

## **Algal and aquatic plant carbon concentrating mechanisms in relation to environmental change**

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### **Abstract.**

Carbon dioxide concentrating mechanisms (also known as inorganic carbon concentrating mechanisms; both abbreviated as CCMs) presumably evolved under conditions of low CO<sub>2</sub> availability. However, the timing of their origin is unclear since there are no sound estimates from molecular clocks, and even if there were, there are no proxies for the functioning of CCMs. Accordingly, we cannot use previous episodes of high CO<sub>2</sub> (e.g. the Palaeocene-Eocene Thermal Maximum) to indicate how organisms with CCMs responded. Present and predicted environmental change in terms of increased CO<sub>2</sub> and temperature are leading to increased CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> and decreased CO<sub>3</sub><sup>2-</sup> and pH in surface seawater, as well as decreasing the depth of the upper mixed layer and increasing the degree of isolation of this layer with respect to nutrient flux from deeper waters. The outcome of these forcing factors is to increase the availability of inorganic carbon, photosynthetic active radiation (PAR) and ultraviolet B radiation (UVB) to aquatic photolithotrophs and to decrease the supply of the nutrients (combined) nitrogen and phosphorus and of any non-aerial iron. The influence of these variations on CCM expression has been examined to varying degrees as acclimation by extant organisms. Increased PAR increases CCM expression in terms of CO<sub>2</sub> affinity, while increased UVB has a range of effects in the organisms examined; little relevant information is available on increased temperature. Decreased combined nitrogen supply generally increases CO<sub>2</sub> affinity, decreased iron availability increases CO<sub>2</sub> affinity, and decreased phosphorus supply has varying effects on the organisms examined. There are few data sets showing interactions among the observed changes, and even less information on genetic (adaptation)

changes in response to the forcing factors. In freshwaters, changes in phytoplankton species composition may alter with environmental change with consequences for frequency of species with or without CCMs. The information available permits less predictive power as to the effect of the forcing factors on CCM expression than for their overall effects on growth. CCMs are currently not part of models as to how global environmental change has altered, and is likely to further alter, algal and aquatic plant primary productivity.

**Keywords** CO<sub>2</sub> concentrating mechanism – combined nitrogen – inorganic carbon – iron – mixing depth - photosynthetically active radiation – phosphorus – temperature – UVA-UVB

### Abbreviations

CCM CO<sub>2</sub> concentrating mechanism

DOC Dissolved organic carbon

PAR Photosynthetically active radiation (400-700 nm)

Rubisco Ribulose bisphosphate carboxylase-oxygenase

UVA Ultraviolet A radiation (320-400 nm)

UVB Ultraviolet B radiation (280-320 nm)

### Introduction

Global and local environmental change is having significant effects on the habitat of algae and aquatic plants, most of which have CO<sub>2</sub> concentrating mechanisms (also known as inorganic carbon concentrating mechanisms: both abbreviated as CCMs) (Giordano et al. 2005; Raven et al. 2005a; Raven 2010, 2011; Reinfelder 2011). Physical and chemical changes have occurred in the last 200 years that have, or may, affect photosynthetic organisms. Further changes are predicted to occur up to 2100. The aim of this paper is to provide a synopsis of what we know of how these forcing factors influence photosynthetic organisms, and the responses of these organisms through regulation and acclimation, using the current genome, or through adaptive genotype modification (Raven and Geider 2003). There is a brief consideration of the possibility of using the response of organisms with CCMs to previous high CO<sub>2</sub> episodes as indicators of what is likely to happen with increasing CO<sub>2</sub> over the next several decades.

The first publications demonstrating the accumulation of inorganic carbon in photosynthesising cells of a cyanobacterium and an alga were published in 1980

(Badger et al. 1980; Kaplan et al. 1980). Since then very significant progress has been made in relating CCMs to the range of kinetics of Rubisco (ribulose biphosphate carboxylase-oxygenase) in cyanobacteria, algae and aquatic plants, to the species of inorganic carbon entering the cells, the roles of carbonic anhydrase and the possibility of C<sub>4</sub>-like photosynthetic metabolism in cyanobacteria, algae and aquatic plants (Giordano et al. 2005; Raven 2010, 2011; Renberg et al. 2010). The molecular genetic basis of CCMs in cyanobacteria is now relatively well understood, with some understanding of the genetic basis of eukaryotic CCMs (Raven 2010, 2011).

To expand slightly on the topic of genomics and diversity of CCMs, Badger et al. (2002, 2003, 2006) and Price et al. (2008) (see also Palinska et al. 2002) consider the diversity of CCMs in cyanobacteria. They distinguish between the ancestral  $\beta$ -cyanobacteria with  $\beta$ -carboxysomes containing Form IB Rubiscos and a range of high and low affinity inorganic C acquisition mechanisms, and the derived  $\alpha$ -cyanobacteria with  $\alpha$ -carboxysomes containing Form IA Rubiscos and a restricted range of inorganic C acquisition mechanisms. The  $\beta$ -cyanobacteria occur in a wide range of inland water and coastal habitats with variable inorganic C availability, while the  $\alpha$ -cyanobacteria (*Prochlorococcus* and many strains of *Synechococcus*) occur in oceanic environments with low nutrient availability and relatively constant inorganic C availability. The extent to which the  $\alpha$ -cyanobacteria show (phenotypic) acclimation decreases with decreasing genome size, but there is a wide range of genotypes adapted to, for example, different depths in stratified areas of the ocean. The rather large areas of the ocean which, on satellite imaging of photosynthetic pigments, are dominated by cyanobacteria contribute about 24% of marine planktonic primary productivity (Uitz et al. 2010).

Less is known at the genetic, and often the physiological, level of CCMs in eukaryotes. For marine phytoplankton there are complete genome sequences for two strains of diatom but still uncertainties about the CCM mechanism(s) used in the Bacillariophyceae (Raven 2010; Reinfelder 2011). Diatom-dominated upwelling areas with a high representation of microplankton account for about 32% of marine planktonic primary productivity (Uitz et al. 2010). Areas of the ocean with a high representation of prymnesiophytes in the nano- and larger picoplankton size range (Jardiller et al. 2009; Liu et al. 2009; Cuvelier et al. 2010) contribute about 44% of marine primary productivity (Uitz et al. 2010). There are no published complete genome sequences for prymnesiophytes (see Cuvelier et al. 2010) which, on the basis of present evidence, have CCMs with lower affinities and/or concentration ratios than do diatoms (Reinfelder 2011). There are four completely sequenced genomes of picoplanktonic prasinophycean green algae, two species of *Ostreococcus* (Palenik et al. 2007) and two species of *Micromonas* (Worden et al. 2009). The genomic data have produced little clarification as to the mechanism of the CCM, characterised physiologically by Iglesias-Rodríguez et al. (1998). Despite dominance of some small

ocean areas by a prasinophycean (*Micromonas pusilla*: Not et al. 2004), the Prasinophyceae do not feature in the spectral types determined by Uitz et al. (2009).

Of particular relevance to the effects of environmental change on CCMs is work on the influence of variations in the availability of inorganic carbon on the expression of CCMs, generally in terms of the affinity for inorganic carbon in photosynthesis and growth, noting that the experimental procedures need careful attention (Hurd et al. 2009; Shi and Morel 2009; Riebesell et al. 2010). There are variations in the details of the responses, but the generalised finding is a decreased affinity for inorganic carbon from cells grown at higher inorganic carbon concentrations (Giordano et al. 2005; Vance and Spalding 2005; Falkowski and Raven 2007; Raven 2010, 2011; Reinfelder 2011). For at least some eukaryotic algae at very high inorganic carbon concentrations there is an essentially complete loss of CCM expression and reliance on diffusive CO<sub>2</sub> transport from the bulk medium to Rubisco (Giordano et al. 2005; Falkowski and Raven 2007). The transition from CCM to diffusive CO<sub>2</sub> entry cannot occur until the external inorganic carbon concentration is higher than a critical value that yields, via the CCM, a steady-state CO<sub>2</sub> concentration around Rubisco which saturates photosynthesis (e.g. Figure 2 of Badger et al. 1980; Kaplan et al. 1980). At external inorganic carbon concentrations lower than the critical value diffusive inorganic carbon flux is outwards (Tchernov et al. 1997, 2003), constituting a leak requiring additional CCM functioning to maintain net inorganic carbon flux. With increasing inorganic carbon concentrations above the critical value there is the possibility, not necessarily realised, for the CCM to be increasingly replaced by diffusive CO<sub>2</sub> entry, with a corresponding saving in energy costs and, probably, in nitrogen and iron requirements (Raven and Johnston 1991; Giordano et al. 2005). The critical external concentration of inorganic carbon varies among organisms with, among other factors, the kinetic properties of Rubisco of an organism. Another factor, discussed in more detail below, is the extent to which the CCM is influenced by PAR (photosynthetically active radiation, 400-700 nm), UVA (ultraviolet A, 320-400 nm) UVB (ultraviolet B, 280 – 320 nm) and the availability of nitrogen, phosphorus and iron.

The elevated atmospheric levels of CO<sub>2</sub> and other greenhouse gases will lead to global warming, with direct influences on the ecophysiology of organisms and/or in their latitudinal or vertical distribution. However, as well as such direct effects of an increase in temperature, there will also be temperature-related effects causing a shallower upper mixed layer in the oceans (Behrenfeld et al. 2006; Doney 2006; Boyce et al. 2010; Steinacher et al. 2010) and in the seasonal epilimnion of lakes (DeStasio et al. 1996). This will cause increases in mean flux of PAR and UVB and decreases in the supply of nutrients (including inorganic carbon) derived from mineralisation in the deep ocean or lake. In this paper, we shall also consider what has been established about the interactions among the environmental factors of temperature, PAR, UVB and nutrients (including inorganic carbon) and CCMs,

acknowledging that these factors have significance for algal growth in a broader context than just their influence on CCMs (Beardall and Giordano 2002; Litchman and Klausmeier 2008; Beardall et al. 2009b; Boyd et al. 2010a; Finkel et al. 2010; Steinacher et al. 2010). First, however, the paper assesses the possibility that previous high CO<sub>2</sub> episodes might shed light on what is happening now and in the next several decades.

### **Previous high CO<sub>2</sub> and temperature episodes: evidence for effects on CCMs?**

It would be very helpful if previous high CO<sub>2</sub> and temperature episodes gave evidence as to the influence of these factors on CCMs. However, there are several reasons why very little progress has been made with this possibility. One problem is that there is no consensus on when the polyphyletic CCMs evolved, although it is clear that the  $\beta$ -cyanobacterial CCM preceded the  $\alpha$ -cyanobacterial CCM (Raven 1997; Badger et al. 2002; Giordano et al. 2005; Riding 2006; Raven et al. 2008; Riding 2009; Raven 2010a). The general assumption is that CCMs evolved in response to low CO<sub>2</sub> availability, with the problem of how CCMs were maintained in intervening high CO<sub>2</sub> episodes (Raven 1997; Badger et al. 2002; Giordano et al. 2005; Raven et al. 2008; Raven 2010, 2011). It might be expected that a CCM origin in low CO<sub>2</sub>, and maintenance in subsequent higher CO<sub>2</sub>, would be most likely in those organisms (cyanobacteria, peridinin-containing dinoflagellates) with maximum specific growth rates that would not be CO<sub>2</sub>-saturated when relying on diffusive CO<sub>2</sub> entry from the low CO<sub>2</sub> environment. The lack of CO<sub>2</sub> saturation is mechanistically imposed by the high CO<sub>2</sub>-saturated specific reaction rate, low CO<sub>2</sub> affinity and low CO<sub>2</sub>-O<sub>2</sub> selectivity of their Rubiscos: see Tcherkez et al. (2006) for mechanistic constraints on the kinetics of Rubisco.

By contrast, the origin of CCMs in low CO<sub>2</sub> episodes and their retention in intervening higher CO<sub>2</sub> episodes might be expected to have a lower likelihood in organisms with Rubiscos with higher CO<sub>2</sub> affinity and higher CO<sub>2</sub>-O<sub>2</sub> selectivity. These organisms are red algae and algae whose plastids arose from red alga endosymbionts with Form ID Rubisco, and to a lesser extent glaucocystophyte and chlorophyll *b*-containing algae with Form IB Rubisco and which have maximum specific growth rate saturated at lower concentrations of CO<sub>2</sub>.

However, these considerations do not take into account temporal and spatial variation in the concentration of CO<sub>2</sub> and more generally the fact that aquatic systems are not necessarily in gaseous equilibrium with the atmosphere. This is especially true in productive freshwaters where high photosynthetic demand can produce extremely low concentrations of CO<sub>2</sub> in surface water during the summer (Maberly 1996), potentially producing an ecological niche for species with constitutive or facultative CCMs. The same can also be true in productive marine systems (Middelboe and Hansen 2007).

Molecular clock approaches are not useful, and there are no unequivocal molecular markers or fossils, e.g. the possible pyrenoids (a component of many eukaryotic CCMs: Badger et al. 1998) in a putative eukaryotic alga from the Neoproterozoic Bitter Springs formation (Oehler 1976, 1977), or in a desmid (Chlorophyta: Charophyceae) from the Devonian of New York State (Barschnagel 1966). While there is now a realisation that CCMs are among the confounding factors in using the  $\delta^{13}\text{C}$  of organic matter in marine sediments to estimate past atmospheric  $\text{CO}_2$  levels (Laws et al. 2002), these  $\delta^{13}\text{C}$  measurements are not of use in dating the time of origin of CCMs or their subsequent degree of expression. By contrast, there is anatomical, molecular clock and natural abundance stable isotope data for the timing of the polyphyletic origin of  $\text{C}_4$  photosynthesis in flowering plants on land (Cerling et al. 1989, 1998; Osborne and Beerling 2006).

## **Chemical and Physical Forcing Factors associated with Global Environmental Change**

### General considerations

The chemical effects of additional (anthropogenic)  $\text{CO}_2$  on surface waters on Earth have been extensively investigated. The physicochemical background of  $\text{CO}_2$  solubility in waters of different salinities and temperatures, and the thermodynamics and kinetics of the dissolved inorganic carbon system ( $\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{HCO}_3^- + \text{H}^+ \leftrightarrow \text{CO}_3^{2-} + 2\text{H}^+$ ), is well known: the principals were established in the nineteenth century. Zeebe and Wolf-Gladrow (2001) give a clear account of the chemistry of the inorganic carbon system in seawater, and the outcome for anthropogenic  $\text{CO}_2$  inputs to the ocean and its biota is considered by Giordano et al. (2005), The Royal Society (2005), Rost et al. 2008, Doney et al. (2009a), Joint et al. (2010) and Reinfelder (2011). The input of  $\text{CO}_2$  to the ocean causes an increase in the concentration of dissolved  $\text{CO}_2$  and a parallel increase in the much lower equilibrium concentration of  $\text{H}_2\text{CO}_3$  and  $\text{H}^+$ . There is a smaller proportionate, but larger in absolute terms, increase in the concentration of  $\text{HCO}_3^-$  and, by contrast, a decrease in the concentration of  $\text{CO}_3^{2-}$ . This perhaps counter-intuitive decrease in an inorganic carbon species as a result of added  $\text{CO}_2$  comes about because at seawater pH the effect of decreasing pH and shifting the chemical equilibrium away from  $\text{CO}_3^{2-}$  is greater than the effect of increasing the overall concentration of inorganic carbon. While the physical chemistry of ocean acidification is well understood, there is debate about the methods that should be used to mimic the phenomenon experimentally (Hurd et al. 2009; Schulz et al. 2009; Shi et al. 2009). Methods that involve shifting the  $\text{CO}_2:\text{HCO}_3^-:\text{CO}_3^{2-}$  equilibrium by adding mineral acids decrease the alkalinity of the medium and so should be avoided.

### Marine phytoplankton

While the physical chemistry of these changes is very well understood, there are significant spatial and temporal variations in the extent of equilibration of  $\text{CO}_2$

between the atmosphere and surface seawater (Doney et al. 2009a, b). On a local scale where there are no major downwellings to and upwellings from the deep ocean, this is a function of the physical factors wind and mixing of the upper layer of the ocean, and of the balance of photosynthesis and respiration in the upper mixed layer. This can involve recycling of inorganic and organic carbon between photolithotrophs and chemo-organotrophs in “recycled production” (Raven and Falkowski 1999; Gruber et al. 2009; Riebesell et al. 2009). Distinct from this and superimposed on it is the global scale thermohaline circulation with the “solubility pump” and the “biological pump” (Raven and Falkowski 1999; Gruber et al. 2009; Riebesell et al. 2009). The “solubility pump” is based on downwelling, in the thermohaline circulation, of cool surface water in which CO<sub>2</sub> is more soluble. The “biological pump” is based on photosynthetic CO<sub>2</sub> assimilation into phytoplankton followed by sinking of particulate organic matter out of the photic zone. Continued photosynthetic primary productivity for this “export production” requires inputs of carbon, nitrogen, phosphorus, iron and other inorganic resources in varying proportions from below the photic zone and input from the land in rivers and in aeolian deposition (Falkowski and Raven 2007). Removal of CO<sub>2</sub> from the upper mixed layer is long term (millennia and longer) for only a very small fraction of the sinking organic matter. The great majority of the sinking organic material is biologically mineralised in the dark parts of the ocean and the resulting CO<sub>2</sub>, NO<sub>3</sub><sup>-</sup>, HPO<sub>4</sub><sup>2-</sup> etc. returns to the surface with deep mixing in winter at high latitudes and, especially, at systems such as the Humboldt and Benguelas upwellings. These upwellings have CO<sub>2</sub> concentrations above the air-equilibrium value, especially as the cool deeper-ocean water warms at the surface at lower latitudes.

Global warming, and with it the warming of the surface ocean, is influencing and will increasingly influence the physical, and consequently the chemical, oceanography of the upper few hundred metres of ocean (Doney 2006; Riebesell et al. 2009; Berger et al. 2010; Boyce et al. 2010). At low latitudes, in the absence of an upwelling, the greater temperature differential between the warm upper mixed layer and the cooler deeper waters decreases the extent of eddy diffusion of nutrients from the deeper phosphate- and combined nitrogen-rich waters to the surface nutrient-poor waters. The impact on the supply of phytoplankton-available iron of such decreased eddy diffusion is much less than for phosphate and combined nitrogen, since phytoplankton-available iron input to the ocean is mainly aeolian (discussed by Archer et al. 2000; Hutchins et al. 2002; Parekh et al. 2004; Boyd 2007; Boyd et al. 2010b). The restriction on the nutrient flux from the deep ocean means even less new productivity and even less potential for continued export production from this already nutrient-limited ocean. The thermocline also shoals so that the upper mixed layer is less deep and, other things being equal, the mean flux of PAR, UVA and UVB incident on phytoplankton cells is higher. In nutrient-deprived conditions this could result in more photoinhibition *sensu lato* by PAR (and UVA), and cause more inhibition by UVB (or greater costs of avoiding this damage) (Beardall et al 2009a).

At higher latitudes with increasing seasonality of electromagnetic energy input to the surface ocean, the depth of the deep mixing in the winter is decreased as the surface ocean stays warmer. Nutrients from the deeper ocean are still supplied to the surface waters, but the mixing depth in the summer as well as in the winter is decreased so that the mean flux of PAR, UVA and UVB incident on the phytoplankton cells is higher. This means that, because of the earlier decrease in the mixing depth, there is the potential for an earlier initiation of the spring bloom of phytoplankton, and due to the higher mean PAR and continued availability of nutrients, higher productivity may occur throughout the summer, resulting in an overall increase in annual production (Doney 2006; Boyce et al. 2010).

A further possible influence of environmental change on the depth of the upper mixed layer and the related changes in mean electromagnetic radiation flux and nutrient availability is a change in the frequency and intensity of storm events (Knutson et al. 2009; Gnanadesikan et al. 2010). Major storms are known to temporarily increase primary productivity in warmer parts of the ocean (e.g. Landry et al. 2008; Chen et al. 2009), estuaries (e.g. Wetz and Paerl 2008) and lakes (e.g. Robarts et al. 1998). There are also recent suggestions that ozone depletion has a role to play in regulation of the Southern Annular Mode and CO<sub>2</sub> drawdown in the Southern Ocean (Lenton et al. 2009), thus providing additional complexity to the interactions among the various physical and biological components of environmental change.

Superimposed on these general effects on the availability of a range of nutrients as a result of changed ocean circulation is the possibility of changes in the availability of particular nutrients as a result of acidification. An example is iron, with very complicated chemistry in the surface ocean (e.g. Boyd et al. 2010b). However, while Shi et al. (2010) suggest that iron limitation of marine phytoplankton growth is likely to increase in some areas, Breitbarth et al. (2010) found increased Fe(II), the most generally accessible form of iron for photosynthetic organisms, in high-CO<sub>2</sub> mesocosms.

### Phytoplankton of Inland Waters

In inland waters, similar biogeochemical cycles occur as described in the oceans but the influence of the terrestrial catchment is much greater with often substantial inputs, via inflowing streams, of phosphorus, nitrogen, silicon, iron and other elements. Inorganic carbon is supplied from weathering of rocks and input of CO<sub>2</sub> derived from breakdown of organic carbon fixed in the catchment. The catchment can also supply large amounts of terrestrially-produced dissolved organic carbon (DOC), often with high short-wavelength absorbance, that can ameliorate harmful effects of UVB (Williamson et al. 1996) but also limit productivity by absorbing PAR (Karlsson et al. 2009). Microbial and photochemical degradation of DOC within a lake is one of the causes of the generally elevated concentrations of CO<sub>2</sub> in many lakes (Tranvik et al. 2009). There is evidence for increasing concentrations of dissolved organic carbon in



northern-hemisphere temperate lakes as the impact of atmospheric acid deposition decreases (Monteith et al. 2007) which could lead to increased concentrations of CO<sub>2</sub> while changes in catchment productivity resulting from land management, atmospheric deposition of nutrients (primarily nitrogen) and climate change could have a range of effects. Overall, inland waters are frequently not in equilibrium with the atmosphere because rates of biological transformation can exceed physico-chemical rates of air-water exchange and so the direct impact of rising atmospheric CO<sub>2</sub> on freshwaters is likely to be smaller than on the ocean.

Loads to inland waters of critical nutrients, such as phosphorus and nitrogen, have increased as a result of Man's activities (Conley et al. 2009). The increased nutrient availability is likely to exceed any reduced summer-supply resulting from reduced rates of eddy-diffusion and may shift species composition towards competitive species that tend to have effective CCMs. For example, it has been suggested that one reason for the competitive advantage of cyanobacteria during the summer in productive lakes lies in their effective CCM that allows them to outcompete other phytoplankton for inorganic carbon (Shapiro 1997). However, there are other possibilities for the dominance of cyanobacteria in inland waters: some of them are diazotrophs (Vitousek et al. 2002), and the high accumulation factor achieved by the CCM of cyanobacteria means that, even granted the low CO<sub>2</sub> affinity and low CO<sub>2</sub>/O<sub>2</sub> selectivity of the Form 1B Rubisco of cyanobacteria, these organisms could have a higher nitrogen use efficiency than other phytoplankton organisms (Raven 1991a,b, Giordano et al. 2005).

#### Marine benthos

Benthic photosynthesis is influenced in the same ways as phytoplankton by the changes in inorganic carbon and direct temperature aspects of environmental change, and by the changes in the availability of combined nitrogen and of phosphate attendant on shoaling of the thermocline. Coastal eutrophication (Conley et al. 2009) may also favour competitive species, such as 'green tide' *Ulva* sp. with effective CCMs, over other species. Since the organisms are attached to the substrate the shallower upper mixed layer does not directly influence the mean flux of PAR, UVA or UVB incident on organisms at a given depth. In the intertidal the higher atmospheric CO<sub>2</sub> has the potential to influence directly emersed photosynthesis (Maberly and Madsen 1990) by the organisms that are not saturated by the current levels of CO<sub>2</sub> (Johnston and Raven 1986; Surif and Raven 1990; Johnston et al. 1992; Beardall et al. 1998).

#### Inland water benthos

Similar considerations apply to inland water benthos as to inland water phytoplankton for the effects of inorganic carbon and direct effects of temperature, and for effects of

mixed layer depth on combined nitrogen and phosphorus supply. Inland water benthos resembles marine benthos as far as PAR, UVA and UVB flux is concerned. A distinction from marine benthos is the absence of tidal effects: any variations in water depths are over much longer time intervals, e.g. drawdown during seasonal or other drawdowns with no guarantee of rapid re-submergence.

### Terrestrial Algae and Hornworts

All terrestrial cyanobacteria (free-living or lichenized) have CCMs, as do some terrestrial free-living and lichenized green algae, and hornworts (Palmqvist 1993; Palmqvist et al. 1994; Smith and Griffiths 1996; Palmqvist et al. 1997; Badger et al. 1997; Meyer et al. 2008; Gadd and Raven 2010; Raven 2010a). Terrestrial cyanobacteria, algae and hornworts are desiccation tolerant and poikilohydric, contrasting with the great majority of terrestrial vascular plant sporophytes: this influences their response to changed CO<sub>2</sub> and temperature regimes (Meyer et al. 2008; Gadd and Raven 2010; Raven and Andrews 2010).

### **Responses of pholothotrophs with and without CCMs to environmental change**

#### General considerations

Almost all of the available data on particular species concern the regulation or acclimation, rather than adaptation (Raven and Geider 2003), of photosynthesis and CCMs in response to the environmental conditions related to global change. Regulation here means the changes to the functioning of the pre-existing proteome (changes in post-translational modification and in ligand concentration) over times of seconds to minutes of a change in conditions: there is not enough time for changes to the proteome, related to changes in transcription and translation. Acclimation is defined as changes in the use of the existing genome by changes in transcription and translation, and hence in the proteome and metabolome (Raven 2010, 2011; Renberg et al. 2010; Wienkoop et al. 2010), in response to changes in the environment; it occurs over time intervals of an hour and longer, and occurs in parallel with, and may modulate, regulation. Adaptation is taken to mean evolutionary changes to the genome in response to changed environmental conditions, with the possibility of more extreme changes to the proteome and metabolome than is the case for acclimation. Adaptation typically occurs over years or longer, and occurs in parallel with, and may modulate, regulation and acclimation.

Work with organisms originating recently from a common ancestor (e.g. a recently established culture starting from a single cell) on changed environmental conditions for times of up to several months is at the level of regulation and acclimation. Culturing organisms under the experimental and control conditions over longer periods (months – years) is subject to unexpected changes in environmental conditions (e.g. equipment failures, interruption of electricity supply) and has rarely been undertaken. An admirable exception is the work of Collins and co-workers on

increased CO<sub>2</sub> concentration for the growth of *Chlamydomonas reinhardtii* (Collins and Bell 2004, 2006; Collins et al. 2006a,b; Bell and Collins 2008; Collins 2010). Collins and Gardner (2009) have discussed analytical procedures for dealing with the different timescales over which different mechanisms of response to environmental change can operate.

In field observations or manipulations of natural populations of a species or assemblages of species of photosynthetic organisms, there is the possibility of selection of genotypes of the species or of species within the assemblage. Examples are experimental mesocosms of plankton subjected to a range of experimental conditions, e.g. variations in CO<sub>2</sub> (Riebesell 2004), transects involving parcels of surface seawater with naturally or experimentally different CO<sub>2</sub> concentrations (e.g. Tortell et al. 2010) or the comparison of areas of benthos influenced over years or more by CO<sub>2</sub> from a seafloor vent compared with a control area nearby (Hall-Spencer et al. 2008). In the cases where natural assemblages are allowed to react to the changed conditions, molecular genetic analysis of at least the dominant organisms are needed to distinguishing genetic adaptation from the selection of pre-existing genotypes. In these cases there are, of course, also regulatory and acclimatory responses to the changed conditions.

We now consider the effects of environmental change on CCMs through the more direct effects of increased CO<sub>2</sub> and temperature, and the more indirect effects on CCMs of a decreased thickness of the upper mixed layer. Decreases in the upper mixed layer thickness and in nutrient transfer from the deep ocean to the low-nutrient upper mixed layer means lower nitrogen and phosphorus (and iron) availability to primary producers in lotic habitats, and a greater mean incident PAR, UVA and UVB flux in planktonic lotic environments. Beardall and Giordano (2002) have previously discussed the role of environmental factors in modulating CCM activity in cyanobacteria and microalgae. Lest CCMs are accorded undue importance, it is necessary to bear in mind how significant the CCM effects are, relative to other aspects of environmental change, in altering the functioning of algae and aquatic plants. It is of interest that the current models of the effects of environmental change on marine primary productivity do not explicitly consider CCMs (Behrenfeld et al. 2006; Doney 2006; Boyce et al. 2010; Henson et al. 2010; Steinacher et al. 2010). The conclusions from the following analysis are summarised in Table 1.

#### Increased CO<sub>2</sub> and corresponding changes in the dissolved inorganic carbon system and pH

Data summarised by Giordano et al. (2005) and Raven et al. (2005a, b) (see also Ratti et al. 2007 and Egge et al. 2009) suggested that cyanobacteria (all with CCMs) and those microalgae with CCMs were almost all saturated for photosynthesis and growth with the inorganic carbon supply in present day air-equilibrated seawater or alkaline inland waters. For macroalgae and normally submerged vascular plants, inorganic

carbon saturation for growth in these conditions was rather less widespread. There are abundant data showing that the affinity of CCMs for inorganic carbon decreases with increasing inorganic carbon concentrations for growth with eventual, at least in some eukaryotes, loss of CCM expression and reliance on diffusive fluxes of CO<sub>2</sub> from the medium to Rubisco (Giordano et al. 2005, Raven 2010, 2011; Wu et al. 2010, Reinfelder 2011).

Subsequent work with laboratory cultures has shown some instances of a lack of CO<sub>2</sub> saturation of CCM-expressing cyanobacteria and microalgae for specific growth rate (from cell counts) and, more generally, for organic carbon production (Fu et al. 2007, 2008; Riebesell et al. 2007; Feng et al. 2008, 2009a,b; Hu and Gao 2008; Iglesias-Rodriguez et al. 2008; Wu et al. 2008; Finkel et al. 2010; Kranz et al. 2010; Levitan et al. 2010). The miss-match between the effects on cell specific growth rate and on organic carbon production means that the cells have an increased cellular organic carbon quota and/or a greater production of extracellular organic carbon (see Raven et al. 2005b; Finkel et al. 2010). Increased CO<sub>2</sub> concentrations could have influences on the cell size of phytoplankton organisms through a smaller restricting effect of diffusion boundary layer thickness which is in turn a function of cell size (Korb et al. 1996, 1998, Finkel et al. 2010). However, there are other ecological and evolutionary constraints on cell size, e.g. acquisition of other nutrients, and the sinking rate of the organism (Raven and Waite 2004, Finkel et al. 2010). Finkel et al. (2005) relate the decreasing mean cell size of marine diatoms through the Cenozoic to the decreasing temperature rather than the decreasing CO<sub>2</sub> concentration over the tens of millions of years. The sinking rate of diatoms can be altered by increased CO<sub>2</sub> via stimulation of the dissolution of the silica frustules (Milligan et al. 2004), although the influence of this effect is relatively small when the mean lifetime of individual planktonic diatom cells is taken into account (Marbá et al. 2007).

These laboratory cultures were grown at saturating levels of PAR with no UVB, and with saturating levels of nutrients other than the varied supply of inorganic carbon. As was mentioned above, and will be discussed in more detail below, limiting PAR and nutrients other than inorganic carbon, and the presence of UVB, alter the operation of the CCM and the dependence of photosynthesis and, where investigated, growth on inorganic carbon availability. Overall, the distinction between the inorganic C dependence of photosynthesis and growth for algae with CCMs and those relying on diffusive transport from the medium to Rubisco is less clear-cut than was previously believed (Raven 2010b).

There is growing evidence that CO<sub>2</sub> generation during intracellular calcification in coccolithophores is not stoichiometrically involved as component of a CCM, or is otherwise necessarily involved in supplying CO<sub>2</sub> to Rubisco (Herfort et al. 2004; Trimborn et al. 2007; Leonardos and Geider 2009). This conclusion means that the variable nature of the reported effects of increased CO<sub>2</sub> on coccolithophore calcification (see Zondervan 2007; Iglesias-Rodriguez et al. 2008; Doney et al.

2009a; Muller et al. 2010) does not directly impact on CO<sub>2</sub> supply to photosynthesis. It is certain that dissolution of coccolith calcite occurs when the medium is undersaturated with respect to calcite (Doney et al. 2009a), although the relatively short mean lifetime of coccolithophores must be remembered (Marbá et al. 2007). Climate change may alter the balance of advantage for species with and without CCMs. A larger fraction of phytoplankton from inland waters than from marine habitats lack CCMs, with the expectation of a lower affinity for inorganic carbon in those species lacking CCMs (Raven et al. 2005a; Raven 2010. 2011). For example Chrysophytes, as a group, appear to lack CCMs (Maberly et al. 2009) and their greater abundance in freshwaters compared to the oceans may result from the frequently elevated concentrations of CO<sub>2</sub> there. Future changes in chrysophyte distribution in freshwaters as a result of environmental change are uncertain and depend strongly on future concentrations of dissolved CO<sub>2</sub>.

### Temperature

Temperature influences algal growth in many ways (Raven and Geider 1988; Finkel et al. 2010), but, on present evidence, it is difficult to tease out specific effects on CCMs (Raven et al. 2002a,b; Finkel et al. 2010). It is clear that CCMs occur in the polar algae tested, all of which are closely related to algae from warmer habitats that have CCMs (Mitchell and Beardall 1996; Beardall and Roberts 1999; see also Tortell et al. 2008a,b), despite arguments that the necessity for CCMs might be less in very cold habitats, at least in eukaryotic algae if not cyanobacteria (Raven and Geider 1988; Raven et al. 2002a,b; Finkel et al. 2010). Attempts to use the natural abundance of stable isotopes of carbon to determine if there is a latitudinal/temperature gradient of the frequency of algae lacking CCMs in the algal flora have been equivocal (Raven 2002a,b).

Turning from these adaptation and biogeographical considerations, the literature is, as far as we can tell, silent on the effects of temperature acclimation on the properties of CCMs. However, there are data on the effects of temperature on the growth rate of cyanobacteria (Fu et al. 2007) and eukaryotic algae (Feng et al. 2008; Fu et al. 2008) at two concentrations of CO<sub>2</sub>: there are clear interactions between temperature and CO<sub>2</sub>. The excellent start made by David Hutchins and collaborators needs extension in terms of the phylogenetic range of organisms investigated and the range of conditions examined. There are also data on CO<sub>2</sub>-temperature interactions for natural phytoplankton assemblages (North Atlantic spring bloom) in the ocean (Feng et al. 2009a). For aquatic organisms lacking CCMs, Maberly (1985) did pioneering work on the interaction of PAR, CO<sub>2</sub> and temperature in photosynthesis of the freshwater moss *Fontinalis antipyretica* that reinforced the idea that the strength of CO<sub>2</sub>-limitation is determined by the level of other environmental factors that control photosynthesis.

All species of cyanobacteria studied so far possess a CCM. Their ecological dominance depends on a number of ecological factors and they are a very obvious symptom of ‘eutrophication’ caused by anthropogenic nutrient loading. Elevated temperatures resulting from climate change have been documented and continued increases forecast with confidence in the future. Since cyanobacteria generally require warm water for growth (Paerl & Huisman 2008) they, and the CCM syndrome, may become more widespread with climate warming.

#### Photosynthetically Active Radiation

Since the pioneering work of Beardall on the cyanobacterium *Anabaena* (Beardall 1991) there have been several investigations of the effect of the flux density of PAR on CCMs, generally as indicated by the half-saturation concentration of inorganic carbon (Giordano et al. 2005; Raven et al. 2005b, 2008; Young and Beardall 2005; Feng et al. 2009a,b; Fu and Han 2010; Kranz et al. 2010; Levitan et al. 2010). The results of these experiments are that CCM expression is decreased by low PAR for photosynthesis of otherwise unacclimated organisms. Similar, but less detailed, data are available for growth of laboratory cultures of cyanobacteria (Fu et al. 2007) and eukaryotic algae (Feng et al. 2008, Fu et al. 2008) and for the Ross Sea phytoplankton (Feng et al. 2009b). For aquatic plants in inland waters low-light can down-regulate Crassulacean Acid Metabolism in *Crassula helmsii* (Klavnsen and Maberly 2010) and *Littorella uniflora* (Madsen 1987) although in the latter species low-light does not cause down-regulation if concentrations of CO<sub>2</sub> are low (Madsen 1987). There are good mechanistic reasons for the down-regulation of CCMs at low photon flux densities for growth (Raven 1990, 1991a,b; Raven and Johnston 1991; Raven et al. 2000, 2002a,b; Fu and Han 2010). The mechanistic reason for down-regulation of CCMs at low PAR is that the energy input to the energized inorganic C influx is lower in low irradiances, while the leakage of CO<sub>2</sub> from the intracellular pool is unaltered, with the reasonable assumption of a constant conductance of the leakage pathway (Raven et al. 2000, 2002a,b). This also helps to explain the greater representation of algae relying on diffusive CO<sub>2</sub> entry rather than CCMs in the subtidal than the intertidal of the marine benthos (Maberly 1990). For C<sub>4</sub> terrestrial flowering plants there is also evidence of increased CO<sub>2</sub> leakage for plants grown at low PAR (Henderson et al. 1992; Tazoe et al. 2008; Pengelty et al. 2010), and there are relatively few low light-adapted C<sub>4</sub> plants (Winter et al. 1982). Fu et al. (2007, 2008) and Feng et al. (2008) also studied the three-way interactions among CO<sub>2</sub>, PAR and temperature.

An increased incident mean flux of PAR with a less-deep mixed layer might increase the chances of photoinhibition, with probable implications for the size spectrum of phytoplankton (Key et al. 2010) and hence for the function of CCMs and also for inorganic carbon transport in organisms lacking CCMs (Beardall et al. 2009b; Finkel et al. 2010). Wu et al. (2010) found that *Phaeodactylum tricorutum* grown at high (101.3 Pa) CO<sub>2</sub> was more sensitive to photoinhibition, and had less non-photochemical

quenching, in high PAR than did cells grown in present day (39.3 Pa) CO<sub>2</sub>: if this is a general phenomenon it will have significant implications as the upper mixed layer shoals in a warmer higher CO<sub>2</sub> world. Wu et al. (2010) also found that growth at the higher CO<sub>2</sub> concentration resulted in a 34% increase in the rate of dark respiration, which the authors relate to an increased rate of biosynthesis under the higher CO<sub>2</sub> conditions (although the growth rate only increased, significantly, by 5.2%) and/or an increased requirement for metabolic energy with a decreased external pH. Collins and Bell (2004) found increased respiration in some of the genotypes of *Chlamydomonas reinhardtii* that had evolved in a 1000-generation exposure to high CO<sub>2</sub>. Poorter et al. (1992) analysed the literature on respiration rates of organs of C<sub>3</sub> and C<sub>4</sub> terrestrial flowering plants. On a leaf area basis both C<sub>3</sub> and C<sub>4</sub> plants showed a significant increase in respiration in increased CO<sub>2</sub>, while on a leaf dry weight basis both groups showed a decrease in respiration rate in high CO<sub>2</sub>, although only the C<sub>4</sub> data set showed a significant difference. The dry weight data are presumably closer to the algal data: if so, they show the opposite effect of increased CO<sub>2</sub> on flowering plants with CCMs and on algae with CCMs.

## Nitrogen

Growth of eukaryotic microalgae under nitrogen-limiting conditions increases the inorganic carbon affinity of CCMs when the nitrogen source is NO<sub>3</sub><sup>-</sup>, although in *Nannochloropsis* sp. there was a decreased inorganic carbon affinity in moving from the second-lowest to the lowest NO<sub>3</sub><sup>-</sup> concentration used, but a decreased inorganic carbon affinity in the single case in which NH<sub>4</sub><sup>+</sup> was the nitrogen source (Giordano et al. 2005; Raven et al. 2005b, 2008; Young and Beardall 2005; Hu and Zhou 2010). There was a decrease in inorganic carbon affinity with decreasing nitrogen supply with NH<sub>4</sub><sup>+</sup> as nitrogen source in *Chlamydomonas reinhardtii* (Giordano et al. 2003), resembling the results with the lowest NO<sub>3</sub><sup>-</sup> concentrations used for *Nannochloropsis* (Hu and Zhou 2010). The increased CCM expression under NO<sub>3</sub><sup>-</sup>-nitrogen limitation accords with mechanistic considerations and with comparisons of C<sub>3</sub> (CO<sub>2</sub> diffusion) and C<sub>4</sub> (a C<sub>4</sub>-cycle based CCM) flowering plants on land (Raven 1990, 1991a,b; Raven and Johnston 1991; Giordano et al. 2005). When nitrogen is not limiting, its chemical form exerts a rather obvious effect, in the green alga *Dunaliella salina*, on the affinity of photosynthesis for inorganic carbon, which was appreciably higher in the presence of NH<sub>4</sub><sup>+</sup> than of NO<sub>3</sub><sup>-</sup> (Giordano and Bowes 1997; Giordano 2001). The decrease in nitrification rates in the ocean as a consequence of ocean acidification (Beman et al. 2010) will decrease the nitrate concentration relative to that of ammonium and organic nitrogen in seawater, with possible influences on the effect of combined nitrogen of CCMs in the future as well as in past high-CO<sub>2</sub> episodes.

## Phosphorus

The three data sets available yield contrasting conclusions. Using *Chlorella*, Kozłowska-Szeremol et al. (2004) found an increased affinity for inorganic carbon

under phosphorus limitation while Beardall et al. (2005) found a decreased affinity in a different strain of *Chlorella* and different experimental conditions. Hu and Zhou (2010) found an increasing inorganic carbon affinity with increasing phosphorus limitation in *Nannochloropsis*. Increasing affinity for inorganic carbon in two of the three P-deficient algae seems at odds with the known decreased efficiency of energy transformation, at least for respiration where there is a lower ATP per glucose oxidised in P-deficient green algae (Theodorou and Plaxton 1993), although less is known of what happens under photosynthetic conditions (Weng et al. 2008).

Some algae may have C<sub>4</sub>-like photosynthesis as (part of) their CCMs, and it is of interest that three C<sub>4</sub> grasses, one each from the three biochemical subtypes, have a high photosynthetic phosphorus use efficiency in units of mol CO<sub>2</sub> fixed per second per mol leaf phosphorus (Ghannoum and Conroy 2007; Ghannoum et al. 2008). There is no clear mechanistic reason for a higher or lower phosphorus requirement of organisms with CCMs compared to those with diffusive CO<sub>2</sub> entry. We know of no information on the affinity for inorganic carbon as a function of phosphorus supply, or of photosynthetic phosphorus use efficiency, for algae lacking CCMs.

## Iron

The only data available for laboratory cultures of algae are those of Young and Beardall (2005), showing that iron deficiency (such as might happen with decreased mixed layer depth) increased CCM expression, consistent with mechanistic predictions (Raven 1990, 1991a,b; Raven and Johnston 1991). The role, if any, of CCMs in iron-light co-limitation of marine primary productivity (Galbraith et al. 2010) has not yet been investigated.

## UVB

Following the pioneering work of Beardall et al. (2002) there have been a number of studies with a variety of algae on the effects of UVB on CCMs, with a range of results (Song and Qiu 2007; Sobrino et al. 2008, 2009; Beardall et al. 2009a,c; Gao et al. 2009; Xu and Gao 2009). There seem to be no data on how UVB influences inorganic C affinity of algae with diffusive CO<sub>2</sub> entry. However, Sobrino et al. (2008) hypothesized that such down-regulation of Rubisco and CA under elevated CO<sub>2</sub> might be responsible for the increase in UVR sensitivity observed under elevated CO<sub>2</sub> conditions. Increased UVB with decreased mixed-layer depth would exacerbate the UVB effects, particularly when taken with enhanced nutrient limitation under these conditions, and both nitrogen and phosphorus limitations have been shown to increase the UVB sensitivity of algal photosynthesis (see Beardall et al. 2009a, and references within). In contrast, almost nothing is known about the interactive impacts of UVR and Fe-limitation on CCMs and CO<sub>2</sub> fixation. Van de Poll et al. (2005) indicated that iron-limited cultures of the Antarctic marine diatom *Chaetoceros brevis* were less sensitive to high levels of PAR and UVR than were iron-replete cultures, an effect possibly due to elevated superoxide dismutase and ascorbate peroxidase (scavengers



of reactive oxygen species) in iron-limited cells (Van de Poll et al. 2009). It may be of interest here that induction of CCMs in *Chlamydomonas* (Im et al. 2003) and *Cyanophora* (Burey et al. 2005) is paralleled by increased expression of genes related to removal of reactive oxygen species. However, these works do not deal with UV: the laboratory culture conditions had no UV radiation.

## UVA

UVA can energize algal photosynthesis (McLeod and Kanwisher 1962; Halldall 1964, 1967; Mengelt and Prézelin 2005; Gao et al. 2007; Xu and Gao 2010), but can also inhibit growth (Callieri et al. 2001; Gao et al. 2007). The action spectrum for induction of  $\text{HCO}_3^-$  utilization in the green freshwater planktonic microalga *Monoraphidium braunii* shows peaks in the blue and UVA regions of the spectrum, as well as an environmentally irrelevant peak in the UVC (Giráldez et al. 1998); the relevance of this to CCM operation with a smaller mixed layer depth and hence increased mean incident blue and UVA requires further investigation.

## Implications for CCM expression in past and future higher $\text{CO}_2$ and warmer worlds

The information discussed in this section shows that the upper layer of natural water bodies will experience an increased concentration of  $\text{CO}_2$  (apart perhaps from productive inland waters) and temperature, and a decreased mixed layer depth with a corresponding increase in the mean PAR, UVA and UVB incident on phytoplankton and a decreased supply of nutrients such as nitrogen, phosphorus and iron for all photosynthetic organisms. These changes in the environment mean that the changed CCM expression, involving decreased inorganic carbon affinity, as a result of increased  $\text{CO}_2$ , will be at least partly offset by the effects of the other environmental changes on CCM expression. There would also be an increase in the critical concentration of external inorganic carbon above which, for a particular organism, the CCM could be partly replaced by diffusive  $\text{CO}_2$  entry to an extent which parallels further increases in external inorganic carbon.

In addition to the implications for the extent of CCM expression in the future there is also the possibility that the indirect effects of warming on CCM expression have influenced CCMs in the past. If, as seems very likely, the polyphyletic CCMs evolved in a low  $\text{CO}_2$  environment prior to that experienced over the past few tens of millions of years (Raven 1997; Badger et al. 2002; Giordano et al. 2005; Riding 2006; Raven et al. 2008; Riding 2009; Raven 2010, 2011), the indirect environmental effects on CCM expression could have aided retention of CCMs through higher  $\text{CO}_2$  and warmer episodes subsequent to the origin of the CCMs. Such effects of the warming and hence shoaling of the upper mixed layer, and the consequent changes in the supply of nutrients, PAR, UVA and UVB, would be particularly significant in organisms with Rubiscos having relatively high  $\text{CO}_2$  affinities and  $\text{CO}_2/\text{O}_2$  selectivities with the consequent possibility of a low critical external inorganic carbon concentration above

which diffusive CO<sub>2</sub> entry could partly replace CCMs. The retention of CCMs would be expected to be less dependent on interactions with the indirect effects of warming in organisms such as cyanobacteria and peridinin-containing dinoflagellates with Rubiscos having lower CO<sub>2</sub> affinities and CO<sub>2</sub>/O<sub>2</sub> selectivities, and hence higher critical external inorganic carbon concentration above which diffusive CO<sub>2</sub> entry could partly replace CCMs. The correlations described here do not necessarily denote evolutionary driving forces related to the retention of CCMs, and such retention might constitute emergent properties. Indeed, it must be emphasised that these suggestions assume no relevant genetic changes since a given CCM evolved until the present when the experimental correlations were observed, and that this will also be the case for the expression and function of this CCM in the future. This obvious lack in the data available for forecasting (and hindcasting) clearly needs addressing (see e.g. Collins and Gardner 2009).

## **Conclusions**

Global environmental change has had, and will increasingly have, effects on CCMs through the more direct effects of increase CO<sub>2</sub> and temperature in all habitats for algae and aquatic plants. There will also be more indirect effects on CCMs in some environments: for lotic habitats a decreased thickness of the upper mixed means a lower combined nitrogen and phosphorus (and iron) availability to primary producers, and a greater mean incident PAR, UVA and UVB flux for planktonic lotic environments. In terrestrial habitats the direct influences of global environmental change on CCMs in algae and hornworts are increases in CO<sub>2</sub> and temperature. It is also important to remember that effects on CCMs are only one component of the response of photosynthetic organisms to environmental change. Current models of the effects of environmental change on marine primary productivity since the start of the industrial revolution, and that are likely to occur by 2100, do not explicitly consider CCMs (Bopp et al. 2004, 2005; Behrenfeld et al. 2006; Doney 2006; Richardson 2008; Boyce et al. 2010; Steinacher et al. 2010). It seems unlikely that incorporating CCMs into the models will alter the conclusions in the way that CCMs are now known to be among the confounding factors in using the  $\delta^{13}\text{C}$  of organic matter in marine sediments to estimate past atmospheric CO<sub>2</sub> levels (Laws et al. 2002).

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## References

- Archer DE, Johnson K (2000) A model of the iron cycle in the ocean. *Global Biogeochem Cycles* 14: 269-279
- Badger MR, Kaplan A, Berry JA (1980) Internal inorganic carbon pool of *Chlamydomonas reinhardtii*. *Plant Physiol* 66: 407-413.
- Badger MR, Andrews TJ, Whitney SM, Ludwig M, Yellowlees D, Leggat W, Price GD (1998) The diversity and coevolution of Rubisco, plastids, pyrenoids chloroplast-based CO<sub>2</sub>-concentrating mechanisms in algae. *Can J Bot* 76: 1052-1071.
- Badger MR, Hanson D, Price GD (2002) Evolution and diversity of CO<sub>2</sub> concentrating mechanisms in cyanobacteria. *Funct Plant Biol* 29: 161-173.
- Badger MR, Price GD (2003) CO<sub>2</sub> concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution. *J Exp Bot* 54: 609-622.
- Badger MR, Price GD, Long BM, Woodger FJ (2006) The environmental plasticity and ecological genomics of the cyanobacterial CO<sub>2</sub> concentrating mechanism. *J Exp Bot* 57: 249-265.
- Barschnagel RA (1966) New fossil algae from Middle Devonian of New York. *Trans Am Microscop Soc* 85: 297-302.
- Beardall, J (1991). Effects of photon flux density on the "CO<sub>2</sub> concentrating mechanism" of the cyanobacterium *Anabaena variabilis*. *J Plankt Res* 13: 133-141
- Beardall J, Beer S, Raven JA (1998) Biodiversity of marine plants in an era of climate change. Some predictions based on physiological performance. *Bot Mar* 66: 407-413.
- Beardall J, Roberts S (1999) Inorganic carbon acquisition by two Antarctic macroalgae, *Porphyra endivifolia* (Rhodophyta: Bangiales) and *Palmaria decipiens* (Rhodophyta: Palmariales). *Polar Biol* 21: 310-315.
- Beardall J and Giordano M. (2002). Ecological implications of algal CCMs and their regulation (review). *Functional Plant Biology* 29: 335-347.
- Beardall J, Heraud P, Roberts S, Shelly K, Stojkovic S (2002) Effects of UV-B radiation in on inorganic carbon acquisition by the marine microalgae *Dunaliella tertiolecta* (Chlorophyceae). *Phycologia* 41: 268-272. doi?

Beardall J, Roberts S, Raven JA (2005) Regulation of inorganic carbon acquisition by phosphorus limitation in the green alga *Chlamydomonas emersonii*. *Can J Bot* 83: 859-864.

Beardall J, Sobrino S, Stojkovic S (2009a) Interactions between impacts of ultraviolet radiation, elevated CO<sub>2</sub>, and nutrient limitation in marine primary producers. *Photochem Photobiol Sci* 8: 1257-1265

Beardall J, Allen D, Bragg J, Finkel ZV, Flynn KV, Quigg A, Rees TAV, Richardson A, Raven JA (2009b) Allometry and stoichiometry of unicellular, colonial and multicellular phytoplankton. *New Phytol* 181: 295-309. Beardall J, Stojkovic S, Larson S (2009c) Living in a high CO<sub>2</sub> world; impacts of global climate change on marine phytoplankton. *Plant Ecol Divers* 2: 191-205. Behrenfeld MJ, O'Malley RT, Siegel DA, McClain CR, Sarmiento JL, Felman GC, Milligan AJ, Falkowski PG, Letelier RH, Bass EH (2006) Climate-driven trends in contemporary ocean productivity. *Nature* 444: 752-755.

Bell G, Collins S (2008) Experimental evolution and global change. *Evolutionary Applications* 1: 3-16.

Beman AM, Chow CE, King ALK, Fend Y, Fuhrman JA, Bates NR, Popp BN, Hutchins DA (2010) Global declines in oceanic nitrification rates as a consequence of ocean acidification. *Proc Natl Acad Sci USA* doi:10.1073/pnas.1011053108

Berger SA, Diehl S, Stibor H, Trommer G, Ruhlenstroth M (2010) Water temperature and stratification depth independently shift cardinal events during plankton spring succession. *Global Change Biol* 16: 1954-1965.

Bopp L, Boucher O, Aumont O, Belviso S, Dufresne J-L, Pham M, Monfray P. 2004. Will dimethylsulphide emissions amplify or alleviate global warming? A model study. *Can J Fish Aquat Sci* 61: 826-835.

Bopp L, Aumont O, Cadule P, Alvain S, Gehlen M. 2005. Response of diatom distribution to global warming and potential implications: a global model study. *Geophys Res Lett* 32: L19606.

Boyce DG, Lewis MR, Worm B (2010) Global phytoplankton decline over the past century. *Nature* 466: 591-596. Boyd PW (2007) Biogeochemistry: iron findings. *Nature* 446: 989-991

Boyd PW, Ibanawi E, Sander SG, Hunter KA, Jackson GA (2010b) Remineralisation of upper ocean particles: Implications for iron biogeochemistry. *Limnol Oceanogr* 55:1271-1288. Boyd PW, Strzepek R, Fu FX, Hutchins DA (2010a) Environmental control on open-ocean phytoplankton groups: now and in the future. *Limnol Oceanogr* 53: 1353-1376. Breitbarth E, Bellerby RJ, Neill CC, Ardelan MV, Meyerhofer M,

Zollner E, Croot PL, Riebesell U (2010) Ocean acidification affects iron speciation during a coastal seawater mesocosm experiment. *Biogeosci* 7: 1063-1073.

Burey SC, Poroyko V, Ergen ZN, Schuller C, Ohnishi N, Fukuzawa H, Bohnert HJ, Löffelhardt W (2007) Acclimation to low [CO<sub>2</sub>] by an inorganic carbon-concentrating mechanism in *Cyanophora paradoxa*. *Plant Cell Environm* 30: 1422-1435.

Callieri C, Morobito G, Huot Y, Neale PJ, Litchman E (2001) Photosynthetic responses of pico- and nanoplanktonic algae to UVB, UVA, and PAR in a high mountain lake. *Aquatic Sci* 63: 286-293.

Cerling TE, Quade J, Wang Y, Bowman JR (1989) Isotopes in soils and palaeosols as ecology and palaeontology indicators. *Nature* 341: 138-139.

Cerling TE, Ehleringer JR, Harris JM (1998) Carbon dioxide starvation, the development of C-4 ecosystems, and mammalian evolution. *Phil Trans R Soc Lond B* 353: 159-170.

Chen YLL, Chen HY, Jan S, Tuo SH (2009) Phytoplankton productivity enhancement and assemblage change in the upstream Kuroshio after typhoons. *Mar Ecol Progr Ser* 385: 111-126.

Collins S (2010) Competition limits adaptation and productivity in a photosynthetic alga at elevated CO<sub>2</sub>. *Proc Roy Soc B* in press doi: 10.1098/rspb.2010.1173

Collins S, Bell G (2004) Phenotypic consequences of 1000 generations of selection at elevated CO<sub>2</sub> in a green alga. *Nature* 431: 566-569.

Collins S, Bell G (2006) Evolution of natural algal populations at elevated CO<sub>2</sub>. *Ecol Lett* 9: 129-135.

Collins S, Gardner A (2009) Integrating physiological, ecological and evolutionary timescales: a Price equation approach. *Ecol Lett* 12: 744-757. Collins S, Sültemeyer D, Bell G (2006a) Rewinding the tape: selection of algae adapted to high CO<sub>2</sub> at current and Pleistocene levels of CO<sub>2</sub>. *Evoln* 60: 1392-1401.

Collins S, Sültemeyer D, Bell G (2006b) Changes in carbon uptake in populations of *Chlamydomonas reinhardtii* selected at high CO<sub>2</sub>. *Plant Cell Environm* 29: 1812-1819.

Conley DJ, Paerl HW, Howarth RW, Boesch DF, Seitzinger SP, Havens KE, Lancelot C, Likens GE (2009) Controlling eutrophication: nitrogen and phosphorus. *Science* 323: 1015-1015.

Cuvelier ML, Allen AE, Maren A, McCrow JP, Messié M, Tringe SG, Woyke T, Welsh RM, Ishoey T, Less J-H, Binder BJ, Dupont CL, Latasa M, Guigard C, Back

KC, Dupont CL, Latasa M, Caleo E, Read B, Lasken RS, Chavez FP, Woprden AT (2010) Targetted metagenomics and ecology of globally important uncultured eukaryotic phytoplankton. *Proc Natnl Acad Sci USA* 107: 14679-14684.

De Stasio BT, Hill DK, Kleinhans JM, Nibbelink NP and Magnuson JJ (1996) Potential effects of global climate change on small north-temperatre lakes: Physics, fish and plankton. *Limnol Oceanog*, 41: 1136-1149.

Doney SC (2006) Plankton in a warmer world. *Nature* 444: 605-696.

Doney SC, Lima L, Feely RA, Glover DM, Lindsay K, Mahowald N, Moore JK, Wanninkhof R (2009b) Mechanisms governing interannual variability in upper-ocean inorganic carbon system and air-sea CO<sub>2</sub> fluxes. *Deep-Sea Res II: Topical Studies Oceanograph* 56: 640-655.

Doney SC, Fabry VJ, Feeley RA, Kleypas JA (2009a) Ocean acidification: the other CO<sub>2</sub> problem. *Annu Rev Mar Sci* 1: 169-192.

Egge JK, Thingstad TF, Larsen A, Engel A, Wohlers J, Bellerby RGJ, Riebesell U (2009) Primary production during nutrient-induced blooms at elevated CO<sub>2</sub> concentrations. *Biogeosci* 6: 877-885.

Falkowski PG, Raven JA (2007) *Aquatic Photosynthesis*, 2<sup>nd</sup> Edition. Princeton University Press, Princeton, NJ, USA

Feng Y, Warner ME, Zhang Y, Sun J, Fu FX, Rose JM, Hutchins DA (2008) Interactive effects of increased pCO<sub>2</sub>, temperature and irradiance on the marine coccolithophore *Emiliana huxleyi* (Prymnesiophyceae). *Eur J Phycol* 43: 87-98.

Feng Y, Hare CE, Leblanc K, Rose JM, Zhang Y, DiTullio GR, Lee PA, Wilhelm RW, Rowe JM, Sun J, Nemek M, Guegen C, Passow U, Benner I, Hutchins DA (2009a) The effects of increased pCO<sub>2</sub> and temperature on the North Atlantic Spring Bloom. I. The phytoplankton community and biogeochemical responses. *Mar Ecol Progr Ser* 388: 13-25.

Feng Y, Hare CE, Rose JM, Hardy SM, DiTullio GR,S, Sun J, Lee PA, Smith WO, Peloquin J, Tozzi MC, Sohst B, Sun J, Zhang Y, Dunbar KB, Long MC, Sohst B, Hutchins DA (2009b) Interactive effects of iron, irradiance and CO<sub>2</sub> on Ross Sea phytoplankton. *Deep Sea Res* 57: 604-620. Finkel ZV, Katz ME, Wright JD, Schofield OME, Falkowski PG (2005) Climatically driven patterns in the size of diatoms over the Cenozoic. *Proc Natnl Acad Sci USA* 102: 8927-8932.

Finkel ZV, Beardall J, Flynn KJ, Quigg A, Rees TAV, Raven JA (2010) Phytoplankton in a changing world: cell size and elemental stoichiometry. *J Plankt Res* 32; 119-137. doi: 10.1093/plankt/fbp098

- Fu FX, Warren ME, Zhang YH, Feng YY, Hutchins DA (2007) Effects of increased temperature and CO<sub>2</sub> on photosynthesis, growth and elemental ratios in marine *Synechococcus* and *Prochlorococcus*. *J Phycol* 43: 485-496.
- Fu FX, Zhang YH, Warner ME, Feng YY, Sun J, Hutchins DA (2008) A comparison of future increased CO<sub>2</sub> and temperature effects on sympatric *Heterosigma akashiwo* and *Prorocentrum minimum*. *Harmful Algae* 7: 76-90.
- Fu XA, Han BP (2010) Response of cyanobacterial carbon concentrating mechanism to light intensity: a simulated analysis. *Chinese J Oceanol Limnol* 28: 478-488.
- Gadd GM, Raven JA (2010) Geomicrobiology of eukaryotic microorganisms. *Geomicrobiol J* 27: 491-519. doi: 10.1080/014910037022
- Galbraith ED, Gnanadedikan A, Dunne JP, Hiscock MR (2010) Regional impacts of iron-light colimitation in a global biogeochemical model. *Biogeosci* 7: 1043-1064.
- Gao KS, Wu Y, Li G, Wu H, Villafañe VE, Helbling EW (2007) Solar UV radiation drives CO<sub>2</sub> fixation in marine phytoplankton: a double-edged sword. *Plant Physiol* 144: 54-59.
- Gao KS, Ruan ZX, Villafane VE, Gattuso JP, Helbling EW (2009) Ocean acidification exacerbates the effect of UV radiation on the calcifying phytoplankton *Emiliana huxleyi*. *Limnol Oceanogr* 54: 1855-1862.
- Ghannoum O, Conroy JP (2007) Phosphorus deficiency inhibits growth in parallel with photosynthesis in a C<sub>3</sub> (*Panicum laxum*) but not two C<sub>4</sub> (*P. coloratum* and *Cenchrus ciliaris*) grasses. *Funct Plant Biol* 34: 72-81.
- Ghannoum O, Paul MJ, Ward JL, Beale MH, Corol D-I, Conroy JP (2008) The sensitivity of photosynthesis to phosphorus deficiency differs between C<sub>3</sub> and C<sub>4</sub> grasses. *Funct Plant Biol* 25: 213-221.
- Giordano M. (2001) Interactions between C and N metabolism in *Dunaliella salina* cells cultured at elevated CO<sub>2</sub> and high N concentrations. *J Plant Physiol* 158: 577-581.
- Giordano M, Bowes G (1997). Gas exchanges, metabolism, and morphology of *Dunaliella salina* in response to the CO<sub>2</sub> concentration and nitrogen source used for growth. *Plant Physiol* 115: 1049-1056.
- Giordano M, Norici A, Forssen M, Eriksson M, Raven JA (2003). An anaplerotic role for mitochondrial carbonic anhydrase in *Chlamydomonas reinhardtii*. *Plant Physiol* 132: 2126-2134.

- Giordano M, Beardall J, Raven JA (2005) CO<sub>2</sub> concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. *Annu Rev Plant Biol* 56: 99-131.
- Giráldez N, Aparicio PJ, Quiñones MA (1998) Blue light requirements for HCO<sub>3</sub><sup>-</sup> uptake and its action spectrum in *Monoraphidium braunii*. *Photochem Photobiol* 68: 420-426.
- Gnanadesikan A, Vecchi GA, Anderson WG, Hallberg R, Emanuel K (2010) How ocean color can steer Pacific tropical cyclones. *Geophys Res Lett* in press. doi: 10.1029/2010GL044514
- Gruber N, Gloor M, Fletcher SEM, Doney SC, Dutkeiwicz S, Follows S, Gerber MJ, Jacobson AR, Joos F, Lindsay K, Meneenlis D, Mouchet A, Muller SA, Sarmieinto JL, Takahashi T (2009) Ocean sources, sinks and transport of CO<sub>2</sub>. *Global Biogeochem Cycles* 23 Art No. GB1005 2009
- Halldall P (1964) Ultraviolet action spectra of photosynthesis and photosynthetic inhibition in a green and red alga. *Physiol Plant* 17: 414-424.
- Halldall P (1967) Ultraviolet action spectra in algology. A review. *Photochem Photobiol* 6: 445-460.
- Hall-Spencer JM, Rodolfo-Metalpa R, Martin S, Ransome E, Fine M, Turner SM, Rawley SJ, Tedesco D, Buia M-C (2008) Volcanic carbon dioxide vents reveal ecosystem effects of ocean acidification. *Nature* 454: 96-99.
- Henderson SA, von Caemmerer S, Farquhar GD (1992) Short term measurements of carbon isotope discrimination in several C<sub>4</sub> species. *Austr J Plant Physiol* 19: 263-285.
- Henson SA, Sarmineto JL, Dunne JP, Bopp L, Lima I, Doney SC, Beaulieu C. (2010) Detection of anthropogenic climate change in satellite records of ocean chlorophyll and productivity. *Biogeosci* 7: 621-649.
- Herfort L, Loste E, Meldrum F, Thake B (2004) Structural and physiological effects of calcium and magnesium in *Emiliana huxleyi* (Lohman) Hay and Mohler. *J Struct Biol* 148: 307-314.
- Hu HH, Gao KS (2008) Impacts of CO<sub>2</sub> enrichment on growth and photosynthesis in freshwater and marine diatoms. *Chinese J Oceanol Limnol* 26:407-414.
- Hu HH, Zhou QB (2010) Regulation of inorganic carbon acquisition by nitrogen and phosphorus levels in the *Nannochloropsis* sp. *World J Microbiol Biotech* 26: 957-961.
- Hurd CL, Hepburn CD, Currie KI, Raven JA, Hunter KA (2009) Testing the effects of ocean acidification on algal metabolism: considerations for experimental design. *J Phycol* 45: 1030-1051.



Hutchins DA, Hare CE, Weaver RS, Zhang Y, Firme GF, DiTullio GR, Alm MB, Risemanr SF, Mauchner JM, Geesey ME, Trick CG, Smith GJ, Rue EL, Conn J, Bruland KW (2002) Phytoplankton limitation in the Humboldt Current and Peru upwelling. *Limnol Oceanogr* 47: 997-1011

Iglesias-Rodriguez MD, Halloran PR, Rickaby REM, Hall IR, Colmenero-Hidalgo E, Gittins JR, Green DRM, Tyrrell T, Gibbs SJ, von Passow P, Rehm E, Armbrust EV, Boessenkoot KP. (2008). Phytoplankton calcification in a high-CO<sub>2</sub> world. *Science* 320: 336-340.

Im CS, Zhnag Z, Shrager J, Chang CW, Grossman AR (2003) Analysis of light and CO<sub>2</sub> regulation in *Chlamydomonas reinhardtii* using genome-wide approaches. *Photosynth Res* 75: 111-125.

Jardiller L, Zubkov MV, Pearman J, Scanlan DJ (2010) Significant CO<sub>2</sub> fixation by small Prymnesiophyceae in the subtropical and tropical Northeast Atlantic Ocean. *ISME J* 4: 1180-1192.

Johnston AM, Raven JA (1986) The analysis of photosynthesis in air and water by *Ascophyllum nodosum* (L) Le Jol. *Oecologia* 69: 288-295.

Johnston AM, Maberly SC, Raven JA (1992) The acquisition of inorganic carbon by four red macroalgae from different habitats. *Oecologia* 92: 317-326.

Joint I, Doney SC, Karl DM (2010) Will ocean acidification affect marine microbes. *ISME J* 2010 7: in press doi: 10.1038/ismej.2010.79

Kaplan A, Badger MR, Berry JA (1980) Photosynthesis and the intracellular inorganic carbon pool in the blue green alga *Anabaena variabilis*: response to external CO<sub>2</sub> concentration. *Planta* 149: 219-226.

Karlsson J, Byström P, Ask J, Ask P, Persson L, Jansson M (2009) Light limitation of nutrient-poor lake ecosystems. *Nature* 460, 506-509.

Key T, McCarthy A, Campbell DA, Six C, Roy S, Finkel ZV (2010) Cell size trade-offs govern light exploitation strategies in marine phytoplankton. *Env Microbiol* 12: 95-104.

Klavsen SK, Maberly SC (2010) Effect of light and CO<sub>2</sub> on inorganic carbon uptake in the invasive aquatic CAM-plant *Crassula helmsii*. *Funct Plant Biol* 37: 737-747.

Knutson TR, McBride JL, Chan J, Emanuel K, Holland G, Landsea C, Held I, Kossin JP, Srivastava AK, Sugi M (2009) Tropical cyclones and climate change. *Nature Geosci* 3: 157-163.

- Korb RE, Raven JA, Johnston AM, Leftley JW (1996) Effect of cell size and specific growth rate on stable carbon isotope discrimination by two species of marine diatom. *Mar Ecol Progr Ser* 143: 283-288.
- Korb RE, Raven JA, Johnston AM (1998). Relationship between aqueous CO<sub>2</sub> concentrations and stable carbon isotope discrimination in the diatoms *Chaetoceros calcitrans* and *Ditylum brightwellii*. *Mar Ecol Progr Ser* 171: 303-305.
- Kozłowska-Szerernos B, Bialuk I, Maleszewski S (2004) Enhancement of photosynthetic O<sub>2</sub> evolution in *Chlorella vulgaris* under high light and increased CO<sub>2</sub> concentration as a sign of acclimation to phosphate deficiency. *Plant Physiol Biochem* 42:403-409.
- Kranz SA, Levitan O, Richter K-U, Prášil O, Berman-Frank O, Rost B (2010) Combined effects of CO<sub>2</sub> and light on the N<sub>2</sub> fixing cyanobacterium *Trichodesmium* IMS101: Physiological responses. *Plant Physiol* 154: 334-345.
- Landry MR, Brown SL, Rii YM, Selph KE, Bidigare RR, Yang EJ, Simmons MR (2008) Depth-stratified phytoplankton dynamics in Cyclone *Opal*, a subtropical mesoscale eddy. *Deep-Sea Res II* 55: 1348-1359.
- Laws EA, Popp BN, Cassar N, Tanimoto J (2002) <sup>13</sup>C discrimination patterns in oceanic phytoplankton: likely influence of CO<sub>2</sub> concentrating mechanisms, and implications for palaeoreconstructions. *Funct Plant Biol* 29: 323-333.
- Lenton A, Codron F, Bopp L, Metzl N, Cadule P, Tagliabue A, Le Sommer J et al (2009) Stratospheric ozone depletion reduces ocean carbon uptake and enhances ocean acidification. *Geophys Res Lett* 36: L12606.
- Leonardos N, Geider RJ (2009) No mechanistic dependence of photosynthesis on calcification in *Emiliana huxleyi* (Haptophyta). *J Phycol* 45: 1046-1051.
- Levitan O, Kranz SA, Spungin D, Prášil O, Rost B, Berman-Frank O (2010) Combined effects of CO<sub>2</sub> and light on the N<sub>2</sub> fixing cyanobacterium *Trichodesmium* IMS101: A mechanistic view. *Plant Physiol* 154: 346-356.
- Litchman E, Klausmeier CA (2008) Trait-based community ecology of phytoplankton. *Annu Rev Ecol Evol Syst* 39: 615-639.
- Liu H, Probert I, Uitz J, Claustre H, Aris-Broseau S, Froda M, Not F, de Vargas C. (2009). Haptophytes rule the waves: extreme oceanic biodiversity in con-calcifying prymnesiphytes explains the 19-Hex paradox. *Proc Natnl Acad Sci USA* 106: 12803-12808.
- Maberly SC (1985) Photosynthesis by *Fontinalis antipyretica*. I. Interaction between photon irradiance, concentration of carbon and temperature. *New Phytol* 100: 127-140.

- Maberly SC (1990) Exogenous sources of inorganic carbon for photosynthesis by marine macroalgae. *J Phycol* 26: 439-449.
- Maberly SC (1996) Diel, episodic and seasonal changes in pH and concentrations of inorganic carbon in a productive lake. *Freshwater Biol* 35: 579-598.
- Maberly SC, Ball LA, Raven JA, Sültemeyer D (2009) Inorganic carbon acquisition by chrysophytes. *J Phycol* 45: 1052-1061.
- Maberly SC, Madsen TV (1990) Contribution of air and water to the carbon balance of *Fucus spiralis*. *Mar Ecol Progr Ser* 62: 175-183.
- Madsen TV (1987) The effect of different growth conditions on dark and light carbon assimilation in *Littorella uniflora*. *Physiol Plant* 70: 183-188.
- Marbà N, Duarte CM, Agusti S. 2007. Allometric scaling of plant life history. *Proc Nat Acad Sci USA* 104: 1577-15780. McLeod GC, Kanwisher J (1962) The quantum efficiency of photosynthesis in the ultraviolet light. *Physiol Plant* 15: 581-586.
- Mengelt C, Prézelin BB (2005) UVA enhancement of carbon fixation and resilience to UV inhibition in the genus *Pseudo-Nitzschia* may provide a competitive advantage in high UV surface waters. *Mar Ecol Progr Ser* 301: 81-93.
- Meyer M, Seibt U, Griffiths H (2008) To concentrate or ventilate? Carbon acquisition, isotope discrimination and physiological ecology of early land plant life forms. *Phil Trans R Soc Lond B* 363: 2767-2778.
- Middelboe AL, Hansen PJ (2007) High pH in shallow-water macroalgal habitats. *Mar Ecol Progr Ser* 338: 107-117.
- Milligan AJ, Varela DE, Brzezinski MA, Morel FMM (2004). Dynamics of silicon metabolism and silicon isotope discrimination in a marine diatom as a function of pCO<sub>2</sub>. *Limnol Oceanogr* 49: 322-329.
- Mitchell C, Beardall J (1996) Inorganic carbon uptake by the sea ice diatom *Nitzschia frigida*. *Polar Biol* 16: 95-99.
- Monteith DT, Stoddard JL, Evans CD, de Wit HA, Forsius M, Høgåsen T, Wilander A, Skjelvåle LB, Jefferies DS, Vuovenman J, Keller B, Kopáček J, Vesely J et al. (2007) Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature* 450: 537-540.
- Muller MN, Schulz KG, Riebesell U (2010) Effects of long-term high CO<sub>2</sub> exposure on two species of coccolithophore. *Biogeosci* 7: 1109-1116.
- Not F, Latasa M, Marie D, Cariou T, Vaulot D, Simon N (2004) A single species, *Micromonas pusilla* (Prasinophyceae), dominates the eukaryotic picoplankton in the Western English Channel. *Appl. Env Microbiol* 70: 4064-4072.

Oehler DZ (1976) Transmission electron microscopy of organic microfossils from late Precambrian Bitter Springs formation in Australia: Techniques and survey of preserved ultrastructure. *J Palaeontol* 50: 90-106.

Oehler DZ (1977) Pyrenoid-like structures in the Precambrian algae from the Bitter Springs formation of Australia. *J Palaeontol* 51: 885-901.

Osborne CP, Beerling DJ (2006) Nature's green revolution: the remarkable evolutionary rise of C<sub>4</sub> plants. *Phil Trans R Soc B* 361: 173-194.

Paerl HW, Huisman J (2008) Blooms like it hot. *Science* 320: 57-58.

Palenik B, Grimwood J, Aerts A, Razé P, Salamov A, Putnam N, Dupont C, Jorgensen R, Denelle E, Rombauts S, Zhu K, Otiillar R, Mechant SS, Padell S, Gasterland T, Napoli C, Gentler K, Manuell A, Tai V, Vallon O, Pignaneau G, Joncek S, Heijde M, Dubchek T, Pazour S, Werner G, Dubchak I, Pazour GJ, van de Peer Y, Moreau M, Grigoriev IV (2007) The tiny eukaryote *Ostreococcus* provides genomic insight into the paradox of the plankton. *Proc Natl Acad Sci USA* 104: 7705-7710.

Palinska KA, Laloui W, Bédu S, Loiseaux-de Goër S, Catssets AM, Rippka R, Tandeau de Marsac N (2002) The signal transducer P<sub>II</sub> and bicarbonate acquisition in *Prochlorococcus marinus* PCC 9511, a marine cyanobacterium naturally deficient in nitrate and nitrite assimilation. *Microbiology* 148: 2405-2412.

Palmqvist K (1993) Photosynthetic CO<sub>2</sub>-use efficiency in lichens and their isolated photobionts. *Planta* 191: 48-56.

Palmqvist K, Samuelsson G, Badger MR (1994) Photobiont-related differences in carbon acquisition among green-algal lichens. *Planta* 195: 70-79.

Palmqvist K, de los Rios A, Ascaso C, Samuelsson G. 1997. Photosynthetic carbon acquisition in the lichen photobionts *Coccomyxa* and *Trebouxia* (Chlorophyta). *Physiol Plant* 101: 67-76.

Parekh P, Follows MJ, Boyle EA (2004) Modelling the global ocean iron cycle. *Global Biogeochem Cycles* 18: GB1002 Pengelty JPL, Sirault XRR, Tazoe Y, Evans JR, Furbank RT, von Caemmerer S (2010) Growth of the C<sub>4</sub>dicot *Flaveria bidentis*: photosynthetic acclimation to low light through shifts in leaf anatomy and biochemistry. *J Exp Bot* 61: 4109-4122

Poorter H, Gifford RM, Kriedemann PE, Wong SCJ 1992. A quantitative analysis of dark respiration and carbon content as factors in the growth-response of plants to elevated CO<sub>2</sub>. *Austr J Bot* 40: 501-513.

Price GD, Badger MR, Woodger FI, Long BM (2008) Advances in understanding the cyanobacterial CO<sub>2</sub>-concentrating mechanism (CCM): functional components, C<sub>i</sub>

transporters, diversity, genetic regulation and prospects for engineering into plants. *J Exp Bot* 59: 1441-1461.

Ratti S, Giordano M, Morese F (2007) CO<sub>2</sub>-concentrating mechanism of the potentially toxic dinoflagellate *Protoceratium reticulatum* (Dinophyceae, Gonyaulacales). *J Phycol* 53: 393-401.

Raven JA (1990) Predictions of Fe and Mn use efficiencies of phototrophic growth as a function of light availability for growth and C assimilation pathway. *New Phytol* 116: 1-18.

Raven JA (1991a) Implications of inorganic carbon utilization – ecology, evolution and geochemistry. *Can J Bot* 69: 908-924.

Raven JA (1991b). Physiology of inorganic C acquisition and implications for resource use efficiency by marine phytoplankton: relation to increased CO<sub>2</sub> and temperature. *Plant Cell Environ* 14: 779-794.

Raven JA (1997) The role of marine biota in the evolution of terrestrial biota: gases and genes – atmospheric composition and evolution of terrestrial biota. *Biogeochem* 39: 139-164.

Raven JA (2010) Inorganic carbon acquisition by eukaryotic algae: four current questions. *Photosynth Res* 106: 123-134. Raven JA. (2011). Carbon. In: B Whitton, ed. *Ecology of Cyanobacteria*, 2<sup>nd</sup> Edition. Springer, Berlin. In press.

Raven JA, Geider RJ (1988) Temperature and algal growth. *New Phytol* 110: 441-461,

Raven JA, Johnston AM (1991) Mechanisms of inorganic carbon acquisition in marine phytoplankton, and their implications for the use of other resources. *Limnol Oceanogr* 36: 1701-1714.

Raven JA, Falkowski PG (1999) Oceanic sinks for atmospheric CO<sub>2</sub>. *Plant Cell Environ* 22: 741-755.

Raven JA, Geider RD (2003) Adaptation, acclimation and regulation of photosynthesis in algae. In: *Photosynthesis in Algae* (eds. AWD Larkum, SE Douglas, JA Raven), pp. 385-412. Kluwer, Dordrecht.

Raven JA, Waite A (2004) Tansley Review: The evolution of silicification in diatoms: inescapable sinking and sinking as escape? *New Phyt* 162: 45-61.

Raven JA, Andrews M (2010) Evolution of tree nutrition. *Tree Physiol* 30: 1050-1071.

Raven JA, Kübler JI, Beardall J (2000). Put out the light, and then put out the light. *J Mar Biol Assoc UK* 80: 1-25.

Raven JA, Johnston AM, Kübler JE, Korb RE, McInroy SG, Handley LL, Scrimgeour CM, Walker DI, Beardall J, Vanderklift M, Fredriksen J, Dunton, KH (2002a) Mechanistic interpretation of carbon isotope discrimination by marine macroalgae and seagrasses. *Funct Plant Biol* 29: 355-378.

Raven JA, Johnston AM, Kübler JE, Korb RE, McInroy SG, Handley LL, Scrimgeour CM, Walker DI, Beardall J, Clayton MN, Vanderklift M, Fredriksen S, Dunton KH (2002b) Seaweeds in cold seas: evolution and carbon acquisition. *Ann Bot* 90: 525-536.

Raven JA, Ball LA, Beardall J, Giordano M, Maberly SC (2005a). Algae lacking carbon concentrating mechanisms. *Can J Bot* 83, 879- 890.

Raven JA, Brown K, Mackay M, Beardall J, Giordano M, Granum E, Leegood RC, Kilminster K & Walker DI (2005b) Iron, nitrogen, phosphorus and zinc cycling and consequences for primary productivity in the oceans, In *Society for General Microbiology Symposium 65 Micro-organisms and Earth systems: Advances in Geobiology* (eds. G M Gadd, K T Semple and H M Lappin-Scott), pp. 247-272. Cambridge University Press, Cambridge.

Raven JA, Cockell CS, La Rocha CL (2008) The evolution of inorganic carbon concentrating mechanisms in photosynthesis. *Phil Trans Roy Soc B* 363: 2641-2650

Renberg L, Johansson AI, Shutova S, Stenlund H, Aksman A, Raven JA, Gardeström P, Moritz T, Samuelsson G. (2010). A metabolomic approach to study major metabolite changes during acclimation to limiting CO<sub>2</sub> in *Chlamydomonas reinhardtii*. *Plant Physiol* 154: 187-196. Reinfelder JR (2011) Carbon concentrating mechanisms in eukaryotic marine phytoplankton *Annu Rev Mar Sci* 3: 291-315.

Richardson AJ. 2008. In hot water: zooplankton and climate change. *ICES J Mar Sci* 65: 279-295.

Riding R (2006) Cyanobacterial calcification, carbon dioxide concentrating mechanisms and Proterozoic-Cambrian changes in atmospheric composition. *Geobiology* 4: 299-316.

Riding R (2009) An atmospheric stimulus for cyanobacterial-induced calcification ca. 350 million years ago? *Palaios* 24: 685-696.

Riebesell U (2004) Effects of CO<sub>2</sub> enrichment on marine phytoplankton. *J Oceanogr* 60: 719-729.

Riebesell U, Schulz KG, Bellerby RGJ, Botros M, Fritsche P, Meyerhöfer M, Neill C, Nondol G, Oschlies A, Wohlers J, Zöllner E (2007) Enhanced biological carbon consumption in a high CO<sub>2</sub> ocean. *Nature* 450:545-548.

Riebesell U, Kortzinger A, Oschlies A (2009) Sensitivities of marine carbon fluxes to ocean change. *Proc Natl Acad Sci USA* 106: 20602-20609. Riebesell U, Fabry VJ, Nansson LN, Gattuso J-P (eds) (2010) Guide to best practices for ocean acidification research and data reporting. 260 pp. Luxembourg: Publications Office of the European Union. <http://www.epoca-project.eu/index.php/guide-to-best-practices-for-ocean-acidification-research-and-data-reporting.htm>

Robarts RD, Waiser MJ, Hadas O, Zophary T, MacIntyre S (1998) Relaxation of phosphorus limitation due to typhoon-induced mixing in two morphologically distinct basins of Lake Biwa, Japan. *Limnol Oceanogr* 43: 1023-1036.

Rost B, Zondervan I, Wolf-Gladrow D (2008) Sensitivity of phytoplankton to future changes in ocean carbonate chemistry: current knowledge, contradictions and research directions. *Mar Ecol Progr Ser* 373: 227-237.

Schulz KG, Ramos JBE, Zeebe RE, Riebesell U (2009) CO<sub>2</sub> perturbation experiments: similarities and differences between dissolved inorganic carbon and total alkalinity manipulations. *Biogeosci* 6: 2145-2153.

Shapiro J (1997) The role of carbon dioxide in the initiation and maintenance of blue-green dominance in lakes. *Freshwat. Biol.* 37: 307-323.

Shi D, Xu Y, Morel FMM (2009) Effects of the pH/pCO<sub>2</sub> control method on medium chemistry and phytoplankton growth. *Biogeosci* 6: 1199-1207.

Shi D, Hopkinson BM, Morel FMM (2010) Effect of ocean acidification in iron availability to marine phytoplankton. *Science* 327: 676-679. Smith EC, Griffiths H. 1996. The occurrence of the chloroplast pyrenoid is correlated with the activity of a CO<sub>2</sub>-concentrating mechanism and carbon isotope discrimination in lichens and bryophytes. *Planta* 198: 6-16.

Sobrino C, Ward ML, Neale PJ (2008) Acclimation of 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.

Sobrino C, Neale PJ, Phillips-Kress JD, Moeller RE, Porter JA (2009) Elevated CO<sub>2</sub> increases sensitivity to ultraviolet radiation in lacustrine phytoplankton assemblages. *Limnol Oceanogr* 54: 2448-2459.

Song YF, Qiu BS (2007) The CO<sub>2</sub> concentrating mechanism in the bloom-forming cyanobacterium *Microcystis aeruginosa* (Cyanophyceae) and effects of UVB

radiation on its operation. *J Phycol* 43: 957-964. Steinacher M, Joos F, Frolicher TL, Bopp L, Cadule P, Cocco V, Doney SC, Gehlen M, Lindsay K, Moore JK, Schneider B, Segsneider J (2010) Projected 21<sup>st</sup> century decrease in marine primary productivity: a multi-model analysis. *Biogeosci* 7: 979-1005.

Surif MB, Raven JA (1990) Photosynthetic gas exchange under emersed conditions in eulittoral and normally submersed members of the Fucales and the Laminariales: Interpretation in relation to C isotope ratio and N and water use efficiency. *Oecologia* 82: 68-80.

Tazoe Y, Hanba YT, Furumoto T, Noguchi K, Terashima I (2008) Relationships between quantum yield for CO<sub>2</sub> assimilation activity of key enzymes and CO<sub>2</sub> leakiness in *Amaranthus cruentus*, a C<sub>4</sub> dicot, grown in high or low light. *Plant Cell Physiol* 49: 19-29.

Tcherkez GGB, Farquhar GD, Andrews TJ (2006) Despite slow catalysis and confused substrate specificity, all ribulose biphosphate carboxylases may be nearly perfectly optimised. *Proc Natnl Acad Sci USA* 103: 7246-7251. Tchernov D, Hassidim M, Luz B, Sukenik A, Reinhold L, Kaplan A (1997) Sustained net CO<sub>2</sub> evolution during photosynthesis by a marine microorganism. *Curr Biol* 7: 723-728.

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

The Royal Society (2005) Ocean acidification due to increased carbon dioxide. Policy Document 12/05. The Royal Society, London.

Theodorou ME, Plaxton WC (1993) Metabolic adaptations of plant respiration to nutritional phosphorus deprivation. *Plant Physiol* 101: 339-344.

Tortell PD, Payne CD, Li Y, Trimborn S, Rost B, Smith WO, Riesselman C, Dunbar RR, Sedwick P, DiTullio GR (2008a) CO<sub>2</sub> sensitivity of Southern Ocean phytoplankton. *Geophys Res Lett* 35: L04605. Tortell PD, Payne C, Guegon C, Strzpek RF, Boyd PW, Rost B (2008b) Inorganic carbon uptake by Southern Ocean phytoplankton. *Limnol Oceanogr* 53: 1266-1278.

Tortell PD, Trimborn S, Li Y, Rost B, Payne CD (2010) Inorganic carbon utilization by Ross Sea phytoplankton across natural and experimental CO<sub>2</sub> gradients. *J Phycol* 46: 433-443. Tranvik LJ, Downing JA, Cotner JB et al. (2009) Lakes and reservoirs as regulators of carbon cycling and climate. *Limnol. Oceanogr.* 54: 2298-2314.

Trimborn S, Langer G, Rost B (2007) Effect of varying calcium concentrations and light intensities on calcification and photosynthesis in *Emiliana huxleyi*. *Limnol Oceanogr* 52: 2285-2293.



- Uitz H, Claustre H, Gentoli B, Stramski D (2009) Phytoplankton class-specific primary production in the world's oceans; seasonal and interannual variability from satellite observations. *Glob Biogeochem Cycles* 24(3): GB 2016
- van de Poll WHV, van Leeuwe MA, Roggeveld J, Buma AGJ (2005) Nutrient limitation and high irradiance acclimation reduce photosynthetically active radiation and UV-induced viability loss in the Antarctic diatom *Chaetoceros brevis* (Bacillariophyceae). *J Phycol* 41:840-850.
- van de Poll WHV, Janknegt PJ, van Leeuwe MA, Visser RJW, Buma AGJ (2009) Excessive irradiance and antioxidant responses of an Antarctic marine diatom exposed to iron limitation and to dynamic irradiance. *J Photochem Photobiol* 94: 32-37.
- Vance P, Spalding MH (2005) Growth, photosynthesis, and gene expression in *Chlamydomonas* over a range of CO<sub>2</sub> concentrations and CO<sub>2</sub>/O<sub>2</sub> ratios: CO<sub>2</sub> regulates multiple acclimation states. *Can J Bot* 83: 796-809.
- Vitousek PM, Cassman K, Cleveland C, Crews T, Field CB, Grimm NB, Howarth RW, Marino R, Martinelli L, Rastetter EB, Sprent JI (2002) Towards an ecological understanding of biological nitrogen fixation. *Biogeochem* 57/58: 1-45.
- Weng XY, Xu HX, Yang Y, Peng HH (2008) Water-water cycle involved in dissipation of excess photon energy in phosphorus deficient rice leaves. *Photosynthetica* 52: 307-313.
- Wetz MS, Paerl HW (2008) Estuarine phytoplankton responses to hurricanes and tropical storms with different characteristics (trajectory, rainfall, winds). *Estuaries Coasts* 31: 419-429.
- Wienkoop S, Weiß J, May R, Kempa S, Irgang S, Recuenco-Munoz L, Pietzke M, Schwemmer T, Rupprecht J, Egenhofer V, Weckwerth W (2010) Targetted proteomics for *Chlamydomonas reinhardtii* combined with rapid subcellular protein fractionation, metabolomics and metabolic flux analysis. *Mol BioSyst* 6: 1018-1031.
- Williamson CE, Sternberger RS, Morris DP, Frost TM, Paulsen SG (1996) Ultraviolet radiation in North American lakes: attenuation estimates from DOC measurements and implications for plankton communities. *Limnol. Oceanogr.* 41: 1024-1034.
- Winter K, Schmitt MR, Edwards GR (1982) *Microstegium vimineum*, a shade adapted C<sub>4</sub> grass. *Plant Sci Lett* 24: 311-318.
- Worden AZ, Lee J-H, Mock T, Rouzé P, Simmons MP, Aerts AL, Allen AE, Cuvellier ML, Denelle E, Everett MV, Foulon E, Grimwood J, Groundlackh H, Henrissat S, Napoli C, McDonald SM, Parker MS, Romnbauts S, Salamov A, von Dassow P, Badger JH, Couinho PH, Demir E, Dubchak I, Genteman C, Eikrem W, Gready JE, John U, Larrier W, Lundquist EA, Lucas S, Maxymer KFX, Moreau H, Not

F, Otilar R, Panaud O, Pangillar J, Paulsen I, Piegu B, Paliakov A, Robbers S, Schmitz J, Toulza E, Wyss T, Zelensky A, Zhou K, van de Peer Y, Grigoriev IV (2009) Green evolution and dynamic adaptation revealed by genomes of the marine picoeukaryotes *Micromonas*. *Science* 324: 268-272.

Wu HY, Zou DH, Gao KS (2008) Impacts of increased atmospheric CO<sub>2</sub> concentration on photosynthesis and growth of micro- and macro-algae. *Sci in China C Life Sci* 51: 1144-1150. Wu Y, Gao K, Riebesell U (2010) CO<sub>2</sub>-induced seawater acidification affects physiological performance of the marine diatom *Phaeodactylum tricorutum*. *Biogeosciences* 7: 2915-2923.

Xu J, Gao GK (2010) Use of UVA energy for photosynthesis in the red macroalga *Gracilaria lemaneiformis*. *Photochem Photobiol* 86: 580-585.

Xu ZG, Gao KS (2009) Impacts of UV radiation on growth and photosynthetic carbon acquisition in *Gracilaria lemaneiformis* (Rhodophyta) under phosphorus-limited and replete conditions. *Funct Plant Biol* 36: 1057-1064.

Young EB, Beardall J (2005) Modulation of photosynthesis and inorganic acquisition in a marine microalga by nitrogen, iron and light availability. *Can J Bot* 83: 917-928.

Zeebe RE, Wolf-Gladrow D (2001) CO<sub>2</sub> in Seawater: Equilibrium, Kinetics, Isotopes. Elsevier Oceanography Series Volume 65. Elsevier, Amsterdam. pp. xiii + 346.

Zondervan I (2007) The effects of light, macronutrients, trace metals and CO<sub>2</sub> on the production of calcium carbonate and organic carbon in coccolithophores – a review. *Deep-Sea Res II* 54: 521-537.

**Table 1** Effects on CCMs of environmental factors, and the direction of change of these environmental factors in algal and aquatic plant habitats with global environmental change. Further details and references are given in the text.

| Factor          | Global change induced modification of algal and aquatic plant environments   | Effects on CCMs  |
|-----------------|--|--|
| CO <sub>2</sub> | Increase in CO <sub>2</sub> in essentially all environments, although less predictable effect in freshwaters which can be out of equilibrium with the atmosphere | Decreased inorganic carbon affinity with growth at high CO <sub>2</sub> ; can be a switch to diffusive CO <sub>2</sub> entry in some eukaryotes<br>No clear data   |
| Temperature     | Increase in temperature in all environments  | No clear data  |
| PAR             | Increase in PAR in lotic planktonic environments   | Decreased inorganic carbon affinity with growth at low PAR   |
| Nitrogen        | Decrease in combined nitrogen in upper mixed layer of lotic environments   | Generally increased inorganic carbon affinity with growth at low NO <sub>3</sub> <sup>-</sup><br>One example each of decreased carbon affinity with growth at lowest NO <sub>3</sub> <sup>-</sup> concentration tested, and with growth over entire NH <sub>4</sub> <sup>+</sup> range tested. |
| Phosphorus      | Decrease in phosphate in upper mixed layer of lotic environments   | Two examples of increased inorganic carbon affinity, one example of decreased inorganic carbon affinity, with growth at low phosphate  |
| Iron            | Probable decrease in iron in upper mixed layer of lotic environments   | One example of increased inorganic carbon affinity with growth at low iron   |
| UVA             | Increase in UVA in lotic planktonic environments, but decrease in sites with increased concentration of DOC  | No data  |
| UVB             | Increase in UVB in lotic planktonic environments, but decrease in sites with   | Variable responses of CCMs with increased UVB flux for   |

increased concentration of DOC

growth.