CSIRO PUBLISHING
www.publish.csiro.au/journals/fpb Functional Plant Biology, 2009, 36, 1057–1064

Impacts of UV radiation on growth and photosynthetic carbon acquisition in *Gracilaria lemaneiformis* (Rhodophyta) under phosphorus-limited and replete conditions

Zhiguang Xu[A] *and Kunshan Gao*[B,C]

[A]Marine Biology Institute, Shantou University, Shantou, Guangdong 515063, China.
[B]State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, Fujian 361005, China.
[C]Corresponding author. Email: ksgao@xmu.edu.cn

Abstract. Solar ultraviolet radiation (UVR, 280–400 nm) is known to negatively affect macroalgal growth and photosynthesis, while phosphorus availability may affect their sensitivity to UVR. Here, we show that UV-A enhanced the growth rate of the red macroalga, *Gracilaria lemaneiformis* Bory de Saint-Vincent under inorganic phosphorus (Pi)-replete but reduced it under Pi-limited conditions. Maximal net photosynthetic rates were significantly reduced by both UV-A and UV-B, but the apparent photosynthetic efficiency was enhanced in the presence of UV-A. The UV-induced inhibition was exacerbated under Pi-limited conditions. The activity of total carbonic anhydrase was enhanced and the photosynthetic affinity for exogenous inorganic carbon (Ci) was raised for thalli grown in the presence of UVR under both Pi-replete and Pi-limited conditions. The relative growth rate was closely related to Ci acquisition capability (V_{max}/K_{DIC}), which was enhanced by UVR exposure under Pi-replete but not significantly affected under Pi-limited conditions.

Additional keywords: photosynthesis, pigment, the ratio of V_{max} to K_{DIC}, UV-absorbing compounds.

Introduction

Solar UV-B irradiance (280–315 nm) has increased due to the thinning of the ozone layer caused by industrial activities (McKenzie *et al.* 2007). Although the rate of increase of chlorine concentration in the stratosphere has declined, mirroring the execution of the Montreal Protocol, the time required for recovery of the ozone layer is unclear and relies on the impacts of climate change on the stratosphere (Weatherhead and Andersen 2006). This increasing UV-B irradiance can cause additional damage to aquatic organisms (Häder *et al.* 2007).

Marine macroalgae are mainly distributed in the intertidal zone and their physiological behaviour is subject to harm caused by solar ultraviolet radiation (UVR; 280–400 nm). This affects the growth (Henry and Van Alstyne 2004), photosynthesis (Aguilera *et al.* 1999; Davison *et al.* 2007), spore germination (Roleda *et al.* 2006) and early development (Jiang *et al.* 2007) of individual species, and even macroalgal community structure (Makarov 1999; Bischof *et al.* 2006). At both molecular and cellular levels, direct UV damage of DNA (Atienzar *et al.* 2000; Pakker *et al.* 2000) and ultrastructure (Holzinger and Lütz 2006) are reported.

In the natural environment, the harmful effects of UVR on macroalgae are often counteracted by protective strategies, such as the accumulation of UV-screening compounds (Karsten and Wiencke 1999; Oren and Gunde-Cimerman 2007) and photorepairing mechanisms (Neale 2000; Häder *et al.* 2002). Mycosporine-like amino acids (MAAs) are known as important UV-absorbing compounds, and their accumulation relates to reduced inhibition caused by UVR in micro- and macroalgae (Oren and Gunde-Cimerman 2007). Repairing mechanisms involve resynthesis of damaged proteins (Vincent and Neale 2000; Häder *et al.* 2002) and photo-repair of DNA (Pakker *et al.* 2000), both of which are energetically expensive (Raven and Beardall 1981).

Availability of phosphorus affects the generation of ATP by cells, either as a substrate for phosphorylation of ADP or through impacting energy metabolism. Therefore, exogenous inorganic phosphorus (Pi) levels can influence the strategies which an organism uses to cope with environmental stresses, such as exposure to UVR. Additionally, phosphorus is involved in many biosynthetic processes, including nucleic acid and membrane synthesis, signalling, and modification of protein activities (Irihimovitch and Yehudai-Resheff 2008). Consequently, the availability of phosphorus can affect the overall response of algae to UVR, and it is important to examine how macroalgal species respond to UVR under P-limited conditions. Sometimes, the availability of Pi is not considered as a growth limiting factor for phototrophic organisms in the sea because of the existence of periplasmic alkaline phosphatase (Tyrrell 1999), the activity of which often increases at low phosphate levels (Hoppe 2003). Although phosphatase has also been found in macroalgae, its activity is usually low and can easily be affected by changes in pH, light, salinity and temperature (Hernández *et al.* 2002) and, therefore, phosphorus limitation does occur in coastal waters (Larned 1998; Hwang *et al.* 2004). On the other hand, in eutrophic waters, nitrogen is highly enriched, and Pi may become limiting for the growth of macroalgae (Sigee 2005). Thus, the growth and photosynthesis of *Gracilaria tikvahiae* McLachlan

10.1071/FP09092 1445-4408/09/121057

and *Gracilaria tenuistipitata* Zhang et Xia are limited under Pi-deficient conditions (Lapointe 1987; García-Sánchez *et al*. 1996). Beardall and Giordano (2002) speculate that phosphorus limitation may affect CO_2 concentrating mechanisms (CCMs) in algae. This hypothesis has been addressed in the microalgae *Chlorella vulgaris* Beijerinck (Kozlowska-Szerenos *et al*. 2000) and *Chlorella emersonii* Shriaiwa and Krauss (Beardall *et al*. 2005), but this has not been documented in any macroalgal species. Additionally, the decline of growth rate and photochemical parameters after Pi starvation is significantly faster in the presence of UV radiation in the microalga, *Dunaliella tertiolecta* Butcher (Shelly *et al*. 2005).

Gracilaria lemaneiformis Bory de Saint-Vincent is commercially farmed for food and agar production in China (Fei 2004). Such a practice has led to relief of eutrophication in Chinese coastal waters. At the same time, however, nutrient limitation may occur due to the speed of growth and density of this species in cultivation. *G. lemaneiformis* is known to be able to use HCO_3^- via periplasmic extracellular carbonic anhydrase (Zou *et al*. 2004). Its daily photosynthetic production is considerably reduced by solar UVR (Gao and Xu 2008), although it can accumulate UV-protective compounds efficiently (Zheng and Gao 2009). Enrichment with nitrate can stimulate the synthesis of UV-absorbing compounds and alleviate UVR-induced inhibition of its photosynthesis and growth (Zheng and Gao 2009). However, little is known concerning how this species responds to solar UVR when grown under P-limited conditions. The aim of this study was to investigate the physiological responses of *G. lemaneiformis* to UVR exposure when grown under Pi-limited and Pi-replete concentrations.

Materials and methods

Thalli of *Gracilaria lemaneiformis* were collected from its farmed area at 0.5 m depth in the Shen'ao bay of Nanao Island (23.3°N, 116.6°E), Shantou, China, on 5 April 2008 and were transported to the laboratory in an insulated cooler (5°C) within 2 h. Healthy individuals were selected and branches of 1 g fresh weight (FW) were grown in quartz tubes containing 250 mL natural seawater enriched with 500 μM nitrate to ensure a nitrogen-replete condition for this species when grown under solar radiation (Zheng and Gao 2009). Biomass density in the culture vessels was maintained at ~4 g FW L^{-1} (a density within the range of the 1–8 g FW L^{-1} found in the farmed area) during the whole experiment by removing partial thalli when the culture medium was renewed every 48 h. The cultures were maintained in a water bath through which seawater was circulated by a refrigerating circulator (CAP-3000, Tokyo Rikakikai Co., Ltd, Tokyo, Japan) to control the water temperature at 21°C so as to reflect the daily average level of the ambient surface seawater temperature. The cultures were aerated with ambient air of 380 ppmv CO_2 at a rate of 0.6 L min^{-1}. Thalli within the tubes were not shaded by each other, resulting in equal exposure to solar radiation.

Enrichment of phosphate

Two inorganic phosphorus (Pi) concentrations, 0.5 μM (L-Pi, natural seawater) and 50 μM (H-Pi, enriched with NaH_2PO_4), were set up in the culture medium, which was renewed every other day. The Pi concentrations in the cultures were reduced to nil in the L-Pi and to ~34 μM in the H-Pi treatment before the renewal of the medium every 48 h. The concentrations of Pi in the L-Pi treatment reflected Pi-limitation, while that in the H-Pi treatment related to Pi-replete conditions (Xu 2007).

Treatment and measurement of solar radiation

Thalli were exposed to three different solar radiation treatments: (i) photosynthetically active radiation (PAR, P treatment), where quartz tubes were covered with Ultraphan 395 film (UV Opak, Digefra, Munich, Germany), transmitting solar radiation above 395 nm; (ii) PAR+UV-A (PA treatment), where quartz tubes were covered with Folex 320 (Montagefolie, Nr. 10155099, Folex, Dreieich, Germany), transmitting solar radiation above 320 nm; and (iii) PAR+UV-A+UV-B (PAB treatment), where quartz tubes were covered with Ultraphan 295 (UV Opak), transmitting solar radiation above 295 nm. The transmission through these cut-off foils and the quartz tubes are reported elsewhere (Zheng and Gao 2009). The cut-off filters equally trim down 4% of the PAR in water due to their reflection (Gao *et al*. 2007). The incident solar radiation was continuously monitored using an Eldonet broadband filter radiometer (Eldonet XP; Real Time Computer, Möhrendorf, Germany), which has three channels for PAR (400–700 nm), UV-A (315–400 nm) and UV-B (280–315 nm) irradiance, respectively (Häder *et al*. 1999). This device has been used worldwide (certificate No. 2006/BB14/1) and was calibrated regularly with the support from the maker every year against a double monochrometor spectroradiometer and a certified calibration lamp. There was about a 5-nm discrepancy between the measured and exposed UV-A waveband; therefore, thalli were actually exposed to ~2% less UV-A and ~4% less PAR compared with the measured irradiances. Daily doses of PAR, UV-A and UV-B during the culture period ranged from 1340.27–6059.15, 198.64–999.93 and 5.27–31.94 kJ m^{-2}, respectively.

Determination of growth rate

Changes in biomass (FW) of thalli were measured every other day during the period of 5–18 April, 2008. Relative growth rate (RGR) was determined for the end period in order to reflect acclimated physiological performance of thalli. $RGR = \ln(W_t/W_0) \times t^{-1} \times 100$, where W_0 and W_t refer to the FW at days 13 and 14, respectively.

Measurement of photosynthesis

Photosynthetic oxygen evolution rate was determined using a Clark-type oxygen electrode (Model 5300, YSI, Yellow Springs, Ohio, USA) at 21°C. PAR was provided by a halogen lamp and measured with a light meter thermometer (QRT1, Hansatech, Norfolk, UK). Different levels (0–600 μmol photons m^{-2} s^{-1}) of PAR were obtained by altering the distance between the light resource and the oxygen electrode chamber. The dark respiration rate (R_d) was measured by covering the chamber with a black sheet of cloth. The apparent photosynthetic efficiency (α) and the PAR-saturated net photosynthetic rate (P_{max}) were estimated from the P-E curves (Henley 1993).

Determination of UV-absorbing compounds (UVACs) and pigment contents

About 0.1 g (FW) of thalli was ground and extracted in 10 mL absolute methanol at 4°C in darkness for 24 h (Gao and Xu 2008). The absorbance of the supernatant after centrifugation (5000*g*, 15 min, 4°C) was measured from 250 to 750 nm using a scanning spectrophotometer (DU 530, Beckman Coulter, Fullerton, CA, USA). Total absorbance of the UVACs was estimated at 325 nm (Dunlap *et al.* 1995); Chl *a* concentration was calculated according to Wellburn (1994); and phycoerythrin (PE) and phycocyanin (PC) were extracted in 0.1 M phosphate buffer (pH 6.8) with 0.2 g FW of thalli (ground with quartz sand), and their contents were determined according to Siegelman and Kycia (1978).

Evaluation of inorganic carbon (Ci) acquisition capability

The activity of carbonic anhydrase (CA) and the relationship of photosynthetic O_2 evolution rate with exogenous Ci concentrations were determined to see the combined effects of UVR and P-limitation on the Ci acquisition processes of *G. lemaneiformis*. The CA activity was assayed by a potentiometric method as described by Giordano and Maberly (1989). Ci-free seawater was prepared by acidifying the seawater with 1 M HCl (pH < 4.0), sparging for at least 1 h with high purity N_2 gas to deplete CO_2 and adjusting the pH back to 8.2 with freshly prepared 1 M NaOH. Tris was added to give a final concentration of 25 mM. For total CA activity, ~0.1 g FW of thalli were ground and then transferred in 5 mL buffered Ci-free seawater at 4°C. The reaction was initiated by gently injecting 1 mL cold (4°C) CO_2-saturated distilled water (pH 3.4) at the vessel containing the Ci-free medium. The time for a drop of 0.6-pH units in the mixtures was recorded. The changes in pH were measured with a pH meter (420A, Orion, Boston, MA, USA), which was calibrated with standard N.B.S. buffer solution (Merck, Darmstadt, Germany). For external CA, thalli instead of the homogenised tissue were used and measured in the same way. The relative enzyme activity (REA) was estimated as: $10 \times (T_b/T_c - 1)$, where T_b and T_c are the time in seconds for the pH drop without and with the algal sample, respectively. Photosynthetic rates at different Ci concentrations were measured at 600 µmol photons m^{-2} s^{-1} of PAR and at 21°C. The different Ci concentrations were obtained by dissolving $NaHCO_3$ in the Tris-buffered Ci-free seawater. The relationship between photosynthesis and Ci concentrations was analysed according to the Michaelis-Menten equation: $V = V_{max} \times [S]/(K_{DIC} + [S])$ (Von Caemmerer and Farquhar 1981), and the maximal rate (V_{max}) and half saturation constant (K_{DIC}) were estimated directly from the curves. The ratio of V_{max} to K_{DIC} was used to evaluate the capacity for Ci acquisition.

Determination of UVR-induced inhibition

Inhibition caused by UVR was estimated as: $(G_P - G_U)/G_P \times 100$, where G_U and G_P represent the RGR of thalli grown under radiation treatments with PA or PAB and without UVR.

Statistical analysis

One-way ANOVA and Tukey's test were used to analyse differences among treatments and the significant level was set at 0.05.

Results

Thalli of *Gracilaria lemaneiformis* grew faster under Pi-replete than Pi-limited conditions either in the absence or presence of UVR (Fig. 1). In contrast to the PAR alone treatment, presence of UV-A or UV-A+B resulted in faster growth rates when thalli were grown under Pi-replete conditions, reflecting an enhancement by UV-A. However, such enhancement was not found under Pi-limited conditions (Fig. 1). Under Pi-limited conditions, UVR brought ~40% inhibition of the RGR, whereas under Pi-replete conditions, UVR enhanced the RGR by ~50%. However, no significant difference was found between PAR+UV-A and PAR+UV-A+B treatments. The RGR was ~30% higher at the H-Pi than at the L-Pi level under PAR, and about twice as high under PAR+UVR.

When photosynthetic responses to PAR levels were analysed (Table 1), both the maximal net photosynthetic rate and dark respiration showed higher values in thalli grown under Pi-replete conditions regardless of the radiation treatments. Under Pi-limited conditions, thalli grown in the presence of UVR showed ~11% lower P_{max} ($P < 0.05$) and 19% higher R_d ($P < 0.01$) in contrast to those grown under PAR alone. At H-Pi, the P_{max} was ~12% lower and the R_d 8% higher in the presence of UVR. However, there was no significant difference between the PAR+UV-A and PAR+UV-A+B

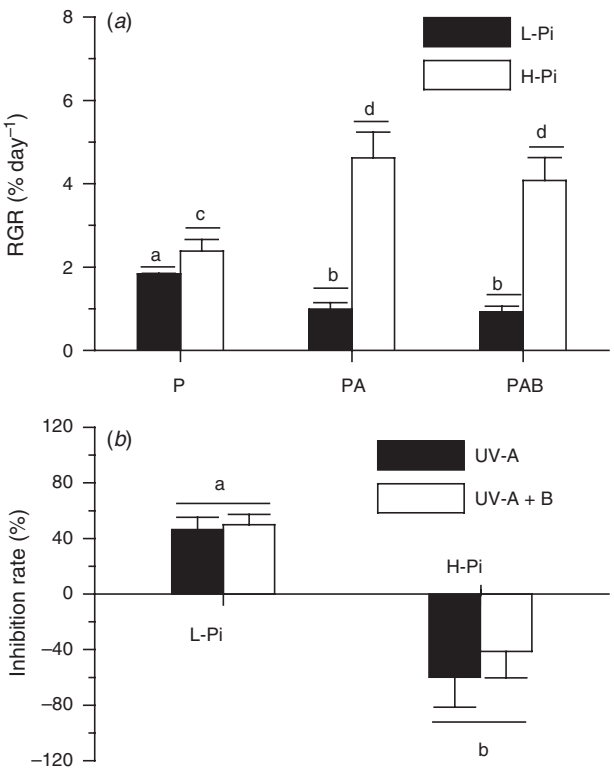

Fig. 1. (*a*) Relative growth rate (RGR) and (*b*) effect of UV-induced inhibition on RGR in *Gracilaria lemaneiformis* grown at low or high phosphate levels under different solar radiation treatments with or without UVR. The RGR was determined at the end of 14 days exposure. Significant ($P < 0.05$) differences among the treatments are indicated by different lowercase letters. Vertical bars represent ±s.d. of the means ($n = 3$).

Table 1. Photosynthetic parameters of *Gracilaria lemaneiformis* thalli grown under different phosphorus and solar radiation treatments

Thalli were cultured for 16 days under Pi-limited (L-Pi) or replete (H-Pi) conditions with or without UVR (P, PAR; PA, PAR+UV-A; PAB, PAR+UV-A+B). P_{max}, the maximal photosynthetic rate (μmol O_2 h^{-1} g^{-1} FW); α, the apparent photosynthetic efficiency (μmol O_2 h^{-1} g^{-1} FW)/(μmol photons m^{-2} s^{-2}); R_d, the dark respiration rate (μmol O_2 h^{-1} g^{-1} FW). Within each column of the data, different superscript letters or letter combinations indicate significant difference at $P = 0.05$. Data are means ± s.d. ($n = 3$)

Treatments		P_{max} (μmol O_2 h^{-1} g^{-1} FW)	α (μmol O_2 h^{-1} g^{-1} FW)/(μmol photons m^{-2} s^{-2})	R_d (μmol O_2 h^{-1} g^{-1} FW)
L-Pi	P	58.06 ± 3.47^{a}	0.29 ± 0.03^{a}	-8.90 ± 0.32^{a}
	PA	51.01 ± 1.14^{b}	0.36 ± 0.01^{b}	-11.44 ± 0.32^{b}
	PAB	51.48 ± 1.26^{b}	0.23 ± 0.02^{c}	-10.62 ± 0.44^{b}
H-Pi	P	78.55 ± 6.59^{c}	0.38 ± 0.04^{b}	-11.50 ± 0.82^{bc}
	PA	69.53 ± 3.19^{d}	0.34 ± 0.01^{b}	-13.03 ± 0.32^{d}
	PAB	69.05 ± 6.48^{d}	0.36 ± 0.01^{b}	-12.39 ± 0.00^{ce}

treatments ($P > 0.05$). The apparent photosynthetic efficiency (α) was higher in thalli grown under Pi-replete than under Pi-limited conditions, except when exposed to PAR+UV-A. The lowest α values were found in thalli grown under Pi-limited conditions in the presence of UV-B (Table 1). On average, the α values were higher by 32% ($P < 0.05$) under PAR alone and by 57% under PAR+UV-A+B treatments ($P < 0.001$) in the H-Pi than in the L-Pi. In the L-Pi, thalli exposed to PAR+UV-A showed higher α values than those grown under PAR alone or PAR+UV-A+B exposures.

UVR exposure and Pi availability affected the pigment content of *G. lemaneiformis* (Fig. 2). The Chl *a* content showed insignificant differences in thalli grown either under different radiation treatments or different Pi concentrations. Both UV-A and UV-A+B significantly decreased the content of PE and PC, respectively (Fig. 2). The enrichment of Pi did not result in any difference in the pigment contents, except that thalli grown under the H-Pi in the presence of UVR showed higher contents of PE.

As indicated by the absorption peak at 325 nm, UV absorbing compounds (UVACs) accumulated more in thalli grown in the presence of UV-A or UV-A+B (Fig. 3). UVR significantly ($P < 0.005$) increased the absorbance of UVACs in thalli in spite of Pi concentrations. No increment in the absorbance was found in the H-Pi-grown thalli compared with the L-Pi ones.

When the photosynthetic rates were determined at different external Ci concentrations under PAR, thalli grown under Pi-replete conditions showed higher V_{max} values than those grown at the limiting Pi level (Fig. 4). On average, the V_{max} was higher under Pi-replete than Pi-limited conditions by 12%, 32% and 37% in thalli grown under PAR, PAR+UV-A and PAR+UV-A+B, respectively (Table 2). Presence of UV-A significantly lowered the level of K_{DIC} both in the Pi-limited and replete cultures, reflecting an enhanced photosynthetic affinity for exogenous Ci (Table 2). The Ci affinity was ~39% higher at replete Pi than at limiting Pi concentrations in thalli grown in the presence of UVR. The efficiency of Ci utilisation, the ratio of V_{max} to K_{DIC}, increased significantly at the replete-Pi level regardless of the solar radiation treatments. Under the PAR and PAR+UVR (PAR+UV-A or PAR+UV-A+B) treatments,

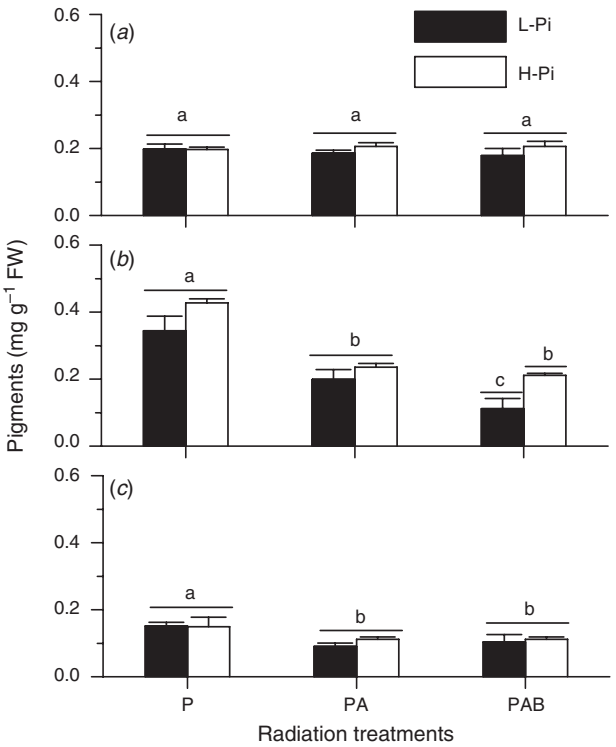

Fig. 2. (*a*) Chlorophyll *a*, (*b*) phycoerythrin (PE) and (*c*) phycocyanin (PC) contents of *Gracilaria lemaneiformis* grown at low and high phosphate levels under different solar radiation treatments with or without UVR. Significant differences between treatments ($P < 0.05$) are indicated by different lowercase letters. Vertical bars represent ±s.d. of the means of four different individuals.

Pi-enrichment raised the V_{max}/K_{DIC} ratio by ~41% and 120%, respectively. There was no significant difference in the V_{max}/K_{DIC} ratio among the radiation treatments at the limiting Pi level, but the difference was significant at the replete Pi level (Table 2). However, presence of UV-B did not significantly affect the V_{max}/K_{DIC} ratio or K_{DIC}. Thalli grown in the presence of UV-A or UV-B showed lower V_{max} by 28% and 7% and increased the photosynthetic Ci affinity by 25% and 6%, respectively. On the other hand, although it was not possible to rule out the external CA activity using the potentiometric method due to very low values (as mentioned by Johnston *et al.* 1992), the activity of the total CA was enhanced in thalli grown at replete Pi and increased in those exposed to UV-A or UV-A+B (Fig. 5). There was no significant difference in the total CA activity between the PAR+UV-A and PAR+UV-A+B treatments, but the difference was significant between the treatments with and without UVR. When the RGR was plotted against the V_{max}/K_{DIC} ratio, a close relationship was established, showing that the RGR increased with increasing V_{max}/K_{DIC} ratios (Fig. 6).

Discussion

In this study, growth of *Gracilaria lemaneiformis* was enhanced under Pi-replete conditions but reduced under Pi-limited conditions by UVR. Pi-enrichment did not affect the accumulation of UVACs, which play protective roles against UVR. Dark respiration increased in thalli exposed to UV-A or

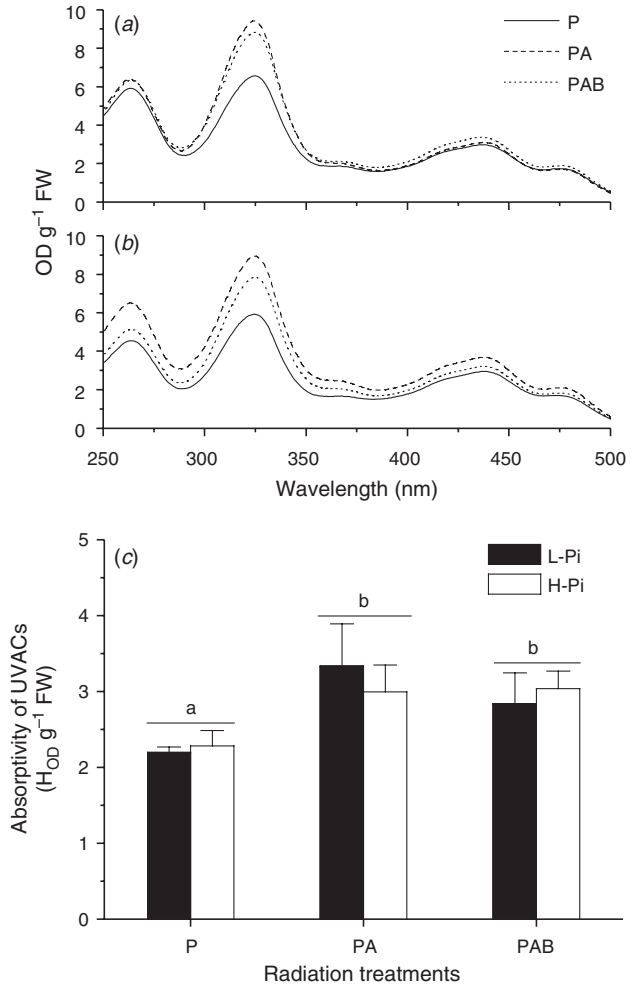

Fig. 3. Absorption spectra of the methanolic extracts from thalli (*a*, L-Pi; *b*, H-Pi) and (*c*) absorbance of the UV-absorbing compounds (UVACs) of *Gracilaria lemaneiformis* grown at low and high phosphate levels under different solar radiation treatments with or without UVR. Significant differences between treatments ($P < 0.05$) are indicated by different lowercase letters. Vertical bars in (*c*) represent ±s.d. of the means ($n = 4$).

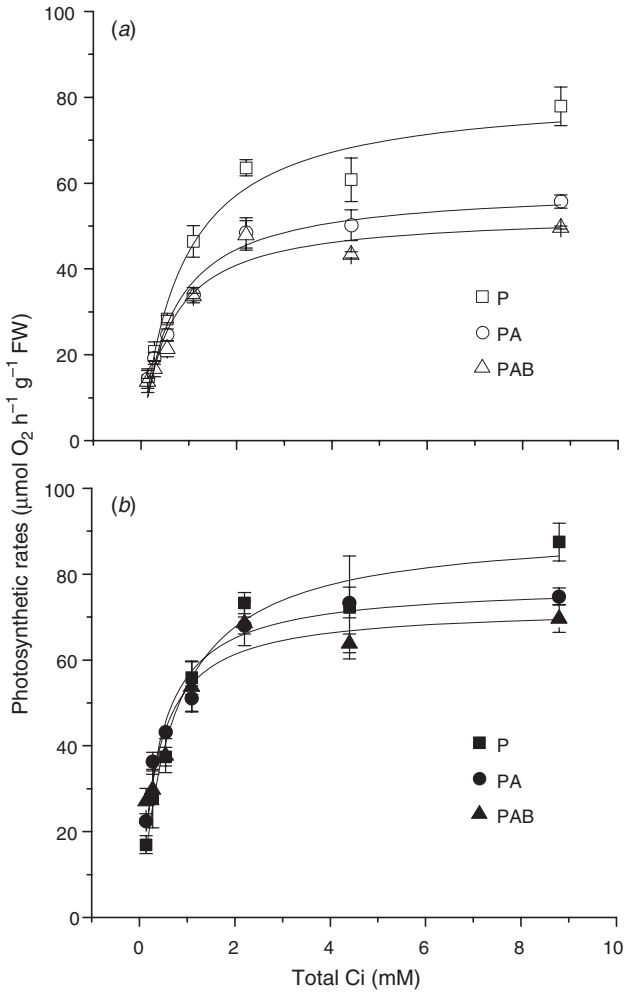

Fig. 4. Photosynthetic oxygen evolution rate as a function of exogenous inorganic carbon concentrations in *Gracilaria lemaneiformis* grown at (*a*) low and (*b*) high phosphate levels under different solar radiation treatments with or without UVR. The curves were determined at 600 μmol photons m^{-2} s^{-1} of PAR. Vertical bars represent ±s.d. of the means ($n = 3$).

UV-A+B to a higher extent at the Pi-replete level, which might supply the increased energy the cells needed to repair UV-induced damage. Presence of UVR significantly increased Ci affinity for photosynthesis, raised the activity of total CA, and enhanced the efficiency of Ci utilisation, to a greater extent under Pi-replete conditions, which must be responsible for the discrepancy between the UV-reduced (L-Pi) and UV-enhanced (H-Pi) growth rates.

Presence of UVR inhibited growth at L-Pi but enhanced it at H-Pi levels, which is not consistent with the UV-reduced maximal photosynthetic rate and the UV-enhanced respiration. However, UV-stimulated activity of total CA subsequently enhanced the efficiency of Ci utilisation under the Pi-replete conditions and thus could have contributed to the reversed effects on the growth caused by UVR. UVR reduced the contents of PE and PC and inhibited photosynthetic O_2 evolution, which is consistent with studies on *Porphyra umbilicalis* L. Kützing and *Laminaria saccharina* (L.) J. V. Lamour. (Aguilera *et al*. 1999; Davison *et al*. 2007).

Under Pi-limited conditions, the presence of UV-A raised the apparent photosynthetic efficiency. Such a UV-A-stimulated photosynthetic rate under PAR-limited conditions is also reported in coastal phytoplankton assemblages (Gao *et al*. 2007), and during twilight periods in *G. lemaneiformis* (Gao and Xu 2008). The photosynthetic efficiency was higher in thalli grown at the Pi-replete than at the Pi-limiting level, however UV-A did not stimulate it as in Pi-limited thalli. The fact that Pi-enrichment stimulated the maximal and light-limited photosynthetic rates might have outweighed the impacts of UV-A on the photosynthetic efficiency. On the other hand, the average daily doses of PAR (3446.78 kJ m^{-2}), UV-A (570.91 kJ m^{-2}) and UV-B (17.62 kJ m^{-2}) were relatively low due to cloud cover, allowing efficient repair of UV-induced damage (Flores-Moya *et al*. 1999; Hanelt and Roleda 2009) and measurable UV-A-enhanced growth rate at a H-Pi level (Fig. 1).

Table 2. Photosynthetic parameters based on the relationship of net photosynthetic rates with inorganic carbon (Ci) concentrations in *Gracilaria lemaneiformis* grown under different phosphorus and solar radiation treatments

Thalli were cultured for 16 days under Pi-limited (L-Pi) or replete (H-Pi) conditions with or without UVR (P, PAR; PA, PAR+UV-A; PAB, PAR+UV-A+B). V_{max} (μmol O_2 h^{-1} g^{-1} FW), the maximum rate of Ci utilisation; K_{DIC} (mmol), Ci concentration (mmol) to achieve half V_{max}; the V_{max}/K_{DIC} ratio, an indicator of carbon acquirement efficiency. Within each column of the data, values with any different superscript letter are significantly different at $P = 0.05$. Data are means ± s.d. ($n = 3$)

Treatments		V_{max} (μmol O_2 h^{-1} g^{-1} FW)	K_{DIC} (mmol)	V_{max}/K_{DIC} (μmol O_2 h^{-1} g^{-1} FW)/(mmol)
L-Pi	P	81.59 ± 4.75^{a}	0.86 ± 0.08^{a}	95.63 ± 5.08^{a}
	PA	58.90 ± 3.42^{b}	0.65 ± 0.09^{b}	92.40 ± 7.58^{a}
	PAB	52.95 ± 1.55^{b}	0.59 ± 0.08^{b}	90.40 ± 10.12^{a}
H-Pi	P	91.22 ± 8.15^{a}	0.70 ± 0.18^{ab}	134.49 ± 24.25^{b}
	PA	77.69 ± 0.47^{c}	0.39 ± 0.02^{c}	200.75 ± 13.07^{c}
	PAB	72.32 ± 3.00^{c}	0.36 ± 0.06^{c}	203.18 ± 38.76^{c}

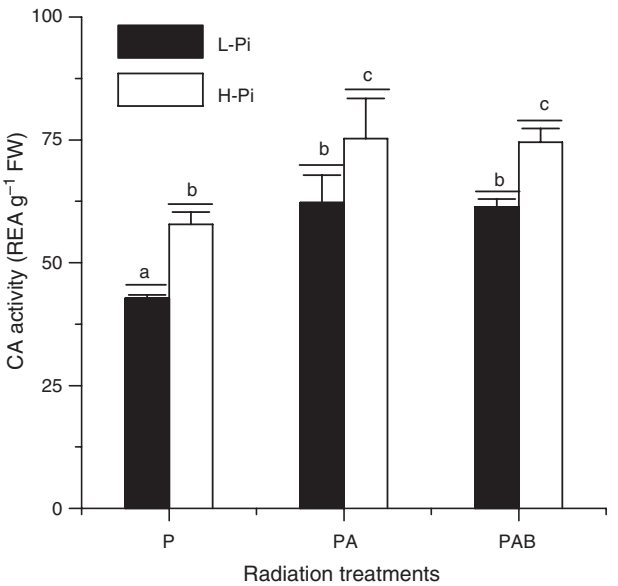

Fig. 5. Relative enzyme activities (REA) of total carbonic anhydrase (CA) in *Gracilaria lemaneiformis* grown at low and high phosphate levels under different solar radiation treatments with or without UVR. Significant differences between treatments ($P < 0.05$) are indicated by different lowercase letters. Vertical bars represent ±s.d. of the means ($n = 4$).

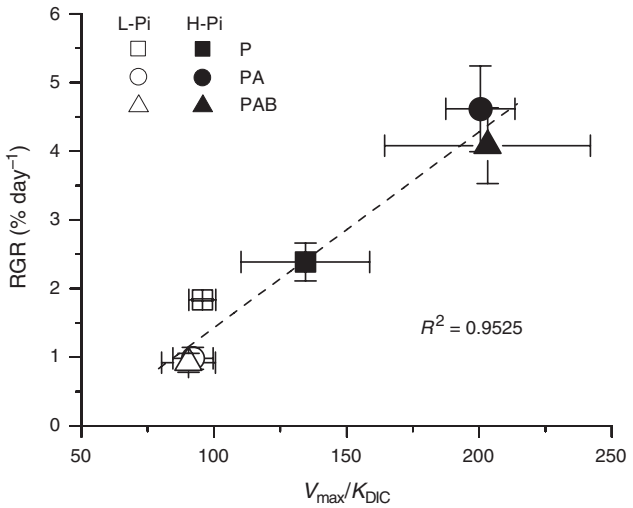

Fig. 6. Relationship of relative growth rate (RGR) with the ratio of V_{max} to K_{DIC} in *Gracilaria lemaneiformis* grown at low and high phosphate levels under different solar radiation treatments with or without UVR. The V_{max}/K_{DIC} ratio was calculated from Fig. 4 and vertical bars represent ±s.d. of the means ($n = 3$).

Such UV-A-induced enhancement of growth is noted in the brown alga *Fucus gardneri* Silva (Henry and Van Alstyne 2004).

UV-enhanced Ci affinity for photosynthesis could be due either to enhanced activity of the CA that catalyses bicarbonate to CO_2 for carboxylation, or to stimulated synthesis of periplasmic proteins associated with bicarbonate pump channels. UV-B-stimulated Ci accumulation is noted in a marine microalga *Dunaliella tertiolecta* Butcher (Beardall *et al.* 2002). Exposure to UV-B increases the photosynthetic Ci affinity in a freshwater cyanobacterium (Song and Qiu 2007), and Wu and Gao (2009) show that numerous periplasmic proteins increase after exposure to moderate levels of UVR.

The UVACs in macroalgae, mainly MAAs, play protective roles against UVR (Oren and Gunde-Cimerman 2007). Zheng and Gao (2009) recently note that enrichment of nitrate significantly raises the content of MAAs in *G. lemaneiformis* and ameliorates the UV-induced harm on photosynthetic performance. In the present study, enrichment of Pi did not result in significant changes in the accumulation of MAAs (Fig. 3). Although presence of UV-A as well as UV-A+B significantly increased the accumulation of MAAs, photosynthetic rate of alga was significantly reduced by UVR. UVR must have induced damage in thalli grown both in the Pi-replete and in Pi-limited conditions, but the repairing efficiency in thalli exposed to replete Pi could be much higher than in the Pi-limited thalli. Thus, UV-A-induced enhancement, as mentioned above, might exceed the UV-related harm, leading to an enhanced growth rate in thalli grown under Pi-replete conditions.

UV-B damages cell membranes (Kramer *et al.* 1991), degrades cell walls and even inhibits protein channels (Murphy 1983; Sobrino *et al.* 2004), thus affecting uptake of nutrients (Hessen *et al.* 1995). Pi and inorganic nitrogen uptake by *G. lemaneiformis* must have been affected by UVR in the present study. Although we did not investigate to what extent the uptake of nutrients would be affected, thalli with a more efficient repairing system would be much less affected. Pi is indispensable for the repair of UV-induced damage to DNA and in the supply of energy for damaged proteins (Murata *et al.* 2007). Pi enrichment is shown to be effective in reducing UV-related damage (Shelly *et al.* 2005) and in accelerating repair (Heraud *et al.* 2005). In the present study, thalli of *G. lemaneiformis* grown under Pi-replete conditions and in the presence of UVR showed a higher respiration rate (Table 1), indirectly reflecting an enhanced supply of energy to cope with UVR-related stress and to support growth.

In coastal waters, the availability of Pi is often low, and most macroalgal species experience P-limitation (Larned 1998; Hwang *et al*. 2004). Temporary inputs of nutrients from runoff or from animal aquaculture occur in the waters adjacent to densely populated areas and therefore lead to eutrophication. Therefore, macroalgae in waters of different nutrient levels can respond to solar UV radiation in different ways, being negatively affected in Pi-limiting waters and positively influenced in Pi-replete areas, especially when solar radiation is at moderate levels. In nutrient-rich waters, *Gracilaria* reaches a physiological status with a high concentration of UVACs (Zheng and Gao 2009) and high Ci utilisation efficiency while exposed to solar UV irradiance (Fig. 6). Pi-enrichment affected photosynthetic Ci utilisation and its response to changes of UVR, reversing the impact of UVR on growth from the negative to the positive (Fig. 1). Response of algal cells to UVR depends on the balance between damage and repair (Häder *et al*. 2002), and the intensity of UVR and the availability of nutrients (such as Pi in this study) determine the balance and therefore the nature of positive or negative impacts.

Acknowledgements

This research was supported by National Basic Research Program of China (2009CB421207), National Natural Science Foundation of China (90411018, 40876058) and '863' project (2006AA10A413) and Ministry of Education (308015). Professor John Hodgkiss is thanked for his assistance with the English in this paper.

References

Aguilera J, Jiménez C, Figueroa F, Lebert M, Häder D (1999) Effects of ultraviolet radiation on thallus absorption and photosynthetic pigments in the red alga *Porphyra umbilicalis*. *Journal of Photochemistry and Photobiology. B, Biology* **48**, 75–82. doi: 10.1016/S1011-1344(99)00015-9

Atienzar FA, Cordi B, Donkin ME, Evenden AJ, Jha AN, Depledge MH (2000) Comparison of ultraviolet-induced genotoxicity detected by random amplified polymorphic DNA with chlorophyll fluorescence and growth in a marine macroalgae, *Palmaria palmata*. *Aquatic Toxicology (Amsterdam, Netherlands)* **50**, 1–12. doi: 10.1016/S0166-445X(99)00100-9

Beardall J, Giordano M (2002) Ecological implications of microalgal and cyanobacterial CO_2 concentrating mechanisms, and their regulation. *Functional Plant Biology* **29**, 335–347. doi: 10.1071/PP01195

Beardall J, Heraud P, Roberts S, Shelly K, Stojkovic S (2002) Effects of UV-B radiation on inorganic carbon acquisition by the marine microalga *Dunaliella tertiolecta* (Chlorophyceae). *Phycologia* **41**, 268–272.

Beardall J, Roberts R, Raven JA (2005) Regulation of inorganic carbon acquisition by phosphorus limitation in the green alga *Chlorella emersonii*. *Canadian Journal of Botany* **83**, 859–864. doi: 10.1139/b05-070

Bischof K, Gómez I, Molis M, Hanelt D, Karsten U, Lüder U, Roleda MY, Zacher K, Wiencke C (2006) Ultraviolet radiation shapes seaweed communities. *Reviews in Environmental Science and Biotechnology* **5**, 141–166. doi: 10.1007/s11157-006-0002-3

Davison JR, Jordan TL, Fegley JC, Grobe CW (2007) Response of *Laminaria saccharina* (Phaeophyta) growth and photosynthesis to simultaneous ultraviolet radiation and nitrogen limitation. *Journal of Phycology* **43**, 636–646. doi: 10.1111/j.1529-8817.2007.00360.x

Dunlap WC, Rae GA, Helbling EW, Villafañe VE, Holm-Hansen O (1995) Ultraviolet-absorbing compounds in natural assemblages of Antarctic phytoplankton. *Antarctic Journal of the United States* **30**, 323–326.

Fei X (2004) Solving the coastal eutrophication problem by large scale seaweed cultivation. *Hydrobiologia* **512**, 145–151. doi: 10.1023/B:HYDR.0000020320.68331.ce

Flores-Moya A, Hanelt D, Lopez-Figueroa F, Altamirano M, Vinegla B, Salles S (1999) Solar UV-B radiation shows beneficial effects on recovery of inhibited photosynthesis in the brown alga *Dictyota dichotoma*. *Journal of Photochemistry and Photobiology. B, Biology* **49**, 129–135. doi: 10.1016/S1011-1344(99)00046-9

Gao K, Xu J (2008) Effects of solar UV radiation on diurnal photosynthetic performance and growth of *Gracilaria lemaneiformis* (Rhodophyta). *European Journal of Phycology* **43**, 297–307. doi: 10.1080/09670260801986837

Gao K, Wu Y, Li G, Wu H, Villafañe VE, Helbling EW (2007) Solar UV radiation drives CO_2 fixation in marine phytoplankton: a double-edged sword. *Plant Physiology* **144**, 54–59. doi: 10.1104/pp.107.098491

García-Sánchez MJ, Fernández JA, Niell FX (1996) Photosynthetic responses of P-deficient *Gracilaria tenuistipitata* under two different phosphate treatments. *Physiologia Plantarum* **96**, 601–606. doi: 10.1111/j.1399-3054.1996.tb00232.x

Giordano M, Maberly SC (1989) Distribution of carbonic anhydrase in British marine macroalgae. *Oecologia* **81**, 534–539. doi: 10.1007/BF00378965

Häder DP, Lebert M, Marangoni P, Colombetti G (1999) ELDONET-European light dosimeter network hardware and software. *Journal of Photochemistry and Photobiology. B, Biology* **52**, 51–58. doi: 10.1016/S1011-1344(99)00102-5

Häder DP, Lebert M, Sinha RP, Barbieri ES, Helbling EW (2002) Role of protective and repair mechanisms in the inhibition of photosynthesis in marine macroalgae. *Photochemical & Photobiological Sciences* **1**, 809–814. doi: 10.1039/b206152j

Häder DP, Kumar HD, Smith RC, Worrest RC (2007) Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. *Photochemical & Photobiological Sciences* **6**, 267–285. doi: 10.1039/b700020k

Hanelt D, Roleda MR (2009) UVB radiation may ameliorate photoinhibition in specific shallow-water tropical marine macrophytes. *Aquatic Botany* **91**, 6–12. doi: 10.1016/j.aquabot.2008.12.005

Henley WJ (1993) Measurements and interpretation of photosynthetic light-response curves in algae in the context of photoinhibition and dial changes. *Journal of Phycology* **29**, 729–739. doi: 10.1111/j.0022-3646.1993.00729.x

Henry BE, Van Alstyne KL (2004) Effects of UV radiation on growth and phlorotannins in *Fucus gardneri* (Phaeophyceae) juveniles and embryos. *Journal of Phycology* **40**, 527–533.

Heraud P, Roberts S, Shelly K, Beardall J (2005) Interactions between UV-B exposure and phosphorus nutrition. II. Effects on rates of damage and repair. *Journal of Phycology* **41**, 1212–1218. doi: 10.1111/j.1529-8817.2005.00149.x

Hernández I, Niell FX, Whitton BA (2002) Phosphatase activity of benthic marine algae. An overview. *Journal of Applied Phycology* **14**, 475–487. doi: 10.1023/A:1022370526665

Hessen DO, Van Donk E, Andersen T (1995) Growth responses, P-uptake and loss of flagella in *Chlamydomonas reinhardtii* exposed to UV-B. *Journal of Plankton Research* **17**, 17–27. doi: 10.1093/plankt/17.1.17

Holzinger A, Lütz C (2006) Algae and UV irradiation: effects on ultrastructure and related metabolic functions. *Micron (Oxford, England)* **37**, 190–207. doi: 10.1016/j.micron.2005.10.015

Hoppe HG (2003) Phosphatase activity in the sea. *Hydrobiologia* **493**, 187–200. doi: 10.1023/A:1025453918247

Hwang R, Tsai C, Lee T (2004) Assessment of temperature and nutrient limitation on seasonal dynamics among species of *Sargassum* from a coral reef in southern Taiwan. *Journal of Phycology* **40**, 463–473.

Irihimovitch V, Yehudai-Resheff S (2008) Phosphate and sulfur limitation responses in the chloroplast of *Chlamydomonas reinhardtii*. *FEMS Microbiology Letters* **283**, 1–8. doi: 10.1111/j.1574-6968.2008.01154.x

Jiang H, Gao K, Helbling EW (2007) Effects of solar UV radiation on germination of conchospores and morphogenesis of sporelings in *Porphyra haitanensis* (Rhodophyta). *Marine Biology* **151**, 1751–1759. doi: 10.1007/s00227-007-0632-1

Johnston AM, Maberly SC, Raven JA (1992) The acquisition of inorganic carbon by four red macroalgae. *Oecologia* **92**, 317–326. doi: 10.1007/BF00317457

Karsten U, Wiencke C (1999) Factors controlling the formation of UV-absorbing mycosporine-like amino acids in the marine red alga *Palmaria palmata* from Spitsbergen (Norway). *Journal of Plant Physiology* **155**, 407–415.

Kozlowska-Szerenos B, Zieliński P, Maleszewski S (2000) Involvement of glycolate metabolism in acclimation of *Chlorella vulgaris* cultures to low phosphate supply. *Plant Physiology and Biochemistry* **38**, 727–734. doi: 10.1016/S0981-9428(00)01175-X

Kramer GF, Norman HA, Krizek DT, Mirecki RM (1991) Influence of UV-B radiation on polyamines, lipid peroxidation and membrane lipids in cucumber. *Phytochemistry* **30**, 2101–2108. doi: 10.1016/0031-9422(91)83595-C

Lapointe BE (1987) Phosphorus- and nitrogen-limited photosynthesis and growth of *Gracilaria tikvahiae* (Rhodophyceae) in the Florida Keys: an experimental field study. *Marine Biology* **93**, 561–568. doi: 10.1007/BF00392794

Larned ST (1998) Nitrogen- versus phosphorus-limited growth and sources of nutrient for coral reef macroalgae. *Marine Biology* **132**, 409–421. doi: 10.1007/s002270050407

Makarov M (1999) Influence of ultraviolet radiation on the growth of the dominant macroalgae of the Barents Sea. *Chemosphere. Global Change Science* **1**, 461–467. doi: 10.1016/S1465-9972(99)00034-3

McKenzie RL, Aucamp PJ, Bais AF, Björn LO, Ilyas M (2007) Changes in biologically-active ultraviolet radiation reaching the Earth's surface. *Photochemical & Photobiological Sciences* **6**, 218–231. doi: 10.1039/b700017k

Murata N, Takahashi S, Nishiyama Y, Allakhverdiev SI (2007) Photoinhibition of photosystem Π under environmental stress. *Biochimica et Biophysica Acta* **1767**, 414–421. doi: 10.1016/j.bbabio.2006.11.019

Murphy TM (1983) Membranes as targets of ultraviolet radiation. *Physiologia Plantarum* **58**, 381–388. doi: 10.1111/j.1399-3054.1983.tb04198.x

Neale PJ (2000) Spectral weighting functions for quantifying the effects of ultraviolet radiation in marine ecosystems. In 'Effects of UV radiation on the marine environment'. (Eds SJ DeMora, M Vernet) pp. 73–100. (Cambridge University Press: Cambridge)

Oren A, Gunde-Cimerman N (2007) Mycosporines and mycosporine-like amino acids: UV protectants or multipurpose secondary metabolites? *FEMS Microbiology Letters* **269**, 1–10. doi: 10.1111/j.1574-6968.2007.00650.x

Pakker H, Beekman CAC, Breeman AM (2000) Efficient photoreactivation of UVBR-induced DNA damage in the sublittoral macroalga *Rhodymenia pseudopalmata* (Rhodophyta). *European Journal of Phycology* **35**, 109–114. doi: 10.1080/09670260010001735691

Raven JA, Beardall J (1981) Respiration and photorespiration. *Canadian Bulletin of Fisheries and Aquatic Sciences* **210**, 55–82.

Roleda MY, Wiencke C, Lüder UH (2006) Impact of ultraviolet radiation on cell structure, UV-absorbing compounds, photosynthesis, DNA damage, and germination in zoospores of Arctic *Saccorhiza dermatodea*. *Journal of Experimental Botany* **57**, 3847–3856. doi: 10.1093/jxb/erl154

Shelly K, Roberts S, Heraud P, Beardall J (2005) Interactions between UV-B exposure and phosphorus nutrition. I. Effects on growth, phosphate uptake, and chlorophyll fluorescence. *Journal of Phycology* **41**, 1204–1211. doi: 10.1111/j.1529-8817.2005.00148.x

Siegelman HW, Kycia JH (1978) Algal biliproteins. In 'Handbook of phycological methods: physiological and biochemical methods'. (Eds JA Hellebust, JS Craigie) pp. 71–79. (Cambridge University Press: Cambridge)

Sigee DC (2005) 'Freshwater microbiology.' 516 pp. (Wiley: Chichester)

Sobrino C, Montero O, Lubián LM (2004) UV-B radiation increases cell permeability and damages nitrogen incorporation mechanisms in *Nannochloropsis gaditana*. *Aquatic Sciences* **66**, 421–429. doi: 10.1007/s00027-004-0731-8

Song Y, Qiu B (2007) The CO_2-concentrating mechanism in the bloom-forming cyanobacterium *Microcystis aeruginosa* (Cyanophyceae) and effects of UVB radiation on its operation. *Journal of Phycology* **43**, 957–964. doi: 10.1111/j.1529-8817.2007.00391.x

Tyrrell T (1999) The relative influence of nitrogen and phosphorus on oceanic primary production. *Nature* **400**, 525–531. doi: 10.1038/22941

Vincent WF, Neale PJ (2000) Mechanisms of UV damage to aquatic organisms. In 'The effects of UV radiation in the marine environment'. (Eds ST de Mora, S Demers, M Vernet) pp. 149–176. (Cambridge University Press: Cambridge)

Von Caemmerer S, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **153**, 376–387. doi: 10.1007/BF00384257

Weatherhead EC, Andersen SB (2006) The search for signs of recovery of the ozone layer. *Nature* **441**, 39–45. doi: 10.1038/nature04746

Wellburn AR (1994) The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of Plant Physiology* **144**, 307–313.

Wu H, Gao K (2009) Ultraviolet radiation stimulated activity of extracellular carbonic anhydrase in the marine diatom *Skeletonema costatum*. *Functional Plant Biology* **36**, 137–143. doi: 10.1071/FP08172

Xu Z (2007) The physiological responses of two economic marine macroalgal species to nutrients supplies in seawater. MSc Thesis, Shantou University, Shantou, China.

Zheng Y, Gao K (2009) Impacts of solar UV radiation on the photosynthesis, growth and UV-absorbing compounds in *Gracilaria lemaneiformis* (Rhodophyta) grown at different nitrate concentrations. *Journal of Phycology* **45**, 314–323. doi: 10.1111/j.1529-8817.2009.00654.x

Zou D, Xia J, Yang Y (2004) Photosynthetic use of exogenous inorganic carbon in the agarophyte *Gracilaria lemaneiformis* (Rhodophyta). *Aquaculture* **237**, 421–431. doi: 10.1016/j.aquaculture.2004.04.020

Manuscript received 27 April 2009, accepted 24 July 2009