

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

John A Raven

Division of Plant Sciences, University of Dundee at SCRI, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK email: j.a.raven@dundee.ac.uk

Mario Giordano

Department of Marine Sciences, Università Politecnica delle Marche, 60131 Ancona, Italy

John Beardall

School of Biological Sciences, Monash University, Clayton, Vic 3800, Australia

Stephen C Maberly

Centre for Ecology and Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, Lancaster LA1 4AP, UK

Abstract.

Carbon dioxide concentrating mechanisms (also known as inorganic carbon concentrating mechanisms; both abbreviated as CCMs) presumably evolved under conditions of low CO₂ 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 CO₂ (e.g. the Palaeocene-Eocene Thermal Maximum) to indicate how organisms with CCMs responded. Present and predicted environmental change in terms of increased CO₂ and temperature are leading to increased CO₂ and HCO₃⁻ and decreased CO₃²⁻ 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 CO₂ 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 CO₂ affinity, decreased iron availability increases CO₂ 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 CO₂ concentrating mechanism – combined nitrogen – inorganic carbon – iron – mixing depth - photosynthetically active radiation – phosphorus – temperature – UVA-UVB

Abbreviations

CCM CO₂ 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 CO₂ 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 CO₂ episodes as indicators of what is likely to happen with increasing CO₂ 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 C₄-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 β -cyanobacteria with β -carboxysomes containing Form IB Rubiscos and a range of high and low affinity inorganic C acquisition mechanisms, and the derived α -cyanobacteria with α -carboxysomes containing Form IA Rubiscos and a restricted range of inorganic C acquisition mechanisms. The β -cyanobacteria occur in a wide range of inland water and coastal habitats with variable inorganic C availability, while the α -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 α -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 CO₂ transport from the bulk medium to Rubisco (Giordano et al. 2005; Falkowski and Raven 2007). The transition from CCM to diffusive CO₂ entry cannot occur until the external inorganic carbon concentration is higher than a critical value that yields, via the CCM, a steady-state CO₂ 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 CO₂ 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 CO₂ 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 CO₂ episodes might shed light on what is happening now and in the next several decades.

Previous high CO₂ and temperature episodes: evidence for effects on CCMs?

It would be very helpful if previous high CO₂ 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 β -cyanobacterial CCM preceded the α -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 CO₂ availability, with the problem of how CCMs were maintained in intervening high CO₂ 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 CO₂, and maintenance in subsequent higher CO₂, would be most likely in those organisms (cyanobacteria, peridinin-containing dinoflagellates) with maximum specific growth rates that would not be CO₂-saturated when relying on diffusive CO₂ entry from the low CO₂ environment. The lack of CO₂ saturation is mechanistically imposed by the high CO₂-saturated specific reaction rate, low CO₂ affinity and low CO₂-O₂ 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 CO₂ episodes and their retention in intervening higher CO₂ episodes might be expected to have a lower likelihood in organisms with Rubiscos with higher CO₂ affinity and higher CO₂-O₂ 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 CO₂.

However, these considerations do not take into account temporal and spatial variation in the concentration of CO₂ 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 CO₂ 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 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 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) CO_2 on surface waters on Earth have been extensively investigated. The physicochemical background of 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 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 CO_2 to the ocean causes an increase in the concentration of dissolved CO_2 and a parallel increase in the much lower equilibrium concentration of H_2CO_3 and H^+ . There is a smaller proportionate, but larger in absolute terms, increase in the concentration of HCO_3^- and, by contrast, a decrease in the concentration of CO_3^{2-} . This perhaps counter-intuitive decrease in an inorganic carbon species as a result of added CO_2 comes about because at seawater pH the effect of decreasing pH and shifting the chemical equilibrium away from 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 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 CO₂ is more soluble. The “biological pump” is based on photosynthetic CO₂ 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 CO₂ 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 CO₂, NO₃⁻, HPO₄²⁻ 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 CO₂ 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 CO₂ 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-CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ affinity and low CO₂/O₂ 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 CO₂ has the potential to influence directly emersed photosynthesis (Maberly and Madsen 1990) by the organisms that are not saturated by the current levels of CO₂ (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 CO₂ 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 CO₂ 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 CO₂ (Riebesell 2004), transects involving parcels of surface seawater with naturally or experimentally different CO₂ concentrations (e.g. Tortell et al. 2010) or the comparison of areas of benthos influenced over years or more by CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ concentration over the tens of millions of years. The sinking rate of diatoms can be altered by increased CO₂ 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 CO₂ generation during intracellular calcification in coccolithophores is not stoichiometrically involved as component of a CCM, or is otherwise necessarily involved in supplying CO₂ 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 CO₂ on coccolithophore calcification (see Zondervan 2007; Iglesias-Rodriguez et al. 2008; Doney et al.

2009a; Muller et al. 2010) does not directly impact on CO₂ 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 CO₂ there. Future changes in chrysophyte distribution in freshwaters as a result of environmental change are uncertain and depend strongly on future concentrations of dissolved CO₂.

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 CO₂: there are clear interactions between temperature and CO₂. 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 CO₂-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, CO₂ and temperature in photosynthesis of the freshwater moss *Fontinalis antipyretica* that reinforced the idea that the strength of CO₂-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 CO₂ 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 CO₂ 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 CO₂ entry rather than CCMs in the subtidal than the intertidal of the marine benthos (Maberly 1990). For C₄ terrestrial flowering plants there is also evidence of increased CO₂ 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 C₄ plants (Winter et al. 1982). Fu et al. (2007, 2008) and Feng et al. (2008) also studied the three-way interactions among CO₂, 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 tricornutum* grown at high (101.3 Pa) CO₂ was more sensitive to photoinhibition, and had less non-photochemical

quenching, in high PAR than did cells grown in present day (39.3 Pa) CO₂: if this is a general phenomenon it will have significant implications as the upper mixed layer shoals in a warmer higher CO₂ world. Wu et al. (2010) also found that growth at the higher CO₂ 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 CO₂ 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 CO₂. Poorter et al. (1992) analysed the literature on respiration rates of organs of C₃ and C₄ terrestrial flowering plants. On a leaf area basis both C₃ and C₄ plants showed a significant increase in respiration in increased CO₂, while on a leaf dry weight basis both groups showed a decrease in respiration rate in high CO₂, although only the C₄ 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 CO₂ 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 NO₃⁻, although in *Nannochloropsis* sp. there was a decreased inorganic carbon affinity in moving from the second-lowest to the lowest NO₃⁻ concentration used, but a decreased inorganic carbon affinity in the single case in which NH₄⁺ 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 NH₄⁺ as nitrogen source in *Chlamydomonas reinhardtii* (Giordano et al. 2003), resembling the results with the lowest NO₃⁻ concentrations used for *Nannochloropsis* (Hu and Zhou 2010). The increased CCM expression under NO₃⁻-nitrogen limitation accords with mechanistic considerations and with comparisons of C₃ (CO₂ diffusion) and C₄ (a C₄-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 NH₄⁺ than of NO₃⁻ (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-CO₂ 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 C₄-like photosynthesis as (part of) their CCMs, and it is of interest that three C₄ grasses, one each from the three biochemical subtypes, have a high photosynthetic phosphorus use efficiency in units of mol CO₂ 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 CO₂ 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 CO₂ entry. However, Sobrino et al. (2008) hypothesized that such down-regulation of Rubisco and CA under elevated CO₂ might be responsible for the increase in UVR sensitivity observed under elevated CO₂ 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 CO₂ 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 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 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 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 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 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 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 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 CO_2 affinities and CO_2/O_2 selectivities with the consequent possibility of a low critical external inorganic carbon concentration above

which diffusive CO₂ 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 CO₂ affinities and CO₂/O₂ selectivities, and hence higher critical external inorganic carbon concentration above which diffusive CO₂ 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 CO₂ 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 CO₂ 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 CO₂ levels (Laws et al. 2002).

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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 ₂	Increase in CO ₂ 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 ₂ ; can be a switch to diffusive CO ₂ entry in some eukaryotes 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 ₃ ⁻ One example each of decreased carbon affinity with growth at lowest NO ₃ ⁻ concentration tested, and with growth over entire NH ₄ ⁺ 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.