



Effects of solar ultraviolet radiation on photosynthesis of the marine red tide alga *Heterosigma akashiwo* (Raphidophyceae)

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Abstract

In order to assess the short- and long-term impacts of UV radiation (UVR, 280–400 nm) on the red tide alga, *Heterosigma akashiwo*, we exposed the cells to three different solar radiation treatments (PAB: 280–700 nm, PA: 320–700 nm, P: 400–700 nm) under both solar and artificial radiation. A significant decrease in the effective quantum yield (Y) during high irradiance periods (i.e., local noon) was observed, but the cells partially recovered during the evening hours. Exposure to high irradiances for 15, 30, and 60 min under a solar simulator followed by the recovery (8 h) under dark, 9 and 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ of PAR, highlighted the importance of the irradiance level during the recovery period. Regardless the radiation treatments, the highest recovery (both in rate and total Y) was found at a PAR irradiance of 9 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, while the lowest was observed at 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. In all experiments, PAR was responsible for most of the observed inhibition; nevertheless, the cells exposed only to PAR had the highest recovery in any condition, as compared to the other radiation treatments. In long-term experiments (10 days) using semi-continuous cultures, there was a significant increase of UV-absorbing compounds (UV_{abc}) per cell from 1.2 to $>4 \times 10^{-6} \mu\text{g UV}_{\text{abc}} \text{cell}^{-1}$ during the first 3–5 days of exposure to solar radiation. The highest concentration of UV_{abc} was found in samples exposed in the PAB as compared to PA and P treatments. Growth rates (μ) mimic the behavior of UV-absorbing compounds, and during the first 5 days μ increased from <0.2 to ca. 0.8, and stayed relatively constant at this value during the rest of the experiment. The inhibition of the Y decreased with increasing acclimation of cells. All our data indicates that *H. akashiwo* is a sensitive species, but was able acclimate relatively fast (3–5 days) synthesizing UV-absorbing compounds and thus reducing any impact either on photosystem II or on growth.

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Keywords: Growth; *Heterosigma akashiwo*; UV-absorbing compounds; Photosynthetic quantum yield; Toxic blooms; UVR

1. Introduction

Solar ultraviolet radiation (UVR, 280–400 nm), and the increase of UV-B radiation (280–315 nm) due to ozone depletion, has a range of effects on phytoplankton [1–3]. They include, among others, the impact on growth, metabolism, motility, photo-orientation, pigmentation and photosynthetic capability of phytoplankton [3–6]. One of the

processes that received particular attention is photosynthesis, as solar energy is directly used by autotrophic organisms, and thus any change on UVR might affect primary production [7].

In short time scale (<day) dynamic photoinhibition or even irreversible photodamage of photosystem (PSII) have been reported in macroalgae and microalgae [8,9]. Photoinactivation, as the definitions proposed in a recent review of Franklin et al. [10], usually occurs when the D1 protein in PSII is damaged, causing a decrease in the electron transport [11,12]. It was found, however, that a fast synthesis of the D1 protein was enough to cope with photodamage,

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at least in *Synchocystis* sp. (PCC6803) and thus the organisms were not inhibited [13]. In addition, any impact of the photosynthetic quantum yield could be potentially recovered (dynamic inhibition) but the recovery time might vary from minutes to several hours after the stress of high irradiance is eliminated [14,15].

When considering relatively long-time scales (several days–weeks), autotrophic organisms might protect themselves through the synthesis UV-absorbing compounds, mainly mycosporine-like amino acids (MAAs) [16]. These compounds absorb UVR at a wavelength range between 310 nm and 360 nm and can be synthesized by many organisms [17–20]. Also many studies [21] have shown that they can be bioaccumulated through the diet by organisms at higher trophic levels that do not have the capacity of synthesis of them. The protective role of MAAs have been shown in previous studies (e.g. [22]); however, the size structure of the community is important as the useful concentration of these UV-absorbing compounds in small cells (e.g., <10–20 μm) would be too high and osmotically disadvantageous [23]. It is still uncertain, however, how different species can utilize them to cope with UVR, their rate of production or even the cell quota amount necessary to confer protection.

Harmful blooms of *Heterosigma akashiwo* have been reported in temperate waters [24,25], especially in Japan [26], New Zealand [27], and China [28]. Some studies showed that *H. akashiwo* had anti-predatory activity and ichthyotoxicity [29]. At the same time, it had been shown allelopathic effects towards other algae, specifically diatoms [30]. The growth and toxicity of *H. akashiwo* can be mediated by hydrogen peroxide [24,31] or viruses [32,33]. In addition, and more relevant to the blooms *H. akashiwo* in a climate change environment, [34] showed that growth and toxicity of this alga was markedly influenced by changes in temperature and light intensity. Although many studies had focused on the ecophysiology of *H. akashiwo*, very little is known about the potential effects of solar UVR on this species. The aim of this study was to assess the short- and long-term impact of UVR on the effective quantum yield and growth of *H. akashiwo*. Our emphasis was on determining two different aspects, on one hand to establish the potential recovery after any damage occurred, and on the other hand the capability of the species to acclimate to an increase in solar irradiance to further prevent cellular damage.

2. Materials and methods

2.1. Species and culture conditions

The experiments to evaluate the effects of UVR on *H. akashiwo* (Hada) Hada were carried out at the Marine Biology Institute, Shantou University, during July–October, 2004 and during April–June, 2005. *H. akashiwo* is a bi-flagellated, unicellular golden-brown microalga (8–25 μm long and 6–15 μm wide) and was obtained from the Institute of

Oceanology, Chinese Academy of Science (Qingdao) and maintained in f/2 medium [35], under cool-white fluorescent light at 60–70 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (12L:12D) and 25 °C in a illuminated growth chamber (White Westinghouse, model 515, USA). The cultures were shaken 2–3 times every day when indoor and aerated continuously when they were exposed outdoor.

2.2. Experimentation

2.2.1. Treatments

Samples were obtained when the culture was in exponential growth, diluted to $0.5\text{--}2.7 \times 10^4$ cells ml^{-1} , and used in short- (< day) and long-term (several days) experiments exposing the cells either to sunlight or using a solar simulator (Sol 1200, Dr. Hönle GmbH, Germany) provided with a 1000 W xenon arc lamp. After dilution the culture was placed in quartz tubes (59 mm in diameter and 350 mm high or 25 mm in diameter and 150 mm high), and maintained in a water bath with running water for temperature control (~ 21 °C). The culture was incubated under three radiation treatments: (1) PAB, tubes covered with a 295 nm cut-off foil (Ultraplan, Digefra, Munich, Germany), transmitting 295–700 nm; (2) PA, tubes covered with 320 nm cut-off foil (Montagefolie, Folex, Dreieich, Germany), transmitting 320–700 nm; and (3) P, tubes covered with a 395 nm cut-off foil (Ultraplan UV Opak, Digefra, Munich, Germany), transmitting 395–700 nm. Duplicate samples were used in all radiation treatments. The transmission spectra of these materials are published in Figueroa et al. [3].

2.2.2. Short-term experiments

These experiments were designed to evaluate the impact of UVR over a daily radiation cycle, the recovery of cells under different light conditions and the influence of cell concentration (shelf-shading) in the observed results. In these experiments three radiation treatments (as mentioned above) were used.

The impact of UVR on diurnal patterns of the effective quantum yield was obtained by exposing *H. akashiwo* cells to solar radiation from 8:00 to 18:00 (local time) and the effective quantum yield monitored every two hours (please see below). This set of experiments were conducted under high (July) and low (October) solar radiation.

The short-term impact of UVR on the photosynthetic quantum yield of *H. akashiwo* was obtained after 15, 30, and 60 min of exposure using a solar simulator. The recovery of the cell was followed for 8 h (measurements every 30–120 min) under three conditions: (a) darkness, (b) low PAR (9 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), and (c) high PAR (100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). The irradiance received during the exposure in the solar simulator were: PAR, 245 W m^{-2} (1142 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), UV-A, 55 W m^{-2} and UV-B, 1.9 W m^{-2} with the culture placed at 120 cm from the light source. The light sources for recovery were cool-white fluorescent tubes.

The importance of shelf-shading was studied by exposing cultures of *H. akashiwo* at three different cell density (0.5 , 1.6 , and 2.7×10^4 cells ml^{-1}) to solar simulator for 60 min at local noon and then following the recovery of the effective quantum yield in low light ($\text{PAR} = 9 \mu\text{mol photons m}^{-2} \text{s}^{-1}$).

2.2.3. Long-term experiments

Semi-continuous cultures were maintained in quartz tubes (59 mm in diameter and 350 mm high) at a cell density of 2.7×10^4 cells ml^{-1} . The cultures were exposed to solar radiation under three radiation treatments, PAB, PA, and P (as explained above), during 10 days (from April 5th to 14th, 2005). In order to keep stable cell density, cell counts were obtained using a Coulter counter (Z^{TM} Series particle counter and size analyzer, USA) and fresh medium added to reach the desired cell concentration. Every day samples were taken from each radiation treatment to determine cell concentration (as explained above), spectral characteristic and concentration of UV-absorbing compounds and photosynthetic pigments. In addition, photosynthetic quantum yield was determined at the beginning, middle (day 5) and at the end (day 10) of the experiment.

2.3. Measurements and determinations

2.3.1. Chlorophyll fluorescence

The optimal quantum yield (F_v/F_m) and other fluorescence parameters were determined using a portable pulse amplitude modulated fluorometer (PAM – WATER-ED, Walz, Germany). The initial fluorescence (F_0 , all reaction centers are open) was induced in dark-adapted cells by a red light source, which was weak enough not to cause the PS II reaction centers to close. The maximal fluorescence (F_m , all reaction centers are closed) was induced on this background by a saturating white light pulse (approximately $5300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) in the presence of a weak measuring actinic light. The variable fluorescence (F_v) was obtained as:

$$F_v = F_m - F_0$$

The optimal quantum yield of PS II was determined after acclimating the cells in darkness for 5 min. The effective quantum yield ($\Delta F/F'_m$) was determined by measuring the instant maximal fluorescence (F'_m) and the steady state fluorescence of light-adapted cells and calculated according to van Kooten et al. [36] and Genty et al. [37], so that,

$$Y = \Delta F/F'_m = (F'_m - F_t)/F'_m$$

Inhibition of Y in the different radiation treatments was calculated as:

$$\text{Inh}(\%) = (Y_p - Y_x)/Y_p * 100,$$

where Y_p indicates the yield values of samples in the P treatment, while Y_x indicates the yield values of samples either in the PA, or PAB treatments.

2.3.2. UV-absorbing compounds and pigments

The spectral characteristics of the cultures were determined by filtering 10–25 ml of culture (the volume filtered varied for the different cell concentrations) on a Whatman GF/F filter, extracting in absolute methanol (5 ml) overnight at 4 °C, and centrifuging (10 min at 1500 g). The absorption of the supernatant measured from 250 to 750 nm using a scanning spectrophotometer (DU530 DNA/Protein Analyzer, Beckman Coulter, USA). The concentration of UV-absorbing compounds was determined from the peak high (310–360 nm) according to Dunlap et al. [38]. In addition, the concentration of chlorophyll-*a* (chl-*a*) was also calculated from the scan using the equation of Porra [39].

2.3.3. Growth rates

Samples were taken daily for cell counts (please see long-term experiments above) and the specific growth rate (μ) was calculated as:

$$\mu = \ln(C_n/C_{n-1})/(t_n - t_{n-1})$$

where C_n and C_{n-1} are the cell concentration (cells ml^{-1}) over the ($t_n - t_{n-1}$) period.

2.3.4. Radiation measurement

Incident solar radiation was continuously monitored using a broadband filter radiometer (ELDONET, Real Time Computer Inc., Germany) that has three channels for UV-B (280–315 nm), UV-A (315–400 nm), and PAR (400–700 nm). The radiometer is permanently installed on the roof of Shantou University (23.3°N, 116.6°E) and records data every minute into a PC. A similar instrument was used to measure the output irradiance of the Hönle solar simulator.

2.3.5. Data analysis

All experiments were done in duplicates for each radiation treatment and for each determination of the effective quantum yield at least 4 measurements were done, so a total of 8 measurements were used to calculate the mean and SD of the data presented. A one-way analysis of variance (ANOVA) was used to determine significant difference among the radiation treatments. The significant level was set at 0.05.

3. Results

3.1. Short-term experiments

Daily cycle: The impact of solar radiation on the effective quantum yield over a daily cycle was assessed under high irradiance conditions during Summer (Fig. 1A and B), and low irradiance conditions during Autumn (Fig. 1C and D). Regardless the differences in solar radiation patterns and intensity between the two seasons, *H. akashiwo* had a significant inhibition of the effective quantum yield with increasing irradiances (i.e., throughout the

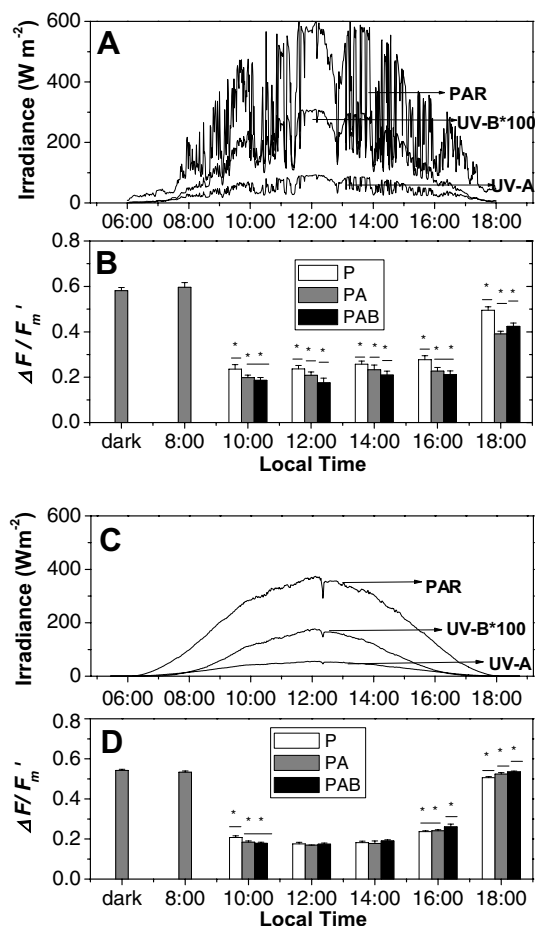


Fig. 1. Irradiance conditions (PAR, UV-A and UV-B) and the diurnal changes in $\Delta F/F'_m$ of *H. akashiwo* cells exposed to solar radiation under the PAB, PA and P treatments. A and B, experiment conducted during July 2004; C and D, experiments conducted during October 2004. The vertical lines in B and D indicate SD ($n = 8$). The asterisks on top of the bars indicate significance level ($P < 0.05$).

day). In both seasons, the bulk of the inhibition was due to PAR, while additional significant inhibition was observed due to UV-A and UV-B radiation at all times during summer (Fig. 1B) but only at some sampling times during autumn (Fig. 1D). The effective quantum yield decreased to 38% (P), 33% (PA) and 28% (PAB) at local noon during summer, and recovered in the late afternoon to 83% (P), 65% (PA) and 70% (PAB) of the initial value (Fig. 1B). The inhibition of the effective quantum yield was to 32% at local noon during autumn (no significant differences among radiation treatments) and the cells recovered in the late afternoon to 98% of the initial value (Fig. 1D).

Exposure/recovery: Cells (0.5×10^4 cells ml^{-1}) were exposed under the solar simulator for 15, 30, and 60 min and their effective quantum yield (Y) measured after the exposure period (Fig. 2). There was a significant decrease of Y in all radiation treatments, but most of the observed inhibition was due to PAR (78%). Additionally, UVR caused a comparatively small inhibition that increased with the exposure time. The samples in the PAB treatment were inhibited by 46.9%, 70.3% and 81.2%, as compared to the P

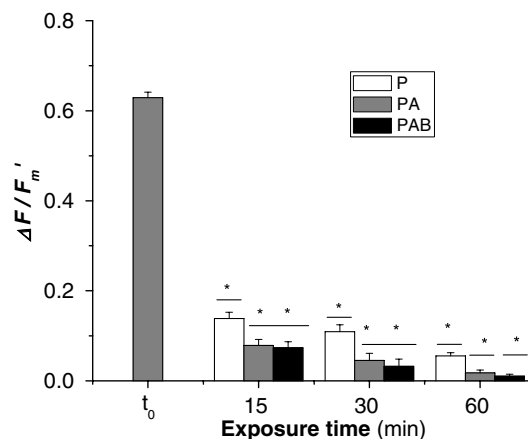


Fig. 2. Effective quantum yield of *H. akashiwo* cells before and after exposure to a solar simulator irradiances for 15, 30 and 60 min under the P (245 W m^{-2}), PA (55 W m^{-2}) and PAB (19 W m^{-2}) treatments. The vertical lines indicate SD ($n = 8$). The asterisks on top of the bars indicate significance level ($P < 0.05$). PAR, 245 W m^{-2} ; UV-A, 55 W m^{-2} ; UV-B, 1.9 W m^{-2} .

treatment, after 15, 30 and 60 min exposure, respectively. The inhibition due to UV-A were 43.1%, 58.5%, and 67.5%, and that by UV-B 3.8%, 11.8%, and 13.8%, respectively, after 15, 30, and 60 min exposure. Of all the UVR inhibition, UV-A was responsible for more than 80% at all exposure times, while UV-B accounted for the rest.

Recovery, after the 15, 30, and 60 min exposure, was followed during 8 hours under three different radiation conditions (Fig. 3). The variation of the effective quantum yield during the recovery period was best fitted with a third degree polynomial function. The reader should be aware that the best fit in other studies [2,5] was obtained with a first-order exponential function. The initial slope (α) of the fitted curves was used as an estimate of the recovery rate of Y , so higher α indicates a faster recovery (please see Table 1). There were significant differences in the recovery among the dark, low PAR and high PAR conditions. The fastest recovery, and higher Y at the end of the recovery period, was achieved under low PAR of $9 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Fig. 3D–F). The only exception was the sample exposed for 15 min under the P treatment (Fig. 3A) that had a higher yield (0.4) at the end of the recovery period than the other samples from the same exposure period (Fig. 3D and G). The lowest recovery was observed in the cells that were under relatively high PAR of $100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Fig. 3G–I). Samples recovering in the dark had an intermediate recovery (Fig. 3A–C). Comparing the rate of recovery (α) for the three radiation treatments (PAB, PA, and P) it is seen (Fig. 3 and Table 1) that the faster recovery was attained in the P samples. While samples additionally exposed to UV-A and UV-B (PA, and PAB treatments, respectively) recovered at lower rates. The samples recovering in the dark that were previously exposed in the P treatment had a significant higher recovery than the ones exposed to UVR, regardless the exposure period (Fig. 3A–C).

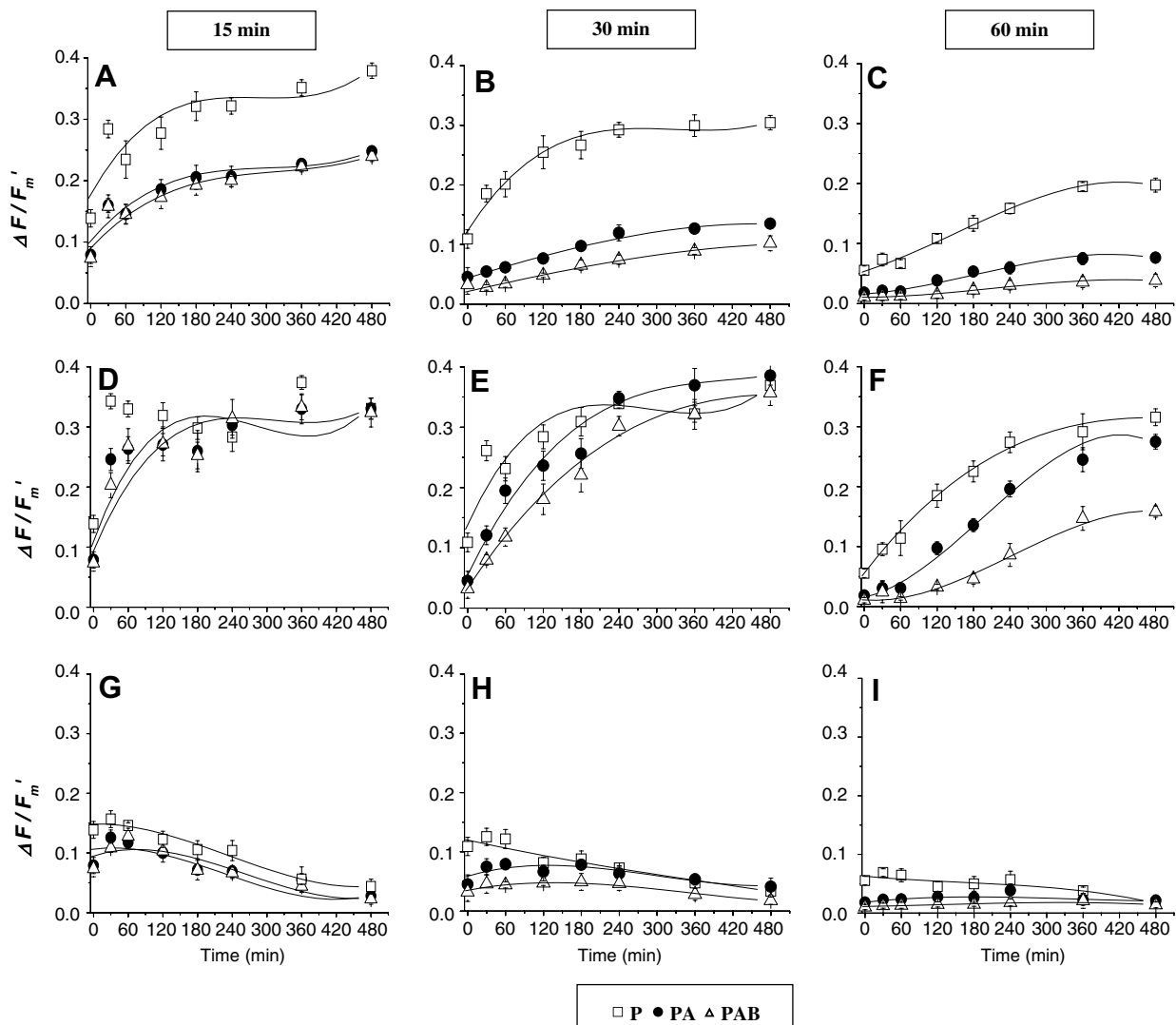


Fig. 3. Recovery of the effective quantum yield of *H. akashiwo* cells after 15 (A, D, and G), 30 (B, E, and H), and 60 (C, F, and I) minutes of exposure (conditions as in Fig. 2). (A–C) Recovery in the dark; (D–F) recovery in PAR = 9 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$; and (G–I) recovery in PAR = 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Values were fitted with a third degree polynomial function. The vertical lines indicate SD ($n = 8$).

Table 1

Recovery rates (initial change of Y per hour) of *H. akashiwo* cells exposed for 15, 30, and 60 min under the solar simulator and recovery under three radiation conditions

Treatment	15 min			30 min			60 min		
	P	PA	PAB	P	PA	PAB	P	PA	PAB
Recovery rates (α)									
Darkness	0.0552	0.0426	0.0390	0.0604	0.0143	0.0103	0.0227	0.0098	0.0041
Low PAR (9)	0.0642	0.0552	0.0612	0.0959	0.0551	0.0517	0.0575	0.0400	0.0202
High PAR (100)	0.0125	-0.0074	-0.0059	-0.0109	0.0006	0.0003	-0.0041	0.0014	0.0007

α values ($\text{Yield} \cdot \text{hour}^{-1}$) were calculated from the adjusted function (explanation in the text). The unit of PAR is $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

We tested the potential short-term effects of self-shading by exposing cultures with three different cell concentrations (0.5 , 1.6 , and 2.7×10^4 cells ml^{-1}) for 60 min under the solar simulator. There was a significant decrease in the photosynthetic quantum yield, after the 60 min exposure, from ca. 0.6 to <0.2 . Most of the observed inhibition was due to PAR, with an additional significant inhibition due to UV-A and UV-B. There were no significant differ-

ences within each radiation treatment with regard to cell concentration. However, the time required for half of recovery ($t_{1/2}$) decreased significantly ($P < 0.05$) (Fig. 4) as a function of the radiation treatment exposure. Cells that received only PAR recovered faster than the ones that received UV-A or UVR. There was also a significant decrease in $t_{1/2}$, within the P and PAB radiation treatments, as a function of cell concentration, indicating that cultures

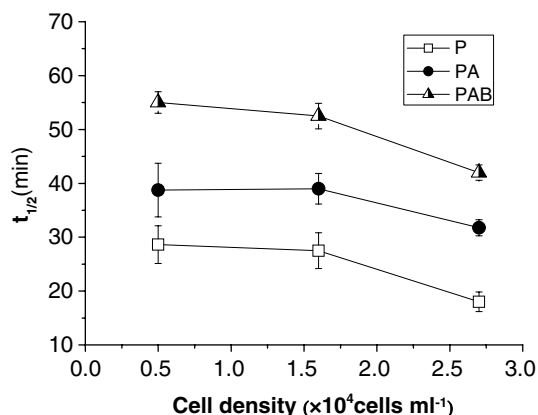


Fig. 4. After exposed *H. akashiwo* cells to solar simulator irradiances for 60 min under the P, PA and PAB treatments, recovery time (minutes) to achieve half of the maximum recovered yield as a function of the previous exposure treatments and cell concentration. The vertical lines indicate SD ($n = 8$). PAR, 245 W m^{-2} ; UV-A, 55 W m^{-2} ; UV-B, 1.9 W m^{-2} .

with high cell density recovered faster than the ones with lower cell density.

3.2. Long-term experiments

The mean daily irradiance values during the long-term experiment were 118, 18.7, and 0.53 W m^{-2} for PAR, UV-A and UV-B, respectively. The specific growth rates (μ) calculated from cell counts from the semi-continuous cultures (maintained at $2.7 \times 10^4 \text{ cells ml}^{-1}$) exposed to solar radiation for 10 days are shown in Fig. 5. Even though there was an initial decrease of μ , most probably reflecting the change of irradiance from the lab (low irradiance) to the outside solar irradiance conditions, it showed a significant increase during the first half of the experiment, from <0.2 to ca. 0.8 at day 5. During the second half (i.e., after day 5) μ was rather constant with the exception of a small decrease at the end of the experiment most probably reflecting a cloudy condition and thus an irradiance decrease during those days. It is interesting to note that during days 2, 3 and 4 the samples receiving UVR had a higher μ than the ones exposed only to PAR, but after this period there were no differences in μ among radiation treatments.

The absorption characteristics of *H. akashiwo* (Fig. 6A) denoted the presence of UV-absorbing compounds with a maximum absorption at 337 nm (inset in Fig. 6A). The concentration of UV_{abc} increased with time, from $1.2 \times 10^{-6} \mu\text{g UV}_{\text{abc}} \text{ cell}^{-1}$ during day one to 2.9, 4.1, and $4.3 \times 10^{-6} \mu\text{g UV}_{\text{abc}} \text{ cell}^{-1}$ at day four for samples under P, PA, and PAB treatments, respectively (Fig. 6B). The specific rates of increase of UV_{abc} within the cells during the first 4 days of experimentation were 0.26, 0.31 and 0.44 for samples in the P, PA, and PAB treatments, respectively. After day 4, there were very little variations in the UV_{abc} content within each radiation treatments suggesting that the culture already acclimated to the new radiation conditions.

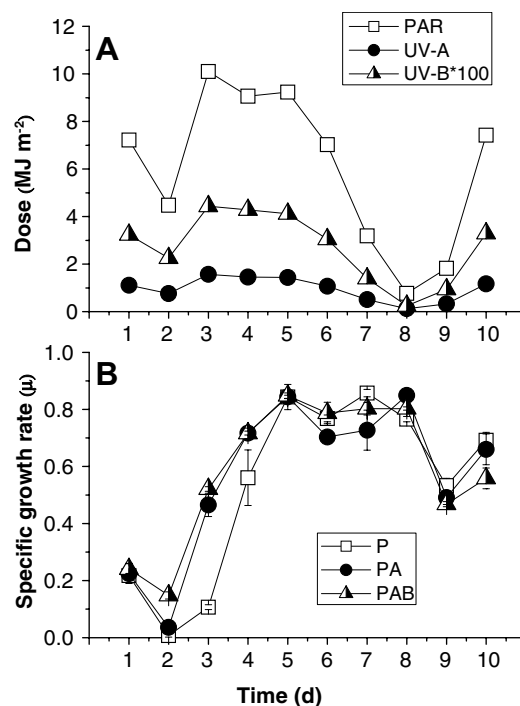


Fig. 5. Daily doses and specific growth rates of *H. akashiwo* cells exposed to solar radiation for 10 days under the PAB, PA and P treatments. The vertical lines indicate SD ($n = 4$).

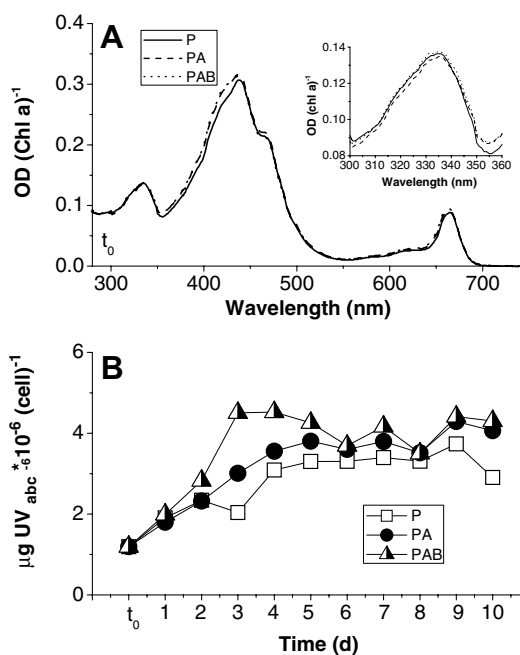


Fig. 6. (A) Absorption spectral characteristics of *H. akashiwo* (methanol extraction) at the beginning of the long-term experiment. The inset shows the absorption spectra from 300 nm to 360 nm. (B) Concentration of UV-absorbing compounds (UV_{abc}) per cell as a function of exposure time and for the different radiation treatments. Cell concentration was maintained at $2.7 \times 10^4 \text{ cells ml}^{-1}$ in the semi-continue cultures.

The first indication that the cells were acclimated after 4–5 days of exposure to solar radiation was provided by the specific growth rates that remained constant after that

day (Fig. 5). The second indication was evident from the UV_{abc} concentration per cell that remained constant also after about day 4. Nevertheless, we conducted short-term experiments exposing samples taken at the beginning of the experiment and after 4 and 9 days of exposure (data not shown). There was a significant decrease in the effective quantum yield when samples from time zero were exposed to solar radiation, as previously described for the daily cycle experiments. There were, however, no significant differences in the quantum yield among radiation treatments after the cells received solar radiation for 4 and 9 days.

4. Discussion

Heterosigma akashiwo is a red tide alga and forms intense Spring blooms that had been reported by many researchers in different areas [24–28], causing huge economic loss in aquaculture. Many studies had focused on the ecophysiology of *H. akashiwo*, however, comparatively very little was done to understand the potential effect of UVR on this species. The data presented here focus on the short- and long-term effect of UVR photosynthesis and growth rate of *H. akashiwo*, so in the next paragraphs we will discuss how this species is affected by solar UVR and also how it can quickly acclimate and thus be resistant high irradiances.

Previous studies [7,18,40–43] have shown that solar UVR can significantly inhibit photosynthesis of aquatic organisms in short time scales (i.e., less than a day). Nevertheless, cells can have different mechanisms to cope with the excessive solar radiation energy in the short-term scale; for example, photoprotection has been shown to be very effective in many algae (e.g. [15]). Photoprotection, based on the xanthophyll cycle, was also found in higher plants [12,14] and refers to dissipation of excitation energy in the antennae [10]. In contrast to this, photoinactivation, due to damage to the D1 protein of PS II, cause a decrease in the electron transport rate [11,12]. In long-term scales (> day, weeks), however, the cells can show different degrees of acclimation after exposure to high radiation through the synthesis of UV-absorbing compounds (e.g. [16]) or a fast synthesis of D1 protein [13,44].

It is expected that during the early phase of the initiation of a bloom increasing stratification of the water column would lead to an increase in solar radiation within the upper mixed layer (UML). For example, it has been shown for the Southern Ocean, that a bloom can not proceed until the ratio between the depth of the UML and the depth of the euphotic zone (E_u) was less than 0.5 [45,46]. At our tropical location in SE China water, the ratio UML/ E_u might be different, however, it would be expected, that any potential blooming cells are thus capable of coping with the increased irradiance. In the present study, we forced the cells to receive a maximum irradiances and thus our data represent the worst case scenario as if the cells were at the surface layer of the water column. Many studies had also shown that photoprotection or photoinactivation

of aquatic organisms can be detected after exposed to solar UVR for short-term period and in general evaluated the recovery in the dark or dim light [47–49]. In the short-term experiments conducted by us, *H. akashiwo* had a light dependency during the recovery, so the relative influence of dynamic and chronic process on recovery will vary with light intensity (Fig. 3). The best recovery in our study was obtained when the cells were exposed to relatively low PAR (i.e., $9 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and the lowest at higher irradiance of $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. This suggests that in the water column recovery would only be attained at a variable depth that would depend on the attenuation coefficient of that particular water column. As the bloom progress, and cell number start to increase, the attenuation coefficient would increase and thus recovery would be achieved at increasing shallower depth. Previous mixing studies [50,51] have shown this dynamic with natural phytoplankton assemblages from different parts of the world.

In addition, with longer time scales, the cells would become more acclimated to the new irradiance conditions and thus the bloom can reach its peak. Our long-term data support the view of the high resilience of *H. akashiwo* to solar ultraviolet radiation through the synthesis of UV-absorbing compounds reaching maximum concentrations after 2 or 3 days of exposure (Fig. 6). Similar results were found by other researchers, that showed an increasing protection of the cell against the deleterious of UVR as the UV_{abc} content increased [16–18,20]. Even though *H. akashiwo*, having a mean cell size of $15 \mu\text{m}$, seems to be in the borderline for an effective use of UV-absorbing compounds [23], our data suggest that these compounds did have influence in photoprotecting the cells during the long-term experiments. Our data also support previous findings [18] that the synthesis of UV-absorbing compounds was an important mechanism for two centric diatom species and that the synthesis was a function of the intensity and radiation quality. This dependence of UV_{abc} synthesis on radiation quality was especially interesting in our results. The faster synthesis of UV_{abc} observed in the samples exposed to full solar radiation (PAB treatment) had direct relationship with the higher specific growth rates (Fig. 5) that these samples had as compared with the other radiation treatments. This indicates that even though *H. akashiwo* cells were sensitive to solar UVR in the short-term exposure (<day 1), they had a high resistance after the long-term exposure, and even more they were able to benefit from the exposure to UVR. Other studies conducted in the area [51] as well as in other parts of the world [52] have previously shown that autotrophic cells can use and benefit from exposure to UVR.

Our data, presented in this study, indicates that the red tide alga *H. akashiwo* had developed mechanisms to cope with increasing solar radiation and that any increase of solar UV-B might have little impact in this toxic species. Future studies should also extend to understand the potential effects of UVR on the toxicity level of this alga as has been done relating toxicity and other environmental variables.

5. Abbreviations

UV _{abc}	UV-absorbing compounds
PAR	photosynthetically active radiation
UVR	UV radiation (280–400 nm)

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