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Combined effects of ocean acidification and solar UV radiation on photosynthesis, growth, pigmentation and calcification of the coralline alga *Corallina sessilis* (Rhodophyta)

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Abstract

Previous studies have shown that increasing atmospheric CO_2 concentrations affect calcification in some planktonic and macroalgal calcifiers due to the changed carbonate chemistry of seawater. However, little is known regarding how calcifying algae respond to solar UV radiation (UVR, UVA + UVB, 280–400 nm). UVR may act synergistically, antagonistically or independently with ocean acidification (high CO_2/low pH of seawater) to affect their calcification processes. We cultured the articulated coralline alga *Corallina sessilis* Yendo at 380 ppmv (low) and 1000 ppmv (high) CO_2 levels while exposing the alga to solar radiation treatments with or without UVR. The presence of UVR inhibited the growth, photosynthetic O_2 evolution and calcification rates by13%, 6% and 3% in the low and by 47%, 20% and 8% in the high CO_2 concentrations, respectively, reflecting a synergistic effect of CO_2 enrichment with UVR. UVR induced significant decline of pH in the CO_2-enriched cultures. The contents of key photosynthetic pigments, chlorophyll a and phycobiliproteins decreased, while UV-absorptivity increased under the high pCO_2/low pH condition. Nevertheless, UV-induced inhibition of photosynthesis increased when the ratio of particulate inorganic carbon/particulate organic carbon decreased under the influence of CO_2-acidified seawater, suggesting that the calcified layer played a UV-protective role. Both UVA and UVB negatively impacted photosynthesis and calcification, but the inhibition caused by UVB was about 2.5–2.6 times that caused by UVA. The results imply that coralline algae suffer from more damage caused by UVB as they calcify less and less with progressing ocean acidification.

Keywords: calcification, CO_2, *Corallina sessilis*, coralline algae, ocean acidification, photosynthesis, UV, UV-absorbing compounds

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Introduction

More than 2 million surveys have shown that the oceans have absorbed more than one-third of the anthropogenic CO_2 released to the atmosphere (Sabine *et al*., 2004). Continuous dissolution of CO_2 from the atmosphere into the oceans is known to affect seawater chemistry, leading to an increase in the concentrations of HCO_3^- and H^+ and a decrease in the concentration of CO_3^{2-} and the saturation state of calcium carbonate (Gattuso *et al*., 1998; Caldeira & Wickett, 2003; Orr *et al*., 2005). The atmospheric CO_2 level is expected to rise to 800–1000 ppmv by the end of this century under a 'business-as-usual' CO_2 emission scenario (Brewer, 1997). The surface ocean is known to have been acidified by 0.1 pH unit (corresponding to a 30% increase of H^+) since 1800 (Orr *et al*., 2005), and that it will be further acidified by 0.3–0.4 U (about a 100–150% increase of H^+) by 2100 (Caldeira & Wickett, 2003). Such an ocean acidifying process may harm marine calcifying organisms by reducing the rate of calcification of their skeletons or shells (Gao *et al*., 1993a; Riebesell *et al*., 2000; Orr *et al*., 2005). However, increased calcification rates have also been reported in the coccolithophore *Emiliania huxleyi* at elevated CO_2 levels (Iglesias-Rodriguez *et al*., 2008b). These controversial results are still being debated (Iglesias-Rodriguez *et al*., 2008a; Riebesell

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et al., 2008). Obviously, indoor controlled experimental systems can lead to different results due to different environmental conditions or treatments. In both kinds of experimental system (Iglesias-Rodriguez *et al*., 2008a; Riebesell *et al*., 2008), low photosynthetically active radiation (PAR, 100–150 $\mu mol\, m^{-2}\, s^{-1}$) was employed. Elevated PAR levels may result in excessive energy, and the presence of UVR may harm the cells, and both may alter the responses of marine calcifiers to ocean acidification.

In the coastal waters adjacent to populated areas, pH varies to a larger extent compared with offshore waters. Nevertheless, calcifying organisms in coastal waters have to tolerate and cope with the chemical changes in the carbonate system as ocean acidification advances (Schneider & Erez, 2006). Recently, CO_2 perturbation experiments show that acidification of coastal water can lead to less calcification of benthic calcifiers (Hall-Spencer *et al*., 2008).

Macroalgae respond to changes both in CO_2 and pH in seawater. Studies on the ecological and physiological impacts of elevated CO_2 concentrations on macroalgae were initiated in the early 1990s (Gao *et al*., 1991, 1993a, b). Growth of *Porphyra yezoensis* juveniles is significantly enhanced with enriched CO_2 to 1000 ppmv (Gao *et al*., 1991). The photosynthetic carbon fixation rate of some intertidal macroalgal species increases during low-tide periods when exposed to air of high CO_2 concentration (Gao *et al*., 1999; Zou & Gao, 2002). On the other hand, a decrease of growth rate caused by elevated CO_2 is also reported in some red algae (Israel *et al*., 1999). A more recent study reports that the growth rates of 13 species (representing the Chlorophyta, Rhodophyta and Phaeophyta) cultivated in normal seawater are comparable to their growth in CO_2-enriched seawater (Israel & Hophy, 2002), and attributes such nonresponsiveness to the presence of CO_2 concentrating mechanisms. Macroalgal species, obviously, may respond to elevated CO_2 levels in different ways.

Increased pCO_2 and decreased pH and CO_3^{2-} are known to negatively affect calcifying macroalgae (Gao *et al*., 1993a; Kuffner *et al*., 2007). It is also shown that the increase of CO_2 concentrations significantly slows down calcification of corals (Leclercq *et al*., 2000). On the other hand, when pH is kept at a constant level, elevated concentrations of dissolved inorganic carbon (DIC) enhance the calcification of the calcifying red alga *Corallina pilulifera* (Gao *et al*., 1993a).

The findings of previous studies are important in understanding how algal calcification, photosynthesis and growth respond to ocean acidification. However, to the best of our knowledge, none of the previous studies have examined the effects of ocean acidification with UV radiation (UVR) being considered. Macroalgae are definitely exposed to UVR because they are usually distributed in shallow waters through which solar UVR can penetrate to a considerable depth (Hargreaves, 2003). In addition, UVB radiation reaching the earth surface has increased due to thinned ozone layer at other latitudes in addition to polar regions (Kerr & McElroy, 1993). Coralline algae are commonly found in shallow coastal waters or coral reefs and may physiologically be sensitive to solar UVR. UVR is known to affect photosynthetic performance (Dring *et al*., 2001), damage DNA molecules (Roleda *et al*., 2006) and affect the growth rate (Gao & Xu, 2008) of macroalgae. Recently, positive effects of UVA are also reported in phytoplankton (Gao *et al*., 2007), cyanobacteria (Wu *et al*., 2005) and macroalgae (Gao & Xu, 2008). Evidently, it is important to examine the ecological effects of elevated CO_2 concentration and/or ocean acidification in the presence of UVR and scrutinize their interactive effects.

The present study aimed to investigate the combined effects of ocean acidification and solar UVR on the coralline alga *Corallina sessilis* to see how its photosynthesis, growth and calcification respond to ocean acidification (high CO_2/low pH conditions) and to see how they would be affected by solar UVR under seawater-acidified conditions.

Materials and methods

Alga and culture

C. sessilis Yendo, a coralline alga, is distributed in the lower parts of the intertidal zone. Plants (2–3 cm in length) of *C. sessilis* growing on floating fish-farming rafts were collected at a depth of 10 cm near Nan'ao island (23.3°N, 116.6°E), Shantou, Guangdong, China, in October 2007 and September 2008. Thalli used in the experiments were carefully selected and cleaned of epiphytes. About 50 individuals (on average, 100 mg fresh weight for each plant) were placed in each quartz tube (Φ5.9 cm, 35 cm long, volume about 1 L) filled with 0.6 L sand-filtered seawater, total of the quartz tubes used were 12. The tubes containing the thalli were maintained in a water tank through which surface seawater was run to control the temperature close to the ambient sea surface temperature (SST) (Fig. 1). The culture seawater was renewed every 48 h and aerated ($0.5\, L\, min^{-1}$) with ambient or CO_2-enriched air. Concentrations of nitrate and phosphate in the seawater used throughout the experimental period were 41.8–43.9 and 0.3–0.5 μM.

Solar radiation treatments and monitoring

The thalli in the quartz tubes were exposed to three different solar radiation treatments: (1) PAR alone (PAR

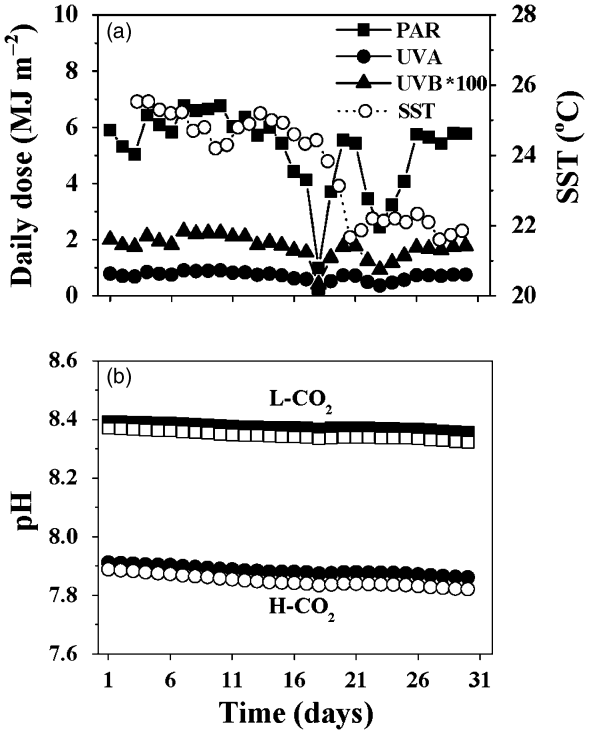

Fig. 1 (a) Changes of daily solar doses of photosynthetically active radiation (PAR), UVA and UVB and of surface seawater temperature (SST); (b) changes of pH in the cultures aerated with ambient (380 ppmv) and enriched (1000 ppmv) CO_2, while exposed to full spectrum of solar radiation (PAR + UVA + UVB, open symbols) or that screened off UVR (PAR, filled symbols) during the period of October 15 to November 13, 2007.

treatment), where the quartz tubes were covered with Ultraphan film 395 (UV Opak, Digefra, Munich, Germany), transmitting solar radiation above 395 nm; (2) PAR + UVA (PA treatment), where the quartz tubes were covered with Folex 320 (Montagefolie, Nr. 10155099, Folex, Dreieich, Germany), which transmits solar radiation above 320 nm; and (3) PAR + UVA + UVB (PAB treatment), where the quartz tubes were covered with Ultraphan 295 (UVT100, Digefra), transmitting irradiance above 295 nm. The transmission spectra of the cut-off filters have been reported elsewhere (Zheng & Gao, 2009). Incident solar radiation was continuously monitored using a broadband ELDONET filter radiometer (Real Time Computer, Möhrendorf, Germany), which has three channels for PAR (400–700 nm), UVA (315–400 nm) and UVB (280–315 nm). This instrument has been internationally recognized (certificate no. 2006/BB14/1) and was calibrated every year with assistance from the maker. The cut-off filters reduce 4% of PAR in water due to reflection (Gao *et al*., 2007). There was about a 5 nm difference between the measured and the exposed UVA waveband. Therefore, the thalli received about 2% less UVA and about 4% less PAR in contrast to the irradiances measured. There was also about a 15 nm difference between the measured and the exposed UVB waveband due to the cut-off by the 295 foil and the thalli received about 10% less UVB in contrast to the UVB irradiance measured.

CO_2 supply and monitoring

The cultures were aerated with ambient (380 ppmv CO_2) or CO_2-enriched (1000 ppmv CO_2) air at 0.5 L min^{-1}. The enrichment of CO_2 to 1000 ppmv was achieved by pumping the air into air bags (about 1 m^3) into which pure CO_2 (99%) had been injected. The air bags were then sealed up and rolled to ensure homogeneous mixture of the gases. The CO_2 concentration in the bags, as measured with an infrared gas analyzer (CGT-7000, Shimadzu, Nakagyo-ku, Japan), was constant within the 12 h during which they were used. During the experiment, at least three air bags were connected in parallel to ensure a continuous aeration each day, and a total of 120 bags of CO_2-enriched air were used.

Determination of individual biomass change

While the thalli were cultured under different levels of CO_2 and different radiation treatments with (PAR + UVA or PAR + UVA + B) or without (PAR) UVR for a period of 30 days in 2007 (October 15 to November 13), changes in individual mass (fresh weight) were assessed every 6 days for five (marked) different plants from each treatment. The fresh weight was determined after blotting the thalli gently with tissue paper.

Measurement of pH

Changes of pH in the cultures were measured using a pH meter (FE20, Mettler Toledo, Greifensee, Switzerland). The pH electrode was calibrated regularly with standard NBS buffer solutions (Oakton, Vernon Hills, IL, USA) to ensure a stable response. From the beginning of the experiment, pH was measured at 08:00, 14:00 and 20:00 hours each day. During the pH measurements, water temperature was recorded simultaneously, and all the pH values were normalized to that at 25 °C on the basis of the relationship of pH and water temperature.

Analysis of carbon components

The content of particulate inorganic carbon (PIC) in the algal tissue was estimated according to Gao *et al*. (1993b) by drying the samples at 85 °C for 24 h, grinding them to a fine powder, dissolving this in HCl solution (1 N), centrifuging at 5000 ***g*** for 10 min after adjusting the pH to 7.0–9.0, measuring the concentrations of

calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions and calculating the contents on the basis of their atomic ratios and the biomass used. The concentrations of the ions in the supernatant were measured with an ion-chromatography unit (LC-10AD$_{VP}$, Shimadzu). The total carbon (TC) and total inorganic carbon (TIC) in the powder were measured with a total organic carbon analyzer (TOC-5000A, Shimadzu), and the particulate organic carbon (POC) was calculated as the difference between the TC and TIC.

Determination of photosynthetic oxygen evolution

A closed system modified from Gao & Umezaki (1989) was used for measuring the photosynthesis of *C. sessilis* in running seawater. The thalli were fixed on stainless-steel wires (without self-shading) and placed in a quartz tube (Φ4 cm, 35 cm long, 0.44 L). The quartz tube was covered with one of the cut-off foils in order to maintain the thalli under the same radiation treatment as in the cultures. Seawater was circulated at 0.6 L min^{-1} using a peristaltic pump (01268-16, Cole-Parmer Instrument Co., Vernon Hills, IL, USA). The concentration of dissolved oxygen in the seawater was monitored with a Clark-type oxygen microelectrode (YSI model 5300, YSI, Yellow Spring, OH, USA) interfaced with an oxymeter (Oxym 5, RealThin-Client, Frankfurt/Main, Germany) connected to a laptop computer. The seawater was renewed at the beginning of each measurement. The net photosynthetic rate was determined from the linear change with time in the dissolved oxygen concentration as follows: P_n (μmol O_2 h^{-1} g^{-1}) = $\Delta O_2 \times 60 \times V \times 1/W$, where ΔO_2 represents the average change in oxygen concentration (μmol L^{-1}) in 1 min during the measuring period; V, the seawater volume (L) in the closed system; W, the dry weight (g) of the samples used. The photosynthetic rates were determined during the period 10:00–14:00 hours for the experiments in both 2007 and 2008.

Determination of total alkalinity (T_A) and calcification rate

The T_A of the seawater was measured using the pH method according to Anderson & Robinson (1946) and was determined at the beginning and at the end of the photosynthetic measurements. The calcification rate (μmol C h^{-1} g^{-1}) of the thalli was then estimated according to the alkalinity anomaly technique (Chisholm & Gattuso, 1991; Gattuso *et al*., 1999) as follows:

$$\Delta CaCO_3 = (T_A f - T_A i) \times 0.5 \times 1000 \times V \times T^{-1} \times W^{-1}$$

where T_Af and T_Ai represent the final and initial total alkalinity of the seawater (mmol L^{-1}), respectively; 0.5, half molar of $CaCO_3$; 1000, a conversion factor (mg–μmol); V (L) of seawater in the closed system; T for the duration (h) of the measurement; and W (g) of the samples. Because uptake of nitrate can affect T_A, the measured values of T_A were corrected (Brewer & Goldman, 1976) before being used to calculate the calcification rates. The calcification rate would have been 0.9–1.9% higher if the effect of nitrate uptake had not been considered.

Determination of UV-induced inhibition of photosynthesis and calcification

Relative inhibition of photosynthesis or calcification caused by UVR was estimated as $(P_{PAR}-P_{PAB}) \times (P_{PAR})^{-1} \times 100\%$, where P_{PAR} represents the rate under PAR alone, while P_{PAB} represents the rate under either PAR + UVA or PAR + UVA + UVB treatments.

Determination of photosynthetic pigments and UV-absorbing compounds (UVACs)

About 0.1 g (fwt) thalli was ground and extracted in 10 mL absolute methanol at 4 °C in darkness for 24 h (Gao & Xu, 2008). After centrifugation at 5000 *g* for 10 min, absorbance of the supernatant was measured from 250 to 750 nm using a scanning spectrophotometer (UV 530, Beckman Coulter, Fullerton, CA, USA). The concentration of chlorophyll *a* (Chl. *a*) and carotenoids were determined according to Wellburn (1994), and the absorptivity of UVACs was obtained based on their absorption peak at 325 nm (Gao & Xu, 2008). To estimate the content of phycoerythrin (PE), about 0.1 g fresh thalli was ground and extracted in phosphate buffer solution (pH 6.8) for 24 h. After centrifugation at 5000 *g* for 10 min, the supernatant was scanned with the same spectrophotometer. The content of PE was determined according to Beer & Eshel (1985).

Statistical analysis

One-way analysis of variance and Tukey's test were used to establish differences among treatments. A confidence level was set at 95%. Standard deviation for UV-induced inhibition was calculated with randomly paired data and subtracting the data of PAR + UVR from that of PAR alone.

Results

During the experimental period of October 15 to November 13, 2007, the incident daily solar radiation doses varied between 985 and 6779 kJ m^{-2} for PAR, 151 and 901 kJ m^{-2} for UVA and 4 and 23 kJ m^{-2} for UVB. The SST fluctuated within a range of 21.6–25.5 °C and sustained at about 22 °C for the last 10 days (Fig. 1a). Under the PAR alone treatment, the CO_2 enrichment (1000 ppmv) reduced the pH by 0.5 U on average

Table 1 Parameters of the seawater carbonate system under the ambient (380 ppmv) and enriched (1000 ppmv) CO_2

	CO_2 (ppmv)	
	380	1000
DIC (μM)	2018 ± 13	2228 ± 11
	(2043 ± 14)	(2252 ± 18)
HCO_3^- (μM)	1836 ± 17	2111 ± 25
	(1855 ± 18)	(2128 ± 23)
CO_3^{2-} (μM)	165 ± 24	85 ± 8
	(171 ± 19)	(92 ± 21)
pCO_2 (μM)	13 ± 4	33 ± 9
	(15 ± 2)	(40 ± 4)
T_A (μM)	2314 ± 19	2312 ± 89
	(2317 ± 61)	(2318 ± 49)
pH	8.4 ± 0.1	7.9 ± 0.1
	(8.3 ± 0.1)	(7.8 ± 0.04)
Ωc	3.8 ± 0.5	1.9 ± 0.2
	3.9 ± 0.4	2.1 ± 0.5

The values for the PAR + UVR treatments were shown in the parentheses in contrast to that for the PAR alone treatments. Total alkalinity (T_A), partial pressure of CO_2 in seawater (pCO_2), pH, salinity and temperature were used to derive all other parameters using a CO_2 system analyzing software (Dickson *et al.*, 2007). The data were based on the measurements on October 30 (day 15) in 2007 and on September 26, 27 (days 10, 11) in 2008, when the surface water temperature ranged 23.1–25.5 °C, and 22.8–26.6 °C, respectively. Data are the means ± SD of six measurements.
DIC, dissolved inorganic carbon; PAR, photosynthetically active radiation; T_A, total alkalinity.

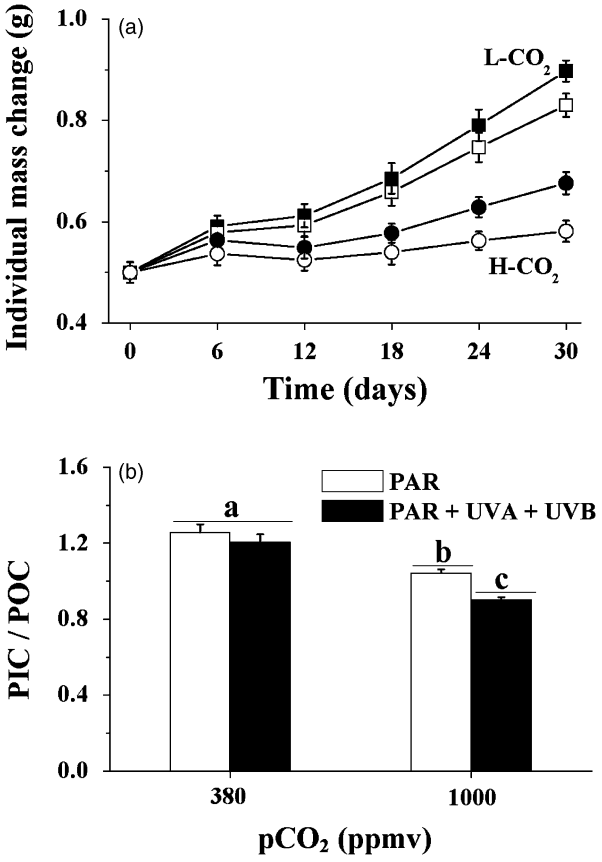

Fig. 2 (a) Change in average individual mass of *Corallina sessilis* over time (October 15–November 13, 2007); (b) PIC/POC ratio at the end of the culture (day 30) in the thalli grown at the low (380 ppmv) and high (1000 ppmv) CO_2 conditions under solar PAR + UVA + UVB (open symbols) or PAR (filled symbols). Data are the means ± SD ($n = 5$). Significant difference among the treatments was indicated as different letters on the bars ($P < 0.05$). PAR, photosynthetically active radiation; PIC, particulate inorganic carbon; POC, particulate organic carbon.

compared with that in the control (380 ppmv CO_2) (Fig. 1b). The difference in the pH values was significant ($P < 0.0001$) between the low and high CO_2 levels throughout the daily cycles in spite of the solar radiation treatments (Fig. 1b). Presence of UVR brought about an insignificant ($P > 0.4$) difference in pH among the treatments at day 1, but significantly ($P < 0.05$) decreased it at day 30 in both the low and high-CO_2 cultures. On average, UVR caused an additional pH drop by 0.03 for the low and 0.04 for the high CO_2 level. Changes in seawater carbonate chemistry showed that, under the PAR alone treatment, the CO_2 enrichment increased the pCO_2 by 154.4%, bicarbonate (HCO_3^-) by 15.1% and the total DIC by 9.9%, but decreased CO_3^{2-} by 48.5% and Ωc by 48.4%. The total alkalinity varied slightly between 2311 and 2318 μM (Table 1). Presence of UVR, however, increased DIC under 380 (or 1000) ppmv CO_2 by 1.3% (1.1%), HCO_3^- by 1.0% (0.8%), CO_3^{2-} by 3.6% (8.2%) and pCO_2 by 15.4% (21.2%). UVR-induced changes were not significantly different ($P > 0.05$) in DIC and HCO_3^-, but the difference in CO_3^{2-} and pCO_2 was significant ($P < 0.01$) (Table 1).

Growth of *C. sessilis* was inhibited at the high CO_2 level either under PAR alone or PAR + UVA + UVB treatments (Fig. 2a). The relative growth rate under PAR alone was 2.1% day^{-1} for the low CO_2, and about 0.9% day^{-1} for the high CO_2 condition during the later phase (12 days later) of the cultures when the mass of the individuals increased steadily. In the presence of UVR, the growth was further reduced either at low or high CO_2 concentration. The rate was 1.6% day^{-1} at low and 0.5% day^{-1} at high CO_2. The ratio of PIC to organic carbon (POC) showed significant ($P < 0.001$) difference between the low and high CO_2 treatments (Fig. 2b). The average PIC/POC ratio was 1.2 under the PAR and 1.1 under the PAR + UVR treatment at low CO_2, while it decreased to 1.0 and 0.9 at high CO_2. At the end of the cultures (day 30), the CO_2

Table 2 Averaged net photosynthetic O_2 evolution and calcification rates of *Corallina sessilis* during noon time period (10:00–14:00 hours) under PAR treatment at day 15 (October 30), 2007 and day 10, 11 (September 26, 27), 2008, when the surface water temperature ranged 23.1–25.5 °C, and 22.8–26.6 °C, respectively

Year	CO_2 (ppmv)	Net photosynthesis ($\mu mol\, O_2\, h^{-1}\, g^{-1}$)	Calcification ($\mu mol\, C\, h^{-1}\, g^{-1}$)
2007	380	34.3 ± 2.6*	nd
	1000	23.5 ± 1.8*	nd
2008	380	35.3 ± 1.2*	30.8 ± 2.3*
	1000	25.7 ± 0.6*	22.9 ± 3.1*

Calcification rate was not measured in 2007 (nd). Data are the means ± SD ($n = 9$) of nine different individuals in three measurements.
*Significant ($P < 0.001$) difference between the CO_2 levels.

enrichment significantly ($P < 0.05$) decreased the PIC/POC ratio by 18.8% under PAR and by 26.3% under PAR + UVR.

When the noontime photosynthetic O_2 evolution was measured between 10:00 and 14:00 hours for the thalli grown at the ambient and enriched CO_2 levels, similar rates were obtained in the 2 years, 2007 and 2008, though the daily average seawater temperature was different by 0.3 °C (Table 2). The high CO_2 and low pH condition significantly ($P < 0.001$) decreased the net photosynthetic rate by 29.3% and the calcification rate by 25.6% under PAR alone treatment compared with the low CO_2/high pH treatment (Table 2). UVR resulted in a significant ($P < 0.05$) inhibition of photosynthetic oxygen evolution between low and high CO_2 conditions (Fig. 3a). The UVR-induced inhibition was 5.7% under the low and 20.2% under the high CO_2 conditions, that is, the enrichment of CO_2 brought about higher ($P < 0.0001$) inhibition of photosynthesis induced by UVR (Fig. 3a). In order to distinguish the impacts of UVA from UVB, the thalli were grown, respectively, under PAR, PAR + UVA or PAR + UVA + UVB, and their photosynthetic oxygen evolution was measured under the corresponding radiation treatments (Fig. 3b). UVA caused less photosynthetic inhibition than UVB ($P < 0.0001$) either under low or high CO_2 conditions (Fig. 3b). The average inhibitions caused by UVA and UVB were 1.9% and 4.8% at the low and 3.1% and 14.2% at the high CO_2 level. The combined inhibition of UVA + UVB (that is UVR) was 6.7% and 17.3% and there was no significant difference ($P > 0.1$) between the 2 years. The calcification rate of *C. sessilis* measured between 10:00 and 14:00 hours on September 26, 27, 2008, was greater ($P < 0.001$) at the low CO_2 than that at the high CO_2 concentration (Table 2), while inhibition caused by UVR was greater ($P < 0.05$) at the high CO_2 level (Fig. 3c). UVA and UVB caused inhibition of calcification by 0.9% and 2.1% at the low and 1.3% and 6.3% at the high CO_2 level, respectively, with higher

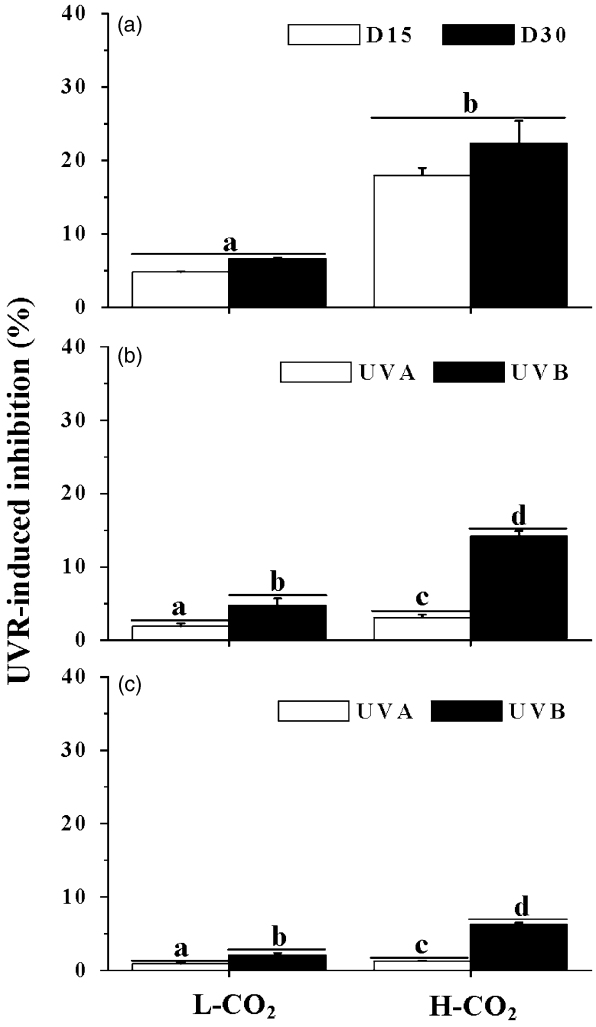

Fig. 3 UV-induced inhibition of photosynthesis and calcification of *Corallina sessilis* around noon period (10:00–14:00) when grown at low (L-CO_2) and high (H-CO_2) CO_2 levels. (a) the UV-induced inhibition of photosynthesis in the thalli grown under the low and high CO_2 conditions at days 15 and 30, both of which were sunny with the solar irradiances ranged from 197–263 $W\, m^{-2}$ for photosynthetically active radiation (PAR), 27–35 $W\, m^{-2}$ for UVA and 0.7–1.1 $W\, m^{-2}$ for UVB, respectively. (b) the photosynthetic inhibition caused by UVA and UVB in the thalli grown under the low and high CO_2 levels for 10–11 days (September 16–27, 2008), measured at days 10 and 11, when average solar irradiance of PAR, UVA and UVB were 243.1, 31.9 and 0.9 $W\, m^{-2}$, respectively. (c) the inhibition of calcification caused by UVA and UVB in the thalli grown under the low and high CO_2 levels for 10–11 days (September 16–27, 2008), also measured at days 10 and 11. Data are the means ± SD ($n = 15$), each representing three measurements for 15 individuals. Different letters in each panel indicate significant difference among the treatments ($P < 0.05$).

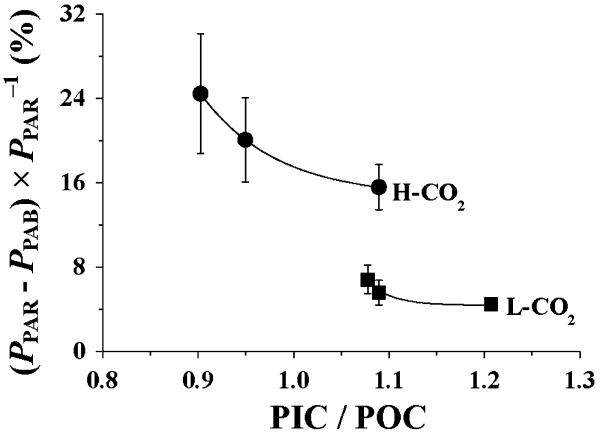

Fig. 4 UVR (UVA + UVB)-induced inhibition (%) of photosynthetic oxygen evolution as a function of the PIC/POC ratios in the thalli of *Corallina sessilis* grown under the low and high CO_2 levels for 1, 15 and 30 days. The relationship was significant under both high ($R^2 = 0.99$) and low ($R^2 = 0.87$) CO_2 levels ($P < 0.05$). Data are the means ± SD ($n = 9$), representing nine individuals in three measurements. PIC, particulate inorganic carbon; POC, particulate organic carbon.

UV-induced inhibition at the high CO_2 level. UVB-induced inhibition was much greater ($P < 0.001$) than that of UVA at the two CO_2 levels for both photosynthesis (Fig. 3b) and calcification (Fig. 3c).

When UVR-induced inhibition of the net photosynthesis was plotted against the PIC/POC ratios of the samples, an exponential decay equation was found, and a negative relationship was established (Fig. 4). The inhibition increased with decreased PIC/POC ratio for the samples grown either at high or low CO_2 levels ($P < 0.01$). The low-CO_2-grown thalli showed higher PIC/POC ratios and lower UVR-induced inhibition (Fig. 4). However, the inhibition was much greater in the thalli grown under the high CO_2 than under the low CO_2 ($P < 0.05$), even at the same PIC/POC ratio (1.1), indicating the involvement of other physiological processes that were sensitive to the combined treatments of high CO_2/low pH and UVR.

The contents of Chl. *a* and PE decreased, while the absorptivity of UVACs increased under the high CO_2 and low pH conditions (Fig. 5). CO_2 enrichment decreased ($P < 0.05$) the content of Chl. *a* by 17.8–21.1% (Fig. 5a), that of PE by 51.2–73.3% (Fig. 5b). The presence of UVR resulted in significant ($P < 0.05$) decrease in PE only under the high CO_2 condition (Fig. 5b). While the contents of carotenoids appeared not to be affected (Fig. 5c), the absorptivity of the UVACs increased by 25.0–32.2% ($P < 0.01$) under the high CO_2 condition, and presence of UVR brought about significant ($P < 0.05$) increase in the absorptivity of the UVACs either under the low or high CO_2 conditions (Fig. 5d).

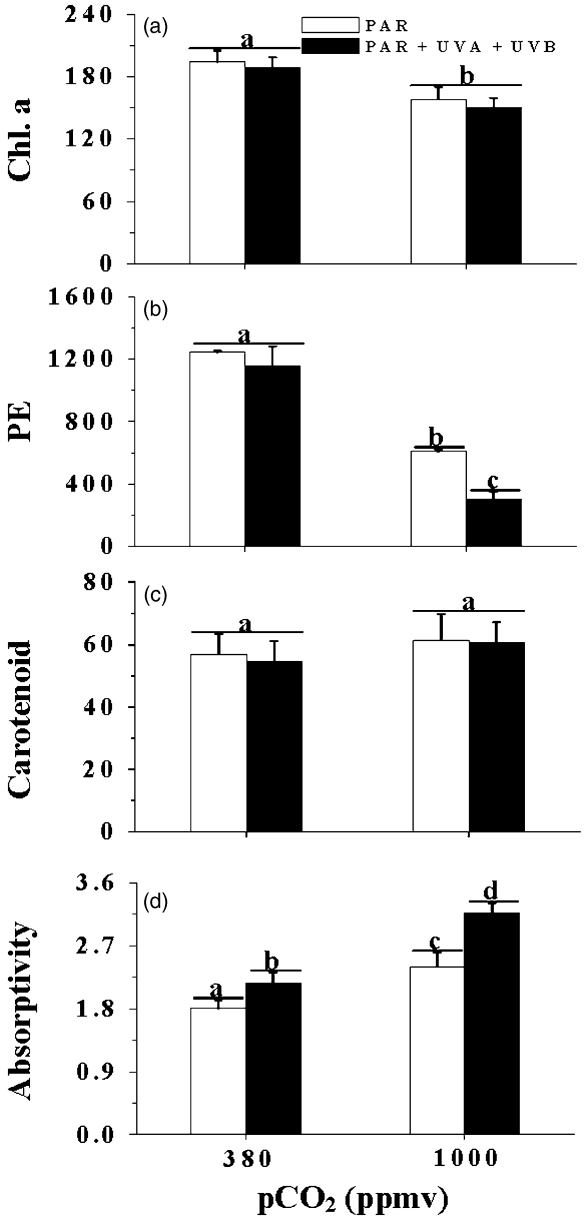

Fig. 5 Contents ($\mu g\, g^{-1}$) of Chl. *a* (a), phycoerythrin (PE) (b), carotenoid (c) and the absorptivity of UVACs (d) in *Corallina sessilis* thalli after grown for 30 days at the low (380 ppmv) and high (1000 ppmv) levels of CO_2 under PAR + UVA + UVB or PAR treatments. Different letters represent significant difference ($P < 0.05$) among the treatments. Data are the means ± SD ($n = 3$), representing three individuals. PAR, photosynthetically active radiation.

Discussion

Under CO_2-enriched conditions, the presence of UVR resulted in greater inhibition of photosynthesis than of calcification by *C. sessilis*. Growth, photosynthesis and calcification rates of *C. sessilis* were inhibited by the

enrichment of CO_2, and addition of UVR led to further inhibition. UVR-induced inhibition of photosynthesis increased under the CO_2-enriched conditions alongside decreased PIC/POC ratios in the algal thalli. The PIC/POC ratio was lower under high-CO_2/low pH conditions as well as in the presence of UVR. The absorptivity of UVACs increased, while those of Chl. *a* and PE contents decreased under CO_2-enriched conditions and in the presence of UVR. The high CO_2/low pH condition (ocean acidification) and solar UVR interacted synergistically to decrease the rates of calcification and photosynthesis of *C. sessilis*, indicating that seawater acidification exacerbated the harm caused by UVR.

The dissolution of CO_2 into seawater by aeration lowers the pH, while photosynthetic carbon removal from the medium increases it, and the pH in the cultures mainly depends on the balance between these two processes (Gao *et al*., 1991). Increased pH values along with increasing solar radiation during daytime could be attributed to the increased photosynthetic removal of inorganic carbon exceeding the rate of CO_2 dissolution in the culture media. The presence of UVR decreased the pH because of its inhibitory effect on photosynthesis which caused less CO_2 removal around noontime. Consequently, presence of UVR up-regulated the concentrations of pCO_2, bicarbonate and carbonate ions (Table 1). The UV-related increase in respiration (Aguilera *et al*., 1999) could also contribute to the observed decrease in pH. UV-induced inhibition of photosynthesis increased from the initial to the end of the experiment, which lasted for 30 days (Fig. 3a). There was a difference in water temperature as high as 4 °C between the start and the end of the cultures (Fig. 1a), and lower photosynthetic rates at the lower temperature must have been responsible for the observed gradual decline of pH (Fig. 1b). On the other hand, calcification may have also influenced the pH because it affects the total alkalinity of seawater, though UV-induced inhibition of calcification might have counteracted this to some extent.

Increased pCO_2 in cultures enhances the growth of some macroalgal species (Gao *et al*., 1991, 1993b; Kübler *et al*., 1999). Even for species that can effectively use bicarbonate, enrichment of CO_2 could still result in enhanced growth, such as in *P. yezoensis* (Gao *et al*., 1991) and *Ulva rigida* (Björk *et al*., 1993), most likely due to the energy saved, which would have been required for bicarbonate utilization. However, acidification of seawater associated with CO_2-enrichment can result in negative effects, thus leading to decreased growth, net photosynthesis or even death of some algae (Israel & Hophy, 2002; Aline *et al*., 2006; Martin & Gattuso, 2009). In the present study, enrichment of CO_2 led to decreased growth rates either under PAR alone or under PAR + UVR treatments. Both photosynthetic O_2 evolution and calcification were inhibited under CO_2-enriched conditions, leading to the decreased growth rate. This finding over a long-term (30 days) acclimation to a simulated ocean acidification condition was not consistent with that observed during a short-term (minutes) acidification using HCl (Borowitzka, 1981). In the articulated coralline algae *Amphiroa* spp., net photosynthesis was higher at acidic pH levels while calcification was higher at alkaline pH levels (Borowitzka, 1981). It is possible that the negative impact of acidification of seawater takes longer time to take place in contrast to the immediate stimulation of photosynthesis by the enriched CO_2 at lower pH levels. The ability of using bicarbonate or CO_2 concentrating mechanisms can be down-regulated in macroalgae that acclimated to high CO_2 (Israel & HopHy, 2002). On the other hand, acidification using acid decreases seawater total alkalinity, while that under high CO_2 does not alter it (Schulz *et al*., 2009). A decreased pH value might have affected nonphotochemical quenching and periplasmic redox activity, both of which can be sensitive to changes in pH. In addition, more energy may be required for the cells to cope with the stress associated with the changes in seawater chemistry, therefore, photosynthetic performance can be down-regulated in the acidification-acclimated thalli.

The UV-induced inhibition of net photosynthesis was negatively related to PIC/POC ratios (Fig. 4), that is, the more PIC the less damage caused by UVR. This reflected a protective role by the calcified layer, which might reduce the UVR effect by a considerable amount. However, there was a discrepancy in the UVR-related inhibition between the low-CO_2-grown and the high-CO_2-grown thalli in that they showed similar PIC/POC ratios (Fig. 4). CO_2 or pH-sensitive physiological processes must have been involved to trigger such difference. Although the irradiance or daily dose of UVA was about 40 times that of UVB during the experimental periods, the former caused much less inhibition of photosynthesis and calcification than the latter (Fig. 3b and c). UVB-induced inhibition of photosynthesis was 262.2% higher and that of calcification was 253.3% higher than that caused by UVA. UVB radiation, although with the strength of $<1.0\%$ of the total solar radiation, can significantly reduce the growth rates of most of the algal species investigated so far (Grobe & Murphy, 1994; Zheng & Gao, 2009). In the present study, the result that UVB-induced inhibition of photosynthesis and calcification was much greater under the high CO_2/low pH conditions implies that seawater acidification interacts with UVB synergistically to affect the physiology of *C. sessilis*. UVB might thus result in more damage to the photosynthetic systems, enzymes and

DNA molecules of algae in a high CO_2 containing ocean. More energy would be required for the marine organisms to repair UV-induced damage under lowered pH levels and this would also stress the cells. Repairing of UVB-induced damage could have been slower at lower temperatures, thus leading to higher inhibition of photosynthesis at day 30 (Fig. 3a).

Calcification of coralline algae requires energy input from photosynthesis (Borowitzka & Larkum, 1976) and depends on pH (de Beer & Larkum, 2001). Lowered pH leads to decreased concentration of CO_3^{2-} and therefore can affect the precipitation of $CaCO_3$ by reducing the saturation state of $CaCO_3$. Because UVR suppresses the photosynthetic rate and therefore reduces the pH in the medium, it could also indirectly affect calcification. However, direct impact of UVR on the calcification of *C. sessilis* may also have been involved.

UVACs, mainly mycosporine-like amino acids (MAAs) in red algae, have maximal absorption spectra within 310–360 nm, and are known to play protective roles against UVR (Oren & Gunde-Cimerman, 2007). Although the composition of UVACs in *C. sessilis* was not identified with high-performance liquid chromatograph, the major peak at 325 nm in the absorption spectra indicates a specific feature of UV absorption represented by MAAs (Gao & Xu, 2008). Accumulation of MAAs can be induced by UVR (Korbee-Peinado *et al*., 2004) or affected by osmotic stress (Oren, 1997) and nutrient availability (Korbee-Peinado *et al*., 2004; Zheng & Gao, 2009). In the present study, enrichment of CO_2 significantly increased the absorptivity of the UVACs in *C. sessilis* (Fig. 5d). The presence of UVR also resulted in an increase in the absorptivity at either low or high CO_2 concentrations. UVB induces more MAAs than UVA (Han & Han, 2005). Increased absorptivity of MAAs under the high CO_2 and low pH condition in the present study could have be attributed to the increased levels of UVR received by the cells due to the thinning of the calcified layer when the thalli calcified less. However, direct impacts of pH reduction on the accumulation of MAAs cannot be ruled out.

Variations in pigment content usually reflect photoacclimation of algae to different light levels. In the present study, when the thalli were grown under CO_2-enriched conditions, the contents of Chl. *a* and PE decreased (Fig. 5a and b). PE and Chl. *a* contents are reduced in *Gracilaria* sp. (Andría *et al*., 2001) and *G. tenuistipitata* (García-Sánchez *et al*., 1994) when they are grown under high CO_2 concentrations. On the other hand, the presence of UVR also decreased the contents of photosynthetic pigments in the present study (Fig. 5a and b). CO_2 enrichment can lower the demand of energy for the HCO_3^- utilization mechanism, leading to down-regulated pigmentation, while UVR can damage pigment molecules and lead to a bleaching effect (Häder & Worrest, 1991). However, in our experiments, the absorptivity of UVACs increased with the enriched CO_2 treatment (Fig. 5d). Increased exposures of the cells to solar radiation under a thinned calcified cover can lead to the development of enhanced protective efficiency. Carotenoids are known to play important roles against high levels of light (Vincent & Roy, 1993). The cells acclimated to high CO_2/low pH conditions might have to raise the carotenoids content when the calcification became less and less. However, in the present study, the content of carotenoids did not increase to a significant amount either at the high CO_2 or in presence of UVR (Fig. 5c). It appeared that, as with UVACs, synthesis of carotenoids responded to changes in solar radiation in a light-quantity-sensitive, but not a UV-sensitive manner.

It is concluded from the present study that UVR and ocean acidification act synergistically to reduce photosynthesis, calcification, light-capturing pigments and growth, and to increase the absorptivity of UV-protective compounds in the coralline alga *C. sessilis*. In view of the continuous increase in atmospheric CO_2 concentration and progressive ocean acidification, coralline algae may calcify and photosynthesize less and less, especially when the harm caused by solar UVR is considered. Although increase in the chlorine concentration in the stratosphere has slowed down, reflecting the execution of the Montreal Protocol, the time said to be required for recovery of the ozone layer is unconvincing and will rely on the impacts of climate change on the stratosphere (Weatherhead & Andersen, 2006). The present study revealed that UVB was responsible for most of the inhibition of photosynthesis and calcification and inhibited the processes to a greater extent under high CO_2/low pH conditions. It is expected that coralline algae will suffer more UV-induced damage if ocean acidification proceeds alongside increased atmospheric CO_2 concentration, and increasing UVB irradiance caused by the reduction of ozone may bring about additional damage to the calcifying algae.

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References

Aguilera J, Karsten U, Lippert H, Vögele B, Philipp E, Hanelt D, Wiencke C (1999) Effects of solar radiation on growth, photosynthesis and respiration of marine macroalgae from the Arctic. *Marine Ecology Progress Series*, **191**, 109–119.

Aline T, Atkinson MJ, Langdon C (2006) Effects of elevated pCO_2 on epilithic and endolithic metabolism of reef carbonates. *Global Change Biology*, **12**, 2200–2208.

Anderson DH, Robinson RJ (1946) Rapid electrometric determination of the alkalinity of sea water using a glass electrode. *Industrial and Engineering Chemistry*, **18**, 767–769.

Andría JR, Brun FG, Pérez-Lloréns JL, Vergara JJ (2001) Acclimation responses of *Gracilaria* sp. (Rhodophyta) and *Enteromorpha intestinalis* (Chlorophyta) to changes in the external inorganic carbon concentration. *Botanica Marina*, **44**, 361–370.

Beer S, Eshel A (1985) Determining phycoerythrin and phycocyanin in aqueous crude extracts of red algae. *Australian Journal of Marine and Freshwater Research*, **36**, 785–792.

Björk M, Haglund K, Ramazanov Z, Pedersén M (1993) Inducible mechanism for HCO_3^- utilization and repression of photorespiration in protoplasts and thalli of three species of *Ulva* (Chlorophyta). *Journal of Phycology*, **29**, 166–173.

Borowitzka MA (1981) Photosynthesis and calcification in the articulated coralline red algae *Amphiroa anceps* and *A. foliacea*. *Marine Biology*, **62**, 17–23.

Borowitzka MA, Larkum AWD (1976) Calcification in the green alga *Halimeda* IV. The action of metabolic inhibitors on photosynthesis and calcification. *Journal of Experimental Botany*, **27**, 894–907.

Brewer PG (1997) Ocean chemistry of the fossil fuel CO_2 signal: the haline signal of ''business as usual''. *Geophysical Research Letters*, **24**, 1367–1369.

Brewer PG, Goldman JC (1976) Alkalinity changes generated by phytoplankton growth. *Limnology and Oceanography*, **21**, 108–117.

Caldeira K, Wickett ME (2003) Anthropogenic carbon and ocean pH. *Nature*, **425**, 365.

Chisholm JRM, Gattuso J-P (1991) Validation of the alkalinity anomaly technique for investigating calcification and photosynthesis in coral reef communities. *Limnology and Oceanography*, **36**, 1232–1239.

de Beer D, Larkum AWD (2001) Photosynthesis and calcification in the calcifying algae *Halimeda discoidea* studied with microsensors. *Plant, Cell and Environment*, **24**, 1209–1217.

Dickson AG, Sabine CI, Christian JR (2007) Guide to best practices for ocean CO_2 measurements. *PICES Special Publication*, **3**, 1–191.

Dring MJ, Wagner A, Lüning K (2001) Contribution of the UV component of natural sunlight to photoinhibition of photosynthesis in six species of subtidal brown and red seaweeds. *Plant, Cell and Environment*, **24**, 1153–1164.

Gao K, Aruga Y, Asada K, Ishihara T, Akano T, Kiyohara M (1991) Enhanced growth of the red alga *Porphyra yezoensis* Ueda in high CO_2 concentrations. *Journal of Applied Phycology*, **3**, 355–362.

Gao K, Aruga Y, Asada K, Ishihara T, Akano T, Kiyohara M (1993a) Calcification in the articulated coralline alga *Corallina pilulifera*, with special reference to the effect of elevated CO_2 concentration. *Marine Biology*, **117**, 129–132.

Gao K, Aruga Y, Asada K, Kiyohara M (1993b) Influence of enhanced CO_2 on growth and photosynthesis of the red algae *Gracilaria* sp. and *G. chilensis*. *Journal of Applied Phycology*, **5**, 563–571.

Gao K, Ji Y, Aruga Y (1999) Relationship of CO_2 concentrations to photosynthesis of intertidal macroalgae during emersion. *Hydrobiologia*, **398–399**, 355–359.

Gao K, Umezaki I (1989) Studies on diurnal photosynthetic performance of *Sargassum thunbergii* [2]. Changes in photosynthesis under natural sunlight. *Japanese Journal of Phycology*, **37**, 89–98.

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.

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.

García-Sánchez MJ, Fernández JA, Niell X (1994) Effect of inorganic carbon supply on the photosynthetic physiology of *Gracilaria tenuistipitata*. *Planta*, **194**, 55–61.

Gattuso J-P, Frankignoulle M, Bourge I, Romaine S, Buddemeier RW (1998) Effect of calcium carbonate saturation of seawater on coral calcification. *Global and Planetary Change*, **18**, 37–46.

Gattuso J-P, Frankignoulle M, Smith SV (1999) Measurement of community metabolism and significance in the coral reef CO_2 source-sink debate. *Proceedings of the National Academic of Sciences USA*, **96**, 13017–13022.

Grobe CW, Murphy TM (1994) Inhibition of growth of *Ulva expansa* (Chlorophyta) by ultraviolet-B radiation. *Journal of Phycology*, **30**, 783–790.

Häder D-P, Worrest RC (1991) Effects of enhanced solar ultraviolet radiation on aquatic ecosystems. *Photochemistry and Photobiology*, **53**, 717–725.

Hall-Spencer JM, Rodolfo-Metalpa R, Martin S *et al*. (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature*, **454**, 96–99.

Han YS, Han T (2005) UV-B induction of UV-B protection in *Ulva pertusa* (Chlorophyta). *Journal of Phycology*, **41**, 523–530.

Hargreaves BR (2003) Water column optics and penetration of UVR. In: *UV effects in aquatic organisms and ecosystems* (eds Helbling EW, Zagarese HE), pp. 59–105. Royal Society of Chemistry, Cambridge, UK.

Iglesias-Rodriguez MD, Buitenhuis ET, Raven JA *et al*. (2008a) Response to comment on ''phytoplankton calcification in a high-CO_2 world''. *Science*, **322**, 1466c, doi: 10.1126/science.1161501.

Iglesias-Rodriguez MD, Halloran PR, Rickaby REM *et al*. (2008b) Phytoplankton calcification in a high-CO_2 world. *Science*, **320**, 336–340.

Israel A, Hophy M (2002) Growth, photosynthetic properties and Rubisco activities and amounts of marine macroalgae grown under current and elevated seawater CO_2 concentrations. *Global Change Biology*, **8**, 831–840.

Israel A, Katz S, Dubinsky Z, Merrill JE, Friedlander M (1999) Photosynthetic inorganic carbon utilization and growth of *Porphyra linearis* (Rhodophyta). *Journal of Applied Phycology*, **11**, 447–453.

Kerr JB, McElroy CT (1993) Evidence for large upward trends of ultraviolet-B radiation linked to ozone depletion. *Science*, **262**, 1032–1034.

Korbee-Peinado N, Díaz RTA, Figueroa FL, Helbling EW (2004) Ammonium and UV radiation stimulate the accumulation of mycosporine-like amino acids in *Porphyra columbina* (Rhodophyta) from Patagonia, Argentina. *Journal of Phycology*, **40**, 248–259.

Kübler JE, Johnston AM, Raven JA (1999) The effects of reduced and elevated CO_2 and O_2 on the seaweed *Lomentaria articulata*. *Plant, Cell and Environment*, **22**, 1303–1310.

Kuffner IB, Andersson AJ, Jokiel PL, Rodgers KS, Mackenzie FT (2007) Decreased abundance of crustose coralline algae due to ocean acidification. *Nature Geoscience*, **1**, 114–117.

Leclercq N, Gattuso J-P, Jaubert J (2000) CO_2 partial pressure controls the calcification rate of a coral community. *Global Change Biology*, **6**, 329–334.

Martin S, Gattuso J-P (2009) Response of Mediterranean coralline algae to ocean acidification and elevated temperature. *Global Change Biology*, **15**, 2089–2100.

Oren A (1997) Mycosporine-like amino acids as osmotic solutes in a community of halophilic cyanobacteria. *Geomicrobiology Journal*, **14**, 231–240.

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

Orr JC, Fabry VJ, Aumont O *et al*. (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*, **437**, 681–686.

Riebesell U, Bellerby RGJ, Engel A *et al*. (2008) Comment on "phytoplankton calcification in a high-CO_2 world". *Science*, **322**, 1466b, doi: 10.1126/science.1161096.

Riebesell U, Zondervan I, Rost B, Tortell PD, Zeebe RE, Morel FMM (2000) Reduced calcification of marine plankton in response to increased atmospheric CO_2. *Nature*, **407**, 364–367.

Roleda MY, Hanelt D, Wiencke C (2006) Growth and DNA damage in young *Laminaria* sporophytes exposed to ultraviolet radiation: implication for depth zonation of kelps on Helgoland (North Sea). *Marine Biology*, **148**, 1201–1211.

Sabine CL, Feely RA, Gruber N *et al*. (2004) The oceanic sink for anthropogenic CO_2. *Science*, **305**, 367–371.

Schneider K, Erez J (2006) The effect of carbonate chemistry on calcification and photosynthesis in the hermatypic coral *Acropora eurystoma*. *Limnology and Oceanography*, **51**, 1284–1293.

Schulz KG, Barcelos e Ramos J, Zeebe RE, Riebesell U (2009) CO_2 perturbation experiments: similarities and differences between dissolved inorganic carbon and total alkalinity manipulations. *Biogeosciences Discussions*, **6**, 4441–4462.

Vincent WF, Roy S (1993) Solar ultraviolet-B radiation and aquatic primary production: damage, protection and recovery. *Environmental Reviews*, **1**, 1–12.

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

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, Villafañe VE, Watanabe T, Helbling EW (2005) Effects of solar UV radiation on morphology and photosynthesis of the filamentous cyanobacterium, *Arthrospira platensis*. *Applied and Environmental Microbiology*, **71**, 5004–5013.

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.

Zou D, Gao K (2002) Effects of desiccation and CO_2 concentrations on emersed photosynthesis in *Porphyra haitanensis* (Bangiales, Rhodophyta), a species farmed in China. *European Journal of Phycology*, **37**, 587–592.