Fundam. Appl. Limnol. Vol. 180/4, 279–290
Stuttgart, May 2012

Article

Growth and photosynthesis of a diatom grown under elevated CO_2 in the presence of solar UV radiation

Xiaojuan Wu[1, §], Guang Gao[1, §], Mario Giordano[2] and **Kunshan Gao[1, *]**

With 2 figures, 2 tables and 2 appendices

Abstract: The combination of elevated CO_2 and the increased acidity in surface oceans is likely to have an impact on photosynthesis via its effects on inorganic carbon speciation and on the overall energetics of phytoplankton. Exposure to UV radiation (UVR) may also have a role in the response to elevated CO_2 and acidification, due to the fact that UVR may variously impact on photosynthesis and because of the energy demand of UVR defense. The cell may gain energy by down-regulating the CO_2 concentrating mechanism, which may lead to a greater ability to cope with UVR and/or higher growth rates. In order to clarify the interplay of cell responses to increasing CO_2 and UVR, we investigated the photosynthetic response of the marine and estuarine diatom *Cylindrotheca closterium* f. *minutissima* cultured at either 390 (ambient) or 800 (elevated) ppmv CO_2, while exposed to solar radiation with or without UVR (UVR, 280–400 nm). After a 6 day acclimation period, the growth rate of cells was little affected by elevated CO_2 and no obvious correlation with the radiation dose (for both PAR and PAR + UV treatments) could be detected. However, the relative electron transport rate was reduced and was more sensitive to UVR in cells maintained at elevated CO_2 as compared to cells cultured at ambient CO_2. The CO_2 concentrating mechanism was down regulated at 800 ppmv CO_2, but was apparently not completely switched off. These data are discussed with respect to their significance in the context of global climate change.

Key words: climate changes, CCM, CO_2, photosynthesis, sunlight, UV.

1 Introduction

In order to make accurate predictions on what is likely to happen to primary producers in the oceans under the "business as usual" scenario (Brewer 1997), the main biological processes that mediate the interplay of rising CO_2, decreasing pH and solar radiation in phytoplankton must be identified, characterized and possibly quantified. The impact of the shoaling of the mixed layer should also be considered, since it will lead to decreased nutrient availability (included inorganic carbon), lower O_2 solubility and increased PAR and UVR exposure (Finkel et al. 2010, Raven et al. 2011), with potentially profound repercussions on inorganic carbon fixation and primary productivity (Beardall et al. 2009, Raven et al. 2011, 2012). This is no easy task, since the effects of these environmental changes are difficult to separate in the real world. For example, the concomitant changes of grazing pressure, potentially due to the impact of higher temperature on grazers (e.g. Feuchtmayr et al. 2010), may substantially complicate the matter.

Authors' addresses:

[1] State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, 361005, China.

[2] Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy.

* Corresponding author; ksgao@xmu.edu.cn

§ Both authors contributed equally to this work

www.schweizerbart.de
DOI: 10.1127/1863-9135/2012/0299
1863-9135/12/0299 $ 3.00

High CO_2 concentration, acidification and stronger water column stratification all have a direct impact on the inorganic carbon speciation, the availability of usable inorganic carbon species and the ability of algae to fix them (Finkel et al. 2010, Raven et al. 2011). Thus, a pivotal role of CO_2 concentrating mechanisms (CCMs, Giordano et al. 2005) in the response to these global changes can be envisaged. It is reasonable to assume that most marine phytoplankters, including diatoms, are capable of increasing the CO_2 concentration in the proximity of rubisco in an energy dependent manner (Giordano et al. 2005, Raven et al. 2005, 2008a, 2008b). The modulation of CCMs is highly dependent on the availability of inorganic C; however, it is not an on/off system and CCMs may have different degrees of plasticity and effectiveness in different organisms and under different conditions (e.g. Beardall & Giordano 2002). Moreover, the presence of an active CCM can enhance nutrient use efficiency (not necessarily by the same extent for all nutrients and in all organisms); for this reason, changes in CCM activity may cause shifts of nutrient limitation and species competitive performance (Beardall & Giordano 2002, Raven et al. 2011, 2012). Some organisms, although in possession of active CCMs, show increased growth rates in the presence of elevated CO_2 (e.g. Riebesell et al. 1993, Ratti et al. 2007, Wu et al. 2010). The physiological bases of this have not been thoroughly investigated, but they may be related to the fact that, at low CO_2, CCMs could ameliorate CO_2 limitation without fully saturating rubisco or to the fact that, when CCMs are down regulated or switched off, a quota of the energy previously invested in their operation and maintenance can be diverted to growth (see Beardall & Giordano 2002 and Raven et al. 2011 for a thorough discussion on this topic). It should also be noticed that, since the energetic cost of running a CCM is not trivial, when light is limiting, algae often have inorganic carbon transport systems with lower capacity and affinity for the carbon species transported (Beardall & Giordano 2002 and references therein). Algae may therefore benefit more from elevated CO_2 when subjected to subsaturating irradiances, although a link between CO_2 uptake and fixation is not an absolute requirement (Tchernov et al. 2003). Reversely, the energy made available from CCM down-regulation would be of little or no use if light is available in large supply. Even the availability of UVR may play a role in CCM, since UV-A appears to stimulate the total activity of carbonic anhydrase measured in whole cells (Wu & Gao 2009). Both UV-A and UV-B have been shown to play a double-edged sword like role, with either inhibitory or stimulatory effects on CCMs depending on the radiation levels (Beardall et al. 2002, Wu & Gao 2009). UVR may affect the CCM by non-specifically damaging DNA and consequently altering the machinery for gene transcription; it could also directly damage membrane proteins involved in the CCM. On the other hand, the UV-induced damage of cell membranes may increase CO_2 permeability, although the probability that such an occurrence effectively compensate for the negative effect of UV damage is extremely small (Sobrino et al. 2008 and references therein). However, a study by Beardall et al (2002) showed that inorganic carbon acquisition was not affected by short-term exposure to UVR. It has also been suggested, that cycling of inorganic carbon associated with the CCM may be involved in the dissipation of excess light energy to reduce photodamage and may thus be uncoupled from CO_2 fixation (Tchernov et al. 2003).

The rise of CO_2 may therefore have a different impact on the growth of organisms with active CCMs, depending on the PAR and UVR irradiance they are exposed to.

To increase understanding of these complex interactions, we investigated the growth and photosynthetic response of the diatom *Cylindrotheca closterium* f. *minutissima* to UVR and elevated CO_2 concentration (and the associated medium acidification) and tested the hypothesis that it is mediated, at least to some extent, by the modulation of the CCMs.

In freshwater and estuarine environments, especially if productive, microalgae can experience pH variation up to 2 units per day and 4 units over a year. The availability of CO_2 will change accordingly (Maberly 1996, Joint et al. 2009). For this reason, the study of the responses of marine algae to changes in CO_2 and pH may also be relevant to understand the physiological processes in freshwater and estuarine algae to diel and seasonal changes in pH and CO_2 (and vice versa). Diatoms are especially interesting, because they are often important components of freshwater and estuarine waters and frequently, the same genus and sometimes the same species can be found in both the ocean and habitats with lower salinity. The species used for this study, *Cylindrotheca closterium* (although not necessarily the genotype we used), has a great ability to cope with low salinity and it is a frequent inhabitant of estuarine waters (Underwood & Provot 2000, Apoya-Horton et al. 2006). *C. closterium* may therefore also represent a good model to study algae inhabiting habitats with lower salinity.

2 Materials and methods

2.1 Indoor cultures

The marine diatom *Cylindrotheca closterium* f. *minutissima* (strain no. NC 060530) was obtained from the Institute of Oceanography (Qingdao), Chinese Academy of Sciences. The cells were grown in 0.5 L conical flasks with f/2 medium (Guillard & Ryther 1962), at 20 °C, a photon flux density (PFD) of 100 µmol photons m^{-2} s^{-1} (12 L:12 D) provided by cool-white fluorescent lights. All cultures were aerated with ambient air filtered through 0.2 µm filters (Millex-RC, Millipore, USA), at a flow rate of about 1 L min^{-1}. The cells were maintained in their exponential growth phase by partially renewing the medium every 24 hours to restore the cell density of the previous day. Indoor cultures were used as inoculants for the outdoor cultures.

2.2 Outdoor cultures

The outdoor cultures were inoculated with cells from the indoor cultures and each experiment was run for 13 days. The same growth medium was used and the same dilution regime was applied as for the indoor cultures. The cultures were kept in quartz tubes (∅ 59 mm, 350 mm in length, 0.5 L), ensuring that cells were exposed to the full spectrum of solar radiation, UV included (PAB treatment). The PAR only treatment was obtained by filtering out the radiation below 395 nm with a cutoff foil (Ultraphan UV Opak, Digefra, Munich, Germany). A circulating water bath was used for temperature control (18.5–21.0 °C).

All the cultures were continuously aerated with 0.2 µm-filtered (Millex-RC, Millipore, USA) air containing either 390 (ambient) or 800 (elevated) ppmv CO_2, at a flow rate of 1 L min^{-1}. The elevated CO_2 concentration mimicked the atmospheric CO_2 predicted for the end of this century, under the A1F1 scenario (Brewer 1997). The CO_2-enriched air was obtained by pumping pure CO_2 and ambient air into air-tight bags; the bags were then vigorously shaken to ensure that the gas mixture was homogeneous; this gas mixture was bubbled inside the culture flasks. The CO_2 concentration was monitored with an infrared gas analyzer (LCA-4, Analytical Development Co, UK).

2.3 Radiation measurements

Incident solar radiation was continuously monitored using a broadband ELDONET filter radiometer (Real Time Computer, Möhrendorf, Germany; Häder et al. 1999). This instrument concomitantly measures three wavebands, 280–315 nm (UV-B), 315–400 nm (UV-A) and 400–700 nm (PAR). The irradiance was measured every second and then integrated over a minute by the computer. The irradiance attenuation exerted by the quartz tube was of about 8 %. A further 4 % reduction in irradiance reaching the cultures occurred in the presence of the cut off filters, i.e. the cells subjected to the PAR only treatment received about 4 % less photons m^{-2} s^{-1} than the cells incubated in the unwrapped tubes in water (Gao et al. 2007).

2.4 Measurements of growth rate

Samples were collected daily in the morning, before and after diluting the cultures. The number of cells was determined by direct counts using an improved Neubauer haemocytometer (XB-K-25, Qiu Jing, Shanghai, China). The specific growth rate (µ) was calculated as follows: $\mu = \ln (C_2 - C_1) / (t_2 - t_1)$, where C_2 and C_1 are the cell concentrations (cells ml^{-1}) at time t_2 (before dilution) and t_1 (after dilution), respectively.

2.5 Measurement of pH and CO_2 and estimation of dissolved inorganic carbon (DIC)

The medium pH was measured at 14:00 with a pH-meter (FE20, Mettler Toledo, Switzerland) that was calibrated daily with standard NBS buffers (Merck, Germany).

The calculation on the inorganic carbon speciation was performed conducted using the dissociation constants in Goyet and Poisson (1989), as described by Ratti et al. (2007)

2.6 Measurement of PSII quantum yield and of electron transport rate

The effective quantum yield of PSII is given by the following equation:

$$\Phi PS = \Delta F / Fm' = (Fm' - Ft) / Fm' \text{ (Genty et al. 1989),}$$

where Fm' is the instant maximal fluorescence and Ft is the steady-state fluorescence of light-adapted cells. Fm' and Ft were determined using a portable pulse amplitude modulated fluorimeter (PAM-WATER-ED, Walz, Effeltrich, Germany). The saturating pulse and actinic light were set at 5000 and 150 µmol m^{-2} s^{-1}, respectively. The measuring beam was 0.3 µmol m^{-2} s^{-1}. The measurements of ΦPS were conducted at least every two hour, between 8:00 and 18:00, at the beginning (day 2), in the middle (day 6) and at the end (day 12) of the experiment.

The relative electron transport rate (rETR) was calculated according to Schreiber et al. (1986) from the following equation:

$$rETR = (\Delta F / Fm') \cdot E_{PAR} \cdot 0.75,$$

where ΔF/Fm' is the effective quantum yield, E_{PAR} is the photon flux density of actinic light, 0.75 is a coefficient which takes into account that roughly 75 % of all absorbed quanta is believed to reach PS II in diatoms (Johnsen & Sakshaug 2007). Other photosynthetic parameters were calculated from the equation by Jassby & Platt (1976):

$$rETR = rETR_{max} \cdot \tanh (\alpha \cdot E / r\,ETR_{max}),$$
$$E_k = rETR_{max} / \alpha,$$

In this equation, $rETR_{max}$ is the maximum relative electron transport rate, α is the relative electron transfer efficiency, E is the irradiance, and E_k is the irradiance at which the onset of light saturation occurs. The determination of rETR was performed at 8:00 at the beginning (day 1) and the mid of the experiment (day 9).

2.7 Determination of photosynthetic O_2 evolution as a function of DIC concentration

To determine the relationship between photosynthesis and DIC concentration in algae that were fully acclimated to the outdoor growth regime, cells (PAR treatment only) were harvested towards the end of the experiment (day 9 and 13) by centrifugation at 4,000 g for 5 min, washed twice in DIC-free sterile growth medium and resuspended in the same medium to a final cell density of 1×10^6 cells ml^{-1}.

The DIC-free medium was prepared by acidifying the culture medium with HCl to pH 3.0, and bubbling with N_2 to remove all the inorganic carbon. The pH was subsequently brought back to 8.2 by the addition of a pre-calculated amount of Bis-Trispropane powder and NaOH pellet (rinsed in HCl to minimize the amount of Ci on their surface), while bubbling with N_2. Net O_2 evolution was measured in the DIC-free medium described above, under continuous stirring, with a Clark-type oxygen electrode (Oxylab, Hansatech Instruments, King's Lynn, UK), placed in a jacketed reaction chamber in which water was circulated at 20 °C.

The cells were exposed to a PAR irradiance of 400 µmol photons m^{-1} s^{-1} (sufficient to saturate photosynthesis) and allowed to photosynthesize until no net O_2 evolution was measured for 5 sequential minutes. In all cases, this condition was attained within 20 min. The photosynthetic rates were measured following incremental additions of $NaHCO_3$ solution. In order to derive the photosynthetic parameters, the data were fitted to a Michaelis-Menten equation:

$$P = P_{max} [DIC] / (K_{0.5}(DIC) + [DIC]),$$

where P is the net photosynthetic rate at a given DIC concentration, P_{max} is the DIC- and light saturated photosynthetic rate, $K_{0.5}$(DIC) is the DIC concentration required for the half of P_{max}.

2.8 Determination of chlorophyll-*a* content

Chlorophyll-*a* was determined for the same cells for which O_2 evolution was measured. The cells were centrifuged at 4,000 g for 5 min. The pellet was then extracted overnight in 100 % methanol. The concentration of chlorophyll-*a* in the supernatant was determined spectrophotometrically according to Porra (2002).

2.9 Statistics

The statistical significance of differences was assessed by a one-way analysis of variance (ANOVA), with a Tukey's post-hoc test for pairwise comparison ($p < 0.05$). Except rETR all measurements were tested in triplicate (i.e. three separate cultures). The measurements on rETR were obtained in duplicate and the results were compared by calculating the average and half range.

3 Results

3.1 Daily radiation dose

The pattern of solar radiation over the 13-day period of the experiment is shown in Figure 1 A. The daily dose of solar PAR ranged from 0.7 to 5.7 MJ m^{-2}, that of UV-A and UV-B ranged from 0.13 to 0.88 and from 0.0032 to 0.026 MJ m^{-2}, respectively (Appendix 1).

3.2 Growth pH

Elevated CO_2 (800 ppmv) caused the pH to decrease significantly ($p < 0.05$) as compared to that at ambient CO_2 (390 ppmv). The pH change was in the order of 0.3–0.4 units (Fig. 1 B), with an average value of about 8.4 ± 0.1 at ambient CO_2 and 8.0 ± 0.1 at elevated CO_2. The presence of UVR did not have an effect on the pH of the growth medium, with the exception of day 12, when the pH was significantly lower in the PAB treatment.

3.3 Growth rates

The specific growth rates (µ) are shown in Fig. 1. In most cases, at both ambient and elevated CO_2, no difference was measured between the growth rate of cells exposed to the PAR-only treatment and those exposed to the full solar spectrum (PAB treatment). For cells grown at ambient CO_2, a significant difference between the growth rate in the absence and in the presence of UVR could be detected only on day 4 ($p = 0.006$) and day 6 ($p = 0.002$). For cells exposed to elevated CO_2, a slight but significant difference was observed on day 2 ($p = 0.004$).

In general, at the beginning of the experiment, cells grown at elevated CO_2 had lower mean growth rates than the cells grown at ambient CO_2, (day 1 to 5, $p = 0.02$, 0.0007, 0.03, 0.04, 0.007, respectively). For the PAB treatment, the difference in µ between ambient and elevated CO_2 levels was statistically significant only on day 2 ($p = 0.001$). On day 6, the cells grown at elevated CO_2 caught up with the cells grown at ambient CO_2. Subsequently, no statistical difference could be detected between the growth rates in the two CO_2 regimes (Fig. 1c).

3.4 Effective quantum yield of photosynthesis

Diel changes of the effective quantum yield (ΦPSII) of *C. closterium* f. *minutissima* were monitored on day 2, 6 and 12 (Fig. 2). During all these days, ΦPSII (Fig. 2 A, B, C) had a reverse relationship with the solar radiation (Fig. 2 D, E, F): On day 6 the solar radiation was more intense than on the cloudy days 2 and 12, and the quantum yield was most strongly depressed from 10:00 am to 3:00 pm.

It is also noteworthy that the responses in quantum yield on day 2 and 12 were rather different, although the doses were comparable (Fig. 1 A). This difference between day 2 and day 12 was probably due to acclimation, which is confirmed by the overall trend of the quantum yield of photosynthesis: on day 2, in agreement with the growth rates, cells grown at elevated CO_2 had a lower quantum yield than cells grown at ambient CO_2 at noon ($p < 0.0001$ for both PAB and PAR). On day 6, the difference in the quantum yield

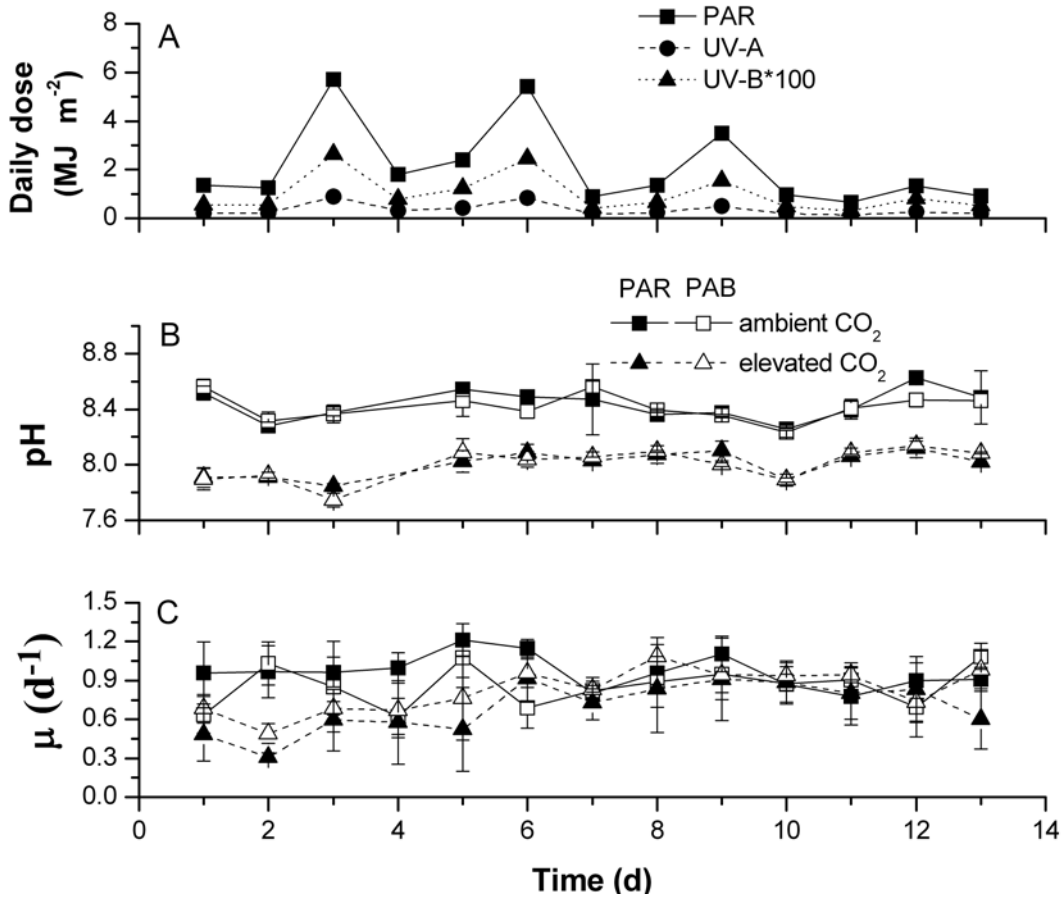

Fig. 1. Radiation doses **(A)**, pH **(B)** and specific growth rates **(C)** in *Cylindrotheca closterium* f. *minutissima* over the 13 days of the outdoor experiment (March 22 – April 3, 2008). The pH and the specific growth rates were measured in the presence of either ambient (390 ppmv) or elevated pCO_2 (800 ppmv), under solar radiation with (PAB) or without UVR (PAR). The results are shown as the mean ± SD (n = 3).

between cells grown under the elevated and ambient CO_2 regime was not significant ($p = 0.05$ under PAR, $p = 0.67$ under PAB). At both CO_2 concentrations, UVR caused a reduction of the quantum yield around mid day ($p < 0.0001$ at ambient CO_2 and at elevated CO_2), (Fig. 2 B). At day 12, the diurnal variation of quantum yield was not related to CO_2 and radiation (Fig. 2 C).

3.5 Relative electron transport rates (rETR)

The response of rETR to irradiance (rETR vs E curve) was measured on a cloudy day (day 1, with daily radiation doses comparable to those of day 2 and 12 used above) and a sunny day (day 9, with a dose similar to that of day 6, Fig. 1 A, Appendix 1). The main parameters of these curves are shown in Table 1. After 9 days, the $rETR_{max}$ increased when compared with $rETR_{max}$ recorded on day 1 under all conditions ($p = 0.04$ at ambient CO_2 under PAB, $p = 0.005$ at elevated CO_2 under PAR), though the differences in rETR of cells grown at ambient CO_2 in the PAR only treatment ($p = 0.06$) and at elevated CO_2 in the presence of UVR ($p = 0.34$) were not significant. Except for cells grown at ambient CO_2 on day 1 ($p = 0.12$), $rETR_{max}$ was lower in the presence of UVR (PAB) than in the PAR only treatment. Both on day1 and day 9, the inhibition of rETR induced by UVR was 13–20 % higher in the cells grown at the elevated CO_2 than in the cells grown at ambient CO_2.

The affinity for photons of the electron transport, as determined from the slope of linear portion of the rETR vs E curve (α), did not show variation between day 1 and 9.

The irradiance at which the onset of light saturation occurred, E_k, increased after 9 days, with comparable values for all conditions, except for the cells cultured at elevated CO_2 under PAR.

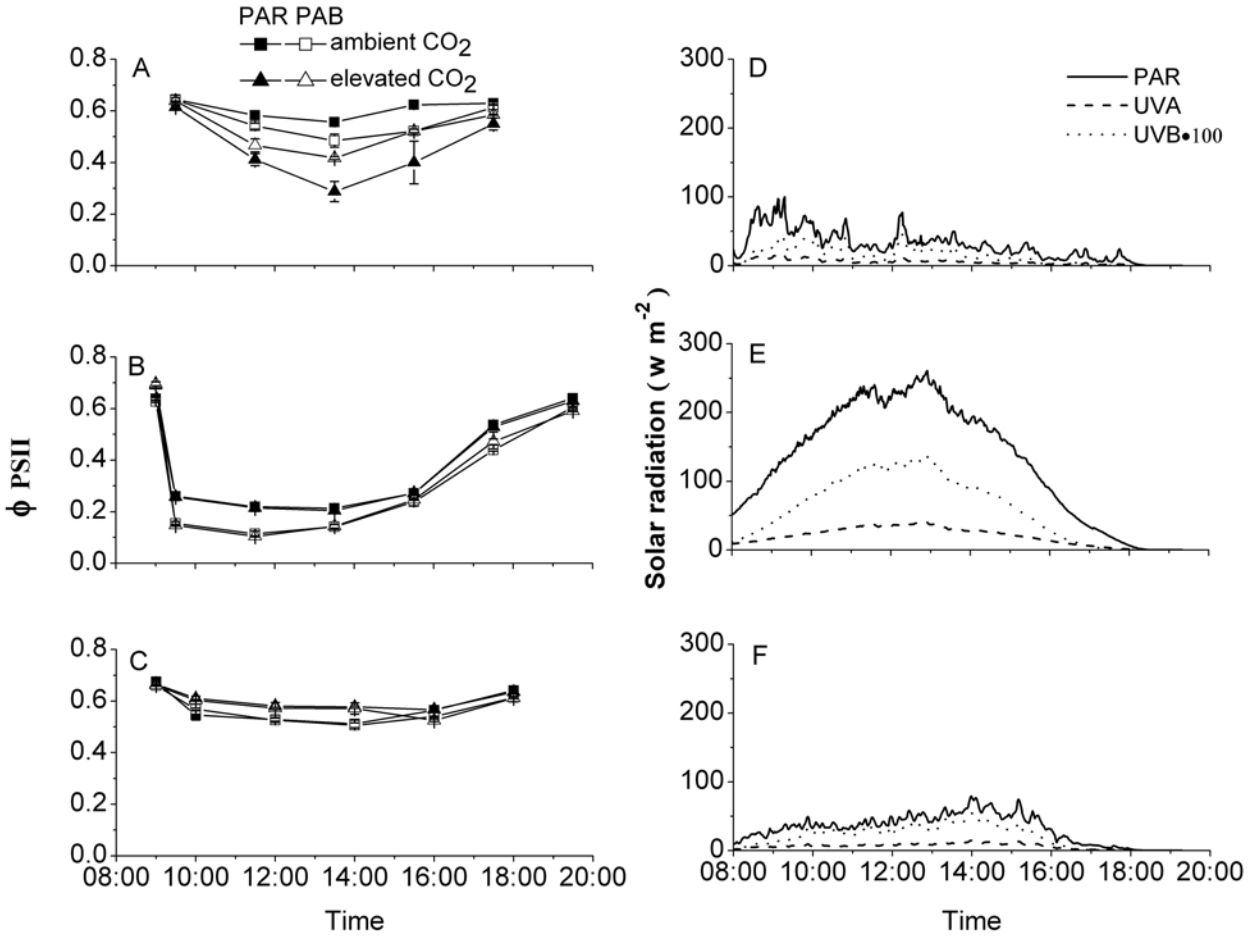

Fig. 2. Diurnal changes in the effective quantum yield of *C. closterium* f. *minutissima* on day 2 **(A)**, day 6 **(B)** and day 12 **(C)** of the same outdoor experiment. The cells were aerated with either ambient (390 ppmv) or elevated pCO_2 (800 ppmv) and exposed to solar radiation, in the presence (PAR) or absence of UV filters (PAB). The daily doses of PAR were 1.258 on day 2 (cloudy), 5.417 on day 6 (sunny) and 1.327 MJ m^{-2} on day 12 (cloudy), respectively. The results are shown as the mean ± SD (n = 3).

Table 1. Photosynthetic parameters derived from the relative electron transport curve in response to irradiance (rETR/E) for *C. closterium* f. *minutissima* grown at either ambient (390 ppmv) or elevated pCO_2 (800 ppmv), in the presence of full solar spectrum (PAB: PAR + UVA + UVB) or without UVR (PAR: PAR alone). The measurements were carried out on day 1 and 9 (solar radiation doses are shown in Fig. 1). $rETR_{max}$, maximum relative electron transfer rate; α, slope of the initial linear part of the rETR/E curve; E_k, irradiance at which the onset of light saturation occurs (μmol·m^{-2}·s^{-1}). Data are shown as the mean ± half range from duplicate cultures. Different superscripts indicate a significant difference ($p < 0.05$) among the treatments on day1 or day 9. Asterisks indicate a significant difference between day 1 and day 9 within one treatment.

Time	Growth conditions		Photosynthetic parameters		
	pCO_2 (ppmv)	Radiation treatment	$rETR_{max}$ (μmol m^{-2}·s^{-1})	α	E_k (μmol m^{-2}·s^{-1})
Day 1	390	PAR	261 ± 9.48^{a}	0.33 ± 0.01^{a}	793 ± 6.63^{a}
		PAB	228 ± 7.82^{ab}	0.32 ± 0.01^{a}	714 ± 8.25^{b}
	800	PAR	231 ± 3.06^{ab}	0.31 ± 0.02^{a}	740 ± 46.06^{a,b}
		PAB	199 ± 3.42^{c}	0.30 ± 0.00^{a}	675 ± 6.51^{b}
Day 9	390	PAR	297 ± 0.74^{a}	0.30 ± 0.00^{a}	989 ± 0.83^{a,*}
		PAB	273 ± 5.20^{b,*}	0.28 ± 0.00^{a}	977 ± 20.18^{a,*}
	800	PAR	275 ± 0.55^{b,*}	0.30 ± 0.01^{a}	927 ± 27.01^{ab}
		PAB	218 ± 14.40^{c}	0.26 ± 0.01^{a}	832 ± 21.17^{b,*}

Table 2. Photosynthetic parameters derived from the photosynthesis vs DIC curve (P/C curve) for *C. closterium* f. *minutissima* grown at either ambient (390 ppmv) or elevated pCO_2 (800 ppmv), under PAR conditions. The P/C curves were determined at a saturating photon flux density of 400 µmol $m^{-2}s^{-1}$, in 20 mM Bis-Trispropane buffered Ci-free reaction medium (pH 8.2), on day 10 for ambient-CO_2 and on day 13 for elevated CO_2. P_{max}, DIC- and light-saturated photosynthetic rates; $K_{1/2}$, Ci concentration at which half P_{max} occurs; β, slope of the linear part of the P/C curve. Data are shown as mean ± SD (n = 3). Different superscripts indicate a significant difference ($p < 0.05$).

	Growth pCO_2 (ppmv)	
	390	800
P_{max} (µmol O_2·(mg chla)$^{-1}$·h^{-1})	640 ± 25.5^a	229 ± 12.7^b
$K_{1/2}$ (DIC) (µM)	112 ± 0.03^a	265 ± 0.07^b
$K_{1/2}$ (CO_2) (µM)	0.70 ± 0.00^a	1.60 ± 0.00^b
$K_{1/2}$ (HCO_3^-) (µM)	98 ± 0.02^a	240 ± 0.06^b
β (µmol O_2·(mg chla)$^{-1}$·h^{-1})/µM DIC)	1065 ± 165.4^a	321 ± 101^b

3.6 Photosynthetic O_2 evolution as a function of DIC concentration (P vs DIC curves) and chlorophyll content

The response of photosynthetic O_2 evolution to external DIC levels was determined under the PAR only treatment, for cells grown at elevated and ambient CO_2 (Table 2). These results show a substantially higher (2.8-fold) maximum O_2 evolution rate for cells grown at ambient CO_2. Both the half-saturation constant, $K_{0.5}$(DIC), and the slope of the linear portion of the P vs DIC curve (β) indicate that the affinity for DIC was 2.4 ($K_{0.5}$(DIC)) to 3.3-fold (β), slope of the initial linear portion of the curve) higher for the cells grown at ambient CO_2 than for the cells grown at elevated CO_2.

The chlorophyll cell content was unaffected by the pCO_2 treatment and was 0.16 ± 0.01 pg $cell^{-1}$ and 0.18 ± 0.02 pg $cell^{-1}$ for cells cultured at ambient and elevated pCO_2, respectively.

4 Discussion

In this work, we attempted to step further from typical laboratory studies, by including the impact of natural solar radiation, with its UV component. The outcome of our study can be summarized in three main points:

1. The elevated CO_2 did not stimulate the growth rate of *C. closterium* f. *minutissima* acclimated to growth under solar radiation, regardless of the presence or absence of UVR.
2. As expected, a down regulation of the CCM was observed at 800 ppmv CO_2. The data on the photosynthetic affinity for CO_2, in combination with the literature information on the affinity for CO_2 of diatoms' rubisco (see below), suggest that this down-regulation was not associated with a complete switch-off of the CCM.
3. Elevated pCO_2 caused a reduction of $rETR_{max}$ both before and after acclimation. The decline of $rETR_{max}$ was more pronounced at elevated CO_2 than at ambient CO_2 ($p = 0.04$ on day1, $p = 0.03$ on day 9), suggesting that the photosynthesis of cells grown at elevated CO_2 was more sensitive to UVR than that of cells grown at ambient CO_2. These effects were not coupled to an equivalent response in terms of growth.

These conclusions certainly need to be tested on a larger number of species, for a longer time and under field conditions. Nevertheless they provide some interesting information on the way to a better understanding of the interactive impact of changes in CO_2, pH and UV.

The growth rate of *C. closterium* was not affected by the change of light regimes, if the cultures were maintained at ambient CO_2 under PAR alone. In the presence of UVR, the cells grown at ambient CO_2 had a more unpredictable behavior, although no clear correlation between the growth rate and the UV dose could be detected: for instance on days 1 and 2 and 4 and 5, the growth rates of *C. closterium* were higher on day 2 ($p = 0.01$) and day 5 ($p = 0.005$) compared with day 1 and day 4, although radiation doses of these days were very similar (Figs 1 A and 2 C). At elevated CO_2, regardless of the radiation treatment, the cells required about 5 days to reach growth rates comparable to those at ambient CO_2. Subsequently, no differences in growth rates were observed between the two CO_2 treatments. Overall, these results suggest that the impact of UVR on growth of *C. closterium* is small and that the mechanisms by which UVR affects growth are not easily described by first order principles. It has been proposed that the presence of UVR would reduce the alkalinization of the water by depressing photosynthesis, potentially exacerbating ocean acidification

(Gao & Zheng 2009). In this study, no consistent difference could be observed between the pH values in the presence and in the absence of UVR, Under fluctuating or reduced levels of solar radiation, UV-A may lead to positive effects on photosynthesis by stimulating photosynthetic carbon fixation (Gao et al. 2007), which would act to set off the UV-B induced harms, therefore, leading to invisible or balanced effects on growth rate (Gao et al. 2007, Xu & Gao 2010). UV-A induced enhancement and UV-B-induced inhibition of growth of a red tide alga was more pronounced under conditions when UVR led to acidification (Chen & Gao 2011). Therefore, balanced effects of UV-A and UV-B might obscure the response in the growth rate of *C. closterium* observed in this study. This conclusion should be investigated in the future since we could not distinguish the effects of UV-A from that of UV-B.

Another conclusion that can be derived from the data set is that long term primary production (in terms of growth) and short term photosynthesis (in terms of electron transfer) are differently affected by UVR. While growth responded little to the radiation regime, the short-term relative electron transport rate and $E_{k,}$ were significantly influenced by UVR, especially at elevated CO_2 (Table 1). These results are in general agreement with those of Sobrino et al. (2009), who showed that, in lakes, elevated CO_2 increased primary production, but made cell photosynthesis more susceptible to UVR. Similar results were obtained for the marine diatom *Thalassiosira pseudonana*, under laboratory conditions (Sobrino et al. 2008). It has been proposed that the greater sensitivity of photosynthesis under elevated CO_2 is associated with an overall down-regulation of the photosynthetic machinery and a concomitant decrease or lower activation state of the repair of UV-dependent damages (Lesser 1996, Litchman et al. 2002, Sobrino et al. 2009 and references therein). It is also worthwhile to mention that, under acidified conditions in the medium, the sensitivity of cells grown under elevated-CO_2 to UV-B and UV-A may show significant discrepancies (Chen & Gao 2011). This different response under UV-A and UV-B may explain why no effects on the growth rate are visible. On the other hand, such combined effects of PAR, UV-A, UV-B and acidification of the medium will rely on the dose of solar radiation (Gao et al. 2012) as well as on nutrients availability (Beardall et al. 2009). Obviously, further investigations on this topic are required to elucidate the involved mechanisms.

The debate on the impact that a CO_2 increase will have on phytoplankton growth and on biomass standing crop is lively and unresolved (Rotty 1980, Hein & Sand-Jensen 1997, Clark & Flynn 2000, Beardall & Giordano 2002, Beardall & Raven 2004, Schippers et al. 2004, Delille et al. 2005, Riebesell et al. 2007, Schulz et al. 2008, Hopkins et al. 2010, also see Geider et al. 2001 for a broader view of this matter and related topics). The difficulty in resolving this matter is partly due to the technical challenge posed by such studies (Hurd et al. 2009) and from a lack of extensive information on the mechanisms associated with acclimation/adaptation to elevated CO_2 and on their modulation. The discrepancies in the data and in their interpretation may also reflect an actual diversity in the response mechanisms. The species-specificity of the responses to elevated CO_2 is implicitly confirmed by the shift in species composition in experiments conducted on natural phytoplankton assemblages (e.g. Tortell et al. 2002, Riebesell et al. 2007). Not surprisingly (see Giordano et al. 2005 and Raven et al. 2011, 2012 for reviews on this topic), the photosynthetic affinity for DIC of *C. closterium* cells measured in this study was appreciably lower (2.4-fold or 3.3-fold, depending on whether measured as $K_{1/2}$(DIC) or as β) in the cells acclimated to elevated CO_2 than in the cells grown at ambient CO_2. This is indicative of a down-regulation of the CCM at 800 ppmv CO_2. The actual growth performance of a species may be directly affected by the degree of deactivation of CCMs, and this may explain some of the differences in the growth responses of microalgae to elevated CO_2. In the case of *C. closterium* f. *minutissima*, the apparent affinity of photosynthesis for CO_2, although appreciably lower at 800 than at 390 ppmv CO_2, still exceeded the typical values recorded for the form ID rubisco of diatoms (on average Km(CO_2) ~30 μM; Raven 1997, Badger et al 1998). In this study the CCM of *C. closterium* was not completely turned off at 800 ppmv CO_2, although it is hard to say to what extent it was still active. If this is true this partial down-regulation of the CCM was not coupled with a change in growth rates, in cells acclimated under solar radiation. The question that arises from these observations is whether the partial CCM down-regulation affects the overall growth performance of cells, reflecting an inefficiency of the system, or it simply responds to the requirement of rubisco for an optimal interaction with the environmental conditions (Tcherkez et al. 2006, Savir et al. 2010), possibly via a CCM modulation process, such as the one mediated by cytosolic cAMP described for the diatom *Phaeodactylum tricornutum* (Harada et al. 2006, Matsuda et al. 2007). We shall state upfront that our data do not allow us to answer this question and we propose this topic as a hypothesis for future

investigations. On the other hand, when CCMs were strongly down-regulated at elevated CO_2 (i.e there was no obvious sign of CCM activity, as shown by a low photosynthetic affinity for Ci and not detectable CA activity; e.g. Giordano & Bowes 1997 for *Dunaliella salina*; Ratti et al. 2007 for *Protoceratium reticulatum*; Spijkerman 2008 for *Chlamydomonas acidophila*), growth was stimulated. Such an enhanced growth rate could be due to the fact that the cells benefited from the energy saved through the CCM down-regulation. In *C. closterium* f. *minutissima*, when cultured under high levels of sunlight, the energy saved from the down-regulation of CCM did not appear to stimulate growth. The unused energy might have contributed to harm the photosynthetic machinery, until cells were fully acclimated to elevated CO_2 (Fig. 2; Table 1). A recent finding that the growth of diatoms grown under high CO_2 was inversely related to PAR levels may aid in understanding the obscured growth rate of this species: Under high levels of sunlight, the benefit of increased CO_2 availability might have been obscured by the stress caused by increased acidity of seawater (Gao et al. 2012). This agrees with the role of the CCM in photoprotection proposed by Tchernov et al. (2003).

In conclusion, two main concepts can be derived from the data presented here: 1) the impact of UVR on photosynthesis does not necessarily result in a decline of the growth rate; 2) the degree of down regulation of the CCM and/or the fate of the energy saved by doing so may affect the growth response of microalgae to elevated pCO_2.

Acknowledgements

This study was supported by National Basic Research Program of China (No. 2009CB421207 and 2011CB200902), Program for Changjiang Scholars and Innovative Research Team (IRT0941), National Natural Science Foundation (No. 40930846, No. 41120164007) and China-Japan collaboration project from MOST (S2012GR0290). M.G.'s work in Xiamen was supported by the visiting professor program (111) from the ministry of Education of China. M.G.'s research was funded by Fondazione Cariverona, Italy, and by the Ministero per le Politiche Agricole e Forestali (MIPAF), Bioforme project. M.G. wishes to thank the "Cenacolo dei Ficofilosofi" for their contribution in the development of some of the ideas contained in this paper. We would like to express our special thanks to three anonymous reviewers for their constructive comments and to Rainer Kurmayer for the editorial contribution.

References

Apoya-Horton, M. D., Yin, L., Underwood, G. J. C. & Gretz, M. R., 2006: Movement modalities and responses to environmental changes of the mudflat diatom *Cylindrotheca closterium* (Bacillariophyceae). – J. Phycol. **42**: 379–390.

Badger, M. R., Andrews, T. J., Whitney, S. M., Ludwig, M., Yellowlees, D. C., Leggat, W. & Price, G. D., 1998: The diversity and coevolution of Rubisco, plastids, pyrenoids and chloroplast-based CO_2-concentrating mechanisms in the algae. – Can. J. Bot. **76**: 1052–1071.

Beardall, J. & Giordano, M., 2002: Ecological implications of algal CCMs and their regulation. – Funct. Plant Biol. **29**: 335–347.

Beardall, J., Heraud, P., Roberts, S., Shelly, K. & Stojkovic, S., 2002: Effects of UV-B radiation on inorganic carbon acquisition by the marine microalga *Dunaliella tertiolecta* (Chlorophyceae). – Phycologia **41**: 268–272.

Beardall, J. & Raven, J. A., 2004: The potential effects of global climate change on microalgal photosynthesis, growth and ecology. – Phycologia **43**: 26–40.

Beardall, J., Stojkovic, S. & Larsen, S., 2009: Living in a high CO_2 world: impacts of global climate change on marine phytoplankton. – Plant Ecol. Divers. **2**: 191–205.

Brewer, P. G., 1997: Ocean chemistry of the fossil fuel CO_2 signal: The haline signal of "business as usual". – Geophys. Res. Lett. **24**: 1367–1369.

Chen, S. W. & Gao, K. S., 2011: Solar ultraviolet radiation and CO_2-induced ocean acidification interacts to influence the photosynthetic performance of the red tide alga *Phaeocystis globosa* (Prymnesiophyceae). – Hydrobiologia **675**: 105–117.

Clark, D. R. & Flynn, K. J., 2000: The relationship between the dissolved inorganic carbon concentration and growth rate in marine phytoplankton. – Proc. R. Soc. Lond. Ser. B Biol. Sci. **267:** 953–959.

Delille, B., Harlay, J., Zondervan, I., Jacquet, S., Chou, L., Wollast, R., Bellerby, R. G. J., Frankignoulle, M., Vieira, B. A., Riebesell, U. & Gattuso, J. P., 2005: Response of primary production and calcification to changes of pCO_2 during experimental blooms of the coccolithophorid *Emiliania huxleyi*. – Global Biogeochem. Cycles. **19**: GB2023.1–14.

Feuchtmayr, H., Moss, B., Harvey, I., Moran, R., Hatton, K., Connor, L. & Atkinson, D., 2010: Differential effects of warming and nutrient loading on the timing and size of the spring zooplankton peak: an experimental approach with hypertrophic freshwater mesocosms. – Plankton. Res. **32**: 1715–1725.

Finkel, Z. V., Beardall, J., Flynn, K. J., Quigg, A., Rees, T. A. V. & Raven, J. A., 2010: Phytoplankton in a changing world: cell size and elemental stoichiometry. – J. Plankton. Res.**32**: 119–137.

Gao, K. S., Wu, Y. P., Li, G., Wu, H. Y., Villafane, V. E. & Helbling, E. W., 2007: Solar UV Radiation Drives CO_2 Fixation in Marine Phytoplankton: A Double-Edged Sword. – Plant Physiol.**144**: 54–59.

Gao, K. S., Xu, J. T., Gao, G., Li, Y. H., Hutchins, D. A., Huang, B. Q., Wang, L., Zheng, Y., Jin, P., Cai, X. N., Häder, D. P., Li, W., Xu, K., Liu, N. N. & Riebesell, U., 2012: Rising CO_2 and increased light exposure synergistically reduce marine primary productivity. – Nature Clim. Change (in press) DOI: 10.1038/NCLIMATE1507.

Gao, K. S. & Zheng, Y. Q., 2009: Combined effects of ocean acidification and solar UV radiation on photosynthesis, growth, pigmentation and calcification of the coralline alga *Corallinasessilis* (Rhodophyta). – Global Change Biol. **16**: 2388–2398.

Geider, R. J., Delucia, E. H., Falkowski, P. G., Finzi, A. C., Grime, P. J. & Grace J., 2001: Primary productivity of planet earth: biological determinants and physical constraints in terrestrial and aquatic habitats. – Global Change Biol. **l7**: 849–882.

Genty, B. E., Briantais, J. M. & Baker, N. R., 1989: Relative quantum efficiencies of the two photosystems of leaves in photorespiratory and nonphotorespiratory conditions. – Plant Physiol. Biochem. **28**: 1–10.

Giordano, M., Beardall, J. & Raven, J. A., 2005: CO_2 concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. – Annu. Rev. Plant Biol. **56**: 99–131.

Giordano, M. & Bowes, G., 1997: Gas exchange and C allocation in *Dunaliella salina* cells in response to the N source and CO_2 concentration used for growth. – Plant Physiol. **115**: 1049–1056.

Goyet, C. & Poisson, A., 1989: New determination of carbonic acid dissociation constants in seawater as a function of temperature and salinity. – Deep-Sea Res. Part I Oceanogr. Res. Pap. **36**: 1635–1654.

Guillard, R. R. L. & Ryther, J. H., 1962: Studies on marine planktonic diatoms. I. *Cyclotella nana* (Hustedt) and *Detonula confervaceae* (Cleve). – Can. J. Microbiol. **8**: 229–239.

Häder, D. P., Lebert, M., Marangoni, R. & Colombetti, G., 1999: ELDONET-European light dosimeter network hardware and software. – J. Photochem. Photobiol. B **52**: 51–58.

Harada, H., Nakajima, K., Sakaue, K. & Matsuda Y., 2006: CO_2 sensing at ocean surface mediated by cAMP in a marine diatom. – Plant Physiol. **142**: 1318–1328.

Hein, M. & Sand-Jensen, K., 1997: CO_2 increases oceanic primary production. – Nature **388**: 526–527.

Hopkins, B. M., Xu, Y., Shi, D., McGinn, P. J. & Morel, F. F. M., 2010: The effect of CO_2 on the photosynthetic physiology of phytoplankton in the Gulf of Alaska. – Limnol. Oceanogr. **55**: 2011–2024.

Hurd, C. L., Hepburn, C. D., Currie, K. I., Raven, J. A. & Hunter, K. A., 2009: Testing the effects of ocean acidification on algal metabolism: considerations for experimental designs. – J. Phycol. **45**: 1236–1251.

Jassby, A. D. & Platt, T., 1976: Mathematical formulation of the relationship between photosynthesis and light for photoplankton. – Limnol. Oceanogr. **21**: 540–547.

Johnsen, G. & Sakshaug, E., 2007: Biooptical characteristics of PSII and PSI in 33 species (13 pigment groups) of marine phytoplankton, and the relevance for pulse-amplitude modulated and fast-repetition-rate fluorometry. – J. Phycol. **43**: 1236–1251.

Joint, I., Karl, D. M., Doney, S. C., Ambrust, E. V., Balch, W., Beman, M., Bowler, C., Church, M., Dickson, A., Heidelberg, J., Iglesias-Rodriguez, D., Kirchman, D., Kolber, Z., Letelier, J., Lupp, C., Maberly, S., Park, S., Raven, J., Repeta, D. J., Riebesell, U., Stewart, G., Tortell, P., Zeebe, R. E. & Zehr, J. P., 2009: Consequences of high CO_2 and ocean acidification for microbes in the global ocean. – Report of Expert Meeting, East-West Center, University of Hawai.

Lesser, M. P., 1996: Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in symbiotic dinoflagellates. – Limnol. Oceanogr. **41**: 271–283.

Litchman, E., Neale, P. J. & Banaszak, A. T., 2002: Increased sensitivity to ultraviolet radiation in nitrogen-limited dinoflagellates: Photoprotection and repair. – Limnol. Oceanogr. **47**: 86–94.

Maberly, S. C., 1996: Diel, episodic and seasonal changes in pH and concentrations of inorganic carbon in a productive lake. – Freshwat. Biol. **35**: 579–589.

Matsuda, Y., Harada, H., Nakajima, K. & Colman, B., 2007: Sensing of elevating CO_2 in a marine diatom molecular mechanisms and implications. – Plant Signal. Behav. **2**: 109–111.

Porra, R. J., 2002: The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b. – Photosynth. Res.**73**: 149–156.

Ratti, S., Morse, D. & Giordano, M., 2007: CO_2 concentrating mechanisms of the potentially toxic dinoflagellate *Protoceratium reticulatum* (Dynophyceae, Gonyaulacales). – J. Phycol. **43**: 693–701.

Raven, J. A., 1997: Putting the C in phycology. – Eur. J. Phycol. **32**: 319–333.

Raven, J. A., Ball, S. C., Beardall, J., Giordano, M. & Maberly, S. C., 2005: Algae lacking carbon concentrating mechanisms. – Can. J. Bot. **83**: 879–890.

Raven, J. A., Cockell, C. S. & De La Rocha C., 2008a: The evolution of inorganic carbon concentrating mechanisms in photosynthesis. – Philos. Trans. R. Soc. London Ser. B **363**: 2641–2650.

Raven, J. A., Giordano, M. & Beardall, J., 2008b: Insights into the evolution of CCMs from comparisons with other resource acquisition and assimilation processes. – Physiol. Plant. **133**: 4–14.

Raven, J. A., Giordano, M., Beardall, J. & Maberly, S. C., 2011: Algal and aquatic plant carbon concentrating mechanisms in relation to environmental change. – Photosyn. Res. **109**: 281–296.

Raven, J. A., Giordano, M., Beardall, J. & Maberly, S. C., 2012: Algal evolution in relation to atmospheric CO_2: carboxylases, carbon concentrating mechanisms and carbon oxidation cycles. – Phil. Trans. R. Soc. B. **367**: 493–507.

Riebesell, U., Schulz, K. G., Bellerby, R. G. J., Botros, M., Fritsche, P., Meyerhofer, M., Neill, C., Nondal, G., Oschlies, A., Wohlers, J. & Zollner, E., 2007: Enhanced biological carbon consumption in a high CO_2 ocean. – Nature **450**: 545–548.

Riebesell, U., Wolf-Gladrow, D. A. & Smetacek, V., 1993: Carbon dioxide limitation of marine phytoplankton growth rates. – Nature **361**: 249–251.

Rotty, R. M., 1980: Uncertainties associated with global effects of atmospheric CO_2. – Sci. Total. Environ. **15**: 73–86.

Savir, Y., Noor, E., Milo, R. & Tlusty, T., 2010: Cross-species analysis traces adaptation of toward optimality in a low-dimensional landscape. – Proc. Nat. Acad. Sci. U.S.A. **107**: 3475–3480.

Schippers, P., Lurling, M. & Scheffer, M., 2004: Increase of atmospheric CO_2 promotes phytoplankton productivity. – Ecol. Lett. **7**: 446–451.

Schreiber, U., Schliwa, U. & Bilger, W., 1986: Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. – Photosynth. Res. **10**: 51–62.

Schulz, K. G., Riebesell, U., Bellerby, R. G. J., Biswas, H., Meyerhofer, M., Muller, M. N., Egge, J. K., Nejstgaard, J. C., Neill, C., Wohlers, J. & Zollner, E., 2008: Build-up and decline of organic matter during PeECE III. – Biogeosciences **5**: 707–718.

Sobrino, C., Neale, P. J., Phillips-Kress, J. D., Moeller, R. E. & Porter, J. A., 2009: Elevated CO_2 increases sensitivity to ultraviolet radiation in lacustrine phytoplankton assemblages. – Limnol. Oceanogr. **54**: 2448–2459.

Sobrino, C., Ward, M. L. & Neale, P. J., 2008: Acclimation to elevated carbon dioxide and ultraviolet radiation in the diatom *Thalassiosira pseudonana*: Effects on growth, photosynthesis, and spectral sensitivity of photoinhibition. – Limnol. Oceanogr. **53**: 494–505.

Spijkerman, E., 2008: What physiological acclimation supports increased growth at high CO_2 conditions? – Physiol. Plant. **133**: 41–48.

Tcherkez, G. G. B., Farquhar, G. D. & Andrews, T. J., 2006: Despite slow catalysis and confused substrate specificity, all ribulose bisphosphate carboxylases may be nearly perfectly optimized. – Proc. Nat. Acad. Sci. U.S.A. **103**: 7246–7251.

Tchernov, D., Silverman, J., Luz, B., Reinhold, L. & Kaplan, A., 2003: Massive light-dependent cycling of inorganic carbon between oxygenic photosynthetic microorganisms and their surroundings. – Photosynth. Res. **77**: 95–103.

Tortell, P. D., DiTullio, G. R., Sigman, D. M. & Morel, F. M. M., 2002: CO_2 effects on taxonomic composition and nutrient utilization in an equatorial pacific phytoplankton assemblage. – Mar. Ecol. Prog. Ser. **236**: 37–43.

Underwood, G. & Provot, L., 2000: Determining the environmental preferences of four estuarine epipelic diatom taxa: growth across a range of salinity, nitrate and ammonium conditions. – Eur. J. Phycol. **35**: 173–182.

Wu, H. Y. & Gao, K. S., 2009: Ultraviolet radiation stimulated activity of extracellular carbonic anhydrase in the marine diatom *Skeletonema costatum*. – Funct. Plant Biol. **36**: 137–143.

Wu, Y. P., Gao, K. G. & Riebesell, U., 2010: CO_2-induced seawater acidification affects physiological performance of the marine diatom *Phaeodactylum tricornutum.* – Biogeosciences **7**: 2915–2923.

Xu, J. T. & Gao, K. S., 2010: UV-A enhanced growth and UV-B induced positive effects in the recovery of photochemical yield in *Gracilaria lemaneiformis* (Rhodophyta). – J. Photochem. Photobiol. B **100**: 117–122.

Submitted: 30 December 2011; accepted: 11 June 2012.

Appendix 1. Daily dose, mean PAR daily radiation and mean PAR radiation between 11:00 and 14:00 (noon radiation) during the 13 days of the *C. closterium* f. *minutissima* outdoor growth experiment (March 22 – April 3, 2008).

Day	Daily dose ($MJ\ m^{-2}\ d^{-1}$)			Mean daily PAR radiation ($\mu mol\ m^{-2}\ s^{-1}$)	Mean noon PAR radiation ($\mu mol\ m^{-2}\ s^{-1}$)
	PAR	UVA	UVB		
1	1.35	0.21	0.0054	152.72	200.22
2	1.26	0.22	0.0055	142.66	166.59
3	5.70	0.88	0.0264	646.97	1171.80
4	1.79	0.30	0.0077	203.47	268.19
5	2.41	0.41	0.0122	272.98	421.07
6	5.42	0.82	0.0244	614.39	1101.05
7	0.89	0.16	0.0041	100.52	109.80
8	1.35	0.23	0.0065	153.11	318.70
9	3.48	0.51	0.0154	395.13	686.36
10	0.96	0.17	0.0047	108.76	175.58
11	0.66	0.13	0.0032	74.36	123.94
12	1.33	0.26	0.0082	150.49	233.58
13	0.92	0.18	0.0051	104.48	167.65

Appendix 2. Cell densities ($10^6 \cdot$ cells $\cdot\ ml^{-1} \pm$ standard deviation) of *C. closterium* f. *minutissima* during the 13 days of the *C. closterium* f. *minutissima* outdoor growth experiment (March 22 – April 3, 2008). PAR, photosynthetic active radiation; PAB, PAR + UV radiation.

Day	Ambient CO_2		Elevated CO_2	
	PAR	PAB	PAR	PAB
1	2.66 ± 0.63	1.90 ± 0.28	1.65 ± 0.36	1.99 ± 0.21
2	2.68 ± 0.53	2.84 ± 0.49	1.36 ± 0.05	1.64 ± 0.13
3	2.64 ± 0.31	2.45 ± 0.80	1.85 ± 0.43	1.99 ± 0.10
4	2.73 ± 0.32	1.88 ± 0.26	1.85 ± 0.58	1.99 ± 0.39
5	3.39 ± 0.44	2.95 ± 0.43	1.75 ± 0.55	2.23 ± 0.66
6	3.16 ± 0.21	2.01 ± 0.34	2.53 ± 0.49	2.61 ± 0.28
7	2.24 ± 0.20	2.28 ± 0.03	2.09 ± 0.29	2.30 ± 0.21
8	2.61 ± 0.24	2.48 ± 0.51	2.41 ± 0.80	2.99 ± 0.44
9	3.04 ± 0.40	2.63 ± 0.54	2.58 ± 0.78	2.59 ± 0.34
10	2.40 ± 0.31	2.43 ± 0.36	2.43 ± 0.15	2.56 ± 0.29
11	2.21 ± 0.46	2.49 ± 0.31	2.26 ± 0.41	2.58 ± 0.16
12	2.48 ± 0.32	2.04 ± 0.46	2.35 ± 0.60	2.10 ± 0.32
13	2.49 ± 0.23	2.98 ± 0.30	1.85 ± 0.42	2.69 ± 0.39