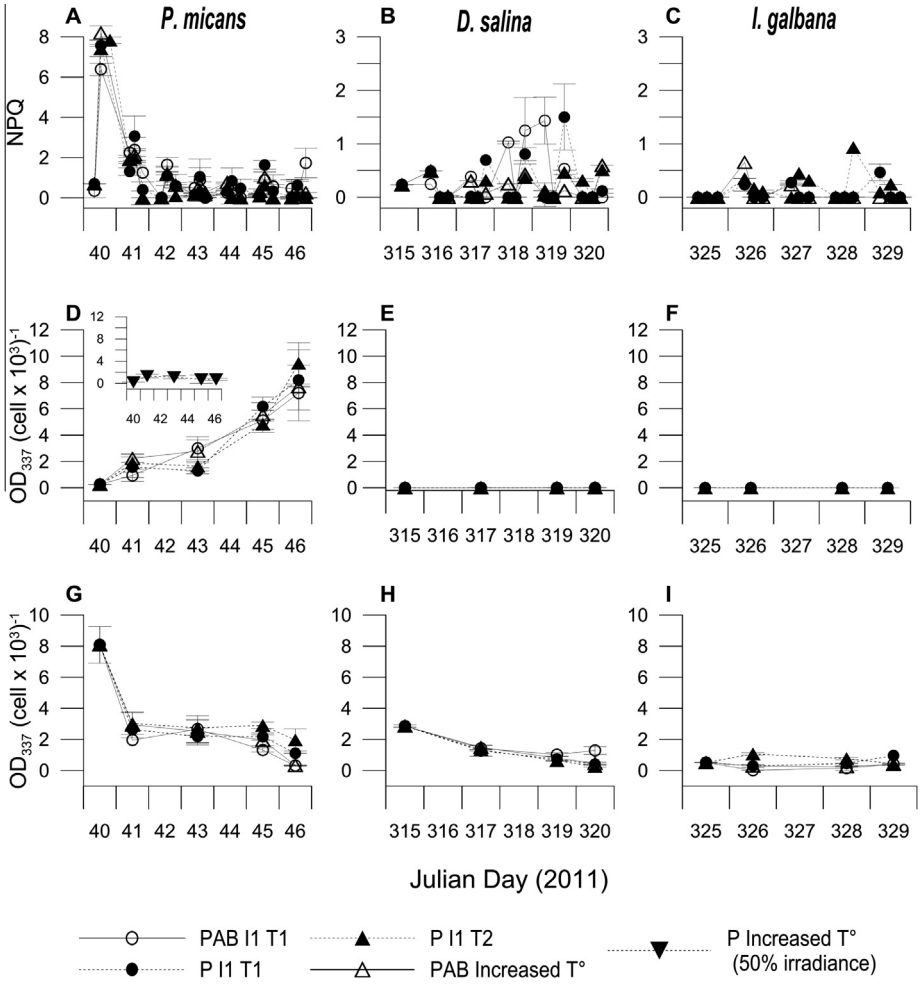

Fig. 4. *Non photochemical quenching* (NPQ; A–C), UVACs concentration by cell ([OD 337 cell^{-1}] * 1000; D–F) and carotenoids concentration by cell ([OD 337 cell^{-1}] * 1000; G–I) in *Prorocentrum micans* (A, D and G), *Dunaliella salina* (B, E and H) and *Isochrysis galbana* (C, F and I) exposed to solar radiation during 5–7 days. Changes in NPQ, UVACs and carotenoids are shown in cells maintained at control temperature (circles) and increased temperature (triangles) under PAB (PAR + UVR; open symbols) or P (PAR; closed symbols). Attenuated intensity radiation treatment (50%) is shown only for UVACs in *P. micans* exposed at P and increased temperature treatments (inset). The lines on top of symbols are the half mean range.

photochemical responses, with exception of the first day, when a pronounced decrease at noon occurred; the other species showed sustained decrease at noon and recovery during the afternoon (*D. salina*) or at the beginning of the next day (*I. galbana*) (Fig. 2). Similarly, Marcoval et al. [35] observed different responses among diatom and dinoflagellate species during a long-term solar radiation exposure: While the Y decrease at noon was pronounced during the whole exposure period in *C. gracilis* and *Thalassiosira fluviatilis*, *Heterocapsa triquetra* and *P. micans* showed lower Y decreases at noon as the experiment progressed, especially in the latter species. Our study demonstrated that PSII inhibition induced by UVR was significant higher in *D. salina* and *I. galbana* than in *P. micans*. All species had similar UVR-induced inhibition at noon but *D. salina* reached inhibition values near zero during the recovery time while *I. galbana* displayed an inhibition of 20% (control temperature) and 60% (increased temperature) lower in comparison to that observed at noon time (Fig. 3). This indicates that photoprotective mechanisms were probably not efficient enough to improve the long-term photochemical responses (*D. salina*) and/or counteract the PSII damage (*I. galbana*). Therefore, the observed responses indicate that the balance between negative (inhibition-damage) and positive (photoprotective-repair mechanisms) effects was different for each species. Some studies demonstrated the higher UVR-tolerance of dinoflagellates in comparison with other groups [35,36] while others have shown that *I. galbana* had a higher UVR sensitivity as compared to diatoms, chlorophytes, and cryptophytes [37,38]. UVR tolerance is achieved through different photoprotective mechanisms, which are species-specific e.g., dinoflagellates have higher concentration and diversity of UVACs, mainly mycosporine-like amino acids – MAAs [14]. In agreement, Marcoval et al. [35] observed that the dinoflagellates *H. triquetra* and *P. micans* exposed to solar UVR reached higher Y values in comparison to the diatoms *C. gracilis* and *T. fluviatilis*, probably due to their higher UVACs concentrations. During our experiment, *P. micans* was the only species that synthesized significant amounts of UVACs (Fig. 4) and showed the best photochemical response and higher UVR tolerance. In fact, UVACs showed a significant (r^2 = 0.96) inverse relation with the Y drop from early morning to noon (ΔY_{8h}–Y_{13h}; Fig. 7). This relationship suggests that higher UVACs content provides photoprotection by minimizing the decrease of Y during morning. Hence, we consider that the UVACs synthesis is a plausible long-term photoprotective mechanism for *P. micans*.

However, other photoprotective mechanisms could help cells to cope with solar radiation exposure, such as xanthophyll cycle. One way to estimate this down-regulation capacity is by analyzing the non-photochemical quenching process (NPQ) which helps to regulate and protect photosynthesis in environments in which light

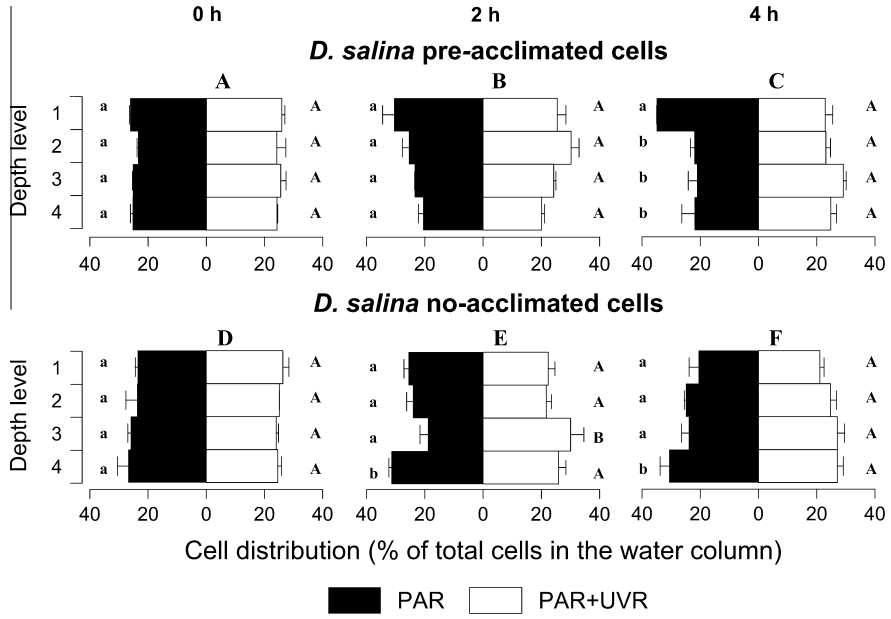

Fig. 5. Percentage of cell abundance of *Dunaliella salina* acclimated (A–C) and no-acclimated cells (D–F) at different depths in the water column: 5, 18, 29 and 40 cm (levels 1, 2, 3 and 4 respectively). Values represent different exposure times: initial (A and D), 2 (B and E) and 4 h (C and F) under a solar simulator. Cells were previously exposed to solar radiation during 5 days (A–C) or maintained in an incubator under dim light (D–F). The lines on top of symbols are the half mean range.

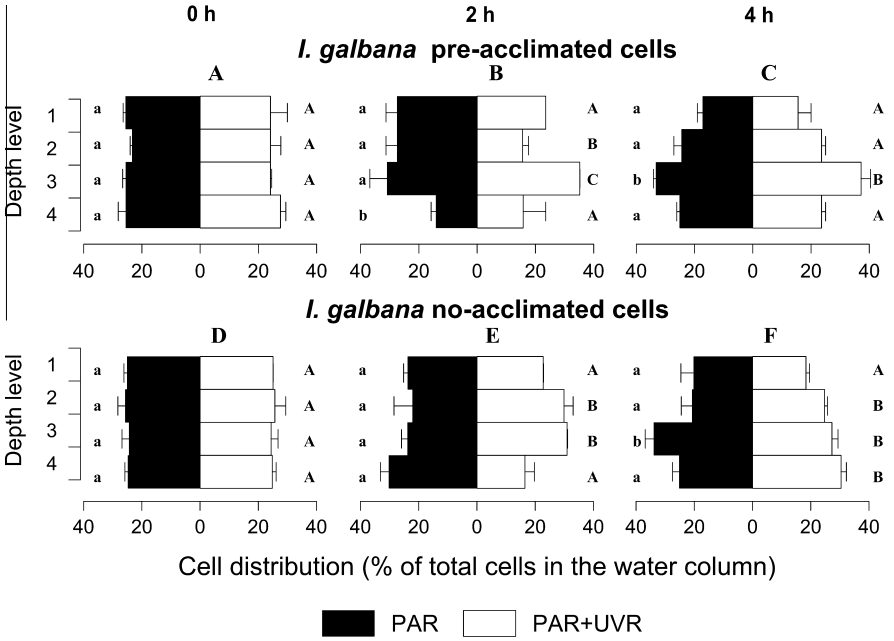

Fig. 6. Percentage of cell abundance of *Isochrysis galbana* acclimated (A–C) and no-acclimated cells (D–F) at different depths in the water column: 5, 18, 29 and 40 cm (levels 1, 2, 3 and 4 respectively). Values represent different exposure times: initial (A and D), 2 (B and E) and 4 h (C and F) under a solar simulator. Cells were previously exposed to solar radiation during 5 days (A–C) or maintained in an incubator under dim light (D–F). The lines on top of symbols are the half mean range.

Table 4
Daily growth rates (μ) based on cell abundance for *Prorocentrum micans*, *Dunaliella salina* and *Isochrysis galbana* incubated at 18 (*P. micans*) or 15 °C (*D. salina* and *I. galbana*) under control temperature – T_1 – and 23 (*P. micans*) or 20 °C (*D. salina* and *I. galbana*) under increased temperature – T_2 – Radiation treatments are: PAB = PAR + UVR; P = PAR only. The values given are the means of duplicates with their half mean range.

Growth rates (d^{-1})	PAB T_1	P T_1	PAB T_2	P T_2
P. micans	0.96 ± 0.06	0.51 ± 0.3	0.80 ± 0.11	0.96 ± 0.07
D. salina	0.32 ± 0.06	0.58 ± 0.12	0.54 ± 0.03	0.66 ± 0.03
I. galbana	n.d.	0.53 ± 0.05	0.54 ± 0.04	0.35 ± 0.06

energy absorption exceeds the capacity for light utilization [39]. Although NPQ is activated in a short time (and considered as a short-term photoprotective mechanism), several studies have demonstrated that the xanthophyll cycle is effective during several days of exposure, e.g. in *T. weissflogii* [10], *D. salina* and *D. tertiolecta* [11,12] xanthophylls decreased the negative UVR and PAR impact on PSII components. In our study, the heat dissipation mechanism, measured as NPQ, was displayed continuously during the whole experiment in *D. salina*, showing maximal peaks at noon (Fig. 4B). On the other hand, *P. micans* displayed lower NPQ values (Fig. 4A), while *I. galbana* did not show a regular pattern (Fig. 4C).

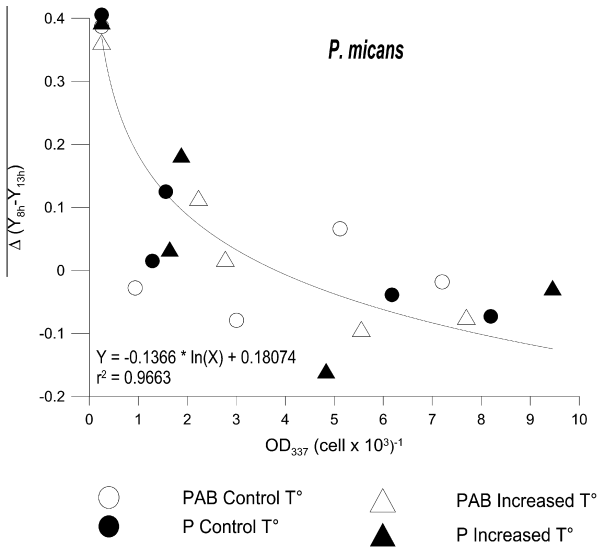

Fig. 7. Relationship between UVACs concentration and Y drop during morning in *Prorocentrum micans* exposed to solar radiation during 7 days. Samples were kept under control (circles) and increased (triangles) temperature, and exposed during daylight to combined PAR and UVR (PAB, open symbols) or PAR only (P, closed symbols).

Probably, the effectiveness of heat dissipation in *D. salina* under stress, i.e. during noon time would contribute to decrease the UVR-induced inhibition during the experiment. On the other hand, *I. galbana* showed a negative balance between inhibition – damage/photoprotective – repair mechanisms, which means that heat dissipation would be not effective enough to prevent PSII damage.

We also noticed that differences in cell size could be a very important factor influencing in photochemical response; large species are usually more resistant to UVR; e.g. in the present study, the most resistant species *P. micans* is also the largest (measured dimensions: length 39–43 μm, width 23–26 μm), while the other 2 species are smaller, they measured around 6–7 μm diameter (*I. galbana*) and 9–11 μm length and 3–5 μm width (*D. salina*). One of the reasons of a higher UVR resistance in larger cells is the chlorophyll package effect: smaller cells (i.e., higher surface-to-volume ratio) needs comparatively less chl *a* per cell as compared to larger cells, while an increase in cell size means a smaller surface-to-volume ratio and thus the need of higher chl *a* content per cell [40]. Moreover, the size dependence of UVR effects are also related to the capacity of cells of synthesizing protective UVACs which is more important in large than in small cells, probably due to the fact that in small ones it would be energetically too costly [41]. As we just already mentioned above, we confirmed that the largest species, *P. micans*, was the only species that showed UVACs synthesis during exposure time and also the highest chl *a* concentration (data not shown).

Some short-term studies have indicated that increase of temperature had a positive effect on photochemical activity, improving the response to PAR and/or UVR [19,20,42]. The last would occur due to higher repair rates of the PSII components under increased temperature, which would result in a positive balance of repair/damage [43]. However, our results show that the temperature effect was species-specific: While the temperature did not have a significant effect on the recovery in *P. micans*, the opposite occurred in *D. salina* and *I. galbana* cells either exposed to solar radiation including or excluding UVR (Figs. 2 and 3). Thus, increased temperature partially counteracted the inhibitory effect of solar radiation on the photosynthesis of the latter two species. These results indicate that beneficial effects of temperature are more evident in cells with a major proportion of damage, i.e. where repair processes induced by temperature are critical- and in a minor proportion in those that develop effective photoprotective mechanisms, e.g., *P. micans.* Similarly, Halac et al. [19] found differences in the antogonistic effects of increased temperature and UVR between two diatom species.

Another outcome of our study was related to the vertical migration responses which were evaluated as a mechanism for avoiding negative radiation effects [7]. Our results showed that *D. salina* cells previously exposed to natural radiation did not evade radiation by means of vertical migration (Fig. 5). Probably, the NPQ activated at noon throughout the long-term exposures in *D. salina* resulted in an effective photoprotection for cells at surface layers and thus, the vertical migration was not so evident in pre-acclimated cells, but was significant in cells not previously exposed to solar radiation. In agreement, Richter et al. [4] found that *D. salina* cells without previous exposure to solar radiation moved to deeper layers at noon. Therefore, although this study demonstrated that *D. salina* is not a UVR-tolerant species, it could have different acclimation status, depending on its previous light history. On the other hand, *I. galbana* cells moved to the deepest layers, whether if they were previously or not exposed to solar radiation. This result is in agreement with the observed lack of effective photoacclimation in *I. galbana* during the exposure to solar radiation in our study.

Finally, the attenuation of radiation intensity – 50% lower than surface irradiance, decreased the PSII inhibition induced by UVR in *D. salina* and *I. galbana* (Fig. 3). Moreover, NPQ in *D. salina* and *I. galbana* (data not shown) or the synthesis of UVACs in *P. micans* (Fig. 4), were almost zero at attenuated radiation. Hence, the cells seem to benefit by this irradiance attenuation as they would not need to activate these photoprotective mechanisms.

In summary, our study demonstrated that among the studied species, *P. micans* was the most tolerant to UVR exposure i.e. it showed the best photochemical response and the capability for synthesizing UVACs. On the other hand, *I. galbana* was the most sensitive to radiation exposure but although it did not synthesize any photoprotective compounds, vertical migration occurred under high irradiances. We also showed that the less UVR-tolerant species would be favored by temperature increase, counteracting in part the negative radiation effect while those synthesizing photoprotective compounds would be not so influenced. Altogether, this study confirmed that the responses of phytoplankton species in a global change scenario would be species-specific and need to be studied using multifactorial approaches, being essential to take into account the combination of different environmental factors for simulating realistic scenarios e.g., radiation attenuation as occurring during mixing. More investigations considering the interaction among radiation, temperature and water column mixing on planktonic organisms and the impact on the aquatic food web and production would contribute to increase the knowledge in global change potential effects in marine systems.

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