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CO_2-driven seawater acidification increases photochemical stress in a green alga

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Increased CO_2 and associated acidification in seawater, known as ocean acidification, decreases calcification of most marine calcifying organisms. However, there is little information available on how marine macroalgae would respond to the chemical changes caused by seawater acidification. We hypothesized that down-regulation of bicarbonate acquisition by algae under increased acidity and CO_2 levels would lower the threshold above which photosynthetically active radiation (PAR) becomes excessive. Juveniles of *Ulva prolifera* derived from zoospores were grown at ambient (390 μatm) and elevated (1000 μatm) CO_2 concentrations for 80 days before the hypothesis was tested. Here, the CO_2-induced seawater acidification increased the quantum yield under low levels of light, but induced higher nonphotochemical quenching under high light. At the same time, the PAR level at which photosynthesis became saturated was decreased and the photosynthetic affinity for CO_2 or inorganic carbon decreased in the high-CO_2 grown plants. These findings indicated that ocean acidification, as an environmental stressor, can reduce the threshold above which PAR becomes excessive.

KEY WORDS: *Ulva prolifera*, CO_2, Photosynthetic performance, Carbon-concentrating mechanisms, Nonphotochemical quenching

INTRODUCTION

Industrialization and the use of fossil fuels, together with deforestation and intensive agricultural activities, have raised atmospheric CO_2 concentrations since the industrial revolution. Atmospheric CO_2 concentrations are expected to rise to 1000 μatm by the year 2100 (A1F1 scenario), with a concomitant decrease in the surface ocean pH of up to 0.4 units (H^+ increase ~ 150%) (Sabine *et al.* 2004; Friedlingstein *et al.* 2006). The current rate at which ocean acidification is occurring may have a profound influence on marine organisms and ecosystems in the future (Guinotte & Fabry 2008). Studies show that ocean acidification decreases calcification of many marine calcifiers (Gao *et al.* 1993; Riebesell *et al.* 2000; Hofmann *et al.* 2010), though increased calcification has also been observed (Iglesias-Rodriguez *et al.* 2008; Rise *et al.* 2009).

Algae have evolved carbon-concentrating mechanisms (CCMs) to ensure high CO_2 concentrations at the active site of the CO_2-fixing enzyme, RuBisCO (Badger *et al.* 1998). A CCM operation is energetically costly (Beardall *et al.* 1998), and higher efficiency of CCMs is usually associated with growth at higher light levels (Beardall 1991; Young & Beardall 2005; Kranz *et al.* 2010). Increased partial pressure or concentration of CO_2 in seawater (pCO_2) to projected levels for the end of 2100 have been shown to stimulate growth (Gao *et al.* 1991) and photosynthesis (Gao *et al.* 1999; Zou & Gao 2002) of some intertidal macroalgae. However, in other studies long-term high CO_2 exposure did not stimulate growth in macroalgal species (Israel & Hophy 2002). Here, we hypothesize that high CO_2-induced ocean acidification can down-regulate carbon acquisition capability or CCMs in macroalgae and that the subsequently saved energy leads to a lower photosynthetically active radiation (PAR) threshold at which PAR becomes excessive, so that the algae experience increased PAR stress under high light conditions. For these studies, we chose a green alga, *Ulva prolifera* (Forsskål) A.P. de Candolle, which is a cosmopolitan species. This alga seems able to withstand tremendous changes in the seawater carbonate system when it forms harmful algal blooms in China (Sun *et al.* 2008).

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MATERIALS AND METHODS

Culture conditions

Samples of *Ulva prolifera* were collected in the intertidal zone around Gaogong Island (34.60°N, 119.18°E), Lianyungang, Jiangsu, China, in June 2009. They were transported to the laboratory in a cooler within 1 day and cleaned of epiphytes. Selected thalli were maintained in the laboratory with filtered seawater (pH 8.1, salinity 30 pss) at 60 μmol m^{-2} s^{-1} illumination (12-h light period). Zoospores were collected and allowed to settle in darkness on glass slides. The attached zoospores were cultured at two different CO_2 concentrations: ambient (390 μatm) and elevated (1000 μatm). Zoospores were grown under 60 μmol m^{-2} s^{-1} illumination (12-h light period) for 80 days before the experiments were carried out. The target pH (pCO_2) in cultures and fresh medium (filtered seawater enriched with 60 μM $NaNO_3$ and 4 μM NaH_2PO_4) was achieved by bubbling premixed air–CO_2 mixtures (393 ± 11 and 1013 ±

Table 1. Seawater carbonate parameters under ambient (390 μatm) and enriched (1000 μatm) CO_2 concentrations for L-C and H-C grown thalli. DIC, pH, salinity, nutrient concentration and temperature were used to derive all other parameters using the CO_2 system analyzing software (CO2SYS) (Lewis & Wallace 1998). Data are the means ± *s* of five measurements. The pH, DIC, HCO_3^- and CO_3^{2-} parameters were significantly different ($P < 0.05$) between the L-C and H-C thalli; there was no significant difference between L-C and H-C for total alkalinity (TA).

	pH National Bureau of Standards	DIC (μM)	HCO_3^- (μM)	CO_3^{2-} (μM)	TA (μM)
L-C	8.20 ± 0.03	2057.3 ± 129.7	1848.4 ± 107.1	196.0 ± 22.7	2323.4 ± 156.3
H-C	7.85 ± 0.02	2238.8 ± 113.3	2106.6 ± 103.7	99.22 ± 9.6	2348.7 ± 124.7

30 μatm) (as recommended in Barry *et al.* 2010) within a plant growth CO_2 chamber (HP1000G-D, Wuhan Ruihua Instrument and Equipment, Wuhan, Hubei, China), which controlled the high CO_2 level with a variation of less than 3%. The culture medium was renewed every 48 h, and the biomass was maintained within a range of 2.0 ± 0.1 g in 1000 ml seawater by removing additional thalli, so that a stable carbonate system was sustained (Table 1). The pH was determined with a pH meter (Benchtop pH510, Oakton, California, USA), and other parameters of the carbonate system were computed with the CO2SYS software (Lewis & Wallace 1998). The concentration of dissolved inorganic carbon (DIC) was measured using an automatic system (AS-C3, Apollo Scitech, Bogart, Georgia, USA) that employs an infrared gas detector (Li-Cor 7000, Li-Cor, Lincoln, Nebraska, USA).

Determination of photochemical performance

Rapid light curves (RLCs) were measured with a xenon-pulse amplitude modulated fluorometer (XE-PAM, Walz, Germany) to determine the photochemical and nonphotochemical responses. The thalli were cut into small segments (about 1.0-cm length) and incubated in filtered seawater medium at 60 μmol m^{-2} s^{-1} and 20°C for 60 min to avoid wound effects and induction effects on the photosystems caused by quasi-dark adaptation during manipulation. RLCs were determined at eight different PAR levels (113, 168, 263, 385, 580, 825, 1187 and 1623 μmol m^{-2} s^{-1}), each of which lasted for 10 s. The relative electron transport rate (rETR) was assessed as: rETR = 0.84 × yield × 0.5 × photon flux density, where the yield represents the effective quantum yield of PSII (F_v'/F_m'); the coefficient 0.5 takes into account that roughly 50% of all absorbed quanta reach PSII; and 0.84 is the absorptance factor (Björkman & Demmig 1987). RLCs were fitted as: $rETR = rETR_{max} \times \tanh(\alpha \times I/rETR_{max})$ according to the photosynthesis light relationship model of Jassby & Platt (1976). Fluorescence induction curves were measured separately on different algal segments after 15 min of dark adaptation. The actinic light levels were set at 596 μmol m^{-2} s^{-1} and the saturating pulse at 4000 μmol m^{-2} s^{-1} for 0.8 s. Nonphotochemical quenching (NPQ) was calculated as: $NPQ = (F_m - F_m')/F_m'$, where F_m represents the maximum fluorescence yield after dark adaptation and F_m' represents the maximum fluorescence yield in the light-adapted state (Genty *et al.* 1989).

Determination of photosynthetic affinity for CO_2

The algal segments were placed in a quartz chamber containing 8 ml of buffered Ci-free seawater, which was prepared by adding 1 M HCl to lower the pH to 2.0, and then sparging for at least 1 h with high purity N_2 gas. A known amount of TRIS was added to give a final concentration of 20 mM, and the pH was then adjusted back to ambient level with freshly prepared 1 M NaOH solution under sparging with N_2. Different aliquots of $NaHCO_3$ stock solution (27.5, 110, 440 mM) were then injected into the quartz chamber to create DIC concentrations (0, 0.14, 0.28, 0.55, 1.1, 2.2, 4.4 mM). Fluorescence induction curves were measured under an actinic light of 596 μmol m^{-2} s^{-1}, which was chosen as a light level above the photosynthesis-saturated light level (which is about 400 μmol photons m^{-2} s^{-1} for maximal O_2 evolution, based on our preliminary tests). Changes in rETR were used to reflect photosynthetic affinity for CO_2 or inorganic carbon (Wu *et al.* 2010). The $K_{1/2}$ values of DIC were calculated by fitting rETR at different DIC concentrations with the Michaelis–Menten formula. Differences among the treatments were tested using one-way analysis of variance (Tukey test) (*s* = standard deviation).

RESULTS

The maximal rETR ($rETR_{max}$) and apparent ETR efficiency (α) were significantly ($P < 0.001$) higher in the high-CO_2 grown thalli (H-C) than in the low-CO_2 grown thalli (L-C) (Fig. 1). The light saturation point (I_k) was significantly ($P < 0.05$) lower in the H-C than in the L-C thalli (Table 2).

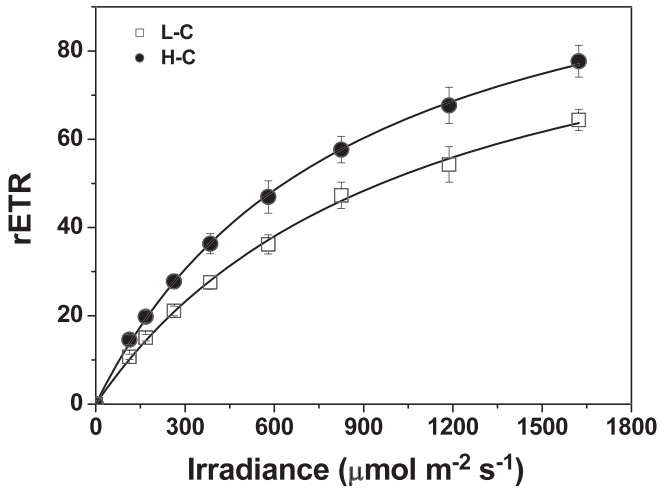

Fig. 1. Rapid light curves of L-C and H-C grown thalli measured in their growth conditions (at their respective CO_2 and pH levels). Vertical bars represent *s*, *n* = 5 (five segments from five different individuals).

Table 2. Parameters derived for L-C and H-C grown thalli from the rapid light curves ($rETR_{max}$, maximal rETR; α, the apparent ETR efficiency; I_k, light saturation point) and the rETR P-C curves (C_{max}, DIC-saturated rETR; $K_{1/2(DIC)}$ and $K_{1/2}(CO_2)$, the DIC and CO_2 concentration required for half maximal ETR). Data from the rapid light curves represent the means of five measurements (five segments from five different individuals). Data from the rETR P-C curves are the means of three measurements (three segments from three different individuals). Standard deviations are given in parentheses. There was a statistical difference ($P < 0.05$) between the L-C and H-C for all parameters.

	$rETR_{max}$	α	I_k	C_{max}	$K_{1/2(DIC)}$	$K_{1/2(CO2)}$
L-C	65.04 (3.72)	0.08 (0.004)	870.02 (66.61)	28.18 (2.18)	0.14 (0.07)	0.87 (0.44)
H-C	76.64 (2.76)	0.10 (0.01)	769.49 (54.06)	41.43 (1.92)	0.33 (0.07)	2.13 (0.45)

The fluorescence induction curve indicated that maximal quantum yield (F_v/F_m) of H-C thalli after 15 min of dark adaptation was significantly lower than that of the L-C thalli ($P < 0.001$), with the actinic light (596 μmol m^{-2} s^{-1}) on, the effective quantum yield (F_v'/F_m') in the H-C was significantly higher (by 20%) than that in the L-C thalli ($P < 0.001$) (Fig. 2). In contrast, the stable NPQ was lower in the H-C thalli (Fig. 3).

At different levels of DIC or CO_2 concentrations but at the same pH (8.2), there was a significant difference in maximum rETR (C_{max}) between the L-C grown and H-C grown thalli, with the latter about 47% significantly higher than the former ($P < 0.01$) (Fig. 4). The $K_{1/2\ (DIC)}$ and $K_{1/2}$ (CO_2) value, derived from rETR P-C curves, were 0.33 mM and 2.13 μM, respectively; these were increased about 130% in the H-C grown thalli (Table 2). This indicates that the photosynthetic Ci affinity was significantly reduced under the high CO_2 and low pH condition.

When the NPQ was examined under Ci-limited and excessive PAR conditions (Figs 5, 6), it increased much faster in the H-C than in the L-C grown thalli at 0.00 or 0.14 mM DIC. The NPQ increased also at 0.55 mM DIC (close to $K_{1/2\ (DIC)}$) but reached a constant value in about 200 s (Figs 5, 6). Importantly, the NPQ of H-C grown thalli was higher than that of L-C grown thalli, reflecting higher sensitivity to light stress.

DISCUSSION

Under low-light growth conditions, thalli of *Ulva prolifera* that developed from zoospores under elevated CO_2 and increased seawater acidity showed higher electron transport rates, quantum yields and lower NPQ compared to those grown at ambient CO_2. However, the CO_2-induced seawater acidification lowered the level of PAR at which light becomes excessive for ETR in *U. prolifera*, and this resulted in an increased NPQ when thalli were exposed to high light levels. This indicates that when acclimated to high CO_2 or low pH conditions the alga can easily become light-stressed. Down-regulation of the CCM could be responsible for this phenomenon because excess energy would no be longer dissipated via the energetically costly CCM.

CCM capacity is known to be down-regulated under high CO_2 (1%–5%) (Raven 1991; Badger & Price 1994). However, little is known about the degree to which the CCM can be down-regulated by levels of CO_2 relevant to predicted global change. In this study, we found that the photosynthetic affinity for CO_2 of *Ulva prolifera* decreased by 57% when grown at 1000 μatm CO_2 for 80 days. Such a down-regulated operation of the CCM in the H-C grown thalli could be responsible for the lowered I_k, the PAR threshold above which light becomes excessive (Table 2). Decreased energy demand due to a down-regulated CCM could be responsible for an observed decrease in Chl *a* and *b* contents in the H-C grown thalli (data not shown). NPQ

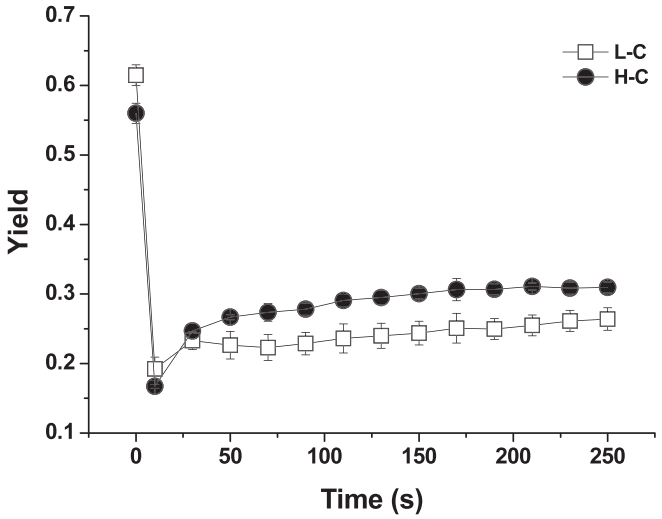

Fig. 2. The effective quantum yield of L-C and H-C grown thalli under their growth levels of CO_2. The induction curves were measured after 15 min of dark adaption. Vertical bars represent *s*, $n = 5$ (five segments from five different individuals).

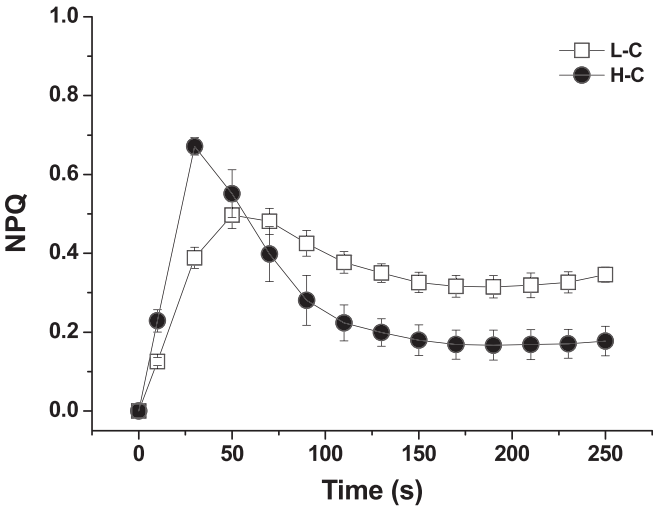

Fig. 3. The NPQ of L-C and H-C grown thalli under their growth levels of CO_2. The induction curves were measured after 15 min of dark adaption. Vertical bars represent *s*, $n = 5$ (five segments from five different individuals).

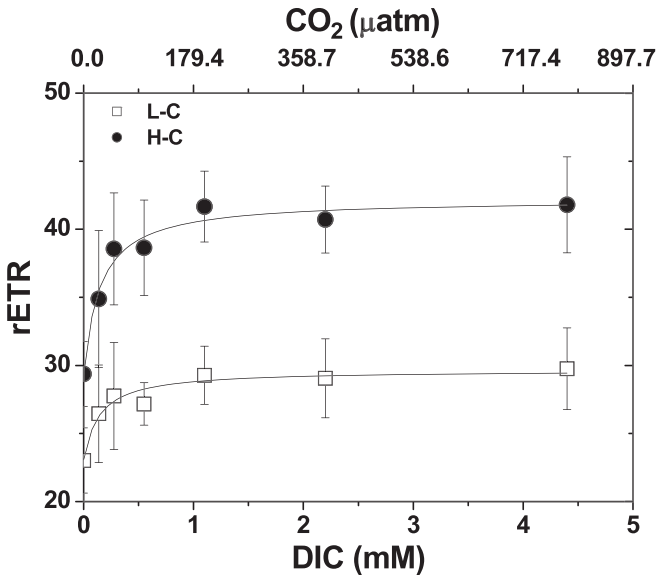

Fig. 4. The rETR of L-C and H-C grown thalli as a function of DIC concentrations in buffered Ci-free seawater at pH 8.2. Vertical bars represent *s*, $n = 3$ (three segments from three different individuals).

was higher in the H-C grown thalli under DIC-limited conditions. The more active CCM in the L-C grown thalli would have consumed more energy and drained more H^+ out of the lumen, which would then have led to decreased NPQ. On the other hand, the H-C grown thalli, with a down-regulated CCM, would have been photoinhibited due to their lowered light threshold and lowered energy dissipation via the down-regulated CCM. Evidentially, our results support our initial hypothesis that down-regulation of CCMs in this alga diminishes energy-dissipation processes, leading to enhanced photoinhibition and increased NPQ. As DIC concentrations increased, NPQ of H-C and L-C grown thalli decreased to a stable level due to increased

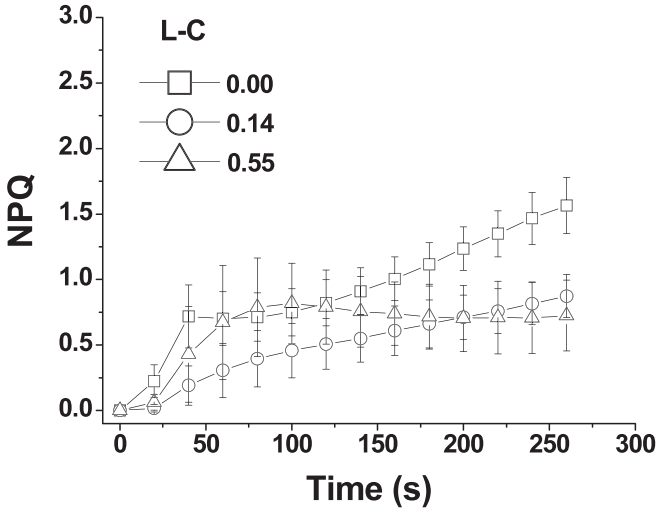

Fig. 5. The NPQ in the L-C thalli when measured in buffered Ci-free seawater or Ci-limited DIC levels (0, 0.14, 0.55 mM) at pH 8.2. Vertical bars represent *s*, $n = 3$ (three segments from three different individuals).

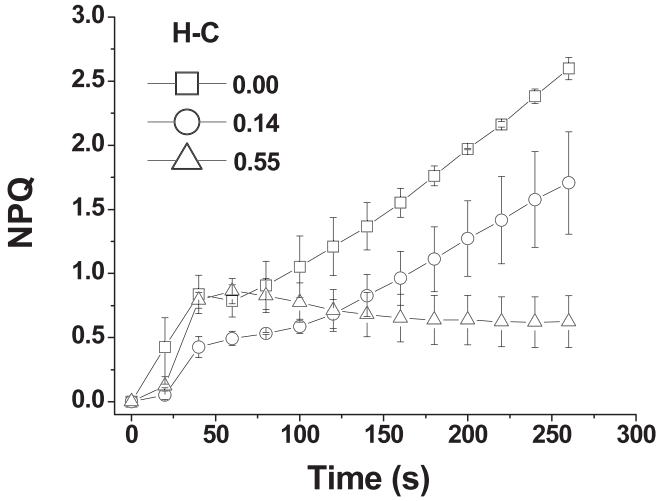

Fig. 6. The NPQ in the H-C thalli when measured in buffered Ci-free seawater or Ci-limited DIC levels (0, 0.14, 0.55 mM) at pH 8.2. Vertical bars represent *s*, $n = 3$ (three segments from three different individuals).

electron sink activity associated with increased carboxylation (Figs 5, 6).

Ongoing ocean acidification, as a potential stressor to marine organisms, may increase the sensitivity of algae to light stress. It has been suggested that CCMs of algae might serve to dissipate excessive light energy (Tchernov *et al.* 1997, 2003), Growth of *Ulva rigida* C. Agardh was enhanced at enriched CO_2 levels (Gordillo *et al.* 2001); although, neutral effects of elevated CO_2 have been reported for several macroalgae grown for long-term periods (Israel & Hophy 2002). The balance of the positive and negative effects determines the net impact of elevated CO_2 on growth. Obviously, physical environments (such as mixing, tidal changes, solar radiation) interact with chemical changes in seawater associated with increasing atmospheric CO_2 concentration to modulate marine primary productivity.

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