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Article

Diurnal light utilization efficiency of phytoplankton is decreased by elevated CO_2 concentration: a mesocosm experiment

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With 8 figures

Abstract: The effects of ocean acidification on marine primary production are of general concern in light of increases in oceanic CO_2 uptake. However, little is known regarding the effects of elevated CO_2 on the physiological performance of phytoplankton in eutrophic waters during the daytime. A mesocosm study was performed to investigate the diurnal photosynthetic behaviour of phytoplankton under ambient (400 µatm; LC) and elevated (1000 µatm; HC) CO_2 concentrations. Elevated CO_2 accelerated the relative electron transport rate (rETR) of phytoplankton and increased the efficiency of photosystem II (PSII) open reaction centers to capture light (F_v'/F_m'), especially after peak irradiance at noon. However, increased light from PSII was not efficiently utilized by photosynthetic carbon fixation but dissipated by other photoprotective pathways. Dissipation helps to retard the photoinhibition rate in the morning and accelerate the PSII activity recovery rate in the afternoon. Under such circumstances, phytoplankton in HC mesocosms decreased their pigment contents and functional absorption cross-section of PSII (σPSII) to reduce light capture and prevent further potential photoinhibion. In general, these intracellular adjustments eventually lead to the total amount of fixed carbon in HC mesocosms decreasing by 31 % per day. Cellular particulate organic carbon (POC) and nitrogen (PON) content was also 26.8 % and 26.4 % lower in HC mesocosms, respectively. These results implied that elevated CO_2 decreases diurnal light utilization efficiency in eutrophic water.

Key words: Diurnal photosynthesis; mesocosm; ocean acidification; phytoplankton

Introduction

Anthropogenic CO_2 was suggested as one of the key sources for ocean carbon sequestration over five decades ago (Revelle et al. 1957). However, the decreased pH of seawater as a result of rising atmospheric CO_2 due to combustion of fossil fuels has only been demonstrated during the past decade (Sabine et al. 2004). This process, known as ocean acidification, is expected to continue for at least two centuries (Caldeira et al. 2003). With the level of atmospheric CO_2 projected to reach up to 1000 µatm by the end of this century, the resulting pH of the surface ocean may decrease by approximately 0.3 to 0.4 units (Caldeira et al. 2003), which represents a 100 % to 150 % increase in H^+ ions. Phytoplankton accounts for half of global primary production and also determines the CO_2 sequestration ability of the ocean (Raven et al. 1999). Each year, approximately 10 gigatons of CO_2 are exported from the surface ocean to the deep sea (Boyd et al. 2007). This ongoing ocean acidification may exert unforeseen effects on ocean biogeochemistry (Falkowski et al. 2008). Thus, a thorough understanding of physiological responses (especially photosynthesis) of phy-

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toplankton to ocean acidification is just around the corner (Gao et al. 2014).

Phytoplankton experiences varying pH and pCO_2 conditions in different regions (especially in intensively mixed coastal seawater) and, in turn, alters seawater carbonate chemistry through photosynthetic carbon removal. Despite the fact that the effects of elevated CO_2 on phytoplankton have been extensively studied, controversy still exists as to whether ocean acidification has positive (Hein et al. 1997; Riebesell et al. 2007), negative (Montechiaro et al. 2010), or neutral effects (Tortell et al. 2000; Tortell et al. 2002; Chen et al. 2003) on phytoplankton cells, in terms of both individual organisms and phytoplankton assemblages (see reviews by Gao et al. 2012 a and Beardall et al. 2014 and references therein). In addition, most studies have been performed under laboratory or shipboard conditions (Wu et al. 2010; Lomas et al. 2012; Li et al. 2014), while little has been documented regarding the response of phytoplankton to ocean acidification in areas of different trophic status.

Among the three major environmental variables that affect phytoplankton, physiology status (solar irradiance, nutrient availability and temperature), solar irradiance has drawn the most attention because of its direct influence on intracellular photosynthetic apparatus (Beardall et al. 2009; Wang et al. 2009; Cunha & Calijuri 2011). Phytoplankton in the water column experiences solar irradiance levels of different orders of magnitude, from dim light in the early morning to extremely high light at noon, and then decreasing solar irradiance towards the end of the day. Cells under low light during the morning are unable to acquire sufficient energy to completely fulfill their metabolic requirements, thus limiting growth and primary production. On the contrary, cells exposed to high levels of solar irradiance at noon will result in a significant loss of photosynthetic production due to photoinhibition. Therefore, a physiological mechanism adjustment that balances the capture and utilization of light is of great significance for phytoplankton survival and competitive strength. However, the balance could be easily affected by other environmental stressors, such as temperature (Hüner et al. 1998). In the present study, both the increase in the CO_2 concentration and corresponding decrease in the pH of seawater may impact the physiological balance of light utilization. Previous studies have shown that increased CO_2 levels significantly improve the photochemical efficiency of *Phaeodactylum tricornutum* under indoor controlled conditions (Wu et al. 2010). However, elevated non-photochemical quenching (NPQ) and intensified photoinhibition under sunlight were also reported due to decreased pH (Gao et al. 2012a). Therefore, it is necessary to investigate the response of phytoplankton to ocean acidification under multiple stressors (Riebesell et al. 2015).

To understand the physiological responses of phytoplankton to elevated CO_2 concentrations under changing marine environments and sunlight, a CO_2-enriched mesocosm experiment was conducted. This work aimed to 1) investigate the diurnal variability in the physiological responses of phytoplankton subjected to elevated CO_2 level, and 2) assess whether seawater acidification has significant impacts on photosynthesis in eutrophic water.

Material and methods

Mesocosm location

This experiment was performed at a facility located in Wuyuan Bay, Xiamen, China (N24° 31′ 48″, E118° 10′ 47″). The facility consisted of a platform that was 28 m × 10 m with three independent laboratories built on and electricity provided by solar panels. The mesocosms consisted of six cylindrical bags that were deployed along the south-facing side of the platform to avoid shading. The bags were made of thermoplastic polyurethane (TPU), which transmits 90 % visible irradiance. Each bag was 3 m deep and 1.5 m wide. The tops of the bags were 50 cm above the water surface and were covered with a TPU dome to avoid contamination from seawater spray and rainfall.

Seawater and phytoplankton species

Six mesocosms were filled simultaneously with local seawater that had been passed through a water purifier (MU801-4 T, Midea). The purifier was equipped with filtration cartridges with a pore size of 0.01 µm to filter out all particles. The filtration lasted for approximately 24 h. Four phytoplankton species, namely *Phaeodactylum tricornutum* (CCMA106), *Thalassiosira weissflogii* (CCMP 102), *Emiliania huxleyi* (CS-369) (calcifying) and *Gephyrocaps oceanica* (NIES-1318) (calcifying), were cultured in the laboratory for 10 days at 20 °C under a light intensity of 150 µmol m^{-2} s^{-1}. The light:dark cycle was 12 L:12D. Before inoculation to the mesocosms, cells were acclimated in the same eutrophic bay seawater with mesocosms. These representative species were chosen because of their widely distribution and potential bloom forming capacity in coastal seawater. Each species had an equivalent Chl-*a* level at the initial phase, with the resulting final total concentration of 5×10^4 cells L^{-1}.

CO_2 manipulation and solar radiation measurement

The seawater pCO_2 was adjusted by bubbling with ambient air (400 µatm CO_2, LC) or CO_2-enriched air at a target concentration (1000 µatm CO_2, HC) which were prepared by a CO_2 Enricher (CE-100 B; Wu Han RuiHua, China). The air containing target CO_2 concentration was delivered into the water

at a flow rate of ~5 L min^{-1} via 6 mm diameter plastic tubing and dispersed using an air stone disk that was placed in the center at the bottom of the mesocosm. The bubbling continued throughout the experiment in order to compensate for inorganic carbon decrease due to photosynthesis. Moreover, the continuous bubbling also stirred the water to prevent cell sedimentation. Incident solar irradiance was continuously recorded by an ELDONET filter radiometer (Real Time Computer, Möhrendorf, Germany). The device has three channels for photosynthetically active radiation (PAR, 400–700 nm), UV-A (UV-A, 315–400 nm) and UV-B radiation (UV-B, 280–315 nm). The temperature and pH in both mesocosms were measured by CTD (RBR) and SeaFET (Satalantic).

Experimental design

After a period of acclimation under each CO_2 treatment, diurnal physiological responses of phytoplankton to acidification were investigated on day six (for at least 8–10 generations) during the exponential growth phase. Each mesocosm was regarded as one treatment and the sampling volume was approximately 200 mL. The sampling depth was near the surface of the enclosed water column.

Pigment content and chlorophyll fluorescence measurement

For determination of Chl-*a*, Chl-*c* and carotenoid contents, cells were sampled (150–200 mL) from each mesocosm every three hours from 06:00 to 18:00 and filtered onto a Whatman GF/F filter (25 mm). The filtered cells were extracted with 4 mL of absolute methanol overnight at 4 °C, centrifuged (10 min at 5000g), and determined by a scanning spectrophotometer (DU800, Beckman Coulter Inc., USA). The calculations of pigment content in the supernatants were based upon previously published methods for Chl-*a* (Porra 2005), Chl-*c* (Rigler 2006) and carotenoids (Strickland et al. 1968).

Chlorophyll fluorescence was determined every 30 min using FIRE (Fluorescence Induction & Relaxation Fluorometer system; Satlantic) from 07:10 to 18:10. All the parameters were measured in an inbuilt cuvette containing phytoplankton cells. After sampling (2 mL) and 15 min dark-adaptation, the maximum quantum yield (F_v / F_m) was measured. The effective quantum efficiency of PSII (F_v' / F_m') was determined immediately after sampling. NPQ was calculated as: NPQ = ($F_m - F_m'$) / F_m'; where F_m represents the maximum fluorescence after dark adaptation, and F_m' represents the maximum fluorescence under growth light condition.

The inhibition (k) and recovery (r) rate of F_v' / F_m' were calculated by exponential functions according to a previous study (Villafañe et al. 2015). The k rate was obtained by fitting F_v' / F_m' data from the initial time (early morning) to noon as

$$F_v' / F_m' = \mathrm{A}e^{-k\,\mathrm{t}},$$

while the r rate was calculated from noon to the last data point measured in the evening as

$$F_v' / F_m' = \mathrm{A}e^{r\,\mathrm{t}}.$$

Here, A is a constant, k and r represents the inhibition and recovery rates, respectively and t is the time. The R^2 of all fits were greater than 0.9.

In addition, the relative electron transport rate (rETR) was calculated as rETR = Yield × PFD, where Yield is the effective quantum efficiency and PFD (photon flux density) is the actinic light intensity which was similar to the level of sunlight at the time of the measurement. The functional absorption cross-section of PSII (σPSII) was monitored every 30 min throughout the daytime.

Diurnal photosynthetic carbon fixation

Diurnal photosynthetic carbon fixation was determined every 3 h from 06:00 to 18:00. Samples (150 mL) from the mesocosms were dispensed into 20 mL quartz tubes, inoculated with 100 µL of a solution containing 5 µCi (0.185 MBq) $NaH^{14}CO_3$ (ICN Radiochemicals, USA), and incubated under solar conditions with three replicates. Two additional tubes were wrapped in foil as dark treatments. All of the tubes were placed into a tank with flow-through seawater to control the temperature (close to that of the seawater in the mesocosms, 27–28.5 °C). After 1 h of incubation, the cells were filtered onto a Whatman GF/F glass fiber filter (25 mm), and then immediately frozen and stored at −20 °C for later analysis. The frozen filter was thawed and placed in a 20 mL scintillation vial and exposed to HCl fumes overnight and then dried (60 °C, 3 h) to expel residual non-fixed ^{14}. Following drying, 5 mL of scintillation cocktail (PerkinElmer, MA, USA) was added to each vial and the radioactivity was counted using a liquid scintillation counter (LS 6500, Beckman Coulter, USA).

Carbonic anhydrase (CA) is of great importance for phytoplankton carbon acquisition (Rost et al. 2003). To assess the role of carbonic anhydrase (CA) in photosynthetic carbon fixation, cells from the LC and HC treatments were sampled and dispensed into 35 mL quartz tubes with or without 6-ethoxyzolamide (EZ). EZ can significantly inhibit the activity of CA. Photosynthetic carbon fixation was measured as described above. The degree of inhibition brought by EZ represents the relative importance of CA in LC and HC treatments during daytime.

Organic particles

Samples (100–200 mL) for particulate organic carbon and nitrogen (POC, PON) were filtered onto pre-combusted (450 °C for 12 h) GF/F filters, and then immediately frozen at −20 °C. For POC and PON determination, filters were dried at 60 °C prior to analysis and subsequently measured by a CHNS/O analyzer (2400 Series II, PerkinElmer).

Statistical analysis

Statistical analysis included one-way ANOVA and Tukey's post-hoc test (for pairwise comparisons) using Origin7.0 and SPSS16.5, with a significance level of $\alpha = 0.05$. All measurements were tested in triplicate (i.e. three separate samples from each mesocosm). The relative inhibition of photosynthetic carbon fixation by EZ in LC and HC mesocosms was calculated as follows: $\mathrm{Inh}_{EZ}(\%) = (Y - Y_{EZ}) / (Y) \times 100$, where Y_{EZ} and Y represents photosynthetic carbon fixation with and without EZ, respectively.

Results

Solar irradiance on the day of the experiment followed a typical diurnal variation. Maximum irradiance inten-

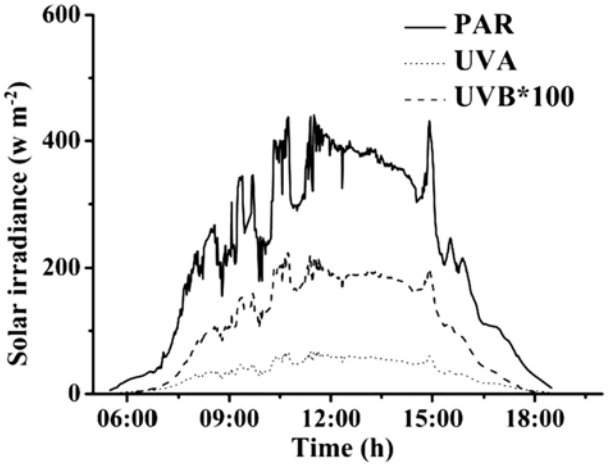

Fig. 1. Fluctuation of the incident solar PAR (400–700 nm), UVA (315–400 nm) and UVB (280–315 nm) during the mesocosm diurnal variation experiments.

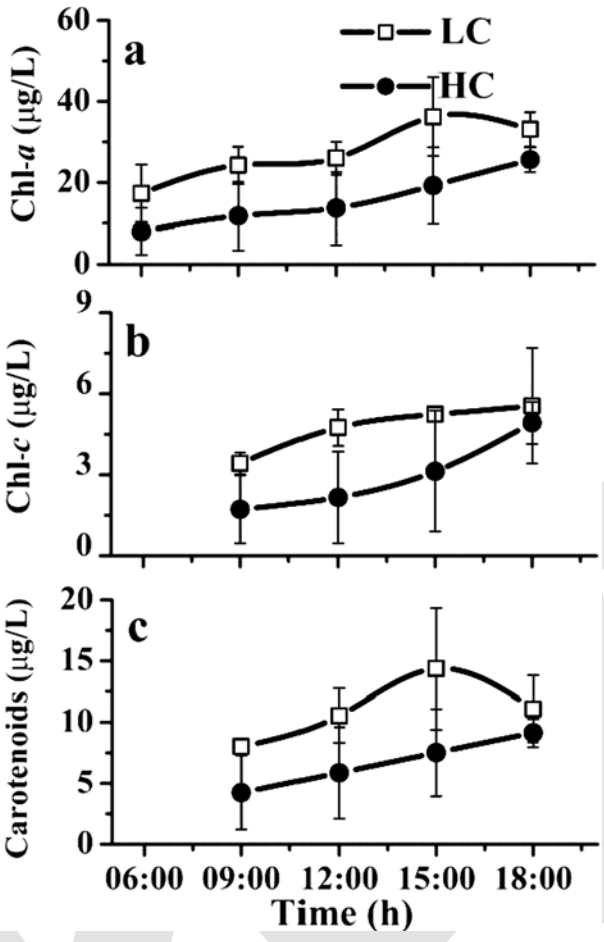

Fig. 2. Diurnal changes in Chl-*a* **(a)**, Chl-*c* **(b)** and carotenoid **(c)** content measured on day six after inoculation in mesocosms aerated with ambient (LC, 400 µatm) and CO_2-enriched (HC, 1000 µatm) air. The vertical lines indicate the standard deviation of the means (n = 3).

sity for PAR, UVA and UVB during noon time was 441.1, 66.9 and 2.2 W m^{-2}, respectively (Fig. 1). The temperature of the enclosed water ranged from 27.5 to 28.5 °C, while the salinity remained relatively stable around 27.4 ‰. After six days acclimation, the pH in LC and HC treatments were stable due to continuous bubbling. On the experiment day (day 6), the pH value was 8.141 ± 0.078 and 7.787 ± 0.057 for LC and HC treatment, respectively. For nitrate and ammonium concentration, the initial value was 30 and 53 µM, respectively. After six days acclimation, the nitrate concentration slightly decreased to 29 and 25 µM for HC and LC treatment, respectively, while the ammonium concentration sharply declined to 13 and 10 µM in HC and LC treatments. The relatively slower nutrient consumption rate in HC treatment was consistent with the lower biomass identified by Chl-*a* contents. Although the initial inoculation Chl-*a* concentrations of the four phytoplankton species were the same, two diatom species had an obvious competitive advantage identified by diagnostic pigment (Fucoxanthin, data not shown) on the day of the experiment. It was worth noting that the two diatom species nearly dominated the phytoplankton community at the end of the mesocosm experiment on day 16.

Pigments

An increased accumulation of pigments was found in both LC and HC treatments during the diurnal period. Chl-*a* concentration (Fig. 2a) nearly doubled during the daytime, while the concentrations of Chl-*c* (Fig. 2b) and carotenoids (Fig. 2c) increased by approximately 38 % and 187 % by the end of the day, respectively. Significantly higher concentrations of pigments were observed in LC mesocosm enclosures compared with HC ones before 15:00 ($p < 0.05$). However, the differences of pigment concentration between the LC and HC diminished toward the end of daytime.

The Chl-*c* / Chl-*a* ratio (Fig. 3a) did not show any diurnal patterns nor have any significant differences between the LC and HC treatments ($p > 0.05$). While the ratio of carotenoids / Chl-*a* (Fig. 3b) had an obvious diurnal variation, with the highest values of 0.43 and 0.41 observed at noon in HC and LC treatments, respectively. Although the difference of carotenoids / Chl-*a* ratio between LC and HC treatments was not significant ($p > 0.05$), a relatively higher value was found in HC mesocosms.

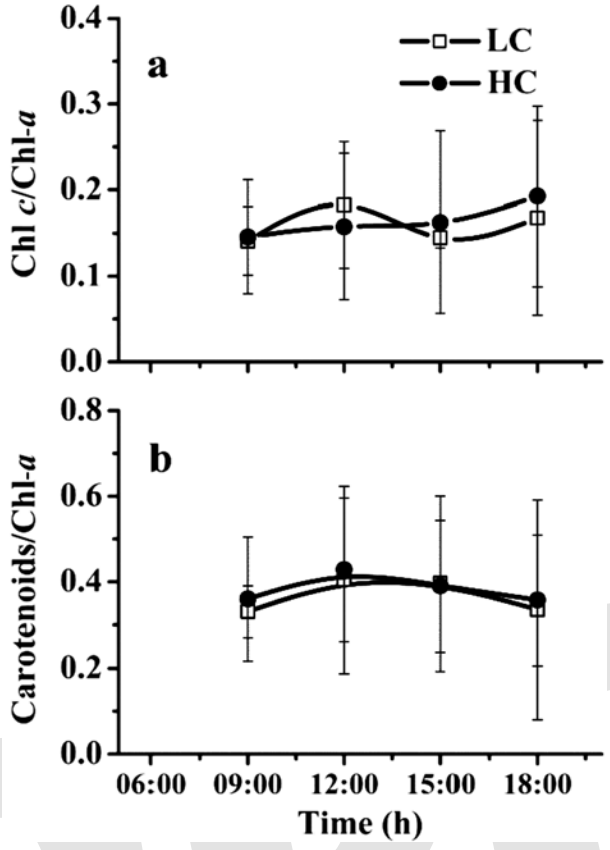

Fig. 3. The ratios of carotenoids to Chl-*a* **(a)** and Chl-*c* to Chl-*a* **(b)** measured on day six after inoculation in mesocosms aerated with ambient (LC, 400 µatm) and CO_2-enriched (HC, 1000 µatm) air. The vertical lines indicate the standard deviation of the means (n = 3).

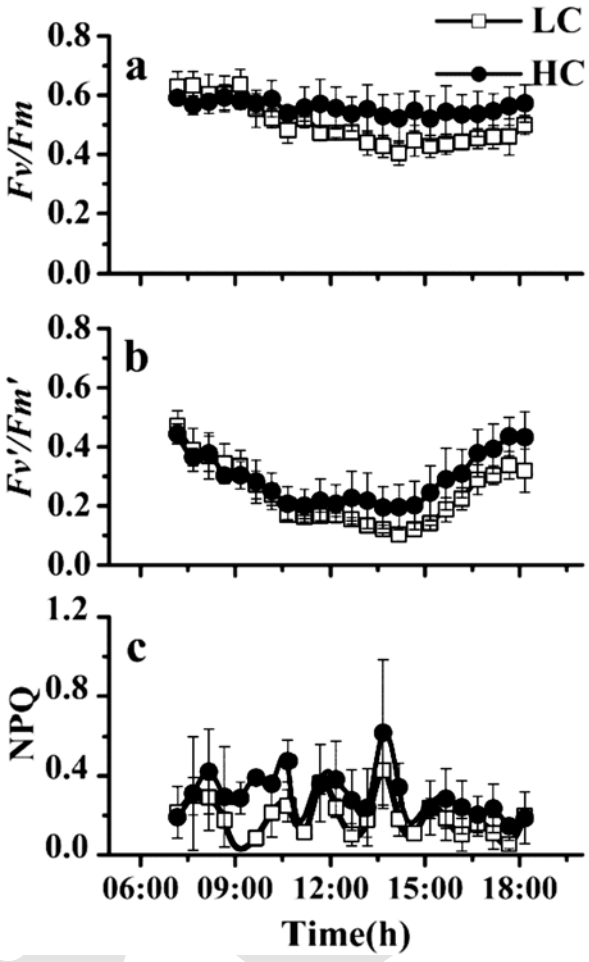

Fig. 4. The maximal **(a)** and effective **(b)** quantum yield, and non-photochemical quenching **(c)** of the phytoplankton cells in response to changing solar radiation under low CO_2 (LC, 400 µatm) and high CO_2 (HC, 1000 µatm) conditions. The vertical lines indicate the standard deviation of the means (n = 3).

Chlorophyll fluorescence

The dark-adapted maximum quantum yield (F_v / F_m) of PSII (Fig. 4a) decreased by approximately 21 % in the LC mesocosm enclosures compared with the initial value ($p < 0.05$). However, such a typical solar light-related decrease of F_v / F_m was not apparent in the HC treatment, suggesting that elevated CO_2 concentration helps to maintain the photochemical activity of PSII. The effective quantum efficiency (F_v' / F_m') (Fig. 4b) almost followed the opposite pattern with sunlight, with the lowest value recorded around 13:30, and relatively higher values observed at dawn and dusk. Similarly, the F_v' / F_m' value was also significantly higher in HC treatments compared with LC ones. At the end of the day, the F_v' / F_m' in the HC treatments nearly recovered to its initial value, while it remained 30 % ($p < 0.05$) lower in LC ones compared with the initial level. Non-photochemical fluorescence quenching (NPQ) showed no clear diurnal pattern (Fig. 4c), but with a somewhat oscillatory variation. However, cells sampled from the HC enclosures generally had relatively higher NPQ values.

CO_2 concentration had significant effects on the k of phytoplankton cells from early morning toward noon, with lower ($p = 0.048$) absolute values of k in samples acclimated to elevated CO_2 concentrations (Fig. 5). The results agreed well with the higher photochemical activity of PSII in HC treatments. On the contrary, the recovery rate was significantly stimulated by elevated CO_2 concentration, with an r value 24.1 % higher ($p = 0.045$) in HC treatments compared with LC ones.

The σPSII gradually decreased toward the middle of the day and then rapidly increased in both LC and HC treatments (Fig. 6a). At the end of the day, σPSII increased by 16 % and 15 % in the LC and HC treatments compared with the initial value, respectively.

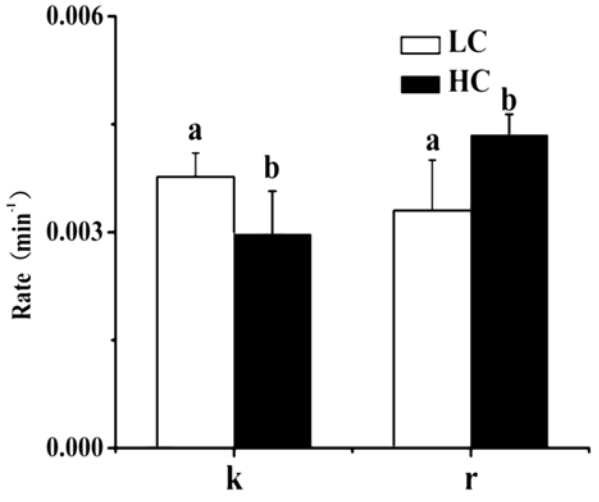

Fig. 5. Inhibition (*k*) and recovery (*r*) rates of effective quantum efficiency of PSII caused by solar radiation under low CO_2 (LC, 400 µatm) and high CO_2 (HC, 1000 µatm) conditions. The vertical lines indicate the standard deviation of the means (n = 3). Different superscript letters represent a significant difference ($p < 0.05$).

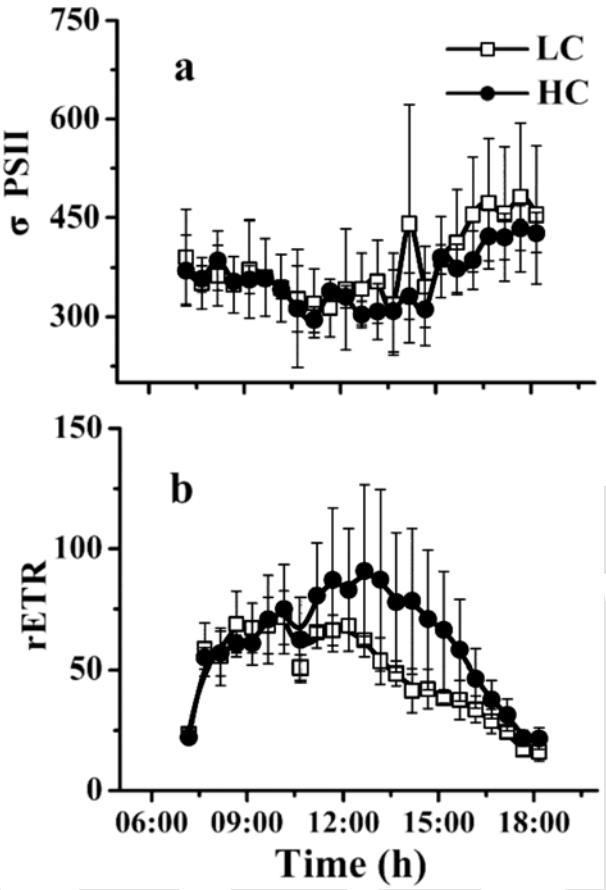

Fig. 6. Diurnal changes in σPSII (Å quanta^{-1}) **(a)** and rETR **(b)** of the phytoplankton cells during the daytime when grown under low CO_2 (LC, 400 µatm) and high CO_2 (HC, 1000 µatm) conditions. The vertical lines indicate the standard deviation of the means (n = 3).

After noon, the σPSII in the LC treatment was relatively higher than that in the HC treatment though not significant ($p = 0.732$). The rETR in both treatment groups increased gradually from 07:10 to noon time, and then decreased towards the end of the day, which followed a typical pattern with sunlight (Fig. 6b). This diurnal variation trend occurred more strongly in HC treatments than that in LC ones ($p = 0.049$).

Carbon fixation

The initial photosynthetic carbon fixation rate was 15.71 and 12.26 µg C·µg Chl-a^{-1}·h^{-1} in LC and HC treatments (Fig. 7a), respectively. Increased solar irradiance at noon resulted in a maximal photosynthetic carbon fixation rate. After noon, the carbon fixation rate decreased markedly, with final values of 2.42 and 1.92 µg C·µg Chl-a^{-1}·h^{-1} in LC and HC treatments, respectively. When all data were integrated to derive a value for the whole-daytime primary production, less carbon (52.98 µg C·µg Chl-a^{-1}·d^{-1}, 31 % lower) was fixed in HC mesocosms compared to LC ones. The role of CA in photosynthetic carbon fixation during a 5 h culture was investigated under full solar radiation. The average photosynthetic carbon fixation rate (Fig. 7b) of LC and HC treatments was 15.88 and 13.43 µg C·µg Chl-a^{-1}·h^{-1}, respectively ($p = 0.531$). The addition of EZ significantly decreased the carbon fixation rate in both CO_2 treatments. Samples in the HC+EZ treatment had a significantly lower carbon fixation rate compared to the LC+EZ treatment ($p < 0.05$). The average level of inhibition of carbon fixation by EZ was 71.54 % and 90.30 % for the LC and HC treatments, respectively ($p < 0.05$) (Fig. 7c).

Particulate organic matter

Cellular particulate organic matters (POM) were significantly influenced by elevated CO_2 concentration. For LC treatments, the average POC (Fig. 8a) and PON (Fig. 8b) content was 3.1 mg L^{-1} and 0.6 mg L^{-1}, respectively. However, elevated CO_2 concentration decreased POC and PON content by 26.8 % ($p < 0.05$) and 26.4 % ($p < 0.05$), respectively. The decreased cellular POC content in HC treatments agreed well with the lower whole-daytime primary production of phytoplankton. There were no significant differences between LC and HC treatments on the POC/PON ratio ($p > 0.05$, Fig. 8c) due to synergistically decreased contents of both POMs.

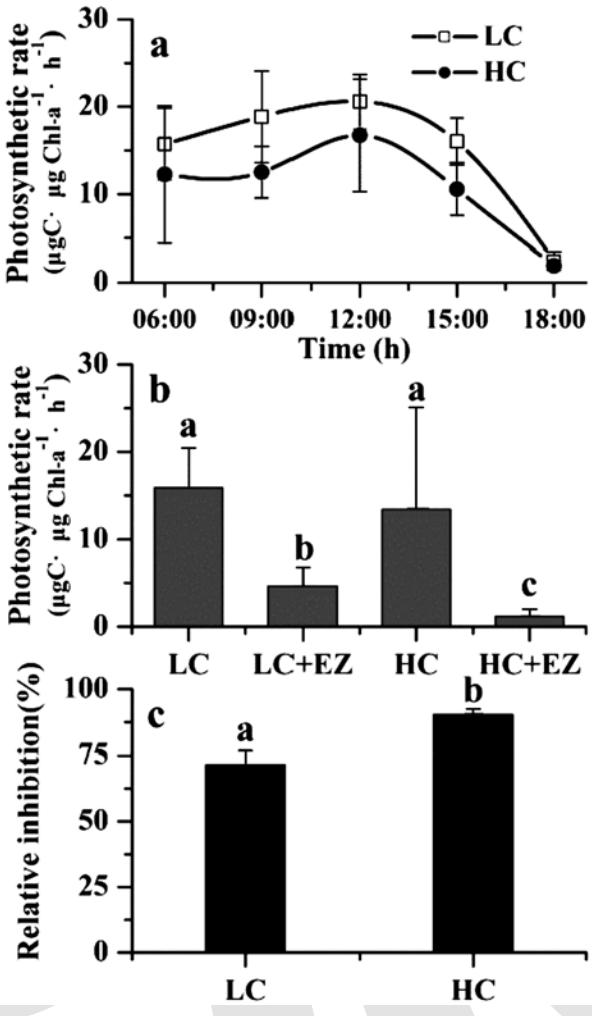

Fig. 7. Diurnal changes in the photosynthetic carbon fixation rate of the phytoplankton cells when measured under incident solar radiation from 6:00 AM to 18:00 PM **(a)**, the cumulative carbon fixation rate in the presence (+) or absence (–) of 6-ethoxyzolamide (EZ) during the period of 10:00 AM to 15:00 PM **(b)**, and the corresponding relative inhibition induced by EZ **(c)**. LC and HC represent low CO_2 (400 μatm) and high CO_2 (1000 μatm), respectively. The vertical lines indicate the standard deviation of the means (n = 3).

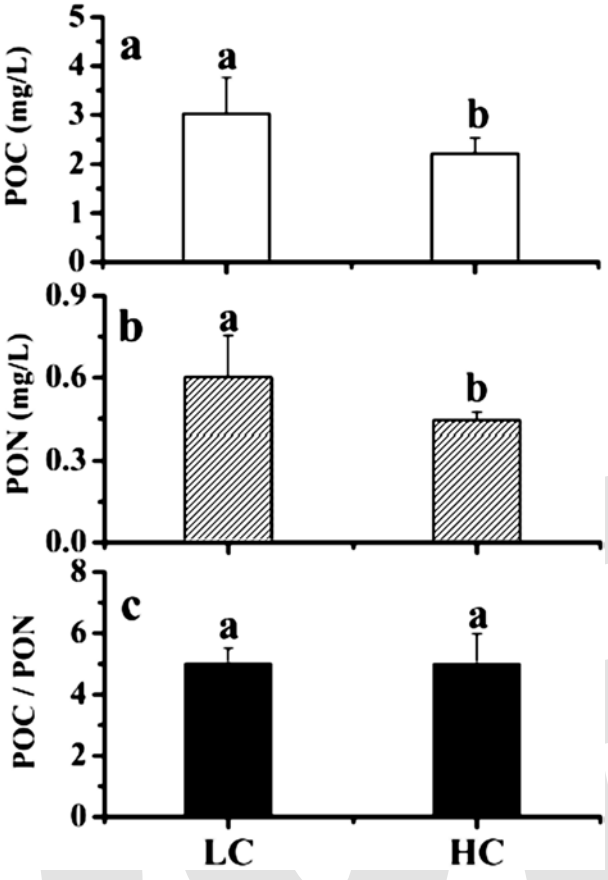

Fig. 8. POC (a), PON (b) content per unit water and POC/PON ratio (c) of mesocosm communities under low CO2 (LC, 400 μatm) and high CO2 (HC, 1000 μatm) conditions after one day diurnal variation experiment acclimation. The vertical lines indicate the standard deviation of the means (n = 3). Different superscript letters represent a significant difference ($p < 0.05$).

Discussion

This study investigated the diurnal photosynthetic performance of phytoplankton during the exponential growth phase in a mesocosm under two levels of CO_2 concentration. Elevated CO_2 concentration accelerated the relative electron transport rate of phytoplankton and increased the efficiency of PSII open reaction centers to capture light. However, increased light from the PSII reaction center was dissipated by other metabolic pathways. These dissipations helped to retard the photoinhibition rate in the morning and accelerate the recovery rate in the afternoon. Accordingly, less light was utilized by photosynthetic carbon fixation in HC mesocosms. The cellular POC and PON content were also significantly lower, suggesting that elevated CO_2 will significantly weaken the capacity of carbon and nitrogen assimilation and subsequent transport from the surface into deep sea by phytoplankton.

Metabolic regulations in response to fluctuating sunlight during daytime play a key role in phytoplankton survival under low or excessive levels of solar irradiance. Any inefficiency in adjustment of intracellular metabolic pathways under rapidly changing light will significantly limit photosynthesis and productivity. In the present study, cells grown under elevated CO_2 levels contained less chlorophyll and had a lower rate of carbon fixation per unit Chl-*a*. While diurnal seawater Chl-*a* contents could be attributed to increased cell density, some of the captured light must have been

diverted to photoprotective pathways, resulting in a less efficient use of the carbon fixed by cells in HC treatments. The dynamic balance among light capture and utilization and/or dissipation can be disturbed by environmental stressors (Hüner et al. 1998). It appeared that elevated CO_2 concentration in the current study resulted in cellular stress among phytoplankton during the exponential growth phase. Previous studies have demonstrated that both an elevation in CO_2 and a corresponding decrease in pH can impact the physiological performance of phytoplankton (Wu et al. 2010; Gao et al. 2012a) and some macroalgae (Xu et al. 2012). In the present study, elevated CO_2 significantly decreased photosynthetic carbon fixation and daytime primary productivity, ultimately resulting in a three-day lag of phytoplankton bloom in HC mesocosms compared with LC ones (Data not shown).

A change in the functional cross-sectional area of PSII (σPSII) provides an additional viewpoint to characterize the physiological response of phytoplankton to environmental changes (Sosik et al. 2002; Suggett et al. 2006; Six et al. 2008). Cells in the water column are able to adjust σPSII for better photoacclimation (Vassiliev et al. 1994). In the current study, the σPSII decreased during the approach to noon to reduce light harvesting, and increased towards the end of the day to capture more light for carbon fixation. These adjustments in σPSII followed a similar pattern in both the LC and HC treatments. However, lower values in HC treatments during late afternoon implied a down-regulation of σPSII induced by a high level of CO_2. Relatively higher rETR and quantum yields observed under elevated CO_2 concentration suggested a higher efficiency of electron flow, reflecting an extra energy cost associated with acidification (Xu et al. 2012). Elevated CO_2 is documented to down-regulate carbon concentration mechanisms (CCMs) (Rost et al. 2003; Yang et al. 2012), and the additional energy saved from this process may intensify photoinhibition under high levels of sunlight (Gao et al. 2012b). Under such circumstances, a decrease in σPSII among phytoplankton in HC treatment could be a strategy to reduce upstream light capture to prevent potential solar irradiance damage.

The decreased carbon fixation rate and increased fluorescence (namely F_v / F_m, F_v' / F_m', and rETR) observed under elevated CO_2 concentrations could be explained by an uncoupling between these processes. It is well recognized that pigment composition, fluorescence, and carbon fixation are not always correlated with each other (Fryer et al. 1998; Kate & Johnson 2000). In certain situations, cells do not choose carbon fixation as the only major sink for accumulated electrons but divert the photosynthetically captured energy to other pathways, such as heat dissipation (NPQ), nutrient uptake and photorespiration (Ort & Baker 2002; Baklouti et al. 2006; Murchie & Niyogi 2011; Su et al. 2012). It is worth mentioning that photorespiration, which shares an enzyme (RuBisCO, ribulose-1,5-bisphosphate carboxylase / oxygenase) with carbon fixation, plays a crucial role in photoprotection owing to its high demand for energy. The increased availability of electrons as a result of photorespiration may have contributed to higher effective quantum efficiency in the HC mesocosms (Raven et al. 2014).

Carbonic anhydrase, which is responsible for the hydration / rehydration of HCO_3^- / CO_2 within phytoplankton cells, plays an important role in CCMs and carbon acquisition (Rost et al. 2003). When CA activity is inhibited by EZ, the instantaneous conversion between HCO_3^- and CO_2 by CA is blocked, and the carbon fixation rate is therefore primarily determined by exogenous CO_2. The cumulative amount of carbon fixed during the day following treatment with EZ was significantly lower in the HC treatment than that in the LC treatment. This observation may indicate that cells in the HC treatment assimilate less aqueous CO_2 from their surroundings than those in the LC treatment. A lower level of CO_2 assimilation may be attributed to changes in the plasma membrane or to the down regulation of active CO_2 uptake. This CO_2 limitation will further decrease the diurnal solar light utilization efficiency due to substrate shortage of RuBisCO. Decreases in pH of seawater also decrease intracellular pH (Anning et al. 1996; Suffrian et al. 2011), and CA may play a role in the removal of excess H^+ in HC treatment. Generally, a larger degree of carbon fixation reduction by the inhibition of CA in HC treatment could be explained by a decreased capacity to buffer against the decrease in cellular pH and a reduction in the endogenous supply of CO_2.

This study was performed in a eutrophic bay, where initial nitrate and ammonium concentration was approximately as high as 30 μM and 53 μM, respectively. The elevated CO_2 concentration and subsequently altered seawater chemistry resulted in changes in diurnal photosynthetic performance, with cells in the HC treatment decreasing solar light utilization efficiency for carbon fixation. Although the high nutrient level can relieve photodamage (Helbling et al. 2010) and maintain a relatively higher level of photochemical efficiency (Moore et al. 2005), elevated CO_2 concentration can still influence photosynthesis in eutrophic seawater. Therefore, phytoplankton in eutrophic sea-

water should not be overlooked when considering the effects of ocean acidification on the primary productivity in different ocean regions.

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