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Combined effects of solar UV radiation and CO_2-induced seawater acidification on photosynthetic carbon fixation of phytoplankton assemblages in the South China Sea

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We carried out short term pCO_2/pH perturbation experiments in the coastal waters of the South China Sea to evaluate the combined effects of seawater acidification (low pH/high pCO_2) and solar UV radiation (UVR, 280–400 nm) on photosynthetic carbon fixation of phytoplankton assemblages. Under photosynthetically active radiation (PAR) alone treatments, reduced pCO_2 (190 ppmv) with increased pH resulted in a significant decrease in the photosynthetic carbon fixation rate (about 23%), while enriched pCO_2 (700 ppmv) with lowered pH had no significant effect on the photosynthetic performance compared to the ambient level. The apparent photosynthetic efficiency decreased under the reduced pCO_2 level, probably due to C-limitation as well as energy being diverged for up-regulation of carbon concentrating mechanisms (CCMs). In the presence of UVR, both UV-A and UV-B caused photosynthetic inhibition, though UV-A appeared to enhance the photosynthetic efficiency under lower PAR levels. UV-B caused less inhibition of photosynthesis under the reduced pCO_2 level, probably because of its contribution to the inorganic carbon (Ci)-acquisition processes. Under the seawater acidification conditions (enriched pCO_2), both UV-A and UV-B reduced the photosynthetic carbon fixation to higher extents compared to the ambient pCO_2 conditions. We conclude that solar UV and seawater acidification could synergistically inhibit photosynthesis.

CO_2, combined effects, pH, phytoplankton, UV

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Solar UV radiation (UVR, 280–400 nm) is one of the most important environmental factors affecting life on the earth. It penetrates pelagic water column up to 60 m [1] and influences marine primary producers [2]. UV-B (280–320 nm) is known to damage DNA [3], photosystems [4] and pigments [5], and affect primary production, species composition and nutrient uptake [6,7]. These UV-related effects differ temporally and spatially [6,8], and the variations have been attributed to the interactive effects of UV with other environmental factors [7]. In natural environments, nutrients, pH, temperature and salinity interact with UV to affect the physiology of phytoplankton [7,9]. Recently, it was demonstrated that the sensitivity of indoor-grown species [10,11] to UV increased when pH decreased, while it decreased under conditions replete with nutrients [12] or at increased temperature levels [3].

Although seawater is naturally buffered by the carbonate system, numerous studies have shown that pH can change significantly in coastal or estuary waters, ranging from 7.0 to 8.5 [13], while typically varying by 0.3 pH units [14] in the open ocean. The seawater carbonate system changes in response to altered seawater pH. The ocean has absorbed more than one third of anthropogenically released CO_2 since the industrial revolution [15], leading to a measurable decline of pH (ocean acidification). It is expected that the oceanic acidity will increase by 120% by the year 2050 [15]. Photosynthesis of marine phytoplankton is supposed to be limited by the present CO_2 level [16] and stimulated

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under increased pCO_2 [17].

The pH changes can affect phytoplankton in different aspects (see review in [18] and references therein). Synthesis of periplasmic proteins can be stimulated or inhibited in a diatom exposed to UVR depending on the UV doses [19]. Phytoplankton cells are often simultaneously exposed to changes in pH and solar UVR, however, little is known about the interactive effects of CO_2-induced pH changes and UV on photosynthetic performance of phytoplankton [20].

The aim of this study is to evaluate the interactive effects of UVR and pH on coastal phytoplankton assemblages in order to understand the relationship of marine photosynthesis with changes in seawater acidity.

1 Materials and methods

A total of 9 experiments were carried out during Julian day 213 to 269 (August 1 to September 26, 2005) in Nan'Ao, an island located in the South China Sea (SCS). The sampling site was 500 m away from the seashore. Water samples were taken around 7∶40 am from the sea surface using a 10-L acid-clean carboy and transported in 15 min to the marine biology station of Shantou University. There, we determined the photosynthetic carbon fixation under pH-perturbed conditions and different radiation treatments (with or without UV).

1.1 pCO_2/pH perturbation and seawater carbonate system

The water samples were pre-filtered through a 180-μm pore size mesh (to remove large zooplankton specimens) before being dispensed into 2 L quartz tubes. These were placed in a water bath for temperature control while exposed to solar radiation. The samples were aerated with air of different levels of CO_2 (190, 380 and 700 ppmv) at a flow rate of 300 mL min^{-1} for 2 h until equilibrium was reached, before being incubated for photosynthetic carbon fixation measurements (see below). The CO_2 concentrations in the aeration were measured with an infrared gas analyzer (CGT-7000, Shimadzu), while the pH changes in the seawater were monitored with a pH meter (420A, Orion). Dissolved inorganic carbon (DIC) of water samples were determined with a total organic analyzer (TOC 5000A, Shimazdu) that automatically measures DIC and total carbon (TC). The reduced (190 ppmv) and enriched (700 ppmv) levels of CO_2 were obtained by mixing the pure CO_2 with CO_2-free air (absorbed through 5 mol L^{-1} NaOH solution) into 1-m^3 plastic bags. A total of two CO_2-enriched and reduced bags were used.

1.2 Solar radiation measurements and treatments

A solar radiometer (ELDONET, Real Time Computer) was placed on the roof of a building within a distance of 30 m from the incubation site. This instrument monitors three wavebands simultaneously, 280–315 (UV-B), 315–400 (UV-A) and 400–700 nm (photosynthetically active radiation, PAR) [21]. The reliability of this device had been certificated with a correspondence error of less than 0.5% in comparison with the most accurate instrument (Certificate No. 2006/BB14/1). The instrument had been calibrated regularly with assistance from the manufacturer.

To determine UV effects upon phytoplankton assemblages, three solar radiation treatments were implemented using UV-cutoff foils:

(i) PAB treatment: PAR + UV-A + UV-B (cells exposed to full spectrum of solar radiation), uncovered quartz tubes;

(ii) PA treatment: PAR + UV-A (cells exposed to irradiance wavelengths above 320 nm), quartz tubes covered with Folex 320 filter (blocks UV-B, 50% transmittance at 320 nm);

(iii) P treatment: PAR (cells exposed to visible light), quartz tubes covered with Ultraphan 395 filter (blocks UVR, 50% transmittance at 395 nm).

The transmission spectra of the filters have been demonstrated elsewhere [22] and there were no significant differences (≤4% transmission) in the PAR levels between the covered and uncovered tubes [23]. There was a 5-nm difference between the measured and exposed UV-A irradiance; which gives rise to about 2% higher measured UV-A than that the cells were actually exposed to. All the incubations were duplicated, and 2 tubes covered with aluminum foil were incubated in the same water bath to determine the fixation in darkness (control).

Different levels of solar radiation were obtained by covering the tubes with nothing or an increasing number of neutral density screens, thus varying irradiance from 100 to 1%.

1.3 Determination of photosynthetic carbon fixation and P *vs*. E curves

The seawater samples equilibrated with air of ambient (380 ppmv), reduced (190 ppmv) or enriched CO_2 (700 ppmv) concentrations, were dispensed into a 20-mL quartz tubes, inoculated with 100 μL-5 μCi (0.185 MBq) of labeled $NaH^{14}CO_3$ (Amersham), and then incubated for 3 h (10∶30–13∶30: centered on local noon). No significant pH drifts (<0.02) were observed after incubations for the photosynthetic measurements. After incubation, samples were filtered on Whatman GF/F glass fiber filters (25 mm). These were placed into 20 mL scintillation vials, exposed to HCl fumes overnight and dried (at 45°C). The amount of incorporated ^{14}C was measured using a liquid scintillation counter (LS 6500, Beckman Coulter) after 3 mL of scintillating cocktail (Hisafe3, Perkin Elmer) was added into each vial. The photosynthetic carbon fixation rate was calculated according to [24].

1.4 Measurements of chlorophyll *a* and species determination

Chlorophyll *a* concentration was determined by filtering 1 L seawater onto a 25-mm GF/F filter, extracting in absolute methanol for 3 h at room temperature (ca. 25°C), centrifuging at 5000 × *g* for 10 min (5804R, Eppendorf), and scanning the supernatant with a spectrophotometer (DU530, Beckman Coulter) between 280–750 nm. The chl *a* concentration (μg mL^{-1}) in the supernatant was estimated according to [25].

The taxonomic analysis of phytoplankton assemblages was carried out using an inverted microscope (IX51, Olympus) after settling 50 mL of sample (fixed with buffered formalin of 0.4% final concentration) for 24 h using an Utermöhl chamber.

1.5 Data analysis

The relative inhibition caused by UV-A, UV-B or their total (UVR) was estimated as follows:

$$\mathrm{Inh_{UVR}} = (P_{\mathrm{PAR}} - P_{\mathrm{UVR}})/P_{\mathrm{PAR}} \times 100\%,$$

$$\mathrm{Inh_{UV\text{-}A}} = (P_{\mathrm{PAR}} - P_{\mathrm{UV\text{-}A}})/P_{\mathrm{PAR}} \times 100\%,$$

$$\mathrm{Inh_{UV\text{-}B}} = \mathrm{Inh_{UVR}} - \mathrm{Inh_{UV\text{-}A}},$$

where P_{PAR}, $P_{\mathrm{UV\text{-}A}}$ and P_{UVR} represent assimilation numbers under P, PA, PAB treatments, respectively.

Photosynthesis *vs.* irradiance curve was fitted as $y = x/(ax^2 + bx + c)$ [26], where y is the photosynthetic carbon fixation (μg C [μg chl a]$^{-1}$ h^{-1}), x is the irradiance (μmol m^{-2} s^{-1}), a, b, and c are the adjustment parameters.

Two-way ANOVA, nonparametric analysis and Kendall test were used to establish significant differences among the treatments (P=0.05).

2 Results

2.1 Carbonate system

The pH and DIC in the water samples reached constant levels in 2 h of aeration with the reduced, ambient and enriched CO_2 concentrations (Table 1). Differences in the pH, CO_2 and DIC were significant (P<0.001) among the treatments. The mean PAR irradiance during incubation ranged 154.4–483.0 W m^{-2} (Figure 1(a)), while the ratios of UV-A or UV-B to PAR were 0.18 (±0.007) and 0.006 (±0.0005) (Figure 1(b)), respectively. The surface seawater temperature ranged from 26.4 to 28.3°C, with a general trend of decreasing from Julian day 213 to 269 (Figure 1(c)).

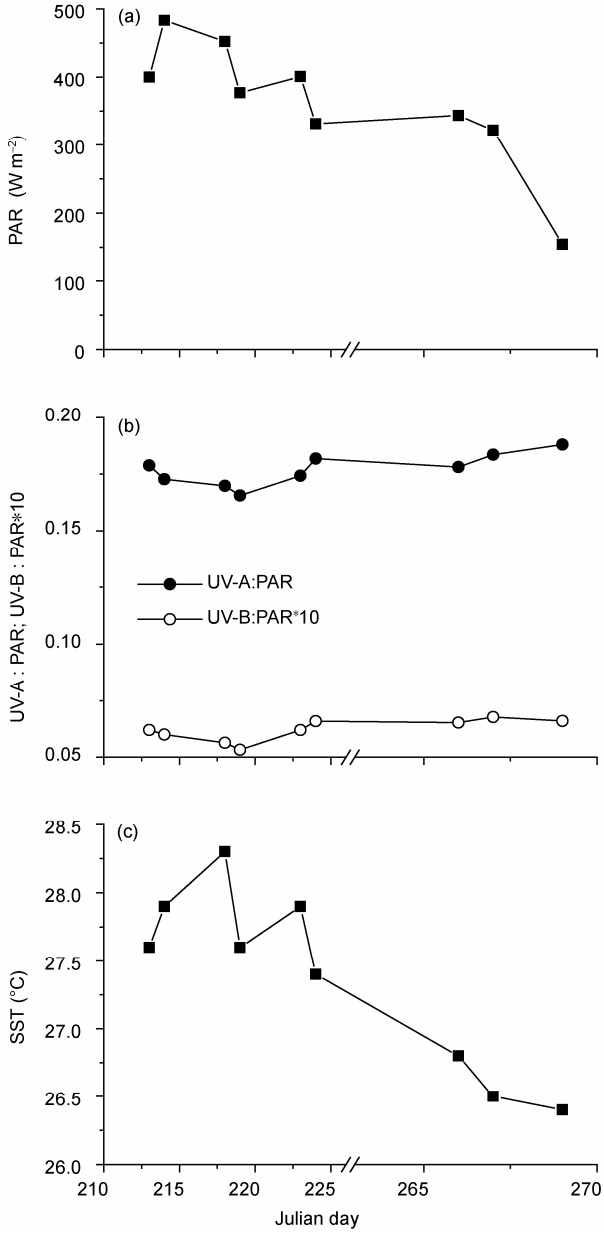

Figure 1 Mean solar PAR irradiance (a), the ratio of UV-A and UV-B to PAR (b) and the surface seawater temperature (c) during the incubation period (10∶30–13∶30) from Julian day 213–269, 2005.

Table 1 Parameters of the seawater carbonate system equilibrated with 190, 380 and 700 ppmv CO_2, respectively[a)]

pCO_2 (ppmv)	pH	DIC (μmol L^{-1})	HCO_3^- (μmol L^{-1})	CO_3^{2-} (μmol L^{-1})	CO_2 (μmol L^{-1})
190	8.38±0.02[a]	1823.2±32.9[a]	1420.2±24.3[a]	398.3±6.9[a]	4.7±0.1[a]
380	8.14±0.02[b]	1943.5±37.3[b]	1670.2±30.7[b]	263.4±4.8[b]	9.9±0.2[b]
700	7.91±0.03[c]	2058.8±49.4[c]	1863.5±42.4[c]	177.1±4.3[c]	18.3±0.4[c]

a) Within each column of the data, values with any different superscript letters are significantly different at P = 0.05 (n = 9).

2.2 Effects of pCO_2/pH and UV on photosynthetic carbon fixation

During the experimental periods (Julian day 213–269, 2005), chlorophyll *a* concentration ranged from 1.1 to 3.3 μg L^{-1} (Figure 2(a)), with the lowest value recorded on Julian day 218, and the highest recorded on Julian day 223. The photosynthetic carbon fixation fluctuated as well, decreasing from Julian day 213 to 269 (Figure 2(b)–(d)). The highest value, 12.8 μg C (μg Chl *a*)$^{-1}$ h^{-1}, was observed at Julian day 214 under 700 ppmv CO_2 and P treatments, while the lowest value, 2.3 μg C (μg Chl *a*)$^{-1}$ h^{-1} at Julian day 269, was under 190 ppmv CO_2 and PAB treatments. For most of the days under P treatments (Figure 2(b)), photosynthetic carbon fixation under 700 ppmv CO_2 was equivalent to the value under the ambient CO_2 level (380 ppmv), while under PA and PAB treatments (Figure 2(c) and (d)), the values under the ambient level were the highest (P<0.05), and those under 190 ppmv CO_2 were the lowest (P<0.02). UVR significantly reduced photosynthetic carbon fixation. To compare the combined effects of different levels of pCO_2 (pH) and UV, the mean assimilation numbers over the study period were compared among the different treatments (Figure 3). Under P treatments, the mean assimilation number was significantly (P<0.005) lower at the reduced than at the ambient or enriched CO_2 levels, while the values under 380 and 700 ppmv CO_2 were comparable (Figure 3(a)). Solar UV-induced inhibition was observed for all treatments, leading to decreased photosynthetic rates. The averaged inhibitions on photosynthesis were 14.1%, 17.5% and 24.7% by UVR, 13.4%, 4.6%, 11.0% by UV-A and 0.7%, 12.9%, 13.7% by UV-B under the reduced, ambient and enriched CO_2 levels (Figure 3(b)), respectively. Presence of UVR caused the highest photosynthetic inhibition under the acidified seawater conditions.

2.3 Photosynthesis versus irradiance curves under varied pCO_2/pH and radiation

The P *vs*. E curves of the phytoplankton assemblages are shown in Figure 4. No photo-inhibition was found under PAR alone (P) treatment up to 1170 μmol m^{-2} s^{-1} (Figure 4(a)), while obvious photo-inhibition was observed under PAR + UV (PAB) treatment (Figure 4(b)). The saturation irradiance (E_k) was comparable between the 380 and 700 ppmv CO_2, while it was the lowest at 190 ppmv, and presence of UVR decreased the E_k values (Table 2). The apparent photosynthetic efficiency was the lowest at the reduced level of CO_2, while comparable between ambient and enriched CO_2 levels, and was enhanced in the presence of UV by 27%, 93% and 124% under the reduced, ambient and enriched CO_2 levels, respectively (Table 2). UVR induced –17.8%, 3.9% and 7.8% inhibition on P_m (the maximal photosynthetic carbon fixation rate) under reduced, ambient and enriched CO_2 levels, respectively.

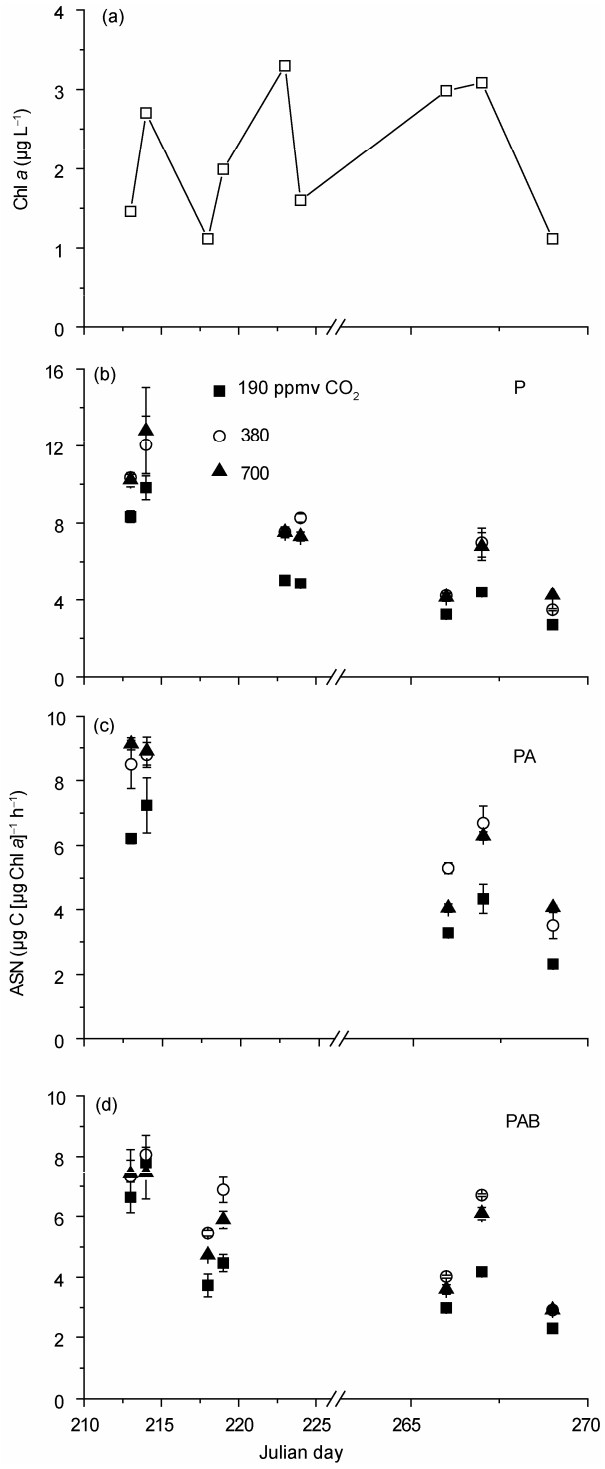

Figure 2 Changes over time in the chl *a* concentration (a) and the photosynthetic carbon fixation rate under P (b), PA (c) and PAB (d) in the seawater equilibrated with 190 (square), 380 (open circle) and 700 (triangle) ppmv CO_2. Vertical bars represent half of the range (n = 2).

The taxonomic composition during the experimental periods was dominated by centric diatoms, mainly *Chaetoceros* sp., *Skeletonema* sp. and *Asterionella* sp.

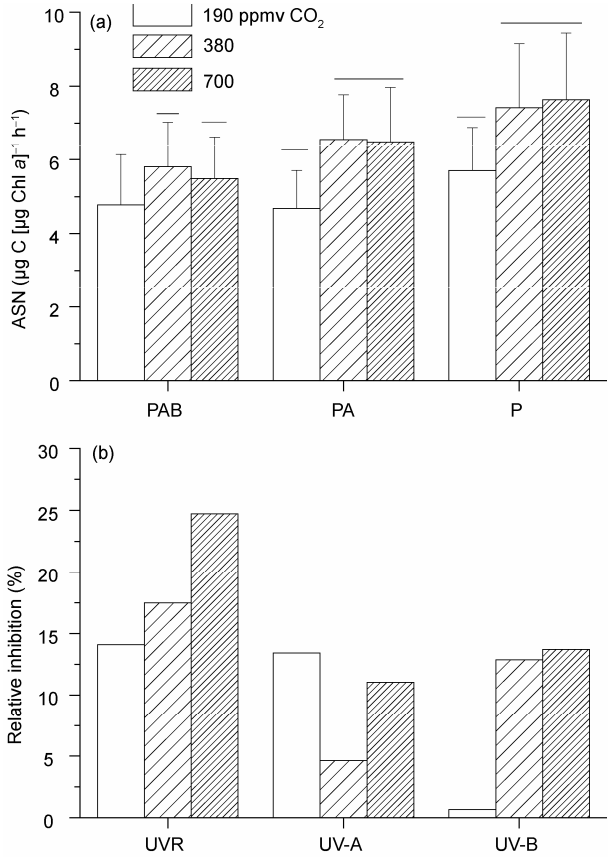

Figure 3 Mean photosynthetic carbon fixation rate under P, PA and PAB treatments in the seawater equilibrated with 190, 380 and 700 ppmv CO_2 (a), and the mean relative inhibition caused by UVR, UV-A and UV-B (b). Vertical bars represent SD (n = 10).

Table 2 Photosynthetic parameters derived from the P *vs.* E curves (Figure 4) for the phytoplankton assemblages in the seawater equilibrated with 190, 380 and 700 ppmv CO_2 and exposed to solar PAR (P) and PAR+UVR (PAB), respectively[a)]

Treatments radiation	pCO_2 (ppmv)	α	E_k	P_m
P	190	0.022±0.001[a]	219.3±7.2[a]	4.71±0.13[a]
	380	0.029±0.003[b]	272.8±34.2[b]	7.68±0.33[b]
	700	0.025±0.003[ab]	298.3±19.6[b]	7.33±0.26[bd]
PAB	190	0.028±0.003[c]	196.1±30.9[a]	5.55±0.26[c]
	380	0.056±0.007[d]	137.7±24.6[c]	7.65±0.34[b]
	700	0.056±0.007[d]	120.0±19.7[c]	6.76±0.26[d]

a) α, the apparent photosynthetic efficiency; P_m, the maximal photosynthetic carbon fixation rate and E_k, the light saturation point. Within each column of the data, values with any different superscript letters are significantly different at P = 0.05 (n = 4).

3 Discussion

CO_2 is regarded as a limiting factor for primary production [27], while elevated CO_2 concentration and reduced pH decreases calcification of calcifying phytoplankton [11,28], and increases the sensitivity of diatoms to UV [10]. In the present study, however, lower CO_2 (higher pH) availability

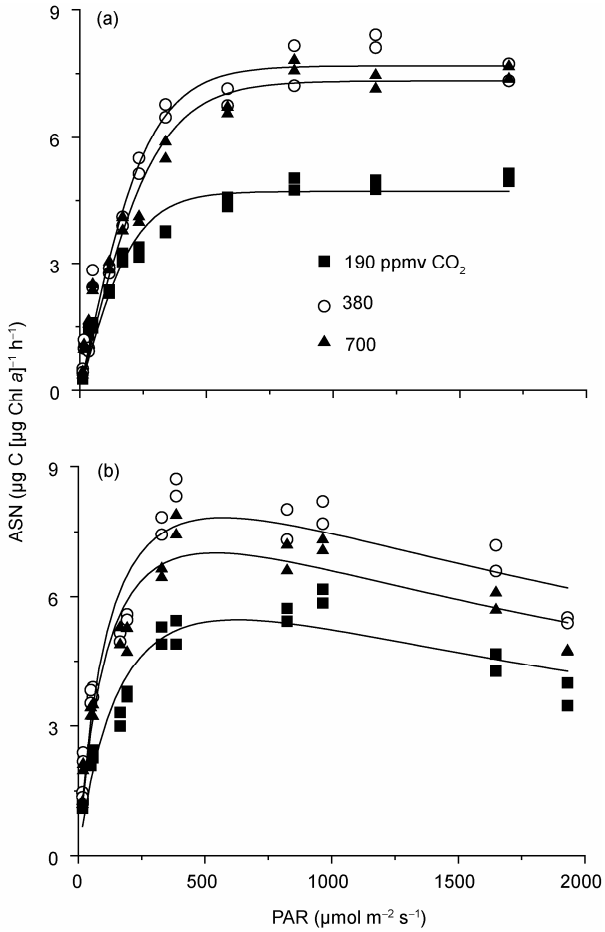

Figure 4 Photosynthetic carbon fixation rate in the seawater equilibrated with 190 (square), 380 (open circle) and 700 (triangle) ppmv CO_2, as a function of the mean PAR irradiance (μmol m^{-2} s^{-1}) under P (a, measured on Julian days 223 and 224, 2005) and PAB (b, measured on Julian days 218 and 219, 2005).

was associated with reduced inhibition of photosynthesis caused by UV-B. Enrichment of CO_2 has been shown to stimulate photoinhibition because of down-regulated CO_2 concentrating mechanisms (CCM) in a cyanobacterium [29]. UV-B was found to stimulate intracellular inorganic carbon (Ci) concentration in a green microalga [30], and both UV-A and UV-B affected the activity of extracellular carbonic anhydrase in a diatom [19]. Thus, UV is most likely to affect CCMs [31] while energizing photosynthesis in phytoplankton [23]. In the present study, decreased photosynthetic efficiency at the reduced CO_2 level implies that light energy might be diverged to up-regulating CCMs. Correspondingly, the lowest inhibition caused by UV-B could be attributed to its contribution to Ci acquisition processes [19,30]. The dominant species during the experimental period were diatoms; CCMs in diatoms are known to respond to changes in CO_2 level within hours, and are more active at reduced levels of CO_2 [32]. Their operation uses additional energy [32] and so UV might have led to reduced inhibition. The CO_2 enrichment did not enhance the photo-

synthesis compared with the ambient CO_2 level; however, the reduced CO_2 level significantly decreased the photosynthetic carbon fixation by up to 37%. This implies that the CCMs could not operate efficiently enough to compensate for the reduced availability of CO_2. Although the cells could adjust their photosynthetic CO_2 affinity in response to changes in pCO_2 during the same period [33], increased operation of CCMs might counteract partially the harms caused by UVR. On the other hand, the photosynthetic carbon fixation rate decreased from Julian day 210 to 270, which could be attributed to the decreased solar radiation (Figure 1(a)). During the experimental period, solar PAR levels at the end day had decreased to about 40% of the initial day, while other factors such as temperature (Figure 1(c)), nutrients [34] and wind speed [35] changed little.

In the present study, pH perturbation with enriched CO_2 enhanced the UV-induced inhibition of photosynthetic carbon fixation rate (Figure 3). The variations in pH and CO_2 availability in coastal waters are influenced by algal photosynthesis; high pH levels are often associated with algal blooms [36,37]. At high pH levels with reduced availability of dissolved CO_2, photosynthesis of phytoplankton can be Ci-limited and their CCMs may become activated or enhanced. The increased requirement of energy for operation of CCMs could aid to minimize the photo-damage to photosystems [38] and thus the UV-inhibition on photosynthetic carbon fixation (Figure 2). The pH level often varies diurnally in coastal areas because of an abundance of both micro- and macro-algae, showing a day (high pH) and night (low pH) reversal pattern [39]. Such diurnal change in pH associated with variation of carbonate system can also affect the response of phytoplankton to solar UVR. The resistance of phytoplankton to solar UV may increase during daytime, especially in afternoon when pH reaches the highest level [39]. In such coastal waters, diurnal variation in pH and the carbonate system can thus regulate the sensitivity of phytoplankton to UV and lessen the UV-induced harms during the daytime. In the upwelling areas, the pH of the seawater is reduced because of the up-mixed low-pH deep water [40]. Solar UV may cause more harm to the cells in these low pH/high CO_2 waters. Phytoplankton cells in coastal waters have shown a higher degree of UV-induced inhibition of photosynthesis after a typhoon, which brought up deep seawater to the surface and lowered the pH [41].

Phytoplankton species have efficient mechanisms to maintain cytosolic pH even when exposed to pH extremes [42], although regulation of intracellular pH has been less studied so far [43]. It has been suggested that the cells may uptake or extrude additional H^+ to maintain internal pH when the external pH changes. Such processes require additional energy [44]. In the present study, lowered pH at the enriched CO_2 level may have negatively affected the phytoplankton cells by consuming the photosynthetic energy to balance the external pH drop, thus counteracting the positive effects of the enriched CO_2 (Figure 3).

The ongoing ocean acidification due to atmospheric CO_2 rise may differentially affect physiology of different phytoplankton species. For CCM-active species, the enriched CO_2/lowered pH condition may not stimulate their photosynthesis, but the acidification can down-regulate their CCMs. For CCM less-active or inactive species, enriched pCO_2/lowered pH may enhance their photosynthesis. Even for CCM-operative species, when light is limiting, CO_2 enrichment can bring about increased photosynthetic C fixation [17] because of the saved energy for active transport of CO_2 or HCO_3^-.

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