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Short- and long-term effects of solar ultraviolet radiation on the red algae *Porphyridium cruentum* (S. F. Gray) Nägeli

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During spring 2002 and fall 2003 we carried out experiment in tropical southern China to determine the short- and long-term effects of solar ultraviolet radiation (UVR, 280–400 nm) on photosynthesis and growth in the unicellular red alga *Porphyridium cruentum*. During the experimentation, cells of *P. cruentum* were exposed to three radiation treatments: (a) samples exposed to PAR (400–700 nm) + UV-A (315–400 nm) + UV-B (280–315 nm) (PAB treatment); (b) samples exposed to PAR + UV-A (PA treatment) and, (c) samples exposed only to PAR (P treatment). To assess the short-term impact of UVR as a function of irradiance, we determined photosynthesis *versus* irradiance (*P vs. E*) curves. From these curves the maximum carbon uptake rate (P_{max}) and the light saturation parameter (E_k) were obtained, with values of ~12.8–14.4 μg C (μg chl *a*)$^{-1}$ h^{-1}, and ~250 μmol m^{-2} s^{-1}, respectively. A significant UVR effect on assimilation numbers was observed when samples were exposed at irradiances higher than E_k, with samples exposed to full solar radiation having significant less carbon fixation than those exposed only to PAR. Biological weighting functions of *P. cruentum* were used to evaluate the UVR impact per unit energy received by the cells; the data indicate that the species is as sensitive as natural phytoplankton from the southern China Sea; however, it is much more resistant than Antarctic assemblages. When evaluating the combined effects of mixing speed and UVR, it was seen that samples rotating fast within the upper mixed layer were less inhibited by UVR as compared to those under slow mixing or in fixed samples. Growth of *P. cruentum* over a week-long experiment was not affected by neither UVR nor UV-A; additionally, low photoinhibition was found at the end as compared to that at the beginning of this experiment. Our results thus indicate that, although on short-term basis *P. cruentum* is affected by solar UVR, it can acclimate to minimize UVR-induced effects when given enough time.

1 Introduction

Solar radiation plays a vital role on autotrophic organisms: On one hand, the portion of the electromagnetic spectrum corresponding to visible wavelengths (PAR, 400–700 nm) is responsible for the bulk of photosynthesis, enabling organisms to obtain its energy, as well as its transferring it to higher trophic levels. On the other hand, ultraviolet radiation-UVR, 280–400 nm–is largely known to produce negative effects, that in aquatic photosynthetic organisms include (among others) a reduction of photosynthetic and growth rates (see review of Villafañe *et al.*[1]) and damage to the DNA molecule (see review of Buma *et al.*[2]).

Organisms have a wide range of responses to UVR exposure. While some species have been shown to be very sensitive even under mild UVR levels, others can be considered very resistant. For example, when addressing UVR-induced photosynthetic inhibition, Helbling *et al.*[3] showed the relatively high resistance of tropical phytoplankton assemblages as compared to those from Antarctica. Instead, when irradiance levels are low or when mixing within the upper mixed layer is relatively fast, longer UVR wavelengths (UV-A, 315–400 nm) can favor photosynthesis.[4,5] Thus, it is obvious that it is not possible to extrapolate the responses of phytoplankton inhabiting a particular aquatic body from results obtained with other organisms or under other radiation conditions.

Numerous studies have addressed the effects of UVR (both UV-A and UV-B, 280–315 nm) on red algae, with most of them recalling for the large variability in responses to these wavelengths[6] because sensitivity and acclimation capacity are species-specific. In this study, we are evaluating the short- and long-term responses to UVR of *Porphyridium cruentum* (S. F. Gray) Nägeli, which is a unicellular red alga with spherical shape that lacks a cell wall. This species has been referenced as a source of several products for food, pharmaceutical and cosmetics purposes, such as arachidonic acid, polysaccharides containing about 10% half-sulfate esters, phycocyanin and phycoerythryn, and antioxidants such as superoxidedismutase; moreover, *P. cruentum* provides relatively large amounts of tocopherol, vitamin K and carotenes.[7] Although several studies have addressed the environmental conditions that would affect biomass and productivity in commercial cultures of *P. cruentum*,[8,9] we are not aware of any of them that had specifically focused on the effects of solar UVR on this species. Montero *et al.*[10] however, have evaluated the responses of *P. cruentum* under artificial UV-B illumination and they have found that exposure to these short wavelengths led to a decrease in the effective quantum yield. However, *P. cruentum* recovered after exposure to white light, thus indicating the presence of repair mechanisms for UVR-induced damage.

The aim of this study is to gain new insights on the autoecology of *P. cruentum* when exposed to ambient levels of UVR, as those naturally received in the tropics. The approach was to evaluate the short- and long-term effects of UVR on photosynthesis (photosynthesis *versus* irradiance (*P vs. E*) relationships, biological weighting functions, combined effects of mixing and UVR) and growth.

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2 Materials and methods

2.1 Culture conditions/study site

The monospecific culture of *Porphyridium cruentum* (S. F. Gray) Nägeli (Rhodophyceae) used in our study was obtained from the Institute of Oceanography (Qingdao), the Chinese Academy of Sciences. Cells of *P. cruentum* were maintained in f/2 medium in a temperature-controlled incubator (LRG-250-G, Zhujiang, Guangdong, China) at 23 °C and under 90 μmol m^{-2} s^{-1} of PAR irradiance, with a photoperiod 12 h-L : 12 h-D. When cells reached the exponential phase of growth, they were used in the experiments as described below. Experiments to evaluate the effects of solar UVR on *P. cruentum* were carried out at the Institute of Marine Biology, Shantou University, Shantou (23.3° N, 116.6° E), the People's Republic of China, during spring (April–June 2002) and fall (October–December 2003).

2.2 Experimentation

Experiments to evaluate short- and long-term effects of solar UVR on photosynthesis and growth of *P. cruentum* were conducted as described below. In addition to measurements of photosynthetic rates, samples were taken at the beginning of all experiments (t_0) to determine chlorophyll *a* (chl *a*), UV-absorbing compounds and cell concentrations (see Section 2.3).

2.2.1 Photosynthesis *versus* irradiance (*P vs. E*) relationships. To assess the short-term impact of solar UVR as a function of the irradiance received by the cells, P. *vs.* E. curves were obtained, and from them the maximum photosynthetic rate (P_{max}) and the light saturation parameter (E_k) were calculated. Samples were put in 20 ml quartz tubes and inoculated with labeled $NaH^{14}CO_3$ to determine photosynthetic rates under three quality radiation treatments, and under seven levels of ambient irradiance. The radiation quality treatments were the following: (1) Duplicate samples that received full radiation (UVR, 280–400 nm, and PAR, 400–700 nm)–uncovered quartz tubes (PAB treatment); (2) Duplicate samples that received UV-A (320–400 nm) and PAR-tubes covered with UV cut-off filter foil (Montagefolie N° 10155099, Folex) (50% transmission at 320 nm) (PA treatment); and (3) Duplicate samples that received only PAR-containers covered with Ultraphan film (UV Opak, Digefra) (50% transmission at 395 nm) (P treatment). The spectra of materials used in our experiments are published elsewhere.[11] Different levels of irradiance were obtained by covering the tubes with none and with an increasing number of neutral density screens up to six layers, thus obtaining a total of seven quantity treatments (*i.e.*, from 100 to <2% of total irradiance). The transmission of the screens was established by measuring the irradiance under them using a filter radiometer (ELDONET, Real Time Computers, Inc., Germany). A tray containing these tubes was then put in a water bath with running water as temperature control, and exposed to solar radiation during 4 h. A total of three experiments were conducted to obtain a mean *P vs. E* curve of *P. cruentum*.

2.2.2 Biological weighting functions (BWFs). To assess the wavelength dependence of photosynthetic inhibition per unit energy and to be able to compare it with that determined in other environments, we conducted experiments to obtain the BWFs of *P. cruentum*. BWFs were determined as follows: Samples were placed in 20 ml quartz tubes and inoculated with labeled $NaH^{14}CO_3$ to measure photosynthetic rates. The tubes (two for each treatment) were placed in a black aluminium frame under the following radiation treatments: uncovered quartz tubes (receiving both UVR and PAR), and quartz tubes covered with various sharp cut-off filters (Schott) cutting incident solar radiation at 295, 305, 320, 360 and 400 nm. The incubations were centered on local noon and lasted 4 h; a total of five experiments were conducted to determine a mean BWF for *P. cruentum*.

2.2.3 Combined effects of UVR and mixing. Within the water column, cells are normally exposed to a fluctuating radiation regime due to mixing within the upper mixed layer (UML). We simulated these irradiance changes by using an experimental device similar to that described by Helbling *et al.*,[5] consisting of one fixed ("static samples") and one rotating system ("moving samples"). Both systems had various layers of neutral density screens that allowed attenuation of incident radiation (from 100 to 6% in five discrete steps) approximately simulating the UVR field received by cells in the upper mixed layer in the coastal waters of the Southern China Sea.[5] Samples were dispensed in 20 ml quartz tubes and inoculated with $NaH^{14}CO_3$ to measure photosynthetic rates under the PAB and P treatments (duplicate samples for each radiation treatment). The tubes in the fixed system received 100, 50, 25, 12.5 and 6% of incident solar radiation during the whole incubation period (2 h). In the moving system, the rotation of the neutral density screens over the samples (*i.e.*, changing of filters from 100 to 6% and back to 100% irradiance) and thus the irradiance was controlled by a stepper motor connected to a microprocessor. The duration of each simulated rotation was 10, 20 and 40 min so that in the experiments that lasted 2 h, phytoplankton experienced 12, 6 or 3 circulations, respectively, within this simulated mixed layer.

2.2.4 Long-term effects of UVR on growth. The potential of *P. cruentum* to acclimate over time to UVR was assessed using long-term experimentation. Samples were put in six 2 l quartz tubes and exposed to the PAB, PA and P treatments (same filters/materials as above). The containers were placed in a water-bath with running water as temperature control, and exposed to natural radiation for one week. Sampling was done on a daily basis to determine chlorophyll *a* (chl *a*), UV-absorbing compounds and cell concentrations as described in Section 2.3. Acclimation to solar UVR during this experiment was established by determining short-term photosynthetic inhibition at the beginning (t_0), and after five days of exposure to solar radiation (*i.e.*, during the exponential growth phase). At t_0 aliquots of the original *P. cruentum* culture were dispensed in 20 ml quartz tubes, inoculated with radiocarbon and exposed to solar radiation under the PAB, PA and P radiation treatments. At day 5, sub-samples from each of these radiation treatments were put in 20 ml quartz tubes, inoculated with radiocarbon and exposed to solar radiation under PAB and P treatments. The tubes were placed in a black aluminium tray in a water bath for temperature control and incubated during 4 h (centered on local noon). One long-term experiment was carried out with *P. cruentum* to determine effects on growth and the ability to acclimate to solar UVR.

2.3 Measurements and analysis

The following measurements and analyses were performed in the samples:

2.3.1 Photosynthetic rates. Samples were inoculated with 0.1 ml −5 μCi (0.185 MBq) of labeled sodium bicarbonate (ICN Radiochemicals). After incubation, samples were filtered onto a Whatman GF/F glass fiber filter (25 mm). The filters were then placed in 7 ml scintillation vials, exposed to HCl fumes overnight, dried, and counted using standard liquid scintillation techniques.[12]

2.3.2 Chlorophyll *a* (chl *a*) and UV-absorbing compounds. Chl *a* and UV-absorbing compounds were measured by filtering a variable volume of sample (50–100 ml) onto a Whatman GF/F glass fiber filter (25 mm), followed by extraction with absolute methanol for 2 h and subsequent determination of the absorbance in a scanning (250–700 nm) spectrophotometer (Shimadzu UV 2501-PC). Chl *a* concentration was calculated from the absorbance using the equation of Wellburn[13] whereas the concentration of UV-absorbing compounds was estimated from the peak height at 334 nm.[14]

2.3.3 Cell counts. Enumeration of *P. cruentum* cells was done under a compound microscope using a 1 ml Sedwick–Rafter chamber and following the methodology described in Villafañe and Reid.[15]

2.3.4 Radiation measurements. Incident solar radiation was continuously measured using a filter radiometer (ELDONET, Real Time Computers, Inc., Germany) which was installed on the roof of the Institute of Marine Biology (Shantou University). The instrument records irradiance in the UV-B, UV-A and PAR wavelength bands with a frequency of one datum per second.

2.3.5 Modeling/statistics. The parameters of the *P vs. E.* curves were obtained using the model of Eilers and Peeters[16] and fitting the data by iteration:

$$P = E/(aE^2 + bE + c)$$

where P is the production (μg C (μg chl a)$^{-1}$ h^{-1}), E is the irradiance (μmol m^{-2} s^{-1}), and a, b and c are the adjustment parameters. The initial slope (*i.e.*, α), the maximum production rate (P_{max}) and the light saturation parameters (E_k) are expressed as a function of a, b and c parameters as follows:

$$E_k = (c/a)^{1/2}$$

$$\alpha = 1/c$$

$$P_{max} = 1/(b + 2\,(ac)^{1/2})$$

The Kruskal–Wallis non-parametric test[17] was used to determine significant differences between the estimated parameters (confidence level = 0.05).

Finally, the BWF curve was obtained by using the BWF-PI model,[18] as our data indicated that photosynthetic inhibition was a function of the irradiance. Photosynthetic inhibition for each wavelength interval (*i.e.*, carbon uptake in the tubes incubated under Schott filters compared to the PAR only control) over the incubation period was expressed as a function of the average irradiance. A detailed spectral irradiance between each filter interval was determined using the STAR software[19] and based on the data obtained with the ELDONET sensor. The spectral dependence of the BWF in the broadband intervals was extracted using the method of Rundel.[20] An exponential decay function (base 10) was used to fit the data in each experiment, and the exponent of the function was expressed as a third degree polynomial function; the best fit was obtained by iteration ($R^2 > 0.95$).

3 Results and discussion

It is widely known that solar radiation, especially UVR, can cause stress to phytoplankton organisms, including inhibition of photosynthetic rates and growth.[1] However, the overall impact of this waveband on phytoplankton is very variable, and it depends on several factors, such as the radiation levels under which the organisms are exposed, their specific sensitivity[1] and their acclimation/repair capacity once the damage has been produced.[2,21] In this paper, we specifically studied the responses to solar UVR of the commercial species *Porphyridium cruentum* when exposed to high natural radiation levels as those received in the tropics.

3.1 Atmospheric conditions

Daily doses of solar radiation throughout the study period (*i.e.*, spring 2002 and fall 2003) are shown in Fig. 1. There was a day-to-day variability in the daily doses due to cloud cover during the study period. Maximum PAR daily doses were ~10 and 8 MJ m^{-2} for spring 2002 and fall 2003, respectively (Fig. 1A). Maximum UV-A and UV-B values during spring 2002 were 1600 and 42 kJ m^{-2}, respectively; and ~1400 and ~30 kJ m^{-2}, respectively during fall 2003 (Fig. 1B and C).

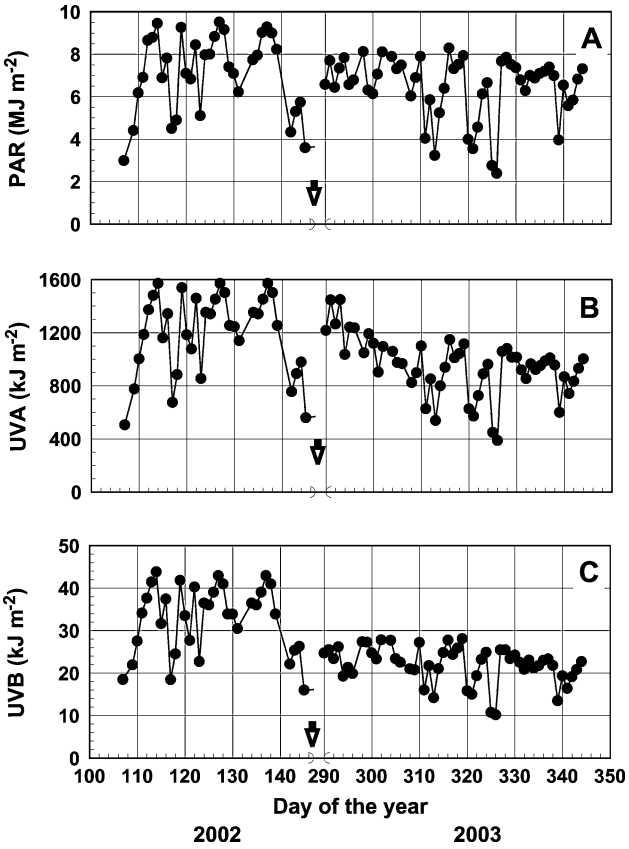

Fig. 1 Daily doses of solar radiation during spring 2002 and fall 2003 at Shantou, China. (A) PAR (400–700 nm, in MJ m^{-2}); (B) UV-A (315–400 nm, in kJ m^{-2}); and (C) UV-B (280–315 nm, in kJ m^{-2}). Days 100 and 290 correspond to April 10, 2002, and October 17, 2003, respectively. Note the axis break indicated with an arrow.

Mean daily doses for PAR, UV-A and UV-B during spring 2002 were 7425, 1252 and 34.4 kJ m^{-2}, respectively, whereas those registered during fall 2003 were slightly lower: 6339, 935 and 21.7 kJ m^{-2}. These relatively high values in this tropical area are rather expected because of the low zenith angles (as compared to those of higher latitudes)[22] as well as because the relatively low ozone concentrations registered throughout the year, with mean values of 285 and 245 Dobson Units for spring 2002 and fall 2003, respectively. As compared to other geographical sites, solar radiation levels in southern China are slightly lower than those registered in the tropical Lake Titicaca in Bolivia (16°S).[23] This tropical lake receives higher radiation fluxes than sea level sites located at comparable latitudes because of its high-altitude location (3800 m a.s.l.); in fact, Blumthaler and Rewald[24] have determined a 10–20% increase in UV-B every 1000 m increase in elevation. Instead, our data on natural radiation levels collected during spring 2002 are about 5 and 2 times higher, for UV-B and UV-A, respectively, than those registered at high latitudes (*i.e.*, Abisko, Sweden, 68° N, web site: www.eldonet.org) at comparable times of the year. Moreover, at the study site in southern China UV-B energy represented ~0.46% of that of PAR, whereas at Abisko it represented ~0.16% of PAR. Thus, the results of the experiments described here represent the worst-case scenario for the UVR impact on *P. cruentum*, as the species has been exposed to the maximum solar irradiances/doses attainable at this sea level site.

3.2 Short-term effects of solar UVR

To obtain information on the photoacclimation status of *P. cruentum* as a function of the irradiance, we determined photosynthesis *versus* irradiance (*P vs. E*) relationships (Fig. 2). These curves are characterized by diverse parameters α (the light limited slope of the *P vs. E* curve), P_{max} (the maximum

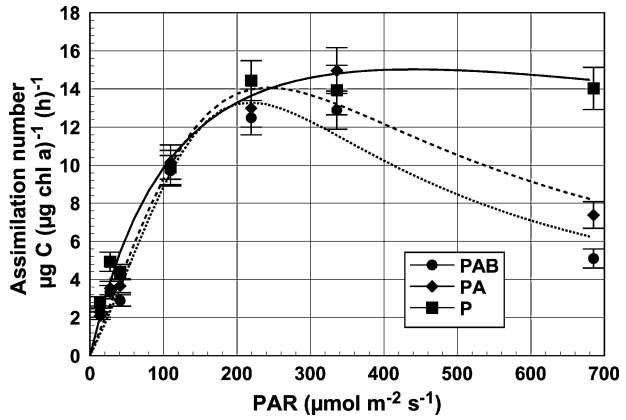

Fig. 2 Phytoplankton assimilation numbers in *P. cruentum* samples (in µg C (µg chl *a*)$^{-1}$ h^{-1}) as a function of the mean PAR irradiance (in µmol m^{-2} s^{-1}). Circles: samples exposed to PAR + UV-A + UV-B (PAB treatment); diamonds: samples exposed to PAR + UV-A (PA treatment); squares: samples exposed to PAR only (P treatment). The data are the mean and standard deviation of three independent experiments.

rate of carbon fixation, *i.e.*, maximum production), E_k (the light saturation parameter, *i.e.*, the intercept between the initial slope of the *P vs. E* curve and P_{max}) and ß (the photoinhibition parameter, *i.e.*, the negative slope of the curve at high irradiances).[25] Previous studies have shown a variety of responses to UVR of these photosynthetic parameters. For example, Furgal and Smith[26] found significant UVR effects on P_{max}, whereas Montecino and Pizarro[27] did not find any impact on phytoplankton assemblages off the Chilean coast. Villafañe *et al.*,[28] working with phytoplankton samples from the Argentinean Sea, found a significant impact of UVR on P_{max} during the pre-bloom period, but not in post-bloom samples. This latter study[28] also showed a significant impact of UVR on E_k in some samples, depending on the previous light history of the cells, which was in turn conditioned by wind speed. In our experiments with *P. cruentum* (Fig. 2), we did not find significant impact ($p > 0.05$) of neither UV-A nor UV-B on both photosynthetic parameters, being P_{max} variable between 12.8–14.4 µg C (µg chl *a*)$^{-1}$ h^{-1} and $E_k \sim 250$ µmol m^{-2} s^{-1}. However, we found significant differences in assimilation numbers between radiation treatments at irradiances higher than E_k. Assimilation numbers of *P. cruentum* receiving only PAR were similar to P_{max} values, indicating the absence of PAR-induced photoinhibition at high irradiances. However, those samples that additionally received UV-A and UV-B, had significant photoinhibition ($p < 0.05$), with assimilation numbers being reduced to 7.4 and 5 µg C (µg chl *a*)$^{-1}$ h^{-1}, respectively, at the highest irradiance used in our experiments (*i.e.*, ~680 µmol m^{-2} s^{-1}). At irradiances of 680 µmol m^{-2} s^{-1}, assimilation numbers decreased by 47 and 16% due to additional UV-A and UV-B exposure, respectively. These differences between radiation treatments, when evaluated together with the mean daily PAR irradiance of 750 µmol m^{-2} s^{-1} (*i.e.*, 163 W m^{-2}) and 650 µmol m^{-2} s^{-1} (*i.e.*, 141 W m^{-2}), for spring 2002 and fall 2003, respectively, clearly suggest that on a short-term basis (*i.e.*, hours), *P. cruentum* is significantly affected by solar UVR. However, since cells had originally a previous light history of relatively low irradiance in the culture chamber (*i.e.*, 90 µmol m^{-2} s^{-1}) it would be plausible that they did not have enough time (*i.e.*, during our short-term incubation) to acclimate to the changes in solar radiation as those occurring in our experiments. Our data, however, suggest a fast acclimation because even though the cells were grown at low PAR irradiances, they had a relatively high E_k (*i.e.*, ~250 µmol m^{-2} s^{-1}) during the incubation period and moreover, there was no photoinhibition due to high PAR; this acclimation however, was not enough to cope with high irradiances of UVR (Fig. 2).

The relative sensitivity towards UVR is generally assessed through the determination of biological weighting functions (BWFs)—functions that quantify the effectiveness of UVR at causing some effect in relation to wavelength,[18] and as they incorporate absolute values of energy, they allow a comparison of responses of species from different environments. The mean BWF for photosynthetic inhibition in *P. cruentum* (Fig. 3) shows that the species is very sensitive at wavelengths lower than ~300 nm. However, this sensitivity decreased sharply with increasing wavelengths. For comparative purposes, we indicated the BWFs of a natural phytoplankton assemblage characteristic from a tropical site in the southern China Sea (Nan'Ao) and from a polar region (Antarctica) (Fig. 3). The biological weights of *P. cruentum* cultures were very similar to those of natural tropical phytoplankton assemblage,[5] suggesting a similar short-term response of cells that were exposed to comparable high natural radiation levels. On the other hand, *P. cruentum* was much more resistant than Antarctic natural assemblages at wavelengths higher than ~300 nm, although more sensitive at lower wavelengths. Thus our results clearly agree with previous findings reporting the relatively high resistance of tropical[3,5,23] as compared to polar species,[3,29,30] probably due to an evolutionary history of adaptation to high radiation levels leading to low damage/high repair rates.[31]

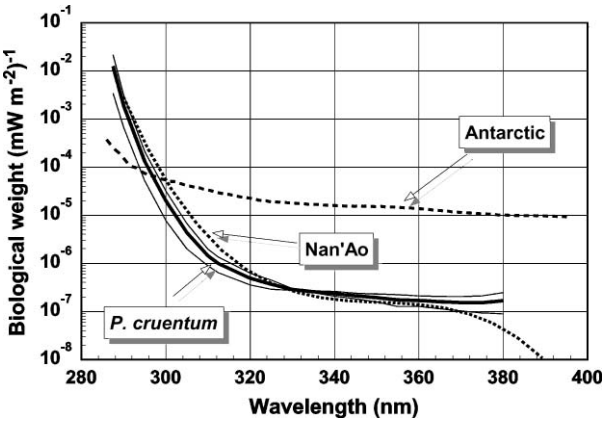

Fig. 3 Mean biological weighting function for *P. cruentum*. For comparison purposes, the BWFs of natural phytoplankton assemblages collected at Nan'Ao (Southern China Sea) and Antarctica are indicated. The thin lines indicate one standard deviation.

Numerous studies have demonstrated that the UVR impact on primary productivity and growth rates can significantly vary if evaluated together with other abiotic parameters, such as temperature,[32,33] nutrient status[34] and mixing.[5,30,35] Here we particularly focused on the interactive effects of UVR and mixing, as in the water column solar radiation attenuates with depth and thus the cells will be exposed to fluctuating radiation regimes within the upper mixed layer (UML). Attenuation of solar radiation in the water column varies from place to place, as it depends on many factors such as dissolved and particulate matter, both of organic and inorganic origin.[36] In our experiments we used published data on the depth and attenuation within the UML at the study area[5] to simulate *in situ* mixing. Photosynthesis of *P. cruentum* cultures were clearly affected by UVR when exposed to a variable irradiance regime (Fig. 4). Since mixing was simulated down to 6% of PAR irradiance, we calculated the integrated inhibition within this UML and compared it to the integrated inhibition of samples exposed at fixed irradiances (*i.e.*, high values in the *y* axis of Fig. 4 indicate higher inhibition in fixed than in rotating samples). Our data support the idea that mixing was beneficial for *P. cruentum* (at least under the simulated mixing speeds used in our experiments), as fixed samples had always higher integrated inhibition due to UVR than those rotating in the water column (*i.e.*, all positive numbers in Fig. 4). However,

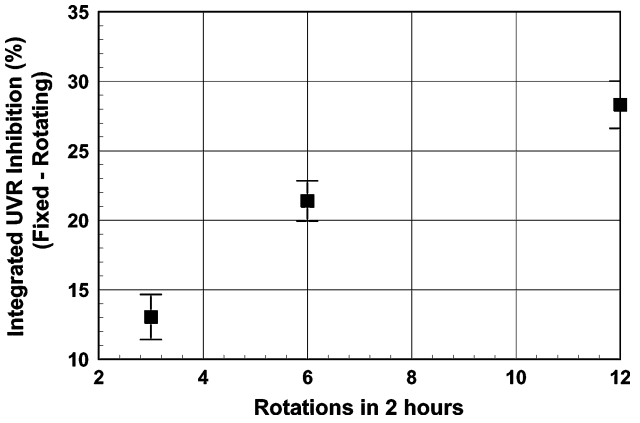

Fig. 4 Inhibition of phytoplankton photosynthesis due to UVR as a function of the number of rotations performed within a simulated UML. Each point represents the difference of the mean integrated (*i.e.*, down to 6% irradiance) UVR inhibition in fixed and in rotating samples. The UVR inhibition was calculated as: $(P_{PAR} - P_{UVR})\ 100/P_{PAR}$, with P_{PAR} and P_{UVR} indicating photosynthetic rates in the treatments that received PAR only or PAR + UVR, respectively.

integrated inhibition of rotating samples varied with the speed of rotation (*i.e.*, frequency change between irradiances): with relatively fast mixing speeds (*i.e.*, twelve rotations of 10 min each, done in 2 h), the rotating samples were much less inhibited, by approximately 28%, than those being fixed in the water column. With slow mixing speeds (*i.e.*, three rotations of 40 min each, done in 2 h), the difference between the integrated UVR-induced inhibition in fixed and rotating samples was significantly lower (*i.e.*, ~13%, $p < 0.05$) than under fast mixing. Finally, at intermediate mixing speeds, the difference in integrated UVR inhibition of photosynthesis between fixed and rotating samples was ~22%. Our results indicating that fast mixing favors photosynthesis (*i.e.*, by reducing the UVR impact on the samples) are clearly in agreement with previous studies carried out with natural phytoplankton populations from the southern China Sea.[5] In that study,[5] the authors reported a similar pattern of higher UVR-induced photosynthesis inhibition under slow mixing speeds, whereas fast mixing not only increased primary productivity but also, phytoplankton were able to use UVR as source of energy. Although we did not test short-term acclimation mechanisms, our data suggest that under fast mixing conditions, dynamic rather than chronic photosynthetic inhibition may occur. In addition, under fast mixing, cells are exposed to a higher frequency of low/high irradiances, which favors the activity of repair mechanisms of the DNA molecule that act once the damage has occurred.[5] This would be especially important for *P. cruentum* cells, which due to their small size (~3–10 μm) might be more vulnerable to UVR-induced DNA damage as compared to larger cells, as seen in studies carried out in temperate marine ecosystems.[37]

3.3 Long-term effects of solar UVR

Even though phytoplankton cells are generally affected by UVR, it is already known that on a long-term basis cells are able to acclimate and thus minimize the damage produced by these wavelengths.[21] Although extensive research has been carried out to address the short-term effects of solar UVR on phytoplankton, the performance of these organisms over longer temporal scales (*i.e.*, days/weeks) has received comparatively much less attention.[23,38–40] Relatively few long-term studies have been done with regard to the impact of UVR on Rhodophyta species- *e.g.*, photoinhibition processes[41] and dynamics of UV-absorbing compounds.[42] Here we evaluated growth over a one-week period when *P. cruentum* cells were exposed to solar UVR (Fig. 5A). Chl *a* concentration either remained relatively constant during the first day of experimentation (PAB treatment) or decreased (PA and P treatments). After this short lag period,

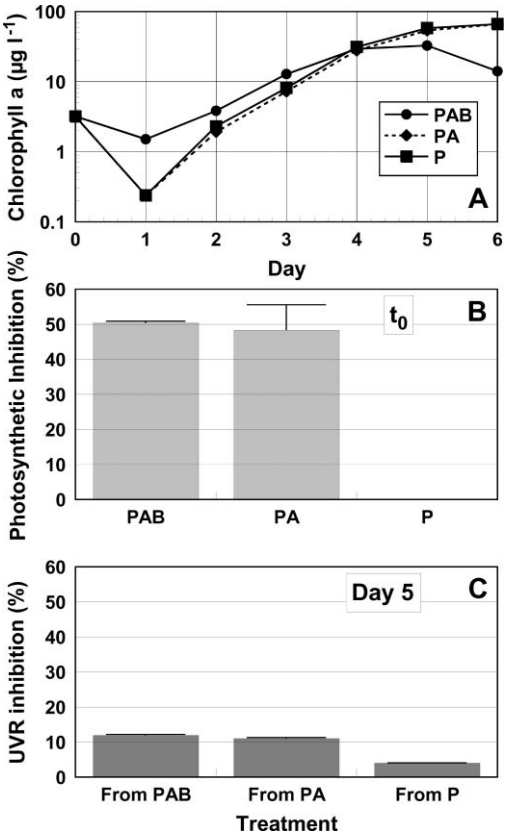

Fig. 5 Growth and photosynthetic inhibition of *P. cruentum* during a one-week long experiment. (A) Growth (as estimated by chl *a* concentrations, in μg l^{-1}) of samples exposed to PAR + UV-A + UV-B (PAB treatment, circles), PAR + UV-A (PA treatment, diamonds) and PAR only (P treatment, squares); (B) Photosynthetic inhibition (%) due to PAR + UV-A + UV-B and PAR + UV-A as compared to the PAR only control at t_0 of the long-term experiment; (C) Photosynthetic inhibition due to UVR at the end of the exponential growth phase for samples that had being previously exposed to PAB, PA and P treatments.

chl *a* concentration increased exponentially, with growth rates of 1.0, 1.56, and 1.58 day^{-1} for the PAB, PA, and P treatments, respectively. Maximum chl *a* concentration after six days of exposure to solar radiation reached values ~66 μg chl *a* l^{-1} in the P and PA treatments, whereas in samples additionally exposed to UV-B, maximum growth was much lower, ~32 μg chl *a* l^{-1}. We also evaluated the acclimation to the solar UVR by determining photosynthetic rates of *P. cruentum* cells both at the beginning and at the end of the exponential growth phase (Fig. 5B and C). At the beginning of the experiment (Fig. 5B), photosynthesis was reduced by ~50% when samples were exposed to either PAR + UV-A or PAR + UVR, indicating a relatively high inhibition that was mostly caused by UV-A. This response is rather similar to that observed in the *P vs. E* curves, although in this latter case UV-B inhibition was much lower (~3% and not significant; $p > 0.05$). The higher UV-A induced photosynthetic inhibition (as compared to that induced by UV-B) seems to be a rather general feature of phytoplankton cells, as seen in numerous studies carried out in diverse marine and freshwater environments of the world.[28,43,44] As the experiment progressed, however (Fig. 5C), there was a clear acclimation to UVR, as evidenced by a significantly lower ($p < 0.05$) photoinhibition (as compared to that determined at t_0, Fig. 5B) when samples from the P, PA and PAB treatments were exposed to full radiation (Fig. 5C). This long-term acclimation to UVR of *P. cruentum* cells is in agreement with studies carried out by Helbling *et al.*[38] with Antarctic marine diatom cultures, who also found lower photoinhibition values at the end of their experiments as compared to that determined at the beginning. It is interesting to note the fact that the PAB treatment had the

lowest growth rate (Fig. 5A) and the highest inhibition at the end of the exponential growth phase (Fig. 5C). However, any damage to the DNA molecule caused by UV-B might not be completely removed and, thus, *P. cruentum* cells had lower growth rates and consequently reaching lower biomass (Fig. 5A). Moreover, our results suggest, that UVR might be affecting in different ways the two main targets—the DNA molecule and photosystems, as previously suggested in studies carried out by Buma *et al.*[37] and Helbling *et al.*[45] in temperate aquatic ecosystems.

A number of mechanisms are proposed to explain the long-term acclimation to UVR of phytoplankton cells.[2,21] One of the most common mechanisms to protect the cells against high UVR levels is through the synthesis of protective UV-absorbing compounds, mainly mycosporine-like amino acids (MAAs) which are commonly found in many Rhodophyta species.[21] However, we did not find significant amounts of them in our *P. cruentum* cultures (data not shown). The virtual absence of these UV-absorbing compounds could be associated to the high energetic cost involved for their synthesis in small-sized cells.[46] We speculate that the acclimation of *P. cruentum* towards high radiation levels, as those registered in our study, might be probably related to an effective dissipation of the excess energy in the photosystem than to DNA damage. Additionally, other mechanisms to minimize UVR impact might act on these cells, as for example through shelf-shading as occurring in mass cultures of *Arthrospira platensis* (Gao, pers. com.).

Based on our results, we conclude that even *P. cruentum* is affected by UVR on short-term basis, it can acclimate relatively fast to the high irradiance conditions prevailing in the tropics, as evidenced in both photosynthetic and growth rates. It is obvious thus that our study has important implications for mass cultivation of this species in outdoor systems. However, in order to fully understand the impact of UVR on *P. cruentum*, further experimentation oriented to determine molecular acclimation mechanisms (*i.e.*, DNA repair) should be done in detail.

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