Impacts of UV radiation on photosynthesis and growth of the coccolithophore *Emiliania huxleyi* (Haptophyceae)

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ABSTRACT

Coccolithophores are exposed to solar UV radiation (UVR, 280–400 nm) in their natural habitats. However, little has been documented on their physiological responses to UVR. We investigated the UV impacts on the photosynthesis, calcification, photochemical efficiency and growth of *Emiliania huxleyi* while culturing the cells under solar radiation. Presence of UVR significantly decreased the rates of photosynthesis and calcification. Shorter wavelengths of UV-B led to more damages to photosynthetic apparatus than to calcifying machinery, while longer wavelengths of UV-A resulted more harms to the calcification. As the cells were grown during long-term exposures to solar radiation, the ratios of repair to UV-related damage increased, indicating their acclimation to UV. The specific growth rate of the acclimated cells was inhibited by natural levels of UVR by about 25%, and the cells became bigger with more coccoliths, reflecting a slower cell division and enhanced calcification per cell, a trade off to counteract the UV-induced harms. The absorptivity of UV-absorbing compounds (peaked at 280 nm) increased tremendously in response to the exposure of UVR. UV-induced stress led to a protective strategy of *E. huxleyi*, sacrificing the growth by allocating energy for accumulation of these compounds and calcification.

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1. Introduction

Coccolithophores influence the global carbon cycle by both photosynthetic carbon fixation and biological calcification. *Emiliania huxleyi*, as the most abundant coccolithophore, is distributed in both nearshore and open oceans and in both temperate and subpolar regions (Holligan et al., 1993). It seems able to adapt to tremendous changes in light and temperature. Studies showed that *E. huxleyi* cells are resistant to high light levels with negligible photoinhibition (Nielsen, 1997), and it has been hypothesized that its coccoliths around the cells can dissipate the high solar radiation (Paasche, 2001). However, other studies showed that the photoinhibition-tolerance of this organism was independent of coccoliths (Nanninga and Tyrell, 1996; Harris et al., 2005), and calcification was supposed not to provide protective functions to dissipate energy under high irradiances (Trimborn et al., 2007).

Solar ultraviolet radiation (UVR, 280–400 nm) is known to affect aquatic primary production (Beardall and Stojkovic, 2006; Häder et al., 2007). It inhibits growth and photosynthesis (Heraud and Beardall, 2000; Gao et al., 2007a; Guan and Gao, 2008; Gao and Ma, 2008), damages proteins and DNA (Karentz et al., 1991; Buma et al., 2000; Grzymski et al., 2001; Bouchard et al., 2005), harms cell membrane (Kramer et al., 1991), degrade cell wall and even inhibit protein channels (Murphy, 1983; Sobrino et al., 2004), thus suppresses nutrient uptakes (Behrenfeld et al., 1995) in phytoplankton. On the other hand, positive effects of UVR have also been documented in algae as well as in higher plants (Yao et al., 2007). In algae, UV-A (315–400 nm) can induce photorepair of DNA damages caused by UV-B (280–315 nm) (Karentz et al., 1991) and enhance photosynthetic carbon fixation (Neori et al., 1988; Barbieri et al., 2002; Helbling et al., 2003; Gao et al., 2007b). In open oceans, biologically effective UV-B radiation often penetrates to depths greater than 20 m (Gieskes and Kraay, 1990; Boelen et al., 1999). In the marine environments, *E. huxleyi* cells are exposed to solar UVR and to more enhanced extents when the water column becomes stratified with shallower UML (upper mixed layer) in the summertime (Balch et al., 1991; Nanninga and Tyrell, 1996).

The growth of *E. huxleyi* is sensitive to UV-B (Buma et al., 2000; Van Rijssel and Buma, 2002); and its cell size and content of photoprotective pigments increase when the cells exposed to UVR (Buma et al., 2000; Garde and Caroline, 2000; Van Rijssel and Buma, 2002). Response of photosynthetic apparatus to changes of light in *E. huxleyi* can differ in different strains, either adjusting the effective absorption cross-sections or altering PSU size (Suggett et al., 2007). However, little is known on the impacts of UVR on calcification of coccolithophores.

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This study aimed to investigate the photosynthesis and calcification of *E. huxleyi* under different radiation treatments with or without UVR and to see how the cells respond to short-term and long-term exposures to UVR.

2. Materials and methods

2.1. Species and culture conditions

E. huxleyi (CS-369) was obtained from CSIRO (Australia) and maintained in K media (Keller et al., 1987), under cool-white fluorescent light at about 50 μmol photons m^{-2} s^{-1} (12L:12D) and 20 °C in growth chamber (GXZ-300D, China). The cells were allowed to acclimate to a PAR level of 400 μmol m^{-2} s^{-1} gradually (the initial phase: cells exposed to 50 μmol m^{-2} s^{-1} for 4 days, the middle phase: renewing the culture medium before exposed to 200 μmol m^{-2} s^{-1} for 4 days, and the third phase: renewing the culture medium again and then exposed to 400 μmol m^{-2} s^{-1}). Before the cells were used for short or long-term experiments, they had been grown at 400 μmol m^{-2} s^{-1} (the PAR inside the glass vessels) for 8 generations (batch culture) on an orbital shaker (ZD-9560, Hualida, China) to avoid sedimentation of cells. Cells in the exponential phase were used for all the experiments or inoculation for outdoor cultures. For the UV-exposures, incubations were carried out in small (2 cm in diameter, 7 cm long) or large (5.9 cm in diameter, 35 cm long) quartz tubes, which were maintained in a water bath for temperature control (20 ± 0.5 °C) using a cooling circulator (CAP-3000, Japan)

2.2. Experimentation

The experiments were carried out at the Marine Biology Institute, Shantou University, during May–July 2007. Short-term exposures were carried out to see the photosynthetic and calcification responses to UV; and long-term exposures were designed to see how the cells acclimate to solar radiation and to what extent the solar UVR affects growth. Photosynthetic carbon fixation, photochemical efficiency (Φ_{PSII}), calcification rate and particulate inorganic carbon (PIC) content per cell were analyzed. Biological weighted function (BWF) was established to distinguish the effects of different UV wavelengths on photosynthesis and calcification.

2.3. Solar radiation monitoring and radiation treatments

Incident solar radiation was continuously monitored using a broadband ELDONET filter radiometer (Real Time Computer, Möhrendorf, Germany) which has 3 channels for photosynthetically active radiation (PAR, 400–700 nm), ultraviolet-A (UV-A, 315–400 nm) and ultraviolet-B radiation (UV-B, 280–315 nm), respectively (Häder et al., 1999). This device has been universally recognized (certificate No. 2006/BB14/1) and was calibrated regularly with the assistance from the maker every year. The cut-off filters reduce 4% of PAR in water due to their reflection (Gao et al., 2007b). There was about 5 nm difference between the measured and exposed UV-A waveband. Therefore, the cells received about 2% less UV-A and about 4% less PAR in contrast to the measured irradiances.

The cells were exposed to the following radiation treatments with or without UVR or UV-A: (1) PAB (PAR + UV-A + B), tubes covered with a 295 nm cut-off foil (Ultraphan, Digefra, Munich, Germany), transmitting irradiances above 295 nm; (2) PA (PAR + UV-A), tubes covered with 320 nm cut-off foil (Montagefolie, Folex, Dreieich, Germany), transmitting irradiances above 320 nm; and (3) P (PAR), tubes covered with a 395 nm cut-off foil (Ultraphan UV Opak, Digefra, Munich, Germany). The transmission spectra of these foils are available elsewhere (Zheng and Gao, 2009). For determina-

tion of the energy-dependant responses to UVR (BWF, biological weighting function) of photosynthetic and calcification, six different radiation treatments were carried out using the cut-off filters (Schott) that cut the solar radiation at 280, 295, 305, 320, 350, and 395 nm (the transmission spectra of these filters have been published elsewhere, Villafañe et al., 2003). The incubations lasted for 3 h for the determination of either the *P–E* curve or the BWF. The irradiances of PAR, UV-A and UV-B for the BWF exposures were 347.8, 69.6 and 2.6 W m^{-2}, respectively.

For the cells to acclimate to the incident solar radiation, a long-term exposure was run during the period of July 8–23, 2007. Solar radiation levels were adjusted using 2 to none layers of neutral density screens so that the cells received 25%, 50% and 100% levels of solar radiation in the initial, middle and later phases, respectively. The initial cell density was set at 2.8×10^6 cells ml^{-1}, diluted to about 5×10^5 cells ml^{-1} in the middle and later phases. To avoid sedimentation of cells, the quartz tubes with caps were shaken three times at dawn, noon and sunset during daytime, respectively. Cells were counted under microscope (BX50F4, Olympus Optical Co. Ltd., Japan) using a haemacytometer.

2.4. Measurements of photosynthesis and calcification

The cells of stable physiological performance (grown at 400 μmol m^{-2} s^{-1} for 8 generations at the exponential phase) were used for measurements of photosynthesis and calcification. Each sample of 20 ml was inoculated with 50 μl of 5 μCi (0.185 MBq) of labeled sodium bicarbonate (ICN Radiochemicals). After the incubations, cells were filtered onto a Whatman GF/F glass fiber filter (25 mm), which was then placed in a 20 ml scintillation vial, exposed to HCl fumes overnight, dried in 45 °C. A parallel sample was also filtered but not exposed to HCl fumes for determination of calcification. In this case, the filter was rinsed 4 times with filtered and sterilized seawater, and 1 careful rim rinse in which the cylindrical tower was removed with vacuum applied (Balch et al., 1996). The radioactivity of the fixed ^{14}C was counted with a scintillation counter (LS 6500 Multi-Purpose Scintillation Counter, Beckman Coulter, USA) after the filter was digested in the cocktail (Wallac Optiphase HiSafe 3, PerkinElmer life and Analytical Sciences, USA). The photosynthetic carbon fixation was estimated according to the radioactivity of HCl-fumed filters. The rate of calcification was determined as the difference between the total (non-CHL-fumed filters) and the photosynthetic carbon fixation (CHL-fumed filters) (Paasche, 1963; Pienaar, 1994).

2.5. Photosynthesis versus irradiance (P–E)

The photosynthesis versus irradiance (*P–E*) curves with or without UVR were determined under three quality radiation treatments as described above and under seven levels of solar radiation by covering the quartz tubes with none or an increasing number of neutral density screens thus varying irradiance from 100% to <2%. The tubes were maintained in a water bath for temperature control (20 ± 0.5 °C) as mentioned above. Incubation under each treatment or radiation level lasted 3 h.

2.6. Biological weighting functions

To assess the sensitivity of photosynthesis and calcification to UVR, BWFs were determined for the cells exposed to solar radiation under six different quality radiation treatments as mentioned above. The BWF curves were obtained by using the BWF-PI model (Neale and Kieber, 2000). The mean energy between each filter interval was calculated using the STAR software (Ruggaber et al., 1994) with the data recorded by the ELDONET filter radiometer. 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 ($R^2 > 0.95$).

2.7. Measurement of particulate inorganic carbon

The amount of PIC was estimated according to Takano et al. (1995). Initially, the total inorganic carbon concentration (IC_1) of the *E. huxleyi* cultures was determined using a total organic carbon analyzer (TOC-5000, Shimazdu Corp., Kyoto, Japan). IC_1 includes both the inorganic carbon of the PIC related to coccoliths and dissolved inorganic carbon (DIC) of the growth medium. Then, the inorganic carbon concentration (IC_2) of the filtrate (Whatman GF/F) was also determined. The PIC content was derived as the difference between IC_1 and IC_2. Since *E. huxleyi* does not possess an efficient CO_2 concentrating mechanism (Raven and Johnston, 1991; Nimer and Merrett, 1992), the influence of intracellular inorganic carbon pool on the estimation of PIC was neglected.

2.8. Determination of photochemical efficiency

The photochemical efficiency or effective quantum yield (Φ_{PSII}) was measured with a pulse-amplitude-modulated (PAM) fluorometer (PAM—WATER-ED, Walz, Germany) according to Genty et al. (1989) as follows: $\Phi_{PSII} = \Delta F/F'_m = (F'_m - F_t)/F'_m$, where F'_m represents the instant maximal fluorescence and F_t the steady state fluorescence of light-adapted cells. The saturating light pulse was 5300 μmol m^{-2} s^{-1} with 0.8 s duration. Measuring light is about 0.3 μmol m^{-2} s^{-1}, and the actinc light 10 μmol m^{-2} s^{-1}.

The rates of UVR-induced damage to photosynthetic apparatus (k, in min^{-1}) and corresponding repair (r, in min^{-1}) were estimated according to the previous studies (Lesser et al., 1994; Heraud and Beardall, 2000). The details were shown in Guan and Gao (2008). UVR-induced inhibition of Φ_{PSII} was calculated as: Inh (%) = ($Y_0 - Y_{10}$) × Y_0^{-1} × 100, where Y_0 indicates the initial (control), while Y_{10} the Φ_{PSII} after 10 min exposures to P or PAB treatments.

2.9. Cell size and growth rates

The cells were examined with a Carl Zeiss microscope (Axioplan 2, Germany) and their sizes were measured by using an Axiovision software. The specific growth rate (μ) was determined as follows: $\mu = \ln(C_a/C_b)/(t_a - t_b)$, where C_a and C_b are the cell concentrations (cells ml^{-1}) after or before 24 h ($t_a - t_b$) incubation, respectively.

2.10. Absorption characteristics of pigments

The absorption of the pigments was 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*) before measuring with a scanning spectrophotometer (Shimadzu UV 250-PC, Japan). Determination of chl-*a* content was carried out according to Strickland and Parsons (1968) after extraction in 90% acetone.

2.11. Data analysis and statistics

The parameters of the *P* versus *E* curves were obtained using the model of Eilers and Peeters (1988) and fitting the data by iteration as follows: Pn = $E/(aE^2 + bE + c)$, where Pn is the photosynthetic rate (pg C cell^{-1} h^{-1}), *E* is the irradiance (μmol photons m^{-2} s^{-1}), and *a*, *b*, and *c* are the adjustment parameters. The initial slope (i.e., α), the maximum photosynthetic rate (P_{max}) and the light saturation point (E_k) were expressed as a function of *a*, *b*, and *c* parameters as $\alpha = 1/c$, $P_{max} = 1/(b + 2(ac)^{1/2})$ and $E_k = (c/a)^{1/2}$, respectively. The parameter

"*a*" is considered as the photoinhibition term (Eilers and Peeters, 1988) or as the function of the exposure time above E_k (Macedo et al., 2002).

A one-way analysis of variance (ANOVA) (Turkey test) was used to determine significant difference among the radiation treatments and a two sample pair-wise *t*-test was used to compare the photosynthetic parameters among the radiation treatments. A confidence level was set at $P = 0.05$.

3. Results

Relationship of photosynthetic carbon fixation with PAR was established under the radiation treatments with or without UVR (Fig. 1). The rate saturated at 450 μmol photons m^{-2} s^{-1} under PAR alone, at 350 μmol photons m^{-2} s^{-1} under PAR + UV-A and at 300 μmol photons m^{-2} s^{-1} under PAR + UV-A + B. Presence of UV-A or UV-A + B enhanced the apparent photosynthetic efficiency (α) and lowered the E_k. The α values were significantly higher in the presence of UV-A or UV-A + B compared with the PAR alone treatment and showed insignificant difference between the UV-A or UV-A + B. Little inhibition of the photosynthetic rate was observed even at the highest PAR irradiance of 1600 μmol photons m^{-2} s^{-1} in the absence of UVR. However, addition of UV-A or UV-A + B resulted in significant reduction (52–62%) of the photosynthetic rate, indicating a UV-induced photoinhibition (Table 1). Such UV-induced photosynthetic inhibition became significant at PAR levels higher than 400 μmol photons m^{-2} s^{-1}. UV-A brought about 52% and UV-B about 10% of the inhibition at the highest PAR level of 1600 μmol photons m^{-2} s^{-1}. The UV-B-induced inhibition was significant (Fig. 1, Table 1). Correspondingly, under the same levels
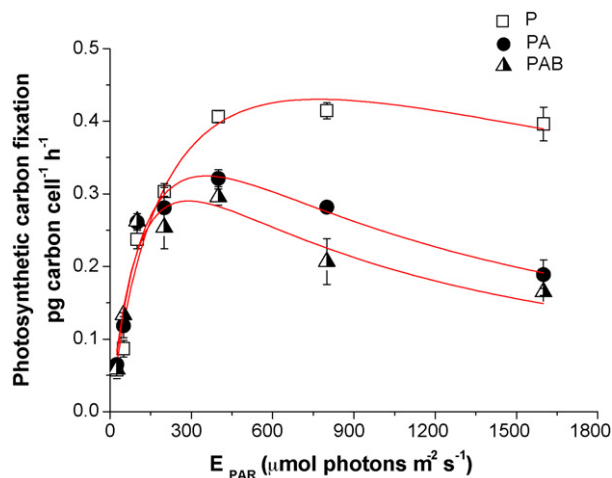

Fig. 1. Photosynthetic carbon fixation of *Emiliania huxleyi* in relation to different radiation treatments with (PA: PAR + UV-A; PAB: PAR + UV-A + B) or without UVR (P). The data are the means + SD of three independent experiments.

Table 1
Photosynthetic parameters (i.e., α, E_k, P_{max}) derived from the *P–E* curves (Fig. 1) of *Emiliania huxleyi* determined with (PAB, PAR + UV-A + B; PA, PAR + UV-A) or without UVR (PAR). Data are the means of 3 repeats *P–E* curves + SD (parenthesis). Different superscript letters (a–c) indicate significance at 95%.

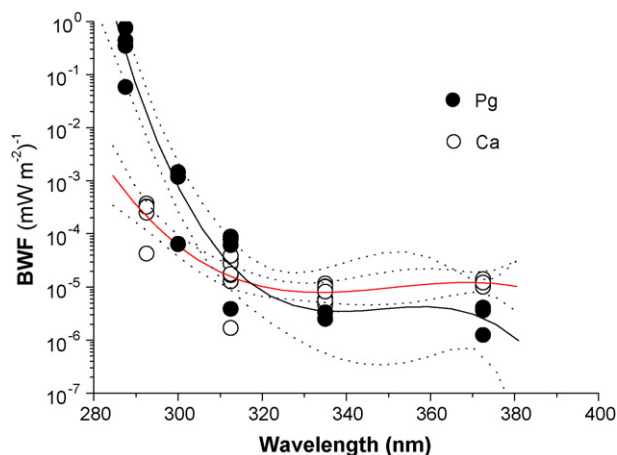|	α	E_k	P_{max}
P	0.0018[a]	239.44[a]	0.43[a]
	(0.0002)	(9.80)	(0.03)
PA	0.0021[b]	152.88[b]	0.33[b]
	(0.0002)	(12.26)	(0.04)
PAB	0.0022[b]	126.47[c]	0.29[c]
	(0.0001)	(3.00)	(0.03)

Fig. 2. Biological weighting functions (BWF) for photosynthetic (Pg) and calcification (Ca) carbon fixation rates of *Emiliania huxleyi* cells while exposed to different wavebands of solar radiation (solar simulator). Data are the means of 4 or 6 samples. The dot lines indicate 95% confidence limit.

of the irradiances, the calcification was inhibited by were 68% by UV-A and 8% by UV-B, respectively.

When energy-dependant responses to UV irradiances (biological weighting function) were analyzed, different UVR wavelengths showed differential impacts to the photosynthetic and to the calcifying process (Fig. 2). Shorter wavelengths of UV-B gave rise to more damages to photosynthetic apparatus than to calcifying machinery, while longer wavelengths of UV-A brought about more harms to the calcification.

When *E. huxleyi* cells were grown under reduced levels of natural solar radiation (Fig. 3), presence of UVR appeared not to result in obvious harm during the initial phase with 25% incident solar radiation. In the middle phase, when the culture was diluted and exposed to 50% incident solar radiation (the exact solar radiation did not increase due to increased cloud cover), presence of UVR led to enhanced growth rate of the cells (Table 2). During the later phase with 100% incident solar radiation, however, the growth rate was significantly reduced in the presence of UVR by 25% compared to PAR alone treatment (Fig. 3A, Table 2). The acclimated cells became tolerant to UV-induced harms in view of the decreased inhibition of the effective quantum yield (Φ_{PSII}) and the increased ratio of repair to damage (Figs. 4 and 5). Exposure of the indoor-grown cells to solar radiation at day 1 brought about 54% and 91% inhibition of the yield in 10 min under the PAR or PAR + UVR treatments, respectively (Fig. 5A). At day 13, however, the difference became insignificant between the PAR and PAR + UVR treatments. Estimated repair rate of PSII was $0.24\,min^{-1}$ at day 1 and $0.36\,min^{-1}$ at day 13, and the

Table 2
The specific growth rates of *Emiliania huxleyi* during the acclimation to incident solar radiation (P, PAR; PAB, PAR + UV-A + B) after shifted from the indoor condition ($400\,\mu$mol photons $m^{-2}\,s^{-1}$, 20 °C). SD was shown in parenthesis ($n = 8$). The initial, middle and final phases correspond to that in Fig. 3. Different superscript letters (a and b) indicate significance at 95%.

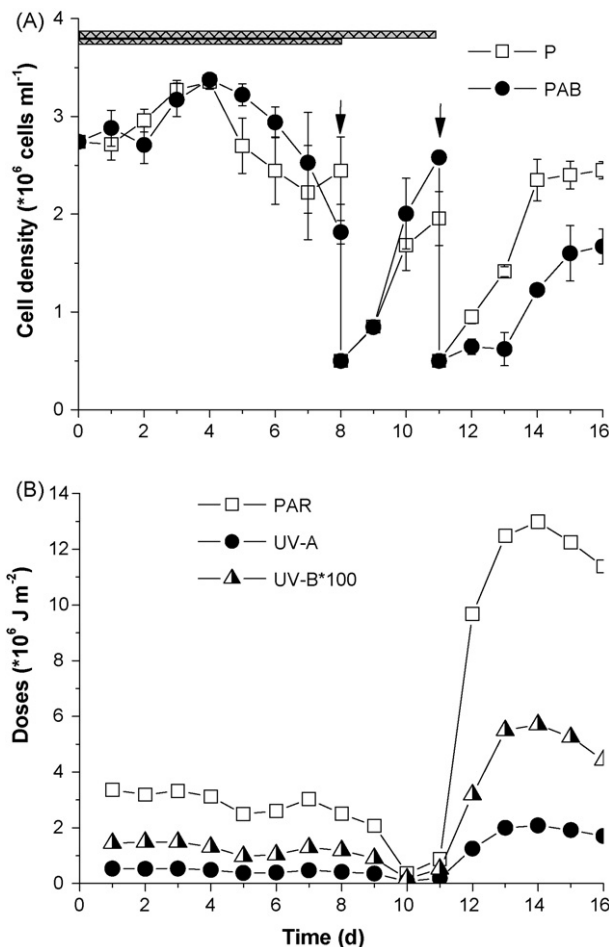|		μ	
Indoor control		0.43	
		(0.02)	
Outdoor condition	Irradiance (%)	P	PAB
Initial phase	25	0.04[a]	0.04[a]
		(0.01)	(0.02)
Middle phase	50	0.45[a]	0.55[b]
		(0.05)	(0.08)
Final phase	100	0.52[a]	0.39[b]
		(0.06)	(0.03)

Fig. 3. Acclimation of *Emilinia huxleyi* cells to solar radiation with (PAB) or without (P) UVR during the period of July 8th–23rd, 2007. (A) Cell density; (B) daily doses of solar PAR, UV-A and UV-B the cells received. Levels of the solar radiation were adjusted using 2 to none layers of neutral density screens to 25% (the initial phase), 50% (the middle phase) and 100% (the final phase). The vertical lines indicate SD ($n = 8$), representing 4 samples from each of 2 cultures. The arrowheads indicate the timing for partial renewal of the fresh medium.

damage rate was $0.27\,min^{-1}$ at day 1 and $0.06\,min^{-1}$ at day 13, respectively. The ratios of repair (r) to damage (k) were 0.89 at day 1 and 5.59 at day 13 under PAR alone (Fig. 5B). Addition of UVR led to reduction of the ratio by 89% at day 1 and 33% at day 13 (Fig. 5B). The ratio at day 13 was 43 times under PAB and 5 times under PAR treatment that at day 1, respectively.

At the end of long-term acclimation experiment, presence of UVR led to bigger cells with more PIC contents per cell, reflecting a slower cell division and enhanced calcification (Fig. 6). The percentage of larger *E. huxleyi* cells (>10 μm) was 16% with UVR (PAB) and 7% without it (Fig. 6A). The percentage of the smaller cells (5–7 μm) was 48% with and 62% without UVR (Fig. 6A). The size fraction of cells with UVR (PAB) is significantly larger than that without UVR (P). The particulate inorganic carbon (PIC) increased in the presence of UVR (Fig. 6B) by 27% compared to that under PAR alone treatment. The absorptivity of the UV-absorbing compounds with the major peak at 270–280 nm increased tremendously in the presence of UVR (Fig. 7).

4. Discussion

Photosynthesis of the *E. huxleyi* cells were found to be more inhibited by UV-B, while their calcification was more sensitive
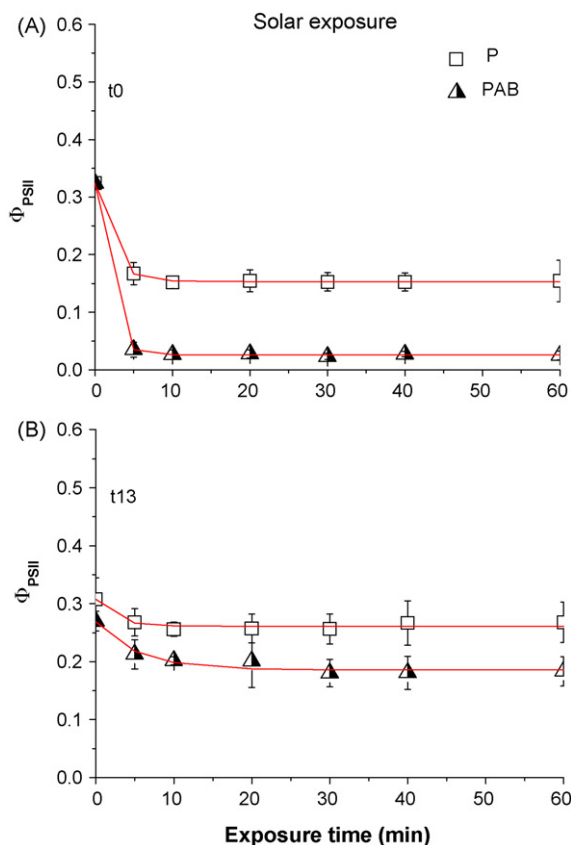
Fig. 4. Changes in the effective quantum yield (Φ_{PSII}) in *Emiliania huxleyi* during 60 min exposures to PAR (P) or PAR + UVR (PAB) before (t0) or after 13 days (t13) acclimation under solar radiation. The vertical lines indicate SD ($n = 8$, 2 cultures, 4 samples from each culture). The mean irradiances during the 60 min exposure (from 11:00 to 12:00) were about 427.00 (PAR), 68.34 (UV-A) and 2.15 Wm^{-2} (UV-B). Difference in PAR between the two days was less than 0.1%.

Fig. 5. UVR-induced inhibition of the Φ_{PSII} of *Emiliania huxleyi* cells in 10 min exposure to solar radiation (A) and the ratio of repair (r) to damage (k) rates of PSII (B) under solar radiation treatments with (PAB) or without (P) UVR. The vertical bars indicate SD ($n = 8$, 2 cultures, 4 samples from each culture). The asterisks mean the significant difference ($P < 0.05$).

to UV-A. Although high levels of UV irradiance led to reduced growth rates, moderate levels of UV-A was found to raise PAR-limited photosynthesis and enhance growth rate under reduced levels of natural solar radiation. The cells acclimated to solar radiation showed much higher absorptivity of UV-absorbing compounds, higher ratios of repair to damage and higher contents of PIC.

UV-induced inhibition of photosynthesis (Fig. 1) could be mostly due to the decreased photochemical efficiency and repair rate (Figs. 4 and 5), which can be attributed to UV-induced damages to D1 protein of PSII. High levels of both UV-A and UV-B irradiances decreased significantly the photosynthetic carbon fixation. However, when the impacts of UV on photosynthesis and calcification were compared on a basis of energy-weighted effect (Fig. 2), UV-A appeared to harm calcification more than photosynthesis. Coccolith vesicle is spatially close to karyon (nuclei), and calcification can be more sensitive to UV-A due to its capability to penetrate deeper inside the cell. The irradiance of UV-B could be more quickly reduced by the coccoliths and chloroplasts. On the other hand, UV-A can be utilized for photosynthetic carbon fixation in *E. huxleyi* (Fig. 2) and other phytoplankton species (Neori et al., 1988; Barbieri et al., 2002; Helbling et al., 2003; Gao et al., 2007b), therefore, its damaging effects could be partially offset by its positive impacts.

The acclimation of the cells during the long exposure led to reduced photoinhibition caused by UVR or PAR. In comparison to the PAR alone treatment, the acclimated cells showed decreased growth rate by about 25% in the presence of UVR (Fig. 3). Such a
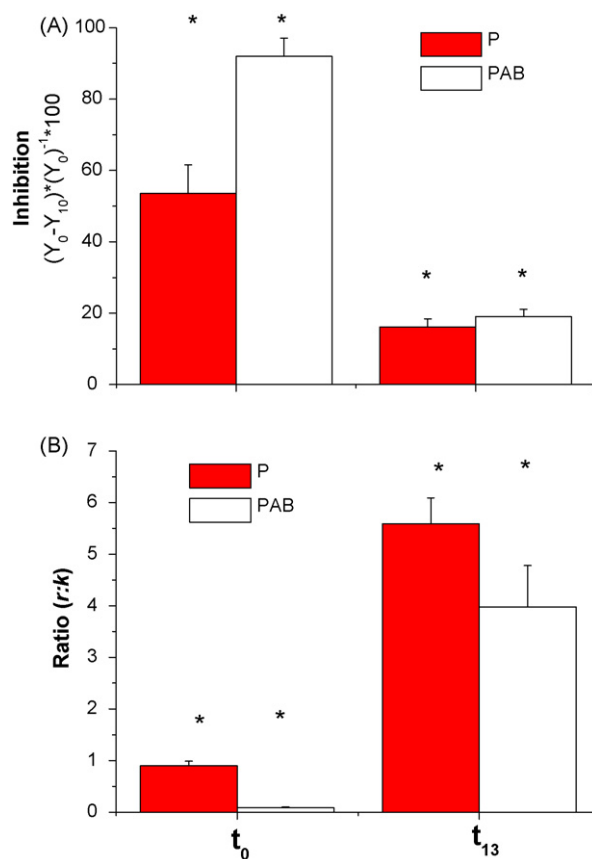
decline in growth rate could be caused directly by UVR and indirectly by consumption of energy for synthesis of UV-absorbing compounds (Garcia-Pichel, 1994), absorptivity of which increased dramatically (Fig. 7) and led to sufficient protection and reduced damage to PSII (Figs. 4 and 5). On the other hand, increased calcification also consumed energy for growth. The cells grown under natural solar radiation did increase their PIC content per cell (Fig. 6). Increased coccolith cover might have also provided some protection from UV due to their absorption and scattering of UVR (Gao et al., 2009). The increased PIC production could be due to UV-induced damages to DNA (Buma et al., 2000; Van Rijssel and Buma, 2002) that led to decreased cell division, which then led to enlarged cells (Fig. 6) and decreased growth rate. According to the previous study, the major strain of *Arthrospira platensis* is more tolerant than that of minor strain (Gao and Ma, 2008).

During the initial phase of the acclimation to solar radiation, cells showed positive growth at the beginning, but the cell numbers declined from day 4, defining negative growth. Depletion of nutrients at the initial high cell density (high cell density at the initial phase was necessary for the cells to survive) could have limited the growth. Preliminary experiments showed that the indoor-grown cells could not survive at densities below 1×10^6 cells ml^{-1} under 50% solar radiation. Increased growth rate under reduced levels of solar radiation in the presence of UVR must be attributed to the enhanced photosynthetic rate by UV-A (Gao et al., 2007b). When the cells were grown under 100% solar radiation, the positive effects from UV-A were offset
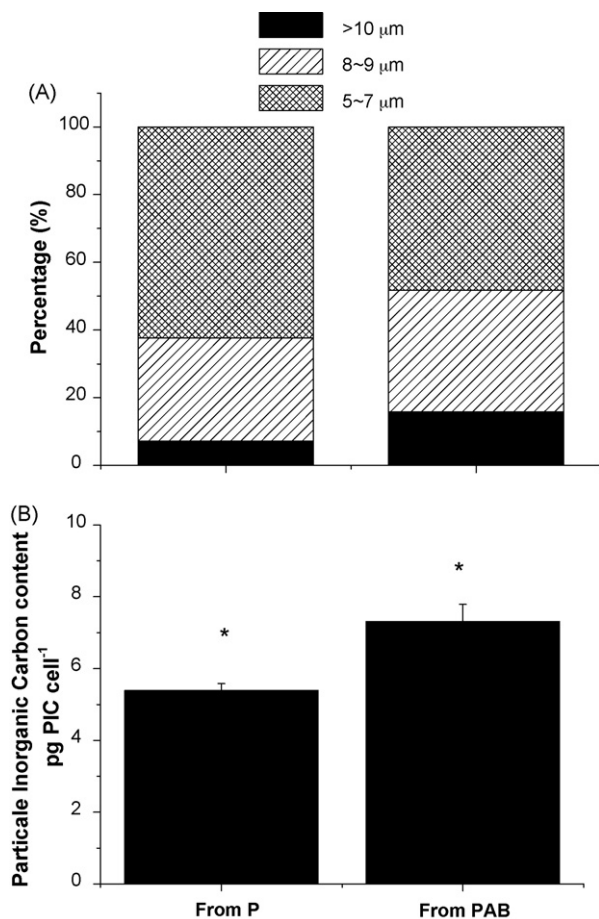
Fig. 6. Size fractions (A) of *Emiliania huxleyi* cells and particulate inorganic carbon content (B, pg PIC cell^{-1}) at the end (t13) of outdoor cultures (Fig. 4) exposed to different solar radiation treatments with (PAB) or without (P) UVR. Significant differences between treatments ($P < 0.05$) are indicated by asterisks. Vertical bars represent $\pm$ SD of the means ($n = 8$, 2 cultures, 4 samples from each culture).

Fig. 7. (A) Absorption spectral characteristics of *Emiliania huxleyi* (methanol extraction) for naked (N-cell) and coccolith-covered (C-cell) cells before (t0, A) and after 13 days (t13, B) exposures to solar radiation with (PAB) or without (P) UVR.

by the inhibiting effects of UV-A and UV-B. The UV-A-induced positive effects are always there, as evidenced under PAR-free irradiation treatment, however, the balance between the positive and negative impacts can lead to positive, neutral or negative impacts of UV. The reduced photoinhibition caused by UVR in the cells that had acclimated to solar radiation could be due to increased repair capacity, increased non-photochemical quenching due to increased pigments, such as UV-absorbing compounds and, decreased damage rate associated with increased calcification that shields off energy. Most algae possess mycosporine-like amino acids (MAAs) with the main absorption peaks at 310–360 nm to provide protection against UV (Sinha and Häder, 2008). However, in *E. huxleyi*, no obvious absorption was found for MAAs, the absorption peak was found at about 280 nm. The compounds of such an abosorption peak are not known, but have been recognized in several algal and cyanobacterial species (Sinha and Häder, 2008).

In offshore seawaters, UV-A penetrates to considerable depths (>50 m). Cells of coccolithophores are more abundant in the UML at about 30 m (Nanninga and Tyrell, 1996). Calcification would be negatively affected by UV-A and UV-B in their natural habitats. Increased stratification of surface waters in the oceans due to global warming will expose the coccolithophores to higher solar radiation (Nanninga and Tyrell, 1996; Boyd and Doney, 2002) than at present due to reduced mixing depth (Young, 1994). Therefore, solar UVR may impose more damages to them.
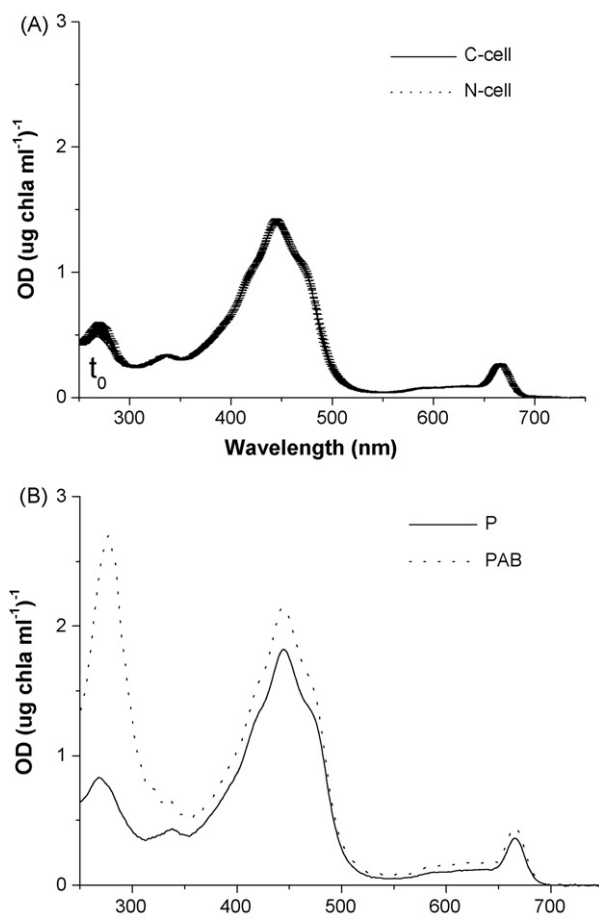
Acknowledgements

References

Balch, W.M., Holligan, P.M., Ackleson, S.G., Voss, K.J., 1991. Biological and optical properties of mesoscale coccolithophore blooms in the Gulf of Maine. Limnol. Oceanogr. 36, 629–643.

Balch, W.M., Fritz, J., Fernandez, E., 1996. Decoupling of calcification and photosynthesis in the coccolithophore *Emiliania huxleyi* under steady-sate light-limited growth. Mar. Ecol. Prog. Ser. 142, 87–97.

Barbieri, E.S., Villafañe, V.E., Helbling, E.W., 2002. Experimental assessment of UV effects on temperate marine phytoplankton when exposed to variable radiation regimes. Limnol. Oceanogr. 47, 1648–1655.

Beardall, J., Stojkovic, S., 2006. Microalgae under global environmental change: implications of growth and productivity, populations and trophic flow. ScienceAsia 32 (Suppl. 1), 1–10.

Behrenfeld, M.J., Hardy, J.T., Lee II, H, 1995. Ultraviolet-B radiation effects on inorganic nitrogen uptake by natural assemblages of oceanic phytoplankton. J. Phycol. 31, 25–36.

Boelen, P., Obernosterer, I., Vink, A.A., Buma, A.G.J., 1999. Attenuation of biologically effective UV radiation in tropical Atlantic waters measured with a biochemical DNA dosimeter. Photochem. Photobiol. 69 (1), 34–40.

Bouchard, J.N., Campbell, D.A., Roy, S., 2005. Effects of UV-B radiation on the D1 protein repair cycle of natural phytoplankton communities from three latitudes (Canada, Brazil, and Argentina). J. Phycol. 41, 273–286.

Boyd, P.W., Doney, S.C., 2002. Modelling regional responses by marine pelagic ecosystems to global climate change. Geophys. Res. Lett. 29 (16), 53-1–153-4.

Buma, A.G.J., van Oijen, T., van de Poll, W., 2000. The sensitivity of *Emiliania huxleyi* (Prymnesiophyceae) to ultraviolet-B radiation. J. Phycol. 36, 296–303.

Eilers, P.H.C., Peeters, J.C.H., 1988. A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton. Ecol. Model. 42, 199–215.

Gao, K.S., Guan, W.C., Helbling, E.W., 2007a. Effects of solar ultraviolet radiation on photosynthesis of the marine red tide alga *Heterosigma akashiwo* (Raphidophyceae). J. Photochem. Photobiol. B: Biol. 86, 140–148.

Gao, K.S., Wu, Y.P., Li, G., Wu, H.Y., Villafañce, V.E., Helbling, E.W., 2007b. Solar UV radiation drives CO_2 fixation in marine phytoplankton: a double-edged sword. Plant Physiol. 144, 54–59.

Gao, K.S., Ma, Z.L., 2008. Photosynthesis and growth of *Arthrospira* (*Spirulina*) *platensis* (Cyanophyta) in response to solar UV radiation, with special reference to its minor variant. Environ. Exp. Bot. 63, 123–129.

Gao, K.S., Ruan, Z.X., Villafañce, V.E., Gattuso, J.P., Helbling, E.W., 2009. Ocean acidification exacerbates the effect of UV radiation on the calcifying phytoplankter *Emiliania huxleyi*. Limnol. Oceanogr. 54 (6), 1855–1962.

Garcia-Pichel, F., 1994. A model for internal self-shading in planktonic organisms and its implications for the usefulness of ultraviolet sunscreens. Limnol. Oceanogr. 39, 1704–1717.

Garde, K., Caroline, C., 2000. The impact of UV-B radiation and different PAR intensities on growth, uptake of ^{14}C, excretion of DOC, cell volume, and pigmentation in the marine prymnesiophyte, *Emiliania huxleyi*. J. Exp. Mar. Biol. Ecol. 247, 99–112.

Genty, B.E., Briantais, J.M., Baker, N.R., 1989. Relative quantum efficiencies of the two photosystems of leaves in photorespiratory and non-photorespiratory conditions. Plant Physiol. Biochem. 28, 1–10.

Gieskes, W.W.C., Kraay, G.W., 1990. Transmission of ultraviolet light in the Weddell Sea: report on the first measurements made in Antarctic. Biomass Newslett. 12, 12–14.

Grzymski, J., Orrico, C., Schofield, O.M., 2001. Monochromatic ultraviolet light induced damage to Photosystem II efficiency and carbon fixation in the marine diatom *Thalassiosira pseudonana* (3H). Photosynth. Res. 68, 181–192.

Guan, W.C., Gao, K.S., 2008. Light histories influence the impacts of solar ultraviolet radiation on photosynthesis and growth in a marine diatom, *Skeletonema costatum*. J. Photochem. Photobiol. B: Biol. 91, 151–156.

Häder, D.P., Lebert, M., Marangoni, R., Colombetti, G., 1999. ELDONET—European light dosimeter network hardware and software. J. Photochem. Photobiol. B: Biol. 52, 51–58.

Häder, D.P., Kumar, H.D., Smith, R.C., Worrest, R.C., 2007. Effect of solar UV radiation on aquatic ecosystems and interactions with climate change. Photochem. Photobiol. 6, 267–285.

Harris, G.N., Scanlan, J.S., Geider, R.J., 2005. Acclimation of *Emiliania huxleyi* (Prymnesiophyceae) to photon flux density. J. Phycol. 41, 851–862.

Helbling, E.W., Gao, K.S., Goncalves, R.J., Wu, H.Y., Villafañce, V.E., 2003. Utilization of solar UV radiation by coastal phytoplankton assemblages off SE China when exposed to fast mixing. Mar. Ecol. Prog. Ser. 259, 59–66.

Heraud, P., Beardall, J., 2000. Changes in chlorophyll fluorescence during exposure of *Dunaliella tertiolecta* to UV radiation indicate a dynamic interaction between damage and repair processes. Photosynth. Res. 63, 123–134.

Holligan, P.M., Fernandez, E., Aiken, J., Balch, W.M., Boyd, P., Burkill, P.H., Finch, M., Groom, S.B., Malin, G., Muller, K., Purdie, D.A., Robinson, C., Trees, C.C., Turner, S.M., van der Wal, P., 1993. A biogeochemical study of the coccolithophore, *Emiliania huxleyi*, in the North Atlantic. Global Biogeochem. Cycles 7, 879–900.

Karentz, D., Cleaver, J.E., Mitchell, D.L., 1991. Cell survival characteristics and molecular responses of Antarctic phytoplankton to ultraviolet-B radiation. J. Phycol. 27, 326–341.

Keller, M.D., Selvin, R.C., Claus, W., Guillard, R.R.L., 1987. Media for the culture of oceanic ultraplankton. J. Phycol. 23, 633–638.

Kramer, G.F., Norman, H.A., Krizek, D.T., Mirecki, R.M., 1991. Influence of UV-B radiation on polyamines, lipid peroxidation and membrane lipids in cucumber. Phytochemistry 30, 2101–2108.

Lesser, M.P., Cullen, J.J., Neale, P.J., 1994. Carbon uptake in a marine diatom during acute exposure to ultraviolet B radiation: Relative importance of damage and repair. J. Phycol. 30, 183–192.

Macedo, M.F., Duarte, P., Ferreira, J.G., 2002. The influence of incubation periods on photosynthesis–irradiance curves. J. Exp. Mar. Biol. 274, 101–120.

Murphy, T.M., 1983. Membranes as targets of ultraviolet radiation. Physiol. Plant. 58, 381–388.

Nanninga, H.J., Tyrell, T., 1996. Importance of light for the formation of algal blooms by *Emiliania huxleyi*. Mar. Ecol. Prog. Ser. 136, 195–203.

Neale, P.J., Kieber, D.J., 2000. Assessing biological and chemical effects of UV in the marine environment: spectral weighting functions. In: Hester, R.E., Harrison, R.M. (Eds.), Causes and Environmental Implications of Increased UV-B Radiation. The Royal Society of Chemistry, Cambridge, pp. 61–83.

Neori, A., Vernet, M., Holm-Hansen, O., Haxo, F.T., 1988. Comparison of chlorophyll far-red and red fluorescence excitation spectra with photosynthetic oxygen action spectra for photosystem II in algae. Mar. Ecol. Prog. Ser. 44, 297–302.

Nielsen, M.V., 1997. Growth, dark respiration and photosynthetic parameters of the coccolithophorid *Emiliania huxleyi* (Prymnesiophyceae) acclimated to different day length-irradiance combinations. J. Phycol. 33, 818–822.

Nimer, N.A., Merrett, M.J., 1992. Calcification and utilization of inorganic carbon by the coccolithophorid *Emiliania huxleyi* Lohmann. New Phytol. 121, 173–177.

Paasche, E., 1963. The adaptation of carbon-14 method for the measurement of coccolith production in *Coccolithus huxleyi*. Physiol. Plant Arum. 16, 186–200.

Paasche, E., 2001. A review of the coccolithophorid *Emiliania huxleyi* (Prymnesiophyceae), with particular reference to growth coccolith formation, and calcification–photosynthesis interactions. Phycologia 40, 503–529.

Pienaar, R.N., 1994. Ultrastructure and calcification of coccolithophores. In: Winter, A., Siesser, W.G. (Eds.), Coccolithophores. Cambridge University Press, Cambridge, pp. 13–39.

Raven, J.A., Johnston, A.M., 1991. Mechanisms of inorganic carbon acquisition in marine phytoplankton and their implications for the use of other resources. Limnol. Oceanogr. 36, 1701–1714.

Ruggaber, A., Dlugi, R., Nakajima, T., 1994. Modelling of radiation quantities and photolysis frequencies in the troposphere. J. Atmos. Chem. 18, 171–210.

Sinha, R.P., Häder, D.P., 2008. UV-protectants in cyanobacteria. Plant Sci. 174, 278–289.

Sobrino, C., Montero, O., Lubian, L.M., 2004. UV-B radiation increases cell permeability and damages nitrogen incorporation mechanisms in *Nannochloropsis gaditana*. Aquat. Sci. 66, 421–429.

Strickland, J.D.H., Parsons, T.R., 1968. A practical handbook of seawater analysis. Pigment analysis. Bull. Fish. Res. Board Canada 167, 49–80.

Suggett, D., Le Floc'H, E., Harris, G.N., Leonardos, N., Geider, R.J., 2007. Different strategies of photoacclimation by two strains of *Emiliania huxleyi* (Haptophyta). J. Phycol. 43, 1209–1222.

Takano, H., Takei, R., Manabe, E., Burgess, J.G., 1995. Increased coccolith production by *Emiliania huxleyi* cultures enriched with dissolved inorganic carbon. Appl. Microbiol. Biotechnol. 43, 460–465.

Trimborn, S., Langer, G., Rost, B., 2007. Effect of varying calcium concentrations and light intensities on calcification and photosynthesis in *Emiliania huxleyi*. Limnol. Oceanogr. 52 (5), 2285–2293.

Van Rijssel, M., Buma, A.G.J., 2002. UV radiation induced stress does not affect DMSP synthesis in the marine prymnesiophyte *Emiliania huxleyi*. Aquat. Microb. Ecol. 28, 167–174.

Villafañe, V.E., Sundbäck, K., Figueroa, F.L., Helbling, E.W., 2003. Photosynthesis in the aquatic environment as affected by UVR. In: Helbling, E.W., Zagarese, H.E. (Eds.), UV Effects in Aquatic Organisms and Ecosystems. The Royal Society of Chemistry, Cambridge, pp. 357–397.

Zheng, Y.Q., Gao, K.S., 2009. Impacts of solar UV radiation on the photosynthesis, growth, and UV-absorbing compounds in *Gracilaria lemaneiformis* (Rhodophyta) grown at different nitrate concentrations. J. Phycol. 45, 314–323.

Yao, Y.N., Xuan, Z.Y., He, Y.M., Lutts, S., Korpelainen, H., Li, C.Y., 2007. Principal component analysis of intraspecific responses of tartary buckwheat to UV-B radiation under field conditions. Environ. Exp. Bot. 61, 237–245.

Young, W.R., 1994. The subinertial mixed layer approximation. J. Phys. Oceanogr. 24, 1812–1826.