

Photophysiological responses of marine diatoms to elevated CO₂ and decreased pH: a review

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Abstract. Diatoms dominate nearly half of current oceanic productivity, so their responses to ocean acidification are of general concern regarding future oceanic carbon sequestration. Community, mesocosm and laboratory studies show a range of diatom growth and photophysiological responses to increasing pCO₂. Nearly 20 studies on effects of elevated pCO₂ on diatoms have shown stimulations, no effects or inhibitions of growth rates. These differential responses could result from differences in experimental setups, cell densities, levels of light and temperature, but also from taxon-specific physiology. Generally, ocean acidification treatments of lowered pH with elevated CO₂ stimulate diatom growth under low to moderate levels of light, but lead to growth inhibition when combined with excess light. Additionally, diatom cell sizes and their co-varying metabolic rates can influence responses to increasing pCO₂ and decreasing pH, although cell size effects are confounded with taxonomic specificities in cell structures and metabolism. Here we summarise known diatom growth and photophysiological responses to increasing pCO₂ and decreasing pH, and discuss some reasons for the diverse responses observed across studies.

Additional keywords: Bacillariophyceae, CO₂, diatom, ocean acidification, photoinhibition, photosynthesis.

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Introduction

Increasing atmospheric CO₂ due to anthropogenic activities affects terrestrial photosynthesis, but is also causing pCO₂ to rise and pH to drop in the surface oceans, which influence marine primary producers (Beardall *et al.* 2009; Riebesell and Tortell 2011; Gao *et al.* 2012a), in a yet more complicated way due to the concurrent changes in seawater chemistry and ocean mixing.

Diatoms are suggested to have evolved between 100 and 200 million years ago (Sims *et al.* 2006), when atmospheric CO₂ is thought to have been ~2000 ppmv (Veron 2008), compared with current levels of ~400 ppmv and projected end-century levels of 750–1000 ppmv. The extant diatoms now contribute ~20% of the organic carbon generated globally each year by photosynthesis (Field *et al.* 1998). They exist both as phytoplankton and as benthic algae. At least some diatom species operate metabolic pathways unusual among studied phytoplankton, including a urea cycle (Allen *et al.* 2011) and a C₄ carboxylation path (Reinfelder *et al.* 2004; Haimovich-Dayana *et al.* 2013). Diatoms run highly efficient CO₂ concentrating mechanisms (CCMs) to achieve a high ratio of carboxylation to oxygenation (Raven *et al.* 2011; Reinfelder 2011). They tolerate high levels of UV radiation (Guan and Gao 2008; Wu *et al.* 2012a), enjoy a low susceptibility to photoinactivation of PSII compared with other phytoplankters (Six *et al.* 2007, 2009; Key *et al.* 2010; Wu *et al.* 2011) and successfully exploit variable light (Lavaud *et al.* 2004,

2007). They are, as a net result, by far the most successful group of eukaryotic aquatic primary producers, not only in terms of primary production but also in their number of species and their capacities to acclimate to environmental changes with diversified metabolisms. Diatom growth rates correlate closely with their size, decreasing almost linearly with the log of increasing cell volume, which ranges across eight orders of magnitude, with cell diameters ranging from ~2 µm to a few mm (Finkel *et al.* 2010). Diatoms have responded to past climate change through successions of differently sized cells, with a trend towards smaller cells under higher temperatures over the past 65 million years (Falkowski and Oliver 2007; Finkel *et al.* 2007).

The ongoing ocean acidification triggered by increasing atmospheric CO₂ concentration alters seawater carbonate chemistry, the availabilities and toxicities of nutrients (Millero *et al.* 2009). Ocean warming will in concert tend to drive increased stratification, decreasing the thickness of the upper mixing layer and lowering transport of nutrients from interior or deeper layers to the surface ocean (Doney 2006; Steinacher *et al.* 2010). These changes will differentially affect differently sized diatom species (Flynn *et al.* 2012), and thereby alter sinking rates and organic carbon export (Finkel *et al.* 2010). Therefore, growth and physiological responses of diatoms to elevated CO₂ concentrations have gained attention (Riebesell *et al.* 1993; Burkhardt and Riebesell 1997; Burkhardt *et al.* 1999). Stimulative, neutral and inhibitory effects of elevated CO₂

concentrations on diatom growth have been reported in different species or even in the same species (Riebesell *et al.* 1993; Burkhardt *et al.* 1999; Chen and Gao 2003; Kim *et al.* 2006; Wu *et al.* 2010; Yang and Gao 2012; Li and Campbell 2013) (Table 1). In this review, we focus on the effects of elevated CO₂ and lower pH on diatom photophysiology, by summarising studies of their growth and photophysiological responses to elevated CO₂ under different experimental conditions, and discuss some potential mechanisms to resolve the diversity of responses observed across studies to date.

Ocean acidification

The oceans are presently absorbing ~25 million tons of CO₂ from the atmosphere each day, an important role in counteracting global warming (Sabine *et al.* 2004). This dissolution of CO₂ from the air is, however, acidifying the oceans (Doney 2006; Gattuso and Hansson 2011).

The exchange of CO₂ between the sea and atmosphere depends on temperature, salinity, physical mixing of seawater, respiration and photosynthesis. Therefore, CO₂ fluxes change horizontally due to physical, chemical and biological properties of waters. When CO₂ dissolves in seawater, it combines with water to form carbonic acid (CO₂ + H₂O → H₂CO₃) which dissociates to bicarbonate (H₂CO₃ → H⁺ + HCO₃⁻), discharging protons (H⁺) and so ultimately attaining a state of new equilibrium. However, as the H⁺ concentration increases with CO₂ dissolution, the H⁺ releases can partially reverse the secondary dissociation reaction, leading to a decrease in carbonate ions (H⁺ + CO₃²⁻ → HCO₃⁻). Typical changes linked with ocean acidification are therefore increased concentrations of pCO₂, H⁺ and HCO₃⁻, decreases in the concentration of CO₃²⁻ and decreases in the CaCO₃ saturation state (Gattuso *et al.* 2010). Since the beginning of the industrial revolution, the pH of oceanic surface seawater has already dropped by ~0.1 unit due to atmospheric CO₂ rise (Caldeira and Wickett 2003),

Table 1. Effects of elevated CO₂ concentrations reported in diatoms

Effects	Authors	Species	Aspects (% change)
Stimulative	(Kim <i>et al.</i> 2006)	<i>Skeletonema costatum</i>	Growth (50%)
	(Wu <i>et al.</i> 2010)	<i>Phaeodactylum tricornutum</i>	Growth (5.2%)
	(King <i>et al.</i> 2011)	<i>Attheya</i> sp.	Growth (31%)
	(Low-Décarie <i>et al.</i> 2011)	<i>Navicula pelliculosa</i>	Growth (100%)
	(Sun <i>et al.</i> 2011)	<i>Pseudo-nitzschia multiseriis</i>	Photosynthesis (21%)
	(Gao <i>et al.</i> 2012b)	<i>Skeletonema costatum</i>	Growth (12%)
	(Gao <i>et al.</i> 2012b)	<i>Phaeodactylum tricornutum</i>	Growth (16%)
	(Yang and Gao 2012)	<i>Thalassiosira pseudonana</i>	Growth (6.5%)
		<i>Thalassiosira pseudonana</i>	Photosynthesis (17%)
	(McCarthy <i>et al.</i> 2012)	<i>Thalassiosira pseudonana</i> CCMP 1014	Growth (50%); PSII electron transport under low light (18%)
Unaffected	(McCarthy <i>et al.</i> 2012)	<i>Thalassiosira pseudonana</i> CCMP 1335	Growth (14%); PSII electron transport under low light (72%)
	(Li and Campbell 2013)	<i>Thalassiosira pseudonana</i> CCMP 1335	Growth under low to optimal light (25%)
	(Riebesell <i>et al.</i> 1993)	<i>Ditylum brightwellii</i>	Growth
		<i>Thalassiosira punctigera</i>	Growth
		<i>Rhizosolenia</i> cf. <i>alata</i>	Growth
	(Chen and Gao 2004a)	<i>Skeletonema costatum</i>	Growth; photosynthesis
	(Kim <i>et al.</i> 2006)	<i>Nitzschia</i> spp.	Growth
	(Crawford <i>et al.</i> 2011)	<i>Thalassiosira pseudonana</i> CCMP 1335	Growth
	(Boelen <i>et al.</i> 2011)	<i>Chaetoceros brevis</i>	Growth
	(Gao <i>et al.</i> 2012b)	Diatoms ^A	Growth (moderate light)
Negative	(Yang and Gao 2012)	<i>Thalassiosira pseudonana</i>	Growth; photoinhibition
	(Ihnken <i>et al.</i> 2011)	<i>Chaetoceros muelleri</i>	Growth (moderate light)
	(Boelen <i>et al.</i> 2011)	<i>Chaetoceros brevis</i>	Growth; photosynthesis
	(Wu <i>et al.</i> 2010)	<i>Phaeodactylum tricornutum</i>	Photoinhibition (14.5%); dark respiration (33.7%)
	(Low-Décarie <i>et al.</i> 2011)	<i>Nitzschia palea</i>	Growth (−67%)
	(Gao <i>et al.</i> 2012b)	<i>Skeletonema costatum</i>	Growth (high light; −12%);
	(Gao <i>et al.</i> 2012b)	<i>Phaeodactylum tricornutum</i>	Growth (highlight; −10%); photorespiration (26%)
		<i>Thalassiosira pseudonana</i>	Growth (high light; −16%); photorespiration (24%)
	(Torstensson <i>et al.</i> 2012)	<i>Navicula directa</i>	Growth (−5%)
	(Yang and Gao 2012)	<i>Thalassiosira pseudonana</i>	Dark respiration (−35%)
	(Ihnken <i>et al.</i> 2011)	<i>Chaetoceros muelleri</i>	Growth (−10%)
	(Li and Campbell 2013)	<i>Thalassiosira pseudonana</i> CCMP 1335	Growth under excess light (−13%)
	(Sobrinho <i>et al.</i> 2008)	<i>Thalassiosira pseudonana</i>	Photoinactivation (25%)
	(Mejía <i>et al.</i> 2013)	<i>Thalassiosira pseudonana</i>	Decreased silicification (12%)
	(Mejía <i>et al.</i> 2013)	<i>Thalassiosira weissflogii</i>	Decreased silicification (90%)

^A*Phaeodactylum tricornutum*, *Skeletonema costatum*, *Thalassiosira pseudonana*.

equivalent to about a 30% increase in the H^+ concentration. With a further increase of CO_2 concentration in the atmosphere to 800–1000 ppmv under the IPCC A1F1 scenario (Houghton *et al.* 2001), by the end of this century, pH of the surface oceans will decrease by another 0.3–0.4 units (Feely *et al.* 2004; Sabine *et al.* 2004; Orr *et al.* 2005), thus, increasing $[H^+]$ by 100–150%. Consequently, organisms in the euphotic zone will be exposed to a higher CO_2 and a lower pH, and their physiologies will respond to changes in seawater carbonate chemistry, as well as to secondary changes in ionic speciation and cell surface chemistry driven by decreasing pH (Millero *et al.* 2009; Flynn *et al.* 2012; Hervé *et al.* 2012; Sugie and Yoshimura 2013). These chemical changes can directly affect physiology of marine organisms (Pörtner and Farrell 2008), but can also indirectly influence organismal responses to other environmental factors including UV radiation (Sobrinho *et al.* 2008; Gao *et al.* 2009; Chen and Gao 2011; Li *et al.* 2012a), light (Bartual and Galvez 2002; Sobrinho *et al.* 2008; McCarthy *et al.* 2012; Li and Campbell 2013), temperature change (Pörtner and Farrell 2008; Zou *et al.* 2011) or nutrients (Burkhardt and Riebesell 1997; Burkhardt *et al.* 1999; Riebesell and Tortell 2011; Li *et al.* 2012b).

Growth responses

Dissolved inorganic carbon (DIC) in surface seawater, at present, is ~100–200 times that of CO_2 in the atmosphere, but most seawater DIC is HCO_3^- , with CO_2 typically accounting for less than 1% in pelagic waters (Gattuso *et al.* 2010). In addition, CO_2 in seawater diffuses ~8000 times slower than in air, which can kinetically limit marine photosynthetic carbon fixation (Raven 1993; Riebesell *et al.* 1993; Morel *et al.* 1994). Growth of diatom species can, in turn, be limited by the availability of CO_2 (Riebesell *et al.* 1993), and oceanic primary production might thus be enhanced by increasing atmospheric CO_2 concentration (Hein and Sand-Jensen 1997; Schippers *et al.* 2004; Riebesell and Tortell 2011). However, the growth rate of diatom-dominated phytoplankton assemblages was not affected by an elevated pCO_2 concentration of 800 μatm during 2–5 days shipboard incubation under ~30% of incident sunlight (Tortell *et al.* 2000). Growth of *Skeletonema costatum* was not stimulated by an enriched CO_2 concentration (800 μatm) under laboratory conditions (Burkhardt and Riebesell 1997; Chen and Gao 2003, 2004a), but was enhanced in a mesocosm at an elevated CO_2 concentration of 750 μatm (Kim *et al.* 2006). Growth of the diatoms *Phaeodactylum tricornutum* (Schippers *et al.* 2004; Wu *et al.* 2010), *Navicula pelliculosa* (Low-Décarie *et al.* 2011) and *Attheya* sp. (King *et al.* 2011) were also enhanced under elevated CO_2 levels under laboratory conditions. However, in the diatom *Chaetoceros muelleri*, low-light treatments showed lower growth rates under elevated CO_2 conditions, but no CO_2 or pH effect was recorded under high light exposure (Ihnken *et al.* 2011). Under similar laboratory conditions, while growth of *Thalassiosira pseudonana* (CCMP 1335) was not stimulated at the elevated CO_2 levels of 760 (Crawford *et al.* 2011) or 1000 μatm (Yang and Gao 2012), *T. pseudonana* (CCMP 1014 and 1335) grew faster under low (McCarthy *et al.* 2012) to moderate light (Li and Campbell 2013) with pCO_2 of 750 μatm ,

but under higher light *T. pseudonana* CCMP 1335 suffered growth inhibition (Li and Campbell 2013). Growth rates of the diatoms *S. costatum* (CCMA110), *P. tricornutum* (CCMA 106) and *T. pseudonana* (CCMP 1335), when grown under different levels of sunlight and elevated CO_2 of 1000 μatm , were stimulated under lower light levels (5–30% surface daytime mean solar PAR), but inhibited under higher light levels, with a drop in the PAR threshold for growth saturation (Fig. 1). These results show that elevated CO_2 and light levels interact to affect diatom growth responses to ocean acidification, which may explain the different results obtained under different experimental setups, at least for the same species (Table 1). Some of these growth responses may relate to CO_2 dependent changes in the cellular susceptibility to photoinactivation of PSII (Li and Campbell 2013) (see below). Since future shoaling of upper-mixed-layer (UML) depths is expected to expose phytoplankton to increased solar irradiance, marine primary producers within UML are expected to suffer from enhanced light stress. However, both low-light CO_2 growth enhancement and high-light CO_2 growth inhibition could occur even within a single daytime solar cycle or a vertical mixing path, making the net community outcomes difficult to predict. Boelen *et al.* (2011) did not find any interactive effects of elevated CO_2 concentration and changing light levels nor fluctuating light on the growth and photosynthesis in the Antarctic diatom *Chaetoceros brevis*. Conversely, frequencies of light fluctuation that mimic different mixing regimes affect a coccolithophore's response to ocean acidification (Jin *et al.* 2013a), implying an interactive effect of light fluctuation and ocean acidification, that could impose an additional layer of influence on the net species and community responses to increasing pCO_2 .

Inorganic carbon acquisition mediated by elevated CO_2

Diatom Rubisco shows comparatively high CO_2 affinity and CO_2/O_2 selectivity, and is served by CO_2 concentrating mechanisms (CCMs) to supply CO_2 to Rubisco, and thereby diminish photorespiration (Roberts *et al.* 2007a). CCMs differ among studied diatom species. *Thalassionema nitzschoides* (Trimborn *et al.* 2009), *Thalassiosira weissflogii* and *P. tricornutum* (Burkhardt *et al.* 2001) actively take up both CO_2 and HCO_3^- , whereas *Thalassiosira punctigera* exclusively uses free CO_2 (Elzenga *et al.* 2000). *T. pseudonana*, though lacking periplasmic (known also as extracellular) carbonic anhydrase (eCA), can take up HCO_3^- directly (Nimer *et al.* 1997; Elzenga *et al.* 2000; Nakajima *et al.* 2013), and indeed uses HCO_3^- as the dominant substrate for photosynthesis even under increased pCO_2 (Hopkinson *et al.* 2013; Isensee *et al.* 2013). The eCA and intracellular carbonic anhydrase (iCA) facilitate C_i acquisition or utilisation by catalysing the inter-conversion of CO_2 and HCO_3^- .

The activity of eCA can be downregulated under elevated CO_2 concentrations relevant to climate change (Burkhardt *et al.* 2001; Chen and Gao 2003; Rost *et al.* 2003; Crawford *et al.* 2011). Therefore, active transport or use of HCO_3^- could be lowered under elevated CO_2 . Different growth conditions can therefore bring about different efficiencies of algal CCMs or changing preferences for CO_2 or HCO_3^- (Korb *et al.* 1997; Nimer *et al.*

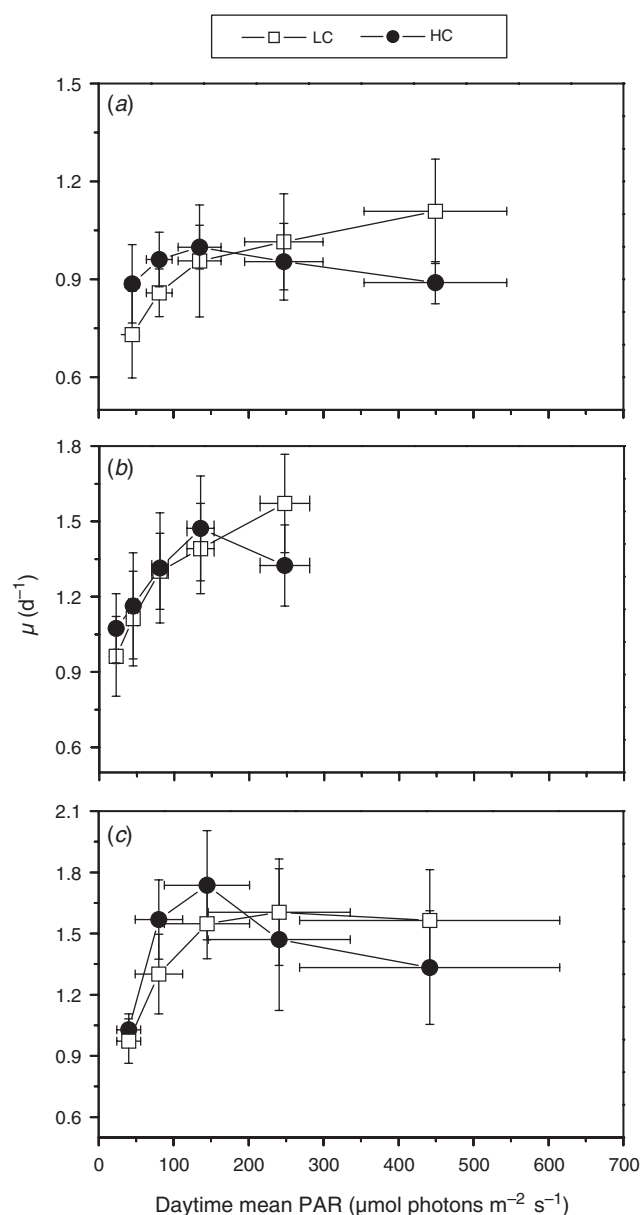


Fig. 1. Light-dependence of diatom growth responses to elevated CO₂ (HC, 1000 μatm, pH_T 7.68) compared with ambient CO₂ level (LC, 390 μatm, pH_T 8.02). Growth rates were stimulated by elevated CO₂ under low to moderate PAR, but inhibited under higher PAR levels. The PAR thresholds for the transition from CO₂ growth stimulation, under lower light, to CO₂ growth inhibition, under higher light, were ~160 for *Phaeodactylum tricornutum* (a), 125 for *Thalassiosira pseudonana* (b) and 178 μmol photons m⁻² s⁻¹, for *Skeletonema costatum* (c). These threshold light levels for the transition for CO₂ growth stimulation to CO₂ growth inhibition correspond to 22–36% of the incident surface solar PAR levels and are equivalent to PAR levels at 26–39 m depth in the South China Sea. The semi-continuous cultures were maintained under the sun and diluted every 24 h to ensure stability of cell density ranges and the seawater carbonate system (from Gao *et al.* 2012b).

1997; Burkhardt *et al.* 2001). In microalgae and in cyanobacteria, very high CO₂ concentrations of up to 50000 μatm (or ppmv in air) turn off CCMs (Kaplan *et al.* 1980; Tsuzuki and Miyachi

1989; Raven 1991; Matsuda *et al.* 2001). CCM induction is closely related to the intracellular Ci pool, ambient CO₂ levels and oxygen availability (Woodger *et al.* 2005). CO₂ levels (up to 1000 μatm) relevant to future CO₂ levels projected for 2100 have been confirmed to partially downregulate CCMs in marine diatoms (Chen and Gao 2003; Trimbom *et al.* 2009; Wu *et al.* 2010, 2012a; Yang and Gao 2012) and to lower reliance upon an intracellular labile carbon pool (Isensee *et al.* 2013). Downregulation of CCMs can include decreased CO₂ affinity resulting in an increased requirement for pCO₂ to support photosynthesis, inhibition of carbonic anhydrase activity, depressed HCO₃⁻ transport, and downregulation of PEPCase and PEPCKase (Reinfelder *et al.* 2000; Giordano *et al.* 2005; Roberts *et al.* 2007a, 2007b; Raven 2010; Reinfelder 2011). Such CCM downregulation was found to be synchronised with diurnal photosynthetic performance in the diatom *S. costatum* (Chen and Gao 2004b).

CCMs in diatoms might connect to multiple metabolic pathways which differ among diatom species of differing physiology or sizes. In *P. tricornutum*, cAMP metabolism is involved to control CCM under elevated CO₂ levels (Harada *et al.* 2006). *T. weissflogii* appears to run a C₃–C₄-intermediate photosynthesis (Roberts *et al.* 2007a), which may concentrate Ci through incorporation into an organic C₄ carbon compound, before Rubisco-aided carboxylation (Reinfelder *et al.* 2000, 2004). However, in *P. tricornutum*, the C₄ path way was recently suggested to contribute to pH homeostasis or excitation dissipation, but not to a CCM function (Haimovich-Dayan *et al.* 2013).

CCMs consume energy (Raven 1991; Bouma *et al.* 1994; Crawford *et al.* 2011; Hopkinson *et al.* 2011). Active uptake of HCO₃⁻ and CO₂ is supported by cyclic and linear electron transport in cyanobacteria (Li and Calvin 1998). Pseudocyclic electron flow through the Mehler reaction can also contribute (Sültemeyer *et al.* 1993). The energisation mechanisms of diatom CCMs are as yet unclear. In addition to the initial uptake, it takes further energy to maintain high intracellular Ci levels by counteracting CO₂ efflux (Sukienik *et al.* 1997; Tchernov *et al.* 1997) although in diatoms tested to date efflux rates appear small (Burkhardt *et al.* 2001; Trimbom *et al.* 2009). The major energy expenditure by the CCMs in diatoms is active Ci transport, and a doubling of ambient [CO₂] could save ~20% of the CCM-related energy expenditure in several diatom species (Hopkinson *et al.* 2011). Under elevated CO₂ concentrations, the growth enhancement under limiting light levels could be partially due to downregulation of CCMs, thereby lowering energy costs (Raven and Johnston 1991; Gao *et al.* 2012b). Alternatively, since CCMs can also be downregulated under low light, elevated pCO₂ could have stimulated the low light growth of diatoms due to both increased availability of CO₂ and savings on the energy cost of CCM operation.

Photosynthetic responses

Light energy captured and delivered via photochemical processes powers the active transport of CO₂ and HCO₃⁻ in cyanobacteria and microalgae (Sültemeyer *et al.* 1993; Sukienik *et al.* 1997; Li and Calvin 1998), and then assimilatory carboxylation. Elevated CO₂ concentration had no significant effect upon pigment

contents nor upon the effective absorbance cross-section serving photosystem II photochemistry (σ_{PSII}) in *S. costatum*, *P. tricornutum* or *T. pseudonana* (Chen and Gao 2004a; Wu *et al.* 2010; Crawford *et al.* 2011; McCarthy *et al.* 2012; Li and Campbell 2013). Furthermore, elevated $p\text{CO}_2$ had only limited effects on levels of the major protein complexes mediating photosynthesis across multiple species of centric diatoms, grown under low to saturating light (McCarthy *et al.* 2012; Li and Campbell 2013).

The photochemical quantum yield of *S. costatum*, *P. tricornutum* and *T. pseudonana* decreases faster with increasing levels of PAR under elevated, than under ambient CO_2 levels (Gao *et al.* 2012b). Non-photochemical quenching (NPQ), however, increases faster in the high- CO_2 grown cells with increasing light levels compared with the ambient CO_2 grown cells (Gao *et al.* 2012b). Modulating NPQ helps diatoms, like other photoautotrophs, to withstand high or fluctuating levels of PAR (Niyogi *et al.* 2005; Lavaud *et al.* 2007; Zhu and Green 2010; Wu *et al.* 2012b). *T. pseudonana* employs NPQ to cope with light stress, even under elevated CO_2 levels, more effectively than does a strain of *P. tricornutum* (Yang and Gao 2012), so that photoinhibition of electron transport was observed in *P. tricornutum*, but not *T. pseudonana*, when grown under elevated CO_2 of 1000 μatm (Wu *et al.* 2010). These differential responses between two model diatoms show taxon-specific mechanisms in coping with the combined impacts of ocean acidification and light stress.

At a functional level, the diatoms *P. tricornutum* and *T. pseudonana* grown under elevated CO_2 of 1000 μatm , at subsaturating photosynthetically active radiation, showed an increase in photosynthetic carbon fixation rate per cell of more than 20% (Wu *et al.* 2010; Yang and Gao 2012). Their growth rate was, however, only enhanced by ~5% in *P. tricornutum* and was unaffected in *T. pseudonana*. Enhanced respiratory and photorespiratory carbon losses under elevated CO_2 are likely responsible for this discrepancy (Wu *et al.* 2010; Gao *et al.* 2012b; Yang and Gao 2012). In the toxic diatom *Pseudo-nitzschia multiseries*, maximum carbon fixation rates per cell also increased with elevated CO_2 levels, although the apparent light use efficiency was not affected (Sun *et al.* 2011). In *Cylindrotheca closterium* f. *minutissima*, when grown at 1000 μatm CO_2 under sunlight, rates of electron transport and O_2 evolution dropped compared with the cells grown at the ambient CO_2 concentration (Wu *et al.* 2012a).

PSII photoinactivation and UV responses

Diatoms, like all photoautotrophs, suffer light- and UV-dependent photoinactivation of their PSII centres (Kok 1956). To maintain their photosynthesis in the face of light-dependent photoinactivation, diatoms must use a metabolically expensive PSII repair cycle (Aro *et al.* 1993) to proteolytically remove photoinactivated protein subunits (Nixon *et al.* 2010; Nagao *et al.* 2012; Campbell *et al.* 2013) and replace them with newly synthesised subunits (Edelman and Mattoo 2008). In comparison with other phytoplankton groups, diatoms enjoy a relatively low susceptibility to photoinactivation of their PSII (Key *et al.* 2010; Wu *et al.* 2011, 2012b). In *T. pseudonana* CCMP 1335, however, the primary susceptibility to photoinactivation

of PSII changes under elevated $p\text{CO}_2$ (Sobrinho *et al.* 2008; McCarthy *et al.* 2012; Li and Campbell 2013). As a net result, cells under high $p\text{CO}_2$ and high light incur an increased metabolic expense to accelerate PSII protein cycling, to counter increased photoinactivation (G Li, DA Campbell, unpubl. data). This increased metabolic cost to maintain PSII function is a possible explanation for the pattern of growth stimulation under elevated $p\text{CO}_2$ under low to moderate light, but growth inhibition under excess light (Gao *et al.* 2012b; McCarthy *et al.* 2012; Li and Campbell 2013). We are as yet unsure as to the mechanism(s) for the changes in susceptibility to primary photoinactivation under elevated $p\text{CO}_2$. Decreased silification under elevated $p\text{CO}_2$ (Mejía *et al.* 2013) might alter cellular optics. Or, a drop in excitation dissipation capacity, as reported in cyanobacteria (Tchernov *et al.* 1997) and now suggested in diatoms (Haimovich-Dayana *et al.* 2013) could result if the CCM is partly downregulated. Evidence in this direction is that the content of the reactive-oxygen toxicity indicator malondialdehyde increases in *T. pseudonana* CCMP 1335 growing under elevated $p\text{CO}_2$ (Li and Campbell 2013), consistent with a downregulation of paths with photoprotective roles under elevated $p\text{CO}_2$.

Solar UV radiation (UVR, 280–400 nm) affects phytoplankton physiology and primary productivity (Häder 2011; and literatures cited therein). In *T. pseudonana*, acclimation to UVR, partially relieved the increased susceptibility to photoinhibition under elevated $p\text{CO}_2$ (Sobrinho *et al.* 2008), consistent with a hormetic protective induction of reactive oxygen species (ROS) detoxification by UV acclimation. The effect of UV-B irradiance (280–320 nm) on *P. tricornutum* was counteracted under ocean acidification conditions (Li *et al.* 2012a). *Cylindrotheca closterium* f. *minutissima* did not show any significant growth response to solar UVR after acclimation to solar radiation, though a combination of UVR and elevated CO_2 concentration led to significant drop in maximal electron transport (Wu *et al.* 2012a).

Respiratory responses

Altered seawater carbonate chemistry due to ocean acidification could perturb energy requirements for the diatom cells, leading to changes in respiration. Mitochondrial respiration indeed increases under ocean acidification conditions of 1000 μatm $p\text{CO}_2$ (pH 7.8) by ~34% in *P. tricornutum* (Wu *et al.* 2010) and by 35% in *T. pseudonana* (Yang and Gao 2012). Increased acidity of seawater associated with increased $p\text{CO}_2$ could disturb cell surface (Flynn *et al.* 2012) or even intracellular pH stability, so that phytoplankton cells may need to allocate additional energy to transport ions against the acid–base perturbation. Cell surface effects of increasing $p\text{CO}_2$ and decreasing pH will vary with cell size and with the co-varying cellular metabolic rate (Flynn *et al.* 2012). Thus, increasing $p\text{CO}_2$ is likely to increase the influences of cell size on phytoplankton responses to environmental forcings (Finkel *et al.* 2010; Flynn *et al.* 2012).

Photorespiration and electron flows to oxygen can be important in photoprotection and short-term responses to excess light in diatoms (Wingler *et al.* 2000; Waring *et al.* 2010). Both *P. tricornutum* and *T. pseudonana* showed enhanced photorespiration by up to 23–27% under elevated

CO₂ or ocean acidification conditions (Gao *et al.* 2012b). Cells of *T. pseudonana* grown under the elevated CO₂ level of 1000 µatm showed higher carbon fixation rates but a lower net O₂ evolution rate compared with cells grown under the ambient CO₂, although the cells exhibited equivalent electron transfer rates from PSII (Yang and Gao 2012), providing evidence for enhanced photorespiratory or pseudocyclic re-consumption of O₂ released from PSII, under ocean acidification. Enhanced excretion of organic compounds due to photorespiration can connect to production of transparent exopolymers in phytoplankton communities that include diatoms (Engel 2002). These metabolic pathways could result in discrepant effects of ocean acidification on different species or under different light levels.

Effects on diatom communities across diverse habitats

Although photosynthesis of diatoms is likely to be stimulated by increased availability of CO₂, lower pH might increase their respiration (Wu *et al.* 2010; Yang and Gao 2012) and their costs for photoprotection, therefore, the net effect of ocean acidification on diatoms will depend on multiple environmental forcings and possibly species-specific metabolic pathways.

Community level responses to rising pCO₂ and temperature vary across oceanic regions. In the north-east Atlantic and North Sea a 50 year (1960–2009) time series survey revealed a decline of dinoflagellate abundance, whereas diatoms showed relatively constant richness (Hinder *et al.* 2012). The transition over the half century was attributed to ocean warming and windy conditions. In contrast, elevated temperatures lowered the short-term abundance of diatoms in a North Atlantic Bloom incubation study (Feng *et al.* 2009), although CO₂ changes had no apparent effect. A shipboard incubation study that examined rising temperature and CO₂ in two natural Bering Sea assemblages also found large community shifts away from diatoms towards nanoflagellates in the ‘greenhouse’ treatment (Hare *et al.* 2007), though diatom-dominated phytoplankton growth increased in the Ross Sea under elevated CO₂ levels (Tortell *et al.* 2008).

Across a CO₂ and pH gradient off the volcanic island of Vulcano (Mediterranean, NE Sicily), periphyton communities altered significantly as CO₂ concentrations increased, with significant increases in chlorophyll *a* concentrations and in diatom abundance (Johnson *et al.* 2013).

Feng *et al.* (2010) found no interactive effects of light and CO₂ on community photosynthesis during an experiment using a Ross Sea diatom/*Phaeocystis* assemblage, but the diatom community structure shifted away from small pennate diatoms towards much larger centric diatoms. In the very different conditions of the South China Sea a shipboard incubation combining elevated CO₂ concentration and near surface solar irradiances, showed decreased photosynthesis while diatom abundance declined (Gao *et al.* (2012b).

In hypoxic seawaters, algae may experience large changes in the ratio of pO₂ to pCO₂ or respiration index (RI = log₁₀ (pO₂/pCO₂)), which is predicted to decline in future oceans (Brewer and Peltzer 2009). Together with lower pH, hypoxic areas represent a future situation of combined ocean acidification and deoxygenation. There has been little documented on the

interactive effects of these two climate-change factors on diatoms nor upon other marine primary producers. However, changes in RI could affect net photosynthesis (Fig. 2) (Gao *et al.* 2012b; Xu and Gao 2012). For diatoms grown under either elevated or ambient levels of CO₂, net photosynthetic O₂ evolution decreases with increased RI (Fig. 2a, b).

Future efforts

Based on the work summarised here, priorities for future study on diatoms include the following.

- (1) Response of frustule mineralisation to elevated CO₂ concentrations. Biogenic silicate content of some diatoms decreases under ocean acidification conditions (Hervé *et al.* 2012; Tatters *et al.* 2012; Mejía *et al.* 2013). However, we do not know the mechanisms involved, nor the interactive effects of multiple factors, such as warming, UV radiation, nutrient limitation, deoxygenation and cell surface pH (Flynn *et al.* 2012; Milligan and Morel 2002) upon silicate mineralisation.
- (2) Higher CO₂ concentrations appear to favour growth enhancement of larger rather than smaller diatoms (Feng *et al.* 2010) (Y Wu, AJ Irwin, D Suggett, D Campbell, ZV Finkel, unpubl. data). Mechanistic studies are needed to examine responses of differently sized diatoms to ocean acidification, to discriminate among direct size effects (Barton *et al.* 2013) and effects of taxonomic distinctions in cell structures (Mitchell *et al.* 2013) or metabolisms.
- (3) Coastal and pelagic water diatoms may react differently to ocean acidification due to their pre-adaptations to different regimes of mixing, nutrient and diel pH changes. In coastal waters, photosynthetic carbon fixation, and night-time respiration per volume of seawater is much higher, leading to high pH during the day and low pH during the night. Little is known about diatom responses to diel pH changes under elevated CO₂ concentrations as well as to diel changes in the respiration index of the water.
- (4) To guide the scope of studies responses of diatoms to ocean acidification should be examined under expected combinations of environmental changes. Although UV radiation appears not to influence the growth of some diatoms under elevated CO₂ level (Wu *et al.* 2012a), UV-B (280–315 nm) seems to counteract some effects of high CO₂ and low pH (Li *et al.* 2012a). Further studies are needed to explore physiological responses of diatoms to reasonable exposures to UV, temperature rise (Shatwell *et al.* 2012), fluctuation of irradiance to model changing mixing conditions, nutrient limitation and deoxygenation under elevated CO₂ and acidification conditions. Hundreds of genes in *P. tricornutum* are upregulated after acclimation to ocean acidification conditions (Y Li, F Su, Y Wu, KJ Wang, K Gao, unpubl. data), but little has been documented on long-term acclimation of open ocean diatoms, and studies on interactions between ocean acidification and the progression from exponential to stationary phase are just beginning (Orellana *et al.* 2013).
- (5) Evolutionary responses to increasing CO₂ concentration in diatoms should be examined for hundreds to thousands of generations. The coastal strain *Thalassiosira pseudonana*

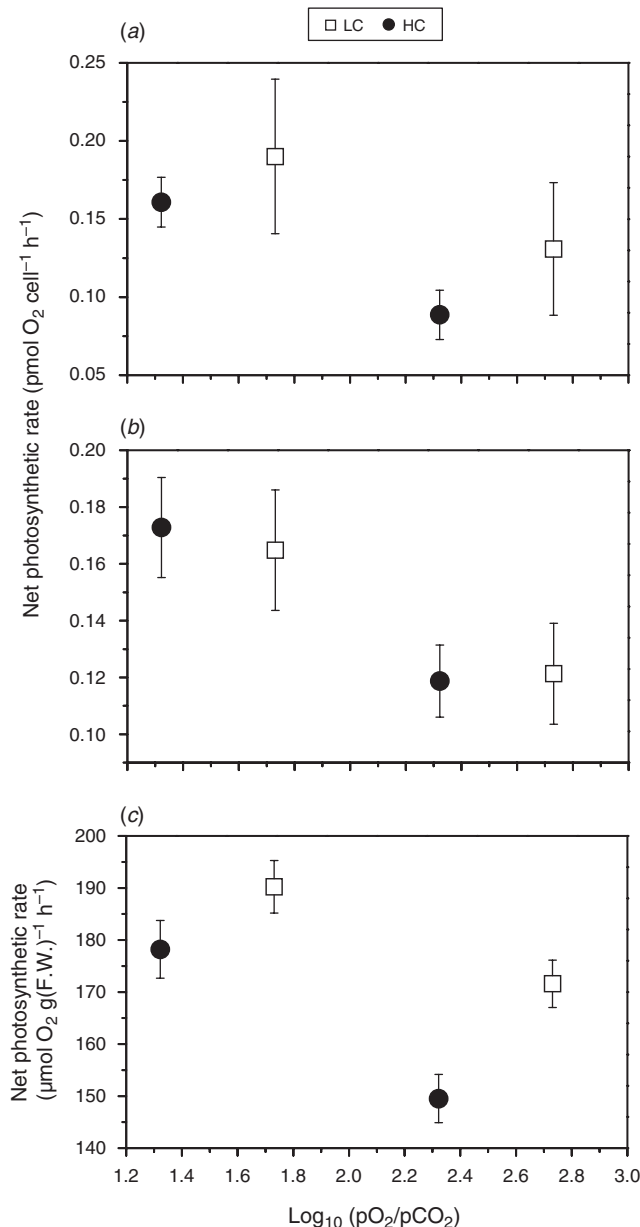


Fig. 2. Decreased net photosynthetic O₂ evolution rates of algae with increased external partial pressure ratio of O₂ to CO₂ or respiration index ($\log_{10}(pO_2/pCO_2)$): (a) diatoms *Thalassiosira pseudonana* and (b) *Phaeodactylum tricornutum* and (c) green alga, *Ulva prolifera*, grown under elevated (HC, 1000 μ atm) or ambient (LC, 390 μ atm) CO₂ levels (re-constructed from Gao *et al.* 2012b; Xu and Gao 2012).

CCMP 1335 showed little evidence of evolutionary adaptation over months of growth at elevated CO₂ (Crawford *et al.* 2011). Evolutionary responses of the freshwater green alga *Chlamydomonas* sp. at high CO₂ demonstrated that some adapted cell lines lost CCM capabilities (Collins and Bell 2004; Collins *et al.* 2006). Lohbeck *et al.* (2012) found that 500 generations of selection at high CO₂ led to recovery of a coccolithophore's growth rates and calcification, although 680 generations of selection

at high CO₂ did not show such a trend in *Gephyrocapsa oceanica* (Jin *et al.* 2013b).

- (6) Monitoring community abundance of diatoms together with other key taxa over longer time scales is important to gain *in situ* information on their responses to environmental changes (Mutshinda *et al.* 2013). These field data obtained from different waters, when combined with mechanistic from controlled experiments would provide valuable insight into future climate change impacts upon phytoplankton communities.

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