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Alleviation of solar ultraviolet radiation (UVR)-induced photoinhibition in diatom *Chaetoceros curvisetus* by ocean acidification

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The study aimed to unravel the interaction between ocean acidification and solar ultraviolet radiation (UVR) in Chaetoceros curvisetus. Chaetoceros curvisetus *cells were acclimated to high CO_2 (HC, 1000 ppmv) and low CO_2 concentration (control, LC, 380 ppmv) for 14 days. Cell density, specific growth rate and chlorophyll were measured. The acclimated cells were then exposed to PAB (photosynthetically active radiation (PAR) + UV-A + UV-B), PA (PAR + UV-A) or P (PAR) for 60 min. Photochemical efficiency (ΦPSII), relative electron transport rate (rETR) and the recovery of ΦPSII were determined. HC induced higher cell density and specific growth rate compared with LC. However, no difference was found in chlorophyll between HC and LC. Moreover, ΦPSII and rETRs were higher under HC than LC in response to solar UVR. P exposure led to faster recovery of ΦPSII, both under HC and LC, than PA and PAB exposure. It appeared that harmful effects of UVR on* C. curvisetus *could be counteracted by ocean acidification simulated by high CO_2 when the effect of climate change is not beyond the tolerance of cells.*

Keywords: acidification, *Chaetoceros curvisetus*, CO_2, ocean acidification, photochemical efficiency, UVR

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INTRODUCTION

The increasingly elevated CO_2 that results from fossil fuel emissions and other human activities is leading to ocean acidification, which has become a serious issue for the ecological environment, because of its detrimental effect on marine organisms, the ocean carbonate system, the marine biogeochemical balance and the marine ecosystem (Turley *et al.*, 2006; Andersson *et al.*, 2011; Kroeker *et al.*, 2013).

It has long been established that elevated CO_2 levels could enhance the photosynthesis, facilitate cell growth and increase cell density of various types of aquatic primary producers in the absence of photoinhibition (Beardall & Raven, 2004; Giordano *et al.*, 2005; Sobrino *et al.*, 2005, 2008). However, high CO_2 levels could also result in a significant reduction in CO_2 uptake, suppress cell growth, and enhance respiration by decreasing ambient pH (Sobrino *et al.*, 2005; Collins *et al.*, 2006; Crawley *et al.*, 2010). Thus, whether high CO_2 levels in oceans would promote phytoplankton productivity remains an open question.

Solar ultraviolet radiation (UVR) is usually defended and absorbed by stratospheric ozone. However, depletion of ozone by industrial activities allows increased UVR irradiance to reach the earth's surface (Häder *et al.* 2007). It has been well-defined that as a natural stress factor for phytoplankton, solar UVR could impair the structure and function of DNA and proteins of phytoplankton (Boelen *et al.*, 2000; Xiong, 2001), and inhibit the photosynthetic activity (Guan & Gao, 2008; Sobrino *et al.*, 2008; Guan & Lu, 2010; Guan *et al.*, 2011), nutrient uptake (Behrenfeld *et al.*, 1995) and growth of phytoplankton (van Rijssel & Buma, 2002; Liang *et al.*, 2006; Guan & Gao, 2010). Furthermore, there is accumulating evidence of the synergistic effect of solar UVR and elevated CO_2 on the growth, composition and productivity of marine primary producers (Beardall *et al.*, 2009; Wu *et al.*, 2010; Chen & Gao, 2011). However, the interaction between CO_2 and UVR on aquatic photosynthetic organisms has yet to be fully understood.

Chaetoceros is known as one of the largest genera of marine planktonic diatoms, including more than 400 species. *Chaetoceros curvisetus* is characterized with curved, spiralling chains. Its cell size and growth rate is associated with concentration of petroleum hydrocarbon, an environmental pollutant (Wang *et al.*, 2004). Notably, a recent study has reported that red tide alga *C. curvisetus* is sensitive to UV radiation, and could produce UV-absorbing compounds and accelerate the repair process of D1 protein so as to acclimate to UV radiation rapidly (Guan *et al.*, 2011). Thus, *C. curvisetus* was used as a model organism in this research. Many factors may influence the interactions between UVR and CO_2, such as species-specificity, the acclimation period, light conditions and experimental methods, thus making research pertaining to the interactions a great challenge. In this study, high CO_2 concentration (HC, 1000 ppmv CO_2) was chosen following previous studies reporting that pH levels will drop to 7.8–7.9 within the

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following 100 years (Caldeira & Wickett, 2003). *Chaetoceros curvisetus* cells were exposed to HC or low CO_2 air concentrations (LC, 380 ppmv) and then treated with solar UVR. We examined the performance of *C. curvisetus* after it had been grown under HC and solar UVR in order to predict the response of this to global climate change.

MATERIALS AND METHODS

Species and culture conditions

Chaetoceros curvisetus was isolated from Tolo Harbour, Hong Kong, China (22°30′N 114°20′E) on 28 February 2009. It was maintained at 20 °C in f/2 medium (Guillard & Ryther, 1962) in a growth chamber (XT5401-CC275TLH, China) at 80 μmol photons m^{-2} s^{-1} under cool-white fluorescent lights (12L: 12D). Cells in exponential phase (2×10^6 cells ml^{-1}) were diluted to 2.2×10^4 cells ml^{-1} with fresh medium for experiments which were conducted in large quartz tubes (5.9 cm in diameter, 35 cm long) maintained in a water bath (20 ± 0.5 °C; CAP-3000, Rikakikai, Tokyo, Japan).

Acidification treatments on cells in the carbon system

Long-term exposure to high CO_2 concentration (HC, 1000 ppmv CO_2) was used to investigate the effect of acidification on *C. curvisetus* cells. Cells were grown in 1 l flasks with HC continuously (300 ml min^{-1}), and cultured in a CO_2 growth chamber (Model EF7, CONVIRON, Canada). Low CO_2 concentration (LC, 380 ppmv CO_2) was considered to be control. LC and HC represented the atmospheric pCO_2 at present and the years around 2100 (pCO_2 800–1000 ppmv, pH 7.8–7.9), respectively (Hughes, 2000; Caldeira & Wickett, 2003). To evaluate alterations in the carbon system, a range of parameters were measured, including pH_{NBS} (National Bureau of Standards), dissolved inorganic carbon (DIC), HCO_3^-, CO_3^{2-} and CO_2. Cells were counted every 2 days by a hemocytometer under light microscopy (BX50F4, Olympus Optical, Japan). After 14 days, chlorophyll a (Chl a) was extracted by absolute methanol (5 ml) overnight at 4 °C, and measured with a scanning spectrophotometer (DU530 DNA/Protein Analyzer, Beckman Coulter, USA). The content of Chl a was calculated by the formula of Porra (2005). Triplicate cultures were set for each treatment.

Solar radiation treatment on acidified cells

After 14 days of acclimation to HC or LC, the cells were used to evaluate the effect of solar UVR on *C. curvisetus*. Outdoor experiments were conducted at Shantou University (23°26′N 116°42′E). Incident solar radiation was continuously monitored using a broadband ELDONET filter radiometer (Real Time Computer, Möhrendorf, Germany), which has three channels, consisting of photosynthetically active radiation (PAR, 400–700 nm), ultraviolet-A (UV-A, 315–400 nm), and ultraviolet-B radiation (UV-B, 280–315 nm) (Häder *et al.*, 1999). This device is universally recognized (certificate No. 2006/BB14/1) and is calibrated regularly. Acidification cells were exposed to the following treatments: (1) PAB (PAR + UV-A + UV-B), tubes covered with 295 nm cut-off filters (Ultraphan, Digefra, Munich, Germany), transmitting irradiances above 295 nm; (2) PA (PAR + UV-A), tubes covered with 320 nm cut-off filters (Montagefolie, Folex, Dreieich, Germany), transmitting irradiances above 320 nm; (3) P (PAR), tubes covered with 395 nm cut-off filters (Ultraphan UV Opak, Digefra, Munich, Germany). The transmission spectra of these filters has been reported previously (Zheng & Gao, 2009). The mean irradiances during 60 min exposure were 172.1 (PAR), 24.8 (UV-A) and 0.7 (UV-B) Wm^{-2}. After exposure to solar P, PA or PAB for 60 min, photochemical efficiency and rapid light curve were measured. Determination of photochemical efficiency (Φ_{PSII}) was carried out under the condition of 10 μmol photons m^{-2} s^{-1}.

Determination of photochemical efficiency

The Φ_{PSII} was measured with a Pulse Amplitude Modulated fluorometer (PAM-Water-ED, Walz, Germany) (Genty *et al.*, 1990). Φ_{PSII} was calculated as:

$$\Phi_{PSII} = \Delta F/F'_m = (F'_m - F_t)/F'_m$$

where F'_m represents the instantaneous maximum fluorescence, F_t represents the steady-state fluorescence of light-adapted cells. Saturating light pulse was 5300 μmol photons m^{-2} s^{-1} with 0.8 s duration. Light at measurement was about 0.3 μmol photons m^{-2} s^{-1}, and the actinic irradiance was 10 μmol photons m^{-2} s^{-1}.

UVR-induced inhibition rate of Φ_{PSII} was calculated as:

$$\text{Inh}\,(\%) = (Y_{PAR} - Y_X) \times Y_{PAR}^{-1} \times 100$$

where Y_{PAR} is the Φ_{PSII} after 1 h exposure to solar PAR, and Y_X is the Φ_{PSII} after 1 h exposure to PA or PAB.

Measurement of relative electron transport rate (rETR)

The protocol of rapid light curve (RLC) measurement included 10 s actinic light steps in 84, 125, 183, 285, 410, 600, 840 and 1200 μmol photons m^{-2} s^{-1}, respectively. This was followed by a 0.8 s saturation light pulse at the end of each light step to record $\Delta F/F'_m$ (Φ_{PSII}). The RLCs were performed before and after 60 min of exposure to P, PA and PAB. The rETR was calculated as:

$$\text{rETR} = \Delta F/F'_m \times 0.5 \times E$$

where E represents the actinic light (incident PAR), 0.5 means 50% incident PAR energy was distributed to PSII (the other 50% assigned to PSI). $\Delta F/F'_m$ represents the photochemical efficiency of PSII.

RLC was arranged to a hyperbolic tangent function (Jassby & Platt, 1976) in order to compare the initial slope and maximal rETR in each treatment.

$$y = P_m \times \tanh\,(\alpha \times x/P_m)$$

where P_m represents the maximal rETR, and α represents the initial slope of rETR curves.

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Data analyses

One-way or two-way analyses of variance followed by *post-hoc* Tukey's test were used to determine significant differences among different treatments, at significance level $P < 0.05$.

RESULTS

The effects of acidification on *C. curvisetus* cells

Several parameters of the carbon system equilibrated by HC and LC were measured at the beginning of the experiment (Table 1). As shown in Figure 1A, pH under HC and LC remained steady. The pH under HC decreased to 7.9, which simulated ocean acidification. Following that, *C. curvisetus* cells were grown under HC or LC. As shown Figure 1B, the cell density under HC and LC remained constant in the first 6 days, and then began to exponentially increase. In the last 2 days, the cell density under HC was significantly elevated compared with that under LC. As shown in Figure 1C, no significant difference was observed in the content of Chl between the simulated ocean acidification (HC) and LC ($P > 0.05$), whereas the specific growth rate under HC was higher than that under LC (Figure 1D).

The effect of solar UVR on acidified cells

After 14 days of acclimation, the Φ_{PSII} (0.54, time zero) of cells in HC was 19% lower in comparison with that in LC (0.68, time zero). In order to further explore the relationship between acidification and solar radiation, outdoor experiments were also performed. In these, the Φ_{PSII} of cells was lower in LC than in HC. When exposed to solar P, PA and PAB, the Φ_{PSII} in LC declined to the lowest level after 5 min, and then remained constant until 60 min (Figure 2A). Besides, significant differences was observed in the inhibition rates of Φ_{PSII} among the three radiation treatments, of which inhibition rates were 71, 79 and 83%, respectively (Figure 2C). On the other hand, the decreasing trend of Φ_{PSII} in HC was similar to that in LC. It dropped to 0.31 after 5 min exposure, and then remained steady (Figure 2B). However, there were no significant

Table 1. Parameters of the carbon system equilibrated with 380 and 1000 ppmv CO_2, respectively.

pCO_2 (ppmv)	pH_{NBS}	DIC (μmol l^{-1})	HCO_3^- (μmol l^{-1})	CO_3^{2-} (μmol l^{-1})	CO_2 (μmol l^{-1})
380	8.22 ± 0.05^a	2023.3 ± 39.4^a	1826.7 ± 23.7^a	183.9 ± 25.9^a	12.7 ± 2.3^a
1000	7.89 ± 0.11^b	2387.9 ± 68.2^b	2248.4 ± 26.5^b	105.3 ± 35.8^b	33.5 ± 9.4^b

Values followed by different superscript letters are significantly different ($P < 0.05$, N = 5).NBS, National Bureau of Standards; DIY, dissolved inorganic carbon.

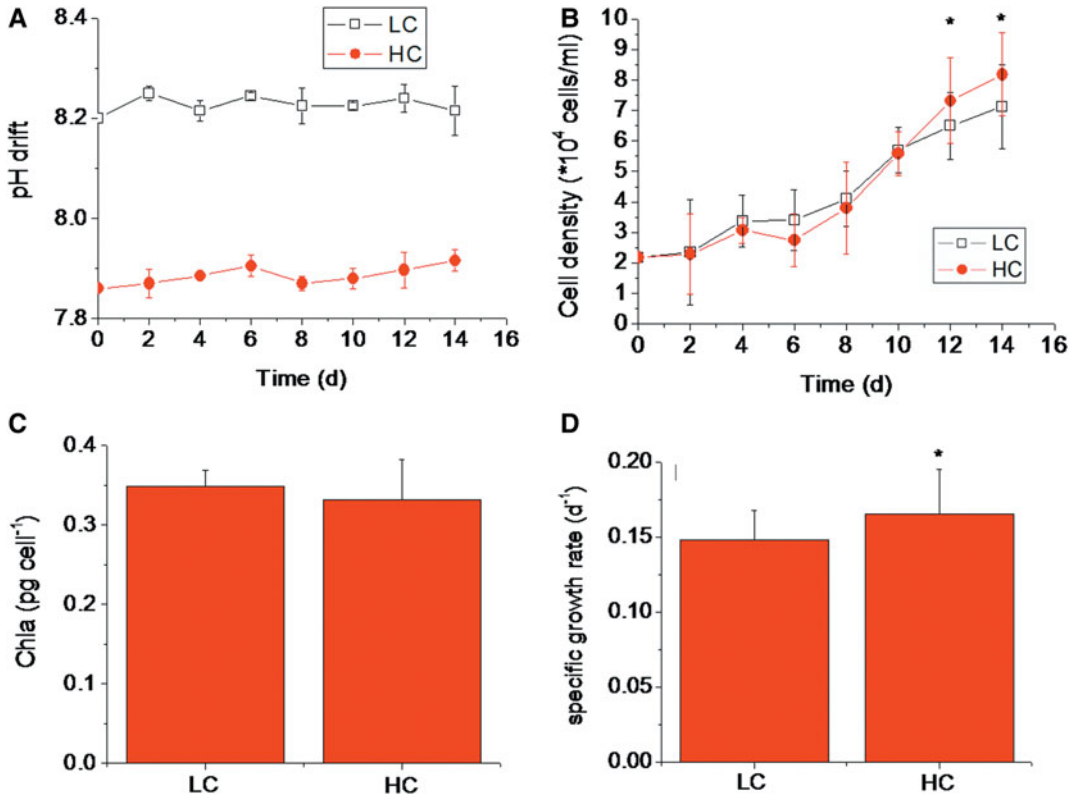

Fig. 1. The effects of acidification on cells and medium during 14 days acclimation to high (pH 7.89) and low (pH 8.22) CO_2 conditions. (A) pH drift of the cultured medium (N = 5); (B) cell density of *C. curvisetus* (N = 8); (C) the content of chlorophyll a in *C. curvisetus* (N = 8); (D) specific growth rate of *C. curvisetus* (N = 8). * represents significant difference ($P < 0.05$).

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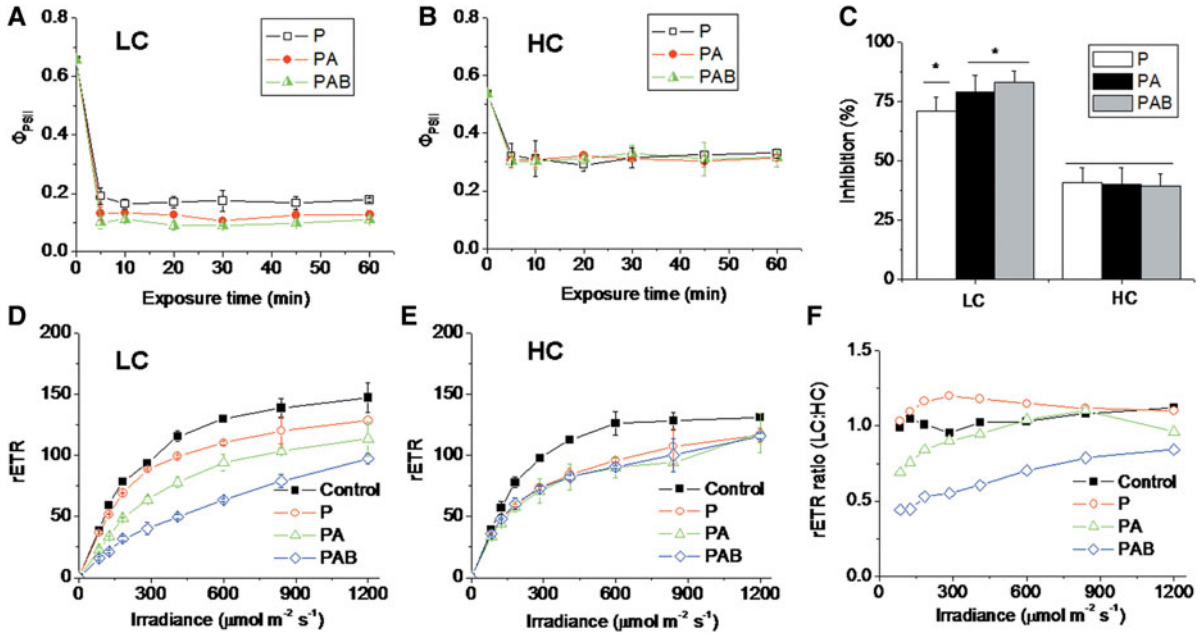

Fig. 2. Φ_{PSII} alterations in *C. curvisetus* during 60 min of exposure to P, PA or PAB after 14 days acclimation to (A) low (LC) and (B) high (HC) CO_2 conditions. (C) The inhibition rate of cells exposed to P, PA, or PAB, N = 8. Rapid light curve of (D) LC and (E) HC cells were measured before (control) and after exposure to P, PA or PAB for 60 min, respectively. (F) The rETR ratio between LC and HC was calculated by the mean data, N = 3. * represents significant difference ($P < 0.05$).

differences in the inhibition rates of Φ_{PSII} under HC among the three radiation treatments (Figure 2C).

In order to evaluate the effect of solar radiation on rETR of cells grown under HC and LC, the rapid light curve was measured. A decreased maximal electron transport rate (P_m) and a slope of the rETR curve (α) were observed in both LC and HC when exposed to solar P (Figure 2D, E, Table 2). Specifically, when compared with controls, P_m declined by 10% (LC) and 27.7% (HC), and α decreased by 14.7% (LC) and 16% (HC), respectively. Moreover, exposure to PA and PAB further enhanced LC or HC-induced decrease of P_m and α, with a more evident decrease in LC than that in HC (P_m: 22.1% *vs* 28.6%; α: 41.8% *vs* 68.2%) ($P < 0.05$) (Table 2). Conversely, rETRs in LC and HC were further elevated in response to solar irradiation. When exposed to PA and PAB, rETRs were significantly lower in LC than in HC. For cells grown under HC, no significant difference was observed in rETRs among the treatments of P, PA and PAB ($P > 0.05$) (Table 2, Figure 2D–F).

Φ_{PSII} recovery

After exposure to solar P, PA or PAB for 60 min, Φ_{PSII} recovery was carried out under 10 μmol photons m^{-2} s^{-1}. As shown in Figure 3, the recovery curve was considered to be an exponential function with time ($R^2 > 0.95$). The initial slope of the fitted curves could be used as an estimate of Φ_{PSII} recovery rate, with a higher initial slope indicating a faster recovery. For cells grown under HC and LC, exposure to P led to faster recovery of Φ_{PSII} than exposure to PA and PAB within 30 min. The Φ_{PSII} in HC achieved the maximum at 60 min and then remained steady. For cells treated with HC, no significant differences were found in Φ_{PSII} among P, PA and PAB. However, accompanied with HC treatment, the Φ_{PSII} of cells exposed to PAB was significantly lower than that of cells exposed to P and PA (Figure 3).

DISCUSSION

Increasing solar UVR caused by decreased thickness of the ozone layer, and ocean acidification caused by elevations of CO_2, have become a threat to marine ecosystems and may seriously impact marine primary producers. It has been established that ocean acidification affects photosynthesis and respiration in phytoplankton, and solar UVR is well known as a natural stress factor (Häder, 2011; Flynn *et al.*, 2012). Recent research reveals complicated interactions between acidified oceans, elevated temperatures and solar UV radiation with unpredictable results (Davis *et al.*, 2013). Examining the effect of ocean acidification on marine primary producers needs to take into consideration light exposure, a warming environment and other factors such as nutrient availability (Gao *et al.*, 2012a). This study focused on the interaction between CO_2 and UVR in *C. curvisetus*. Specifically, the results showed that HC could promote the growth of *C. curvisetus* cells in medium in comparison with LC. In the context of exposure to solar UVR, Φ_{PSII} and rETR was much lower in LC than in HC. These differences indicate that the deleterious effects of UVR on *C. curvisetus* might be counteracted by ocean acidification if the effect is not beyond cell tolerance.

In this study, high concentrations of CO_2 led to increased growth of *C. curvisetus* in the absence of photoinhibition. Similar results were also found in different species by multiple previous studies. For instance, it has been reported that UV-B-induced harm on *Phaeodactylum tricornutum* photosynthesis is ameliorated by increased pCO_2 and lower PH (Li *et al.*, 2012). Consistently, higher CO_2 in the air could give rise to an increase in the photosynthesis rates of *Nannocloris atomus* exposed to photosynthetically active radiation (Sobrino *et al.*, 2005). UVR pre-treatment could partly counteract elevated CO_2-induced photoinhibition in *Thalassiosira pseudonana* (Sobrino *et al.*, 2008). This phenomenon may be caused by the lower concentration of CO_2 (1%

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Table 2. The photosynthetic parameters of the rapid light curve (α, P_m, E_k) in LC and HC acclimated cells.

CO_2	Treatments	α	P_m	E_k (P_m/α)
LC	Control	0.44101[a](0.02345)	140.15606[a](3.66183)	317.8070[a](8.291)
	P	0.40804[b](0.02335)	120.07989[b](3.23172)	294.2846[b](4.271)
	PA	0.25661[c](0.01041)	109.64655[c](2.65932)	427.2887[c](6.172)
	PAB	0.14003[d](0.00932)	100.11272[d](6.64122)	714.9377[d](15.125)
HC	Control	0.47082[e](0.01093)	128.67703[e](1.34934)	273.3041[e](9.362)
	P	0.34328[f](0.02898)	107.19921[f](4.40237)	312.2792[f](10.981)
	PA	0.33326[f](0.03652)	101.60884[f](5.34189)	304.8936[f](9.897)
	PAB	0.35567[f](0.03687)	101.83479[f](4.88898)	286.3182[g](10.434)

The values followed by different superscripts indicate significant difference ($P < 0.05$), N = 3. The data in parentheses are the SD.

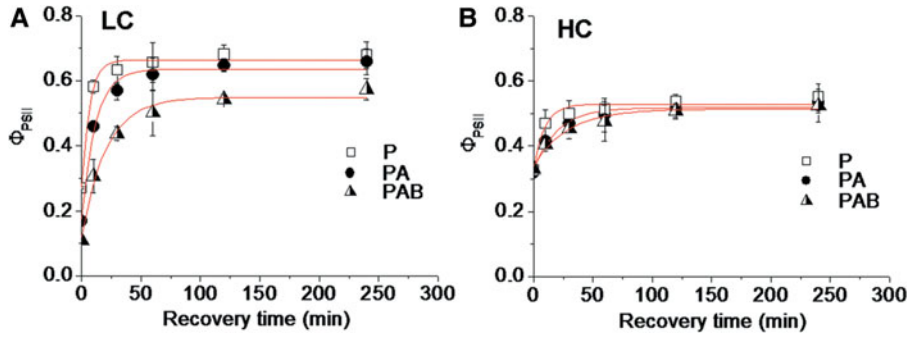

Fig. 3. Φ_{PSII} recovery in *C. curvisetus* cells under 10 μmol photons m^{-2} s^{-1} after 60 min exposure to solar P, PA or PAB (N = 8). Cells were acclimated in (A) LC and (B) HC CO_2 conditions for 14 days in advance of exposure to solar radiation.

DIC, 5–25 μM) (Millero, 1995) than the Michaelis constant ($Km(CO_2)$) (20–70 μM) of Rubisco in the water (Badger *et al.*, 1998), which inhibited photosynthesis, but did not slow down growth (Riebesell *et al.*, 1993; Rost *et al.*, 2003). Lower E_k and higher α in HC might be another reason responsible for the higher growth rate compared with that in LC. However, the photosynthetic rhythms of *Skeletonema costatum* are not affected by CO_2 enrichment during light periods (Chen & Gao, 2004).These conflicting results may be due to discrepancies in species studied and radiation levels. Conversely, negative effects on marine primary producers by ocean acidification have also been reported (Mathis *et al.*, 2011; Gao *et al.*, 2012b). Consequently, accumulating studies lead to a conclusion that whether the acidified ocean has positive or negative effects depends on the species specificity of marine primary producers, and the balance between faster photosynthesis by increased CO_2 and enhanced respiration by decreased ambient pH (Crawley *et al.*, 2010; Chavez *et al.*, 2011; Koch *et al.*, 2013).

Positive interactions between acidified conditions and UVR were also found in this study. Ocean acidification appeared to inhibit the UVR-induced photoinhibition in *C. curvisetus* cells, consistent with previous research on *P. tricornutum* (Li *et al.*, 2012) and *Nannochloropsis gaditana* (Sobrino *et al.*, 2005). Moreover, there is evidence that the net effect of an acidified ocean on red tide alga *Phaeocystis globosa* might be dependent on solar radiation exposure to a large extent (Chen & Gao, 2011).The positive effect induced by HC may be attributed to lower σPSII due to less UV energy, and other biochemical and physiological alterations occurring in cells, such as decreased rETR (P_m) (Wu *et al.*, 2012). It indicates that a proportion of UVR energy was not consumed for emitting pigments, but for repairing damaged protein or DNA. Apart from that, faster non-photochemical quenching could also protect cells against UV radiation (Li *et al.*, 2012). Therefore, UVR exposure could lead to alleviated photoinhibition in HC. As expected, a faster recovery rate was also observed when HC-acclimated cells were transferred into low light conditions. However, the physiological recovery of cells in the current environment could be delayed by shallow mixed layers resulting from global warming.

CONCLUSIONS

Ocean acidification could inhibit the UVR-induced photoinhibition in *C. curvisetus* which might counteract the detrimental effects of both ocean acidification and solar UVR if the effect was not beyond the tolerance of cells.

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