Vol. 23: 109–118, 2015
doi: 10.3354/ab00619

AQUATIC BIOLOGY
Aquat Biol

Published online January 22

FEATURE ARTICLE

Photochemical responses of the diatom *Skeletonema costatum* grown under elevated CO_2 concentrations to short-term changes in pH

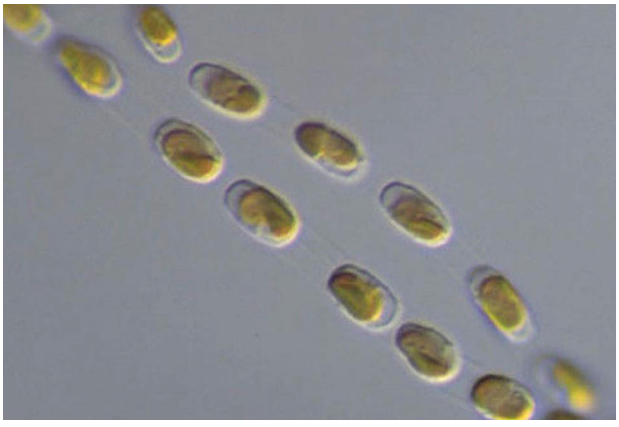
Ying Zheng[1], Mario Giordano[2], Kunshan Gao[1,*]

[1]State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361005, PR China
[2]Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy

ABSTRACT: Variability in pH is a common occurrence in many aquatic environments, due to physical, chemical and biological processes. In coastal waters, lagoons, estuaries and inland waters, pH can change very rapidly (within seconds or hours) in addition to daily and seasonal changes. At the same time, progressive ocean acidification caused by anthropogenic CO_2 emissions is superimposed on these spatial and temporal pH changes. Photosynthetic organisms are therefore unavoidably subject to significant pH variations at the cell surface. Whether this will affect their response to long-term ocean acidification is still unknown, nor is it known whether the short-term sensitivity to pH change is affected by the pCO_2 to which the cells are acclimated. We posed the latter open question as our experimental hypothesis: Does acclimation to seawater acidification affect the response of phytoplankton to acute pH variations? The diatom *Skeletonema costatum*, commonly found in coastal and estuarine waters where short-term acute changes in pH frequently occur, was selected to test the hypothesis. Diatoms were grown at both 390 (pH 8.2, low CO_2; LC) and 1000 (pH 7.9, high CO_2; HC) µatm CO_2 for at least 20 generations, and photosynthetic responses to short-term and acute changes in pH (between 8.2 and 7.6) were investigated. The effective quantum yield of LC-grown cells decreased by ca. 70% only when exposed to pH 7.6; this was not observed when exposed to pH 7.9 or 8.2. HC-grown cells did not show significant responses in any pH treatment. Non-photochemical quenching showed opposite trends. In general, our results indicate that while LC-grown cells are rather sensitive to acidification, HC-grown cells are relatively unresponsive in terms of photochemical performance.

KEY WORDS: Diatoms · Ocean acidification · pH · CO_2 · Photosynthesis

*Corresponding author: ksgao@xmu.edu.cn



Cells of the marine diatom *Skeletonema costatum* subjected to acute changes in pH.
Photo: Anonymous

INTRODUCTION

The concentration of H^+ in water (and its negative log, the pH) are subjected to dynamic changes that occur over time scales ranging from seconds to seasons. This phenomenon is especially obvious in coastal waters (Hinga 2002, Cornwall et al. 2013). In intertidal pools, pH variations of up to 3 units over 24 h have been observed, mostly due to biological activities (Poole & Raven 1997). In highly productive coastal waters, estuarine waters or coral reef systems, conspicuous diel pH variations of between 0.3 and 0.5 units over 24 h have been documented (Gao et al. 2005, Gray et al. 2012), although the range of these variations is strongly influenced by hydrodynamics and by the rates of exchange with the bulk seawater (Middelboe & Hansen 2007, Unsworth et al. 2012). In

Publisher: Inter-Research · www.int-res.com

oligotrophic open oceans, however, daily and yearly pH variations are typically limited to a few tens of a unit (Dore et al. 2009). Superimposed on such 'natural' variations, the average pH of the world's oceans is declining at a rate of about 0.002 yr^{-1}, which could lead to a potential drop of 0.3 to 0.4 units by 2100, as a consequence of the increasing atmospheric CO_2 concentration associated with anthropogenic CO_2 emissions (IGBP et al. 2013). As the H^+ concentration increases with CO_2 dissolution, H^+ releases can partially reverse the secondary dissociation reaction of the seawater carbonate system, leading to a decrease in carbonate ions ($H^+ + CO_3^{2-} \rightarrow HCO_3^-$). Therefore, typical changes linked to ocean acidification (OA) are increased concentrations of pCO_2, H^+ and HCO_3^-, decreased concentrations of CO_3^{2-} and decreases in the $CaCO_3$ saturation state (Gattuso et al. 2010). These changes will lead to a decrease in the alkalinity of seawater, and the consequent decrease in buffering capacity will increase the magnitude of short-term pH changes in the future. In combination with eutrophication and hypoxia events, anthropogenic acidification may occur earlier and at a more accelerated rate in coastal and estuarine waters than in pelagic environments (Cai et al. 2011).

It is known that changes in external pH may have repercussions on cytosolic pH (Raven 2013 and references therein) and cell homeostasis (Montechiaro & Giordano 2010, Giordano 2013, Fanesi et al. 2014). Flynn et al. (2012) showed that the pH at the cell surface of phytoplankton can deviate appreciably from that in bulk seawater, and increasingly so with an increase in metabolic activities and with the thickness of the diffusion boundary layer, which, in the size range of phytoplankton, is a direct function of cell dimension. Thus, in the proximity of the cell, pH can vary across a broad range in a relatively short term depending on the extent of biological (photosynthesis or respiration) and physical (e.g. boundary layer thickness and diffusional gradients) processes. Little is known about how longer-term changes in average bulk water pH may affect organisms already subject to large diel pH variations in their microenvironment.

In coastal waters and estuaries (e.g. Changjiang Estuary, Wang 2002 and Jiulong River estuary, Li et al. 2011) where *Skeletonema costatum* is usually the most abundant algal species, pH can vary by up to 0.5 units d^{-1} (Hinga 2002, Gao et al. 2005). *S. costatum* responds rapidly to changes in pH or pCO_2 by down-regulating the activity of periplasmic carbonic anhydrase within 60 to 120 min (Chen & Gao 2003, Wu & Gao 2009). Elevated pCO_2 was shown not to stimulate its growth in the lab (Chen & Gao 2003) or under high solar irradiance (Gao et al. 2012); however, enhanced growth of *S. costatum* was observed under elevated CO_2 levels in a mesocosm study (Kim et al. 2006). Here, we hypothesize that acclimation to increased CO_2 may decrease the sensitivity of *S. costatum* to acute pH variations. For this reason, we acclimated *S. costatum* to growth in media equilibrated with gas phases containing either 390 (present) or 1000 µatm pCO_2 (the level predicted at the end of this century; IPCC 2007); we then exposed these cultures to acute acidification and measured their photosynthetic responses in terms of Photosystem II (PSII) variable fluorescence.

MATERIALS AND METHODS

Culture conditions

The diatom *Skeletonema costatum* (Grev.) Cl. (CCMBP 112) was pre-cultured at 20°C under a photon flux density (PFD) of 100 µmol photons m^{-2} s^{-1}, with a 12 h light:12 h dark photoperiod. The cells were grown in filtered and autoclaved seawater collected in the South China Sea, supplemented with nutrients using f/2 medium (Guillard & Ryther 1962). Culture CO_2 was maintained in equilibrium with gas phases containing either 390 (pH 8.2; LC) or 1000 (pH 7.9; HC) µatm CO_2 in a CO_2 plant growth chamber (HP1000G-D, Wuhan Ruihua Instrument and Equipment), with variations <4%. For all treatments, 3 independent cultures were used as replicates. Prior to the experiments, the cells were grown for at least 20 generations (about 15 d), under either LC or HC; the cultures were diluted every 24 h to maintain cell concentrations between 2×10^4 and 1.5×10^5 ml^{-1}. This daily dilution and cell density maintained the cells in mid-exponential growth phase and minimized changes in nutrient availability and seawater carbonate chemistry (see Table S1 in the Supplement at www.int-res.com/articles/suppl/b023p109_supp.pdf). A pH meter (Benchtop pH 510, Oakton), calibrated daily with standard buffers (NBS, Hanna) was used to measure the pH in the cultures before and after dilution. Other parameters of the seawater carbonate system were calculated using CO2SYS software (Table S1; Lewis & Wallace 1998), taking into account salinity (35 psu), total dissolved inorganic carbon (DIC), pH, nutrient concentrations and temperature (20°C); DIC was determined with a DIC analyzer (AS-C3, Apollo SciTech). The equilibrium constants K_1 and K_2 for carbonic acid dissociation and K_B for

boric acid were determined according to Roy et al. (1993) and Dickson (1990), respectively.

Cells were harvested by filtration on cellulose membranes (Xinya), washed with 10 ml of growth medium and then resuspended in 100 ml of medium in which the pH had been adjusted by the addition of appropriate amounts of HCl to obtain the 3 experimental pH levels of 7.6, 7.9 and 8.2. These cell suspensions were transferred to sterile 100 ml light-transparent polyethylene syringes. The syringes were maintained under the same conditions used for the pre-cultures. We opted to use syringes because they allowed for the rapid collection of samples and the maintenance of a negligible air space (only the needle tip of the syringes), and thus minimal CO_2 leakage.

Variable fluorescence measurements

At time zero (t_0), a 2 ml aliquot was collected from the syringe and incubated in the dark for 15 min in a quartz tube and then directly placed in a Xe-PAM (Walz) holder for variable fluorescence measurements. Subsequently, 2 ml aliquots were transferred to quartz tubes after 2 (t_2), 4 (t_4), 6 (t_6), 15 (t_{15}), 30 (t_{30}), 45 (t_{45}) and 60 (t_{60}) min since the transfer into the syringes, and subjected to the measurements without prior dark adaptations. Samples were also collected after 15, 30, 45 and 60 min, and dark-adapted for 15 min to obtain maximum quantum yield of fluorescence (F_v/F_m). This parameter was derived from the fluorescence induction curves (Dorigo & Leboulanger 2001) and obtained according to the following equation (Genty et al. 1989):

$$F_v/F_m = (F_m - F_o) / F_m \quad (1)$$

where F_m is the maximum fluorescence measured after a saturating flash of 5000 µmol photons m^{-2} s^{-1}, and F_o is the basal fluorescence of PSII with measuring light of 0.1 µmol photons m^{-2} s^{-1}.

The light-adapted cells were used to determine the effective quantum yield of fluorescence ($\Delta F/F_m'$). This parameter was also derived from the induction curves, but in this case the cells were exposed to actinic light with an irradiance similar to that used for growth (100 µmol photons m^{-2} s^{-1}). The induction curve was conducted as follows: the first saturating pulse (5000 µmol photons m^{-2} s^{-1}) was provided after irradiating the cells with measuring light for 10 s; the actinic light was turned on 10 s after the first saturating pulse, then after a further 10 s and at 10 s intervals, for a sequence of 10 saturating pulses. The $\Delta F/F_m'$ values were obtained from the last 1 or 2 saturating pulses when the F_m' was steady by application of the following equation (Genty et al. 1989):

$$\Delta F/F_m' = (F_m' - F_t) / F_m' \quad (2)$$

where F_m' is the instant maximum fluorescence and F_t is the steady-state fluorescence under the actinic light.

Non-photochemical quenching (NPQ), which provides an indication of regulated thermal energy dissipation, was estimated using the following equation (Bilger & Björkman 1990):

$$NPQ = (F_m - F_m') / F_m' \quad (3)$$

The relative electron transport rate (rETR) was obtained from the rapid light curves (White & Critchley 1999), which were measured under the same experimental conditions used for the induction curve and calculated as (Genty et al. 1989):

$$rETR = E_{PAR} \times \Delta F/F_m' \times 0.75 \quad (4)$$

where E_{PAR} is the irradiance of the actinic light and 0.75 is a factor used to take into account that, in diatoms, approximately 75% of the photons reach PSII (Johnsen & Sakshaug 2007). For the rapid light curves, samples were subject to a series of saturating pluses (5000 µmol photons m^{-2} s^{-1}) in the presence of incremental actinic light PFD (0, 125, 185, 285, 410, 600, 840, 1200 and 1650 µmol photons m^{-2} s^{-1}) at 10 s intervals.

Other photosynthetic parameters were obtained from the rapid light curves, which were fitted by the mathematical model of Jassby & Platt (1976):

$$rETR = rETR_{max} \times \tanh(\alpha \times E / rETR_{max}) \quad (5)$$

$$E_k = rETR_{max} / \alpha \quad (6)$$

where E is the photon flux density (µmol m^{-2} s^{-1}), α the initial slope of the rapid light curve and E_k is the photon flux density at which the onset of photosynthesis light saturation occurs.

Statistics

The results are presented as means ± SD of measurements, conducted on samples collected from 3 independent cultures (n = 3). For the rapid light curves, the Jassby & Platt (1976) model was fitted to individual curves, and averages and standard deviations of the photosynthetic parameters were then calculated from 3 independent cultures (n = 3). The statistical significance of differences was assessed by a 3-way repeated measured ANOVA, using pH (df = 2), CO_2 (df = 1) and time (df = 7 for non-dark adapted

cells or df = 4 for dark-adapted cells) as variables, with a Tukey's post-hoc test for comparison. Differences were considered significant at $p < 0.05$.

RESULTS

Quantum yield and NPQ

In the LC-acclimated cells, $\Delta F/F_m'$ of PSII showed an initial increment for the first 5 min when the cells were exposed to acute acidification (Fig. 1A). When the cells were incubated at pH 7.6, after the initial increase $\Delta F/F_m'$ decreased rapidly for about 30 min ($p = 0.001$), then plateaued at about 34% of the maximum value. When the cells were incubated at pH 7.9 and 8.2, $\Delta F/F_m'$ did not vary significantly ($p = 0.612$). In the HC-acclimated cells, the $\Delta F/F_m'$ value also underwent a slight increase at the beginning, but then reached a steady value and did not change, even in the lowest pH treatment ($p = 0.981$; Fig. 1B). F_v/F_m showed a similar change pattern, except that the drop in pH to 7.6 led to less of a decline in the dark-adapted cells but more reduction in the light-adapted ones in LC-acclimated cells ($p < 0.001$; Fig. 1A,C). If the variations were expressed as a function of the total time at a given pH, the rate of yield decrease was smaller in dark-adapted (0.00475 min^{-1}) than in light-adapted cells (0.0056 min^{-1}).

NPQ values in the LC-grown cells exposed to pH 8.2 and 7.9 (Fig. 2A) remained low (always below 0.4), with no statistical difference between the values measured at these 2 pH levels ($p = 1.000$). At pH 7.6, however, NPQ increased from ca. 0.4 to 3.4 over 60 min ($p = 0.003$). Interestingly, unlike $\Delta F/F_m'$, NPQ did not plateau. In the HC-acclimated cells, NPQ did not change as obviously as in the LC-grown cultures, even when the cells were subjected to pH 7.6

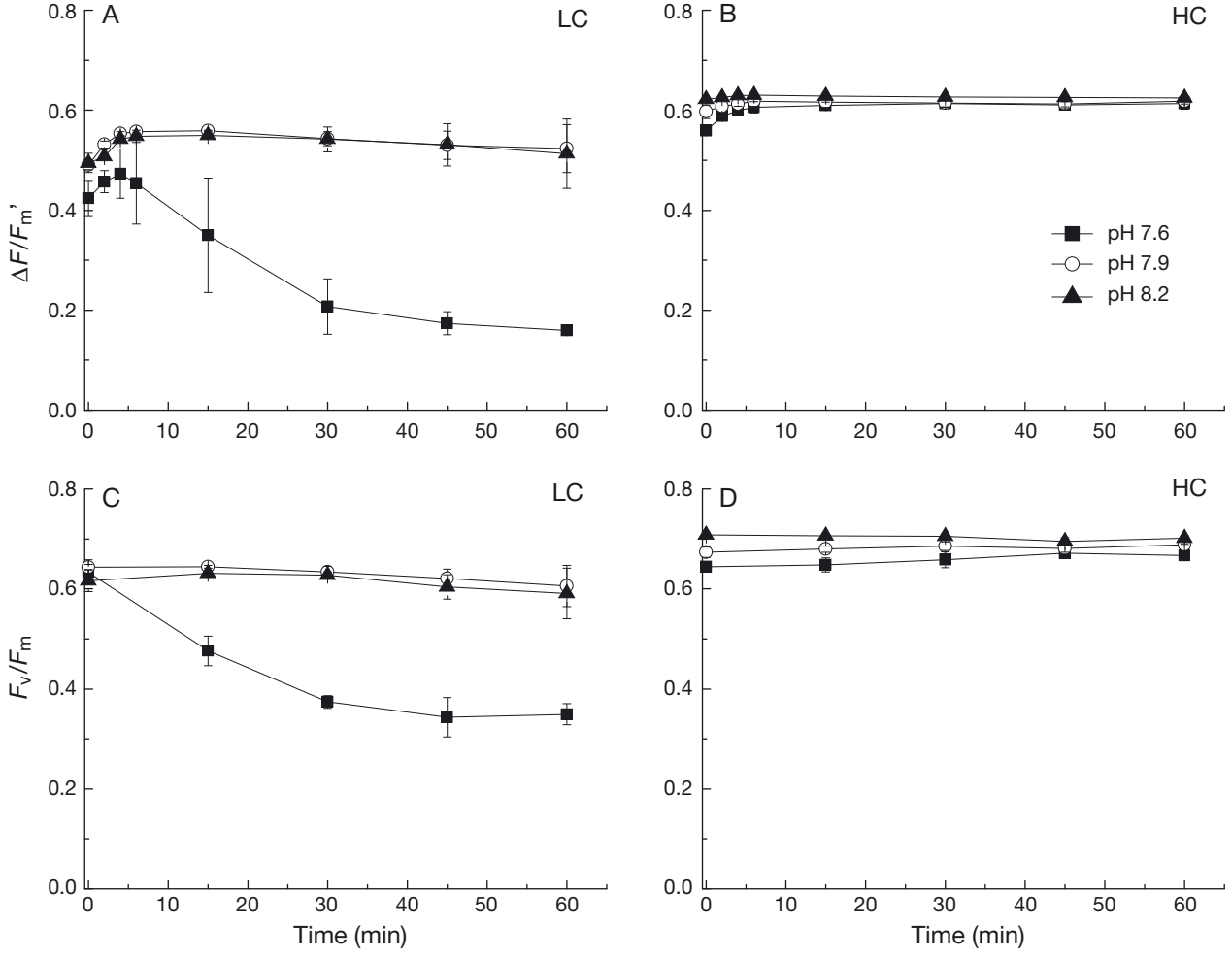


Fig. 1. (A,B) Effective ($\Delta F/F_m'$) and (C,D) maximum (F_v/F_m) quantum yield of PSII of *Skeletonema costatum* cells acclimated to (A,C) low (390 µatm; LC) or (B,D) high (1000 µatm; HC) CO_2 levels and grown for 1 h at pH treatment levels of 7.6, 7.9 or 8.2. Error bars: SD of 3 independent measurements (n = 3 cultures)

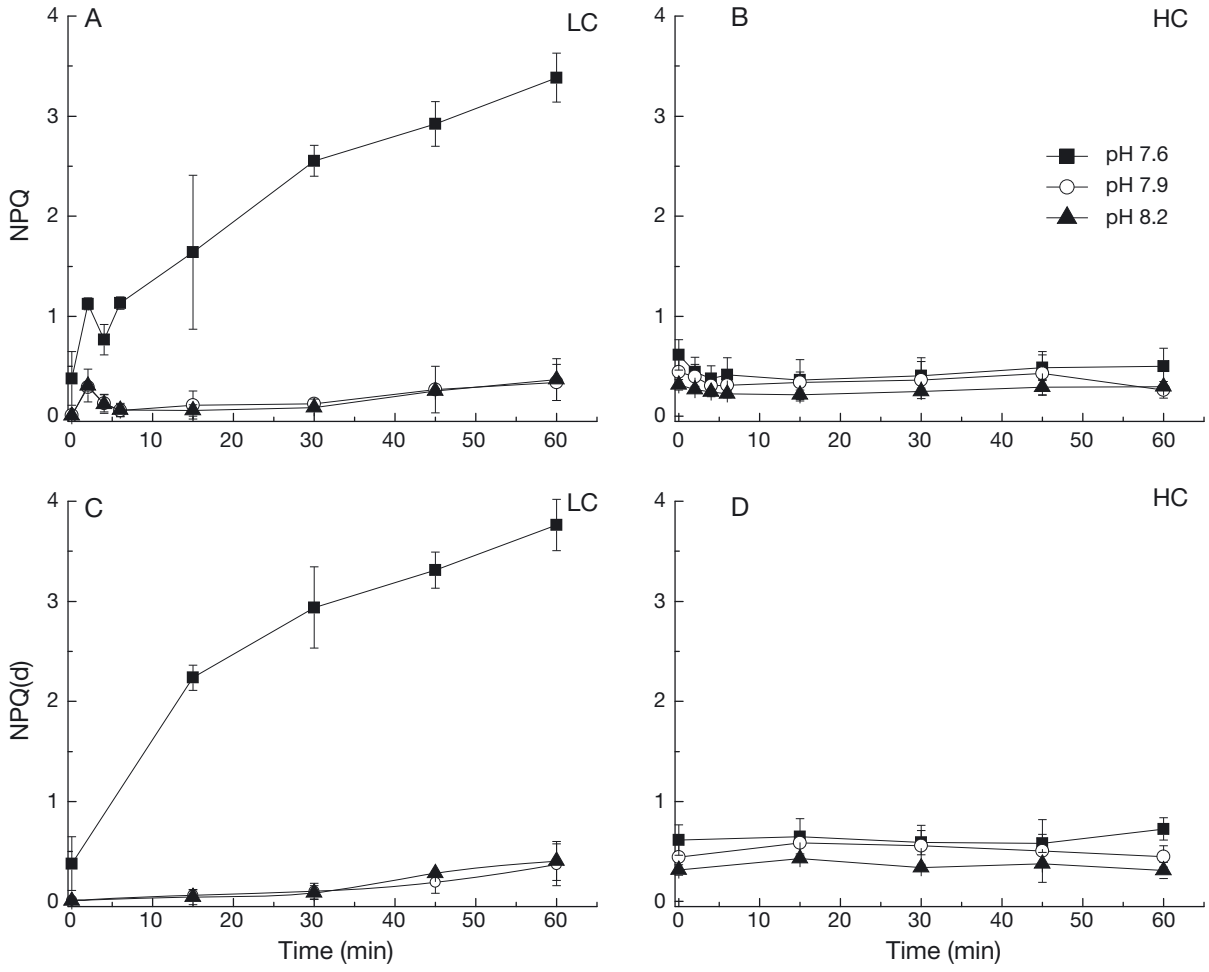


Fig. 2. Non-photochemical quenching (NPQ) of light-adapted (A,B) and dark-adapted (C,D) *Skeletonema costatum* cells acclimated to (A,C) low (390 µatm; LC) or (B,D) high (1000 µatm; HC) CO_2 levels, grown for 1 h at pH treatment levels of 7.6, 7.9 or 8.2. Error bars: SD of 3 independent measurements (n = 3 cultures); NPQ(d) is the NPQ value of dark-adapted cells

(Fig. 2B). Similar trends were observed for the NPQ of the cells that were dark-adapted for 15 min, after having been in the light for 15, 30, 45 or 60 min (Fig. 2C). In the HC-grown cells, however, the drop of pH to 7.6 did enhance the NPQ by 53.8 to 93.8% ($p = 0.001$) in the light-acclimated cells, and by 51.2 to 135.5% ($p = 0.001$) in the dark-adapted cells compared to the pH 8.2 treatment.

A comparison of $\Delta F/F_m'$ of LC- and HC-grown cells indicated that the yield of the former was always lower than that of the latter ($p < 0.001$). At pH 7.9 and 8.2, $\Delta F/F_m'$ was about 20% lower in LC-grown cells and this difference did not vary over time; at pH 7.6, the difference between the $\Delta F/F_m'$ of LC- and HC-grown cells was much higher, and after 60 min the value for HC-grown cells was 285% of that of their LC counterparts (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/b023p109_supp.pdf). At pH 7.9 and 8.2, NPQ was higher in HC- than in LC-grown cells by about 130% ($p < 0.001$). Only at pH 7.6 was the NPQ of LC-grown cells much higher (almost by one order of magnitude) than that of cells acclimated to HC (Fig. S2 in the Supplement).

Light response curves

The rETR did not show major differences as a function of time of sampling and pH treatment, but showed that dark-adapted cells (included those at t_0 in Fig. 3) tended to have lower rETR at saturating light than light-adapted cells ($p < 0.001$; Figs. 3 & 4). HC-grown cells always had higher $rETR_{max}$ than LC-grown cells ($p < 0.001$; Fig. S3 in the Supplement). α, which represents the apparent light-use efficiency for the rETR, was 60 to 79% higher in the HC- than in the LC-grown cells (Fig. 5, Fig. S3C). In the LC-grown cells, the α value was fairly constant within a

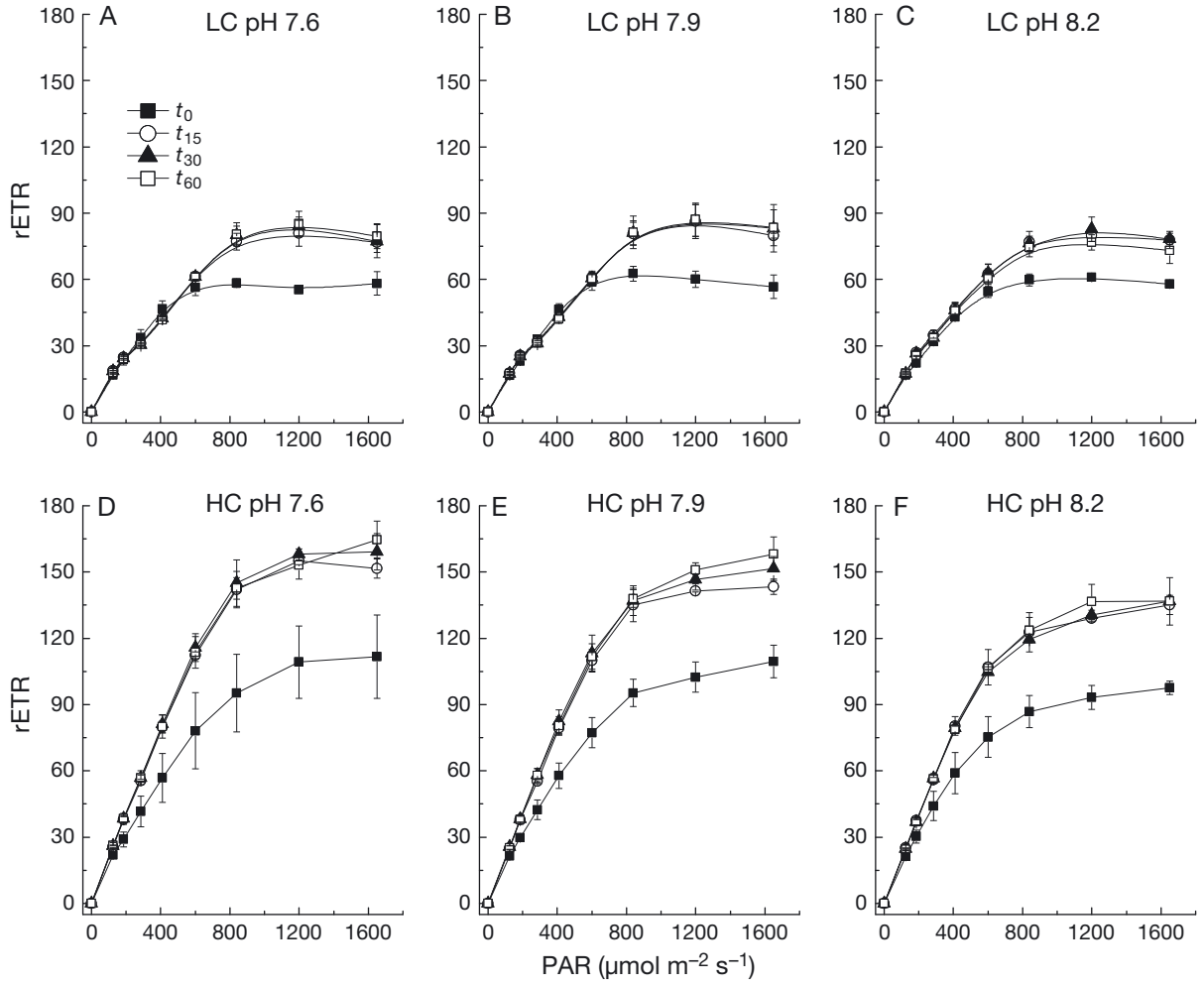


Fig. 3. Relative electron transport rate (rETR) from rapid light curves of *Skeletonema costatum* cells acclimated to (A–C) low (390 µatm; LC) or (D–F) high (1000 µatm; HC) CO_2 levels, grown for 1 h at pH treatment levels of 7.6, 7.9 or 8.2 and measured at time points 0 (t_0), 15 (t_{15}), 30 (t_{30}) and 60 (t_{60}) min. Error bars: SD of 3 independent measurements (n = 3 cultures)

60 min period (only a modest decline was observed at pH 7.9). In the HC-grown cells, however, α increased over the first 15 min and then remained constant (Fig. 5). At the beginning of the pH treatments (t_0), higher light levels were required to saturate rETR in HC- than in LC-grown cells; this difference was not observed at subsequent times (Fig. S3A,B).

DISCUSSION

The main finding of this work is that *Skeletonema costatum* cells are well equipped to maintain photochemical performance in response to acute pH changes when acclimated to growth at CO_2 concentrations and pH values on the order of those expected for the next 100 yr (i.e. in the HC treatment). Acute exposures to acidification at pH 7.6 had different impacts on the cells that were acclimated to present conditions (LC) than from those acclimated to elevated CO_2 (HC) concentrations, with the former being much more susceptible to changes in their quantum yield (which decreased upon acidification) and NPQ (which increased upon acidification). The HC-acclimated cells also showed higher light-use efficiency for rETR.

S. costatum has been shown not to express periplasmic (external) carbonic anhydrase when the medium CO_2 concentration was raised to the HC levels used for this study (Chen & Gao 2003). This is usually considered as a line of evidence that energy-dependent CO_2 acquisition is turned off or strongly down-regulated (Giordano et al. 2005). Under these conditions, diffusional influx of CO_2 into the cells may become relevant, provided that cellular consumption takes the internal CO_2 concentration below the ambient concentration (Raven et al. 2014). The rate of CO_2

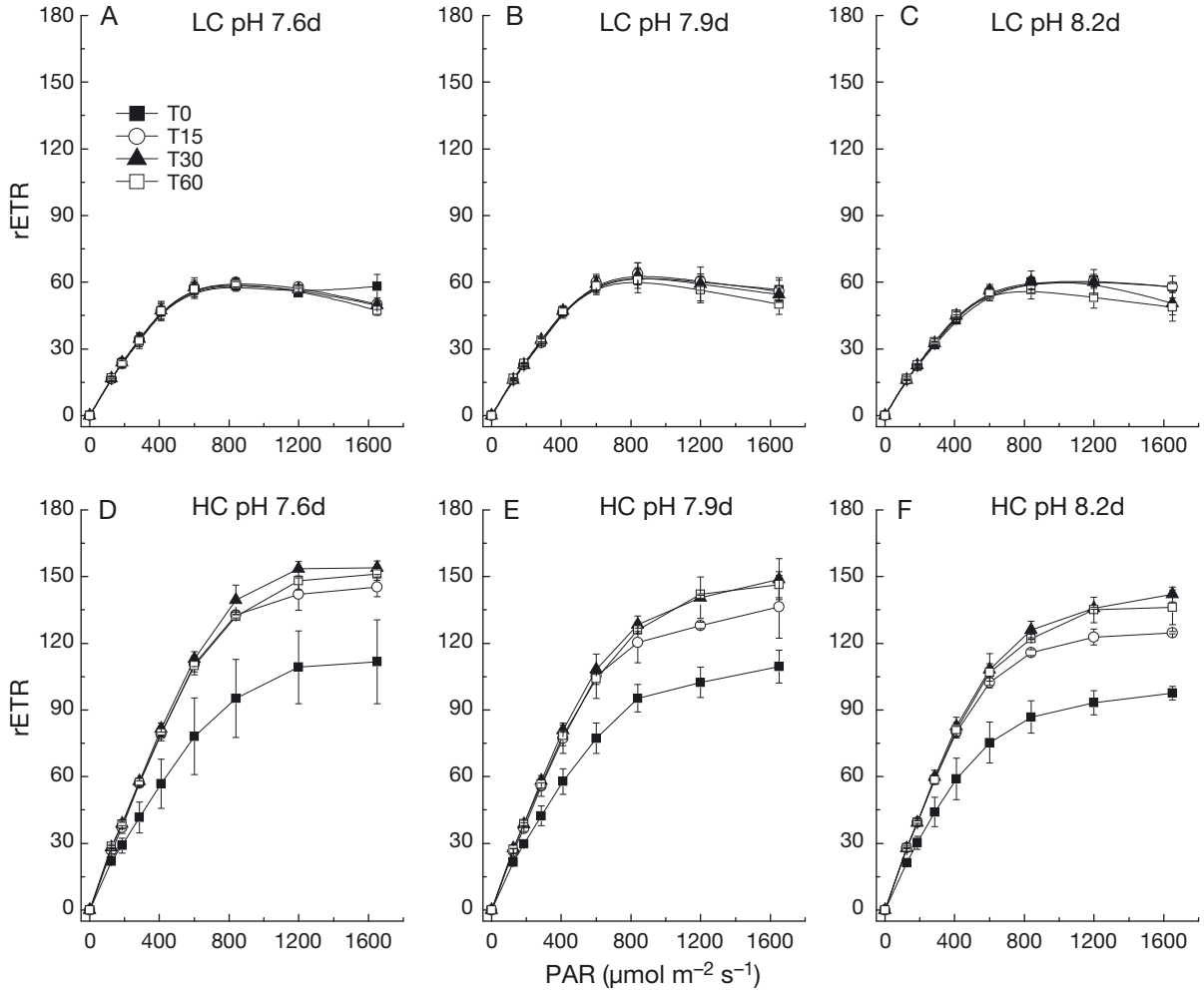


Fig. 4. Relative electron transport rate (rETR) in the *Skeletonema costatum* cells that had been dark-adapted for 15 min ('d'). Other information regarding symbols and panels is the same as in Fig. 3

diffusion is a direct function of the concentration gradient across the membrane, according to Fick's first law (Fick 1855). Also, the absolute CO_2 concentration in the medium under the conditions in which photosynthesis prevails over respiration cannot be higher than that allowed by the equilibrium between the gaseous and liquid phase, according to Henry's law (Henry 1803). Therefore, assuming that the intracellular pH is relatively constant and that the CO_2 concentration within the cell is not at equilibrium with the external environment due to positive net photosynthetic CO_2 consumption, the acidification of the external medium, in concomitance with a higher pCO_2 in the gas phase, would favor a diffusional flux of CO_2 into the cell. The same would not occur in the LC-grown cells, where the presence of an active CO_2 concentrating mechanism (CCM; Giordano et al. 2005) makes diffusional CO_2 influx unlikely (since the internal CO_2 concentration is higher than that in the medium). Therefore, HC-grown cells may benefit more from acidification than LC-grown cells. The down-regulation of the CCM due to elevated (but not saturating) CO_2 is also likely to enhance photorespiration. The enlargement of the sink for electrons constituted by photorespiration (Raven et al. 2014) may contribute to the explanation of the higher effective quantum yield and light-use efficiency in HC-grown cells (Figs. 1, 5 & S1), and the higher NPQ of LC-grown cells (Fig. 2) at the lower pH (7.6) treatment. This result implies that HC-acclimated cells can reduce their sensitivity to acute pH stress. Increased NPQ with time in the LC-grown cells when exposed to pH 7.6 (Fig. 2A,C) reflects enhancement of q_E (energy-dependent quenching, a key component of NPQ) with down-regulation of CCMs in this species. A drop of pH by 0.4 units was shown to completely turn off the activity of periplasmic carbonic anhydrase (CAe) within 120 min (Wu et al. 2010), which has been considered a key indicator of

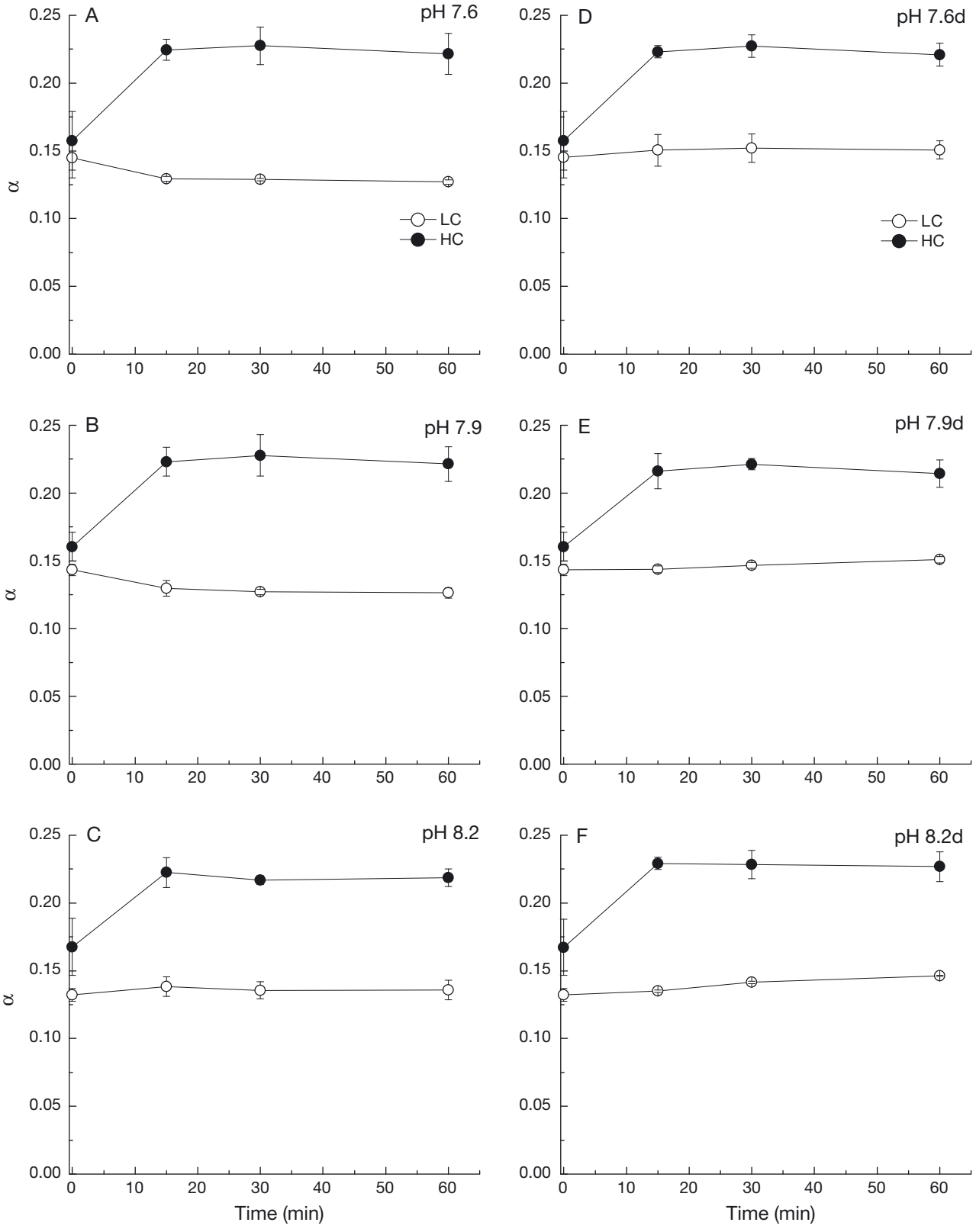


Fig. 5. Initial slope of rapid light curve (α) of (A–C) non-dark adapted and (D–F) dark-adapted ('d') *Skeletonema costatum* cells acclimated to low (390 µatm; LC) and high CO_2 (1000 µatm; HC), grown for 1 h at pH treatment levels of 7.6, 7.9 or 8.2. Error bars: SD of 3 independent measurements (n = 3 cultures)

down-regulation of CCMs, since this enzyme plays an important role in active acquisition of inorganic carbon (Reinfelder 2011) and in intracellular pH regulation (Badger & Price 1994). The HC-acclimated cells did not show a decrease in quantum yield when exposed to different levels of pH (Fig. 1B,D), which implies that energy transfer efficiency was not affected by the acid–base perturbation. When exposed to lowered pH, the HC-grown cells have a high diffusive CO_2 supply to maintain photochemical machinery; when exposed to elevated pH (8.2) with reduced availability of CO_2, up-regulation of CCMs or active pumping of bicarbonate may meet the photosynthetic requirements for inorganic carbon. The fact that NPQ increased rather than decreased with increasing variable fluorescence (Figs. 2B,D & S2) implies incompatibleness of heat dissipation and energy transfer, which usually reverse. Active pumping of bicarbonate or photorespiration may be responsible for this phenomenon, since these processes enhance energy flow but may not directly affect NPQ, which is sensitive to acidic stress (Goss & Garab 2001).

We are well aware of the limits of our relatively simple and short-term experiment; nevertheless, our results indicate that diel pH variations in coastal waters, as reflected in the acute pH drop treatment, can alter the photosynthetic performance of *S. costatum*, and that acclimation to elevated pCO_2 may decrease the impact of ocean acidification on this diatom. The fact that *S. costatum* is a common inhabitant of coastal and estuarine waters (Wang 2002, Li et al. 2011) can at least partially be attributed to its capability to cope with rapid pH changes in these environments. While coastal and estuary waters are predicted to be acidified faster through anthropogenic activities (Sunda & Cai 2012), we might predict that *S. costatum* would still cope well with these changes in coastal and estuary habits based on our experimental data. Certainly, multifactorial experiments, comprising concomitant and realistic variations of pCO_2, pH and warming are required to further clarify the complex interplay among the different facets and consequences of an increase in pCO_2.

With progressive changes such as OA and warming, the abundance of diatoms may increase or decrease in different latitudes or different waters of constant or fluctuating seawater carbonate chemistry. While diatom abundance in the northeast Atlantic and North Sea increased during a half-century time period (Hinder et al. 2012), global phytoplankton biomass in the world's oceans has been shown to decline (Boyce et al. 2010). While there is still a controversy over whether diatoms will benefit from or be harmed by OA (as reviewed by Gao & Campbell 2014), beneficial or inhibitory effects of OA on some diatoms appear to depend on levels of light exposures (Gao et al. 2012). In coastal waters, phytoplankton cells usually experience dramatic changes in pH/pCO_2 due to biological production and consumption, and exposure to a low pH/high CO_2 milieu can affect their physiological performance when the cells move (e.g. some dinoflagellates) or are mixed in with waters of different acidities. Although the present study showed the tolerance of *S. costatum* to acidic stress (especially in those acclimated to high CO_2 conditions), a mechanistic understanding of different diatoms' responses to acidic stress is essential in order to predict the general responsive patterns of this group to ocean changes.

Acknowledgements. This study was supported by the National Natural Science Foundation (NSFC, Nos. 41430967 and 41120164007), the Joint project of the NSFC and Shandong province (Grant No. U1406403), the Strategic Priority Research Program of the Chinese Academy of Sciences (Grant No. XDA11020302), State Oceanic Administration (SOA, GASI-03-01-02-04) and the China-Japan collaboration project from MOST (S2012GR0290). M.G. was funded by the Cariverona Foundation (project Biomasse dioggiedi domani), by the Italian Ministry for Agriculture (MIPAF, Bio-forme project), by the Italian Ministry of Foreign Affairs (MAE, Joint Italian–Israel Cooperation Program) and by the Assemble program of the European Union (ECCOCREP project). The academic visit of M.G. to Xiamen University was supported by the 111 Program from the Ministry of Education.

LITERATURE CITED

Badger MR, Price GD (1994) The role of carbonic anhydrase in photosynthesis. Annu Rev Plant Biol 45:369–392

Bilger W, Björkman O (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. Photosynth Res 25:173–185

Boyce DG, Lewis MR, Worm B (2010) Global phytoplankton decline over the past century. Nature 466:591–596

Cai WJ, Hu X, Huang WJ, Murrell MC and others (2011) Acidification of subsurface coastal waters enhanced by eutrophication. Nat Geosci 4:766–770

Chen X, Gao K (2003) Effect of CO_2 concentrations on the activity of photosynthetic CO_2 fixation and extracellular carbonic anhydrase in the marine diatom *Skeletonema costatum*. Chin Sci Bull 48:2616–2620

Cornwall CE, Hepburn CD, McGraw CM, Currie KI and others (2013) Diurnal fluctuations in seawater pH influence the response of a calcifying macroalga to ocean acidification. Proc R Soc Lond B Biol Sci 280:20132201

Dickson AG (1990) Standard potential of the reaction: AgCl (s) +1/2 H_2 (g) = Ag (s) + HCl (aq), and the standard acidity constant of the ion HSO_4^- in synthetic seawater from 273.15 to 318.15 K. J Chem Thermodyn 22:113–127

Dore JE, Lukas R, Sadler DW, Church MJ, Karl DM (2009) Physical and biogeochemical modulation of ocean acidi-

fication in the central North Pacific. Proc Natl Acad Sci USA 106:12235–12240

Dorigo U, Leboulanger C (2001) A pulse-amplitude modulated fluorescence-based method for assessing the effects of photosystem II herbicides on freshwater periphyton. J Appl Phycol 13:509–515

Fanesi A, Raven JA, Giordano M (2014) Growth rate affects the responses of the green alga *Tetraselmis suecica* to external perturbations. Plant Cell Environ 37:512–519

Fick A (1855) On liquid diffusion. Philos Mag J Sci 10:30–39

Flynn KJ, Blackford JC, Baird ME, Raven JA and others (2012) Changes in pH at the exterior surface of plankton with ocean acidification. Nat Clim Change 2:510–513

Gao K, Campbell DA (2014) Photophysiological responses of marine diatoms to elevated CO_2 and decreased pH: a review. Funct Plant Biol 41:449–459

Gao X, Song J, Li X, Yuan H, Li N (2005) Spatial and temporal variations in pH and total alkalinity at the beginning of the rainy season in the Changjiang Estuary, China. Acta Oceanol Sin 24:68–77

Gao K, Xu J, Gao G, Li Y and others (2012) Rising CO_2 and increased light exposure synergistically reduce marine primary productivity. Nat Clim Change 2:519–523

Gattuso JP, Gao K, Lee K, Rost B, Schulz KG (2010) Approaches and tools to manipulate the carbonate chemistry. In: Riebesell U, Fabry VJ, Hansson L, Gattuso JP (eds) Guide to best practices for ocean acidification research and data reporting. Publications Office of the European Union, Luxembourg, p 41–52

Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990:87–92

Giordano M (2013) Homeostasis: an underestimated focal point of ecology and evolution. Plant Sci 211:92–101

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

Goss R, Garab G (2001) Non-photochemical chlorophyll fluorescence quenching and structural rearrangements induced by low pH in intact cells of *Chlorella fusca* (Chlorophyceae) and *Mantoniella squamata* (Prasinophyceae). Photosynth Res 67:185–197

Gray SE, DeGrandpre MD, Langdon C, Corredor JE (2012) Short-term and seasonal pH, pCO_2 and saturation state variability in a coral-reef ecosystem. Global Biogeochem Cycles 26:GB3012, doi:10.1029/2011GB004114

Guillard RR, Ryther JH (1962) Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (cleve) gran. Can J Microbiol 8:229–239

Henry W (1803) Experiments on the quantity of gases absorbed by water, at different temperatures, and under different pressures. Philos Trans R Soc Lond 93:29–276

Hinder SL, Hays GC, Edwards M, Roberts EC, Walne AW, Gravenor MB (2012) Changes in marine dinoflagellate and diatom abundance under climate change. Nat Clim Change 2:271–275

Hinga KR (2002) Effects of pH on coastal marine phytoplankton. Mar Ecol Prog Ser 238:281–300

IGBP, IOC, SCOR (International Geosphere-Biosphere Program, Intergovernmental Oceanographic Commission, Scientific Committee on Oceanic Research) (2013) Ocean acidification: summary for policymakers. In: Broadgate W, Gaffney O, Isensee K, Riebesell U, Urban E, Valdés L (eds) Third symposium on the ocean in a high-CO_2 world. International Geosphere-Biosphere Programme, Stockholm

IPCC (Intergovernmental Panel on Climate Change) (2007) Climate change 2007: the physical science basis. In: Solomon Q, Qin D, Manning M, Chen Z and others (eds) Contribution of Working Group I to the 4th assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

Jassby AD, Platt T (1976) Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. 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

Kim JH, Lee K, Shin K, Kang JH and others (2006) The effect of seawater CO_2 concentration on growth of a natural phytoplankton assemblage in a controlled mesocosm experiment. Limnol Oceanogr 51:1629–1636

Lewis E, Wallance DWR (1998) Program developed for CO_2 system calculations. ORNL/CDIAC-105. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, TN

Li G, Gao K, Yuan D, Zheng Y, Yang G (2011) Relationship of photosynthetic carbon fixation with environmental changes in the Jiulong River estuary of the South China Sea, with special reference to the effects of solar UV radiation. Mar Pollut Bull 62:1852–1858

Middelboe AL, Hansen PJ (2007) High pH in shallow-water macroalgal habitats. Mar Ecol Prog Ser 338:107–117

Montechiaro F, Giordano M (2010) Compositional homeostasis of the dinoflagellate *Protoceratium reticulatum* grown at three different pCO_2. J Plant Physiol 167:110–113

Poole L, Raven J (1997) The biology of Enteromorpha. Prog Phycol Res 12:1–148

Raven JA (2013) Half a century of pursuing the pervasive proton. Prog Bot 74:3–34

Raven JA, Beardall J, Giordano M (2014) Energy costs of carbon dioxide concentrating mechanisms in aquatic organisms. Photosynth Res 121:111–124

Reinfelder JR (2011) Carbon concentrating mechanisms in eukaryotic marine phytoplankton. Annu Rev Mar Sci 3: 291–315

Roy RN, Roy LN, Vogel KM, Porter-Moore C and others (1993) The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperature 0 to 45°C. Mar Chem 44:249–267

Sunda WG, Cai WJ (2012) Eutrophication induced CO_2-acidification of subsurface coastal waters: interactive effects of temperature, salinity, and atmospheric P_{CO_2}. Environ Sci Technol 46:10651–10659

Unsworth RK, Collier CJ, Henderson GM, McKenzie LJ (2012) Tropical seagrass meadows modify seawater carbon chemistry: implications for coral reefs impacted by ocean acidification. Environ Res Lett 7:024026

Wang J (2002) Phytoplankton communities in three distinct ecotypes of the Changjiang Estuary. J Ocean Univ Qingdao 32:422–428

White AJ, Critchley C (1999) Rapid light curves: a new fluorescence method to assess the state of the photosynthetic apparatus. Photosynth Res 59:63–72

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

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

Editorial responsibility: Max Tilzer, Konstanz, Germany

Submitted: June 23, 2014; Accepted: December 10, 2014
Proofs received from author(s): January 15, 2015