Praeger Review: Effects on marine algae of changed seawater chemistry with increasing atmospheric CO 2

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EFFECTS ON MARINE ALGAE OF CHANGED SEAWATER CHEMISTRY WITH INCREASING ATMOSPHERIC CO_2

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ABSTRACT

The acid–base relations of plant (including algal) environments are complex, comprising geological processes as modified by biology including, especially over the last 200 years, man. Some habitats (e.g. high intertidal rockpools and some freshwater bodies) have pH variations of up to three units over a diel cycle as a result of photosynthesis and respiration. Other habitats, e.g. nutrient-poor open ocean habitats, have diel variations that are more than an order of magnitude smaller. Anthropogenic influences on acid–base relations of different habitats include the input to the atmosphere of gases that dissolve to produce acidic solutions. The quantitatively predominant gas is CO_2, but SO_2, NO_x and NH_y (via nitrification) can also be significant. The influence of the acidic gases in aquatic habitats (including the upper layers of peat bogs) and on terrestrial photosynthetic organisms alters the inorganic carbon speciation and pH around the photosynthetic cells. The calcified coralline marine red macroalgae, with benthic and unattached (maerl) life forms, have extracellular calcification; their calcification rate will decline in the future, with a more CO_2-rich ocean and decreasing CO_3^{2-} concentrations. The marine planktonic coccolithophores have intracellular calcification, though the coccoliths themselves occur externally. While many coccolithophores show decreased calcification with increasing external CO_2 and the attendant decrease in external CO_3^{2-}, this is not universal. For both coralline red algae and coccolithophores the external $CaCO_3$ will dissolve when seawater becomes undersaturated with respect to the relevant crystal form of $CaCO_3$. Overall, the effects of increased CO_2 alone are negligible or result in increased growth of non-calcified algae, while there is most generally a decreased growth of calcified algae.

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INTRODUCTION

The acid–base properties of the environment of plants and algae are complex and ecologically important. These acid–base properties are often encapsulated in the pH value, though this is just one outcome of often complex chemistry and biochemistry. Of particular interest is the inorganic carbon system, i.e. CO_2–H_2CO_3–HCO_3^-–CO_3^{2-}–$CaCO_3$ with the associated H^+ and OH^-, and the cycling of its components through the biosphere and the Earth's crust. This sort of cycling was first proposed by the 'father of geology', James Hutton (Hutton 1795; 1889). On an abiotic Earth, but with the present atmospheric composition, the main acid–base interaction would be the chemical weathering of silicates consuming H_2O and atmospheric CO_2, producing soluble cations, bicarbonate and silicic acid. These would reach the sea in rivers, and would ultimately be precipitated as $CaCO_3$ and $Si(OH)_4$. After subduction, reverse weathering and vulcanism return CO_2 to the atmosphere and silicate volcanic rocks to the land surface. Although the precipitation of $CaCO_3$ generates CO_2, there is a net CO_2 removal from the atmosphere in the overall process of chemical weathering on land, and $CaCO_3$ precipitation in the ocean that is approximately balanced over the long term (many millions of years) by CO_2 output from vulcanism in the exogenic cycle (Garrels and Lerman 1981; Berner and Berner 1996). Biology on Earth increases the rate of chemical weathering by transfer of photosynthate into the upper layers of rock followed by production of CO_2 by respiration, so that the CO_2 concentration within the rock is higher than that at air equilibrium. Biota also catalyse the sedimentation of $CaCO_3$ and SiO_2 in the ocean, with photosynthetic organisms playing a leading role: diatoms dominate SiO_2 sedimentation and have decreased the surface ocean $Si(OH)_4$ to well below saturation with respect to diatom opal, while coccolithophores are major contributors to sedimentation of $CaCO_3$, although the present

surface ocean is oversaturated with respect to coccolithophore calcite.

Some marine habitats, such as high intertidal rockpools, have pH variations of up to three units over a diel cycle as a result of photosynthesis and respiration (Poole and Raven 1997), and there are rather smaller variations in shallow-water macroalgal habitats with more exchange with bulk seawater (Middelboe and Hansen 2007). Other habitats, e.g. nutrient-poor open ocean habitats, have diel and seasonal variations that are more than an order of magnitude smaller (Dore *et al.* 2009).

Anthropogenic addition of CO_2 to the atmosphere is partly removed by net ecosystem productivity on land and by solution in the surface seawater, the rest remaining in the atmosphere. Solution of CO_2 in seawater alters the speciation of the dissolved inorganic carbon system (Table 1). The dissolved CO_2 hydrates to form hydrated CO_2 and some H_2CO_3 (Adamczyk *et al.* 2009), which increases the concentration of these two molecular species (Table 1). The increased concentration of H_2CO_3 dissociates to form HCO_3^- and H^+, while hydroxylation of hydrated CO_2 also forms HCO_3^-: both reactions increase the H^+/OH^- and the concentration of HCO_3^-. Despite the increase in HCO_3^- and in total dissolved inorganic carbon, the increased H^+ means that the ratio of HCO_3^-/CO_3^{2-} rises to an extent that corresponds to a decrease in the concentration of CO_3^{2-} (Zeebe and Wolf-Gladrow 2001; Raven *et al.* 2005; Doney *et al.* 2009). This anthropogenic 'ocean acidification' has been mimicked in many experiments in order to determine its effect on photosynthetic and non-photosynthetic organisms (Hurd *et al.* 2009; Shi *et al.* 2009; Riebesell *et al.* 2010). Given the prevailing Ca^{2+} concentration, the CO_3^{2-} concentration in surface seawater is still saturated with respect to calcite, though aragonite is undersaturated in parts of the Arctic Ocean as a result of increased fresh (melt) water inputs (Chierichi and Fransson 2009; Yamamoto *et al.* 2009). The effects of the changes in ocean chemistry attendant on anthropogenic increases in CO_2 have been subject to a several reviews: recent meta-analyses have been published by Hendriks *et al.* (2010) (see also Dupont *et al.* (2010) and Hendriks and Duarte (2010)) and by Kroeker *et al.* (2010). It must be borne in mind, however, that the statistical analysis component of meta-analyses needs very careful attention (e.g. Gurevitch and Hedges 1999; Hedges *et al.* 1999). What is unequivocal is that there are significantly different effects for different processes and groups of organisms, and the results reported here are robust.

Increasing atmospheric CO_2 also influences inland waters, with consequent decreases in pH, although small or enclosed water bodies may become supersaturated with CO_2 as a result of input of ground water containing organic and inorganic carbon due to terrestrial photosynthesis in the catchment (Maberly 1996). Such water bodies will presumably be less influenced by increased atmospheric CO_2 than large inland water bodies and the open ocean.

Other atmospheric anthropogenic inputs—NO_x/HNO_3 and SO_2/H_2SO_4 (Fowler *et al.* 2007)—can cause localised increases in H^+/OH^- in the surface ocean (Doney *et al.* 2007). These compounds are produced naturally, by lightning (NO_x/HNO_3) and oxidation of biogenic dimethylsulphide (SO_2/H_2SO_4). Their anthropogenic production comes from high-temperature combustion (NO_x/HNO_3) and from sulphur in coal and some ship fuels (SO_2/H_2SO_4) (Fowler *et al.* 2007). When these compounds, by dry or wet deposition, enter surface seawater they are, or can form, the strong acids

Table 1—Changes in surface ocean inorganic carbon chemistry at four atmospheric CO_2 mol fractions, assuming equilibrium with the atmosphere and a total alkalinity 2324μmol kg^{-1} at a temperature of 18°C. Adapted from Raven *et al.* (2005) and Adamczyk *et al.* (2009).

	Pre-industrial	*Present*	*2 × pre-industrial*	*3 × pre-industrial*
Atmospheric CO_2*	280	380	560	840
Surface ocean CO_2†	9	13	19	28
Surface ocean H_2CO_3†				
Surface ocean HCO_3^-†	1766	1876	1976	2070
Surface ocean CO_3^{2-}†	225	185	141	103
Surface ocean total dissolved inorganic carbon	2003	2065	2136	2133
Surface ocean pH	8.18	8.07	7.92	7.77

HNO_3 and H_2SO_4. The essentially complete dissociation of these acids resembles the addition of a strong acid (HCl) in many experiments to determine the effects of ocean acidification on photosynthetic (and other) organisms (Hurd *et al.* 2009; Shi *et al.* 2009; Riebesell *et al.* 2010). This differs from the method involving CO_2 addition because there is no increase in total inorganic carbon in the system, so that HCO_3^- decreases rather than increases as CO_2 increases. Doney *et al.* (2007) show that the effect of these strong acids on the ocean H^+/OH^- are small relative to those of anthropogenic CO_2: the effects are also more localised because production of those compounds is, like that of CO_2, localised, but the strong acids and their precursors have much shorter atmospheric lifetimes than does CO_2. The strong acid, HNO_3, is also a result of nitrification of NH_y that, in the form of NH_3, is a weak base. Atmospheric NH_y is produced naturally from wildfire and decomposition of animal faeces and urine on land, and can be produced anthropogenically by low-temperature combustion of coal. Intensive animal production is also a significant source of NH_y, as is land-spreading of slurry and related waste products (Hyde *et al.* 2003; DEFRA 2005). The extent of surface ocean acidification from this source is limited by the short atmospheric lifetime of NH_y, the photoinhibition of nitrification, and the decreased nitrification in an ocean at lower pH (Beman *et al.* 2011). The deposition of combined nitrogen from the atmosphere into the surface ocean, and especially parts of the coastal ocean, is more important to algae nutritionally than with respect to ocean acid–base relations (Paerl 1997; Doney *et al.* 2007). The prevailing wind direction relative to sources of atmospheric combined nitrogen means that the coastal waters of Ireland, and especially western Ireland, are less influenced by such nitrogen inputs than are some other coastal waters, e.g. the North Sea (Paerl 1997). The very high sulphate concentration in seawater means that sulphur inputs to seawater are nutritionally insignificant for algae.

An influence of inputs of strong acid input from the atmosphere similar to that found in seawater is found in inland waters, including mires, and in soils that have acid–base buffering that is of a similar magnitude to, or higher than, that of seawater (Maberly 1996). By contrast, soils and inland waters with a low acid–base buffering have their H^+/OH^- relations significantly influenced by atmospheric inputs of strong acids (Fowler *et al.* 2007; Battarbee 2010). There are direct influences of the nitrogen and sulphur inputs to ecosystems (e.g. Fowler *et al.* 2007; Jones *et al.* 2007a; 2007b; Giordano *et al.* 2008), but the acid–base impact is also significant.

INFLUENCE OF ANTHROPOGENIC CARBON DIOXIDE ON PHOTOSYNTHESIS AND GROWTH BY MARINE ALGAE

The increasing atmospheric CO_2 has variable effects on the photosynthesis and growth of marine algae. Almost all marine phytoplankton organisms have CO_2-concentrating mechanisms (CCMs) and have a higher affinity for inorganic carbon than would be expected for an otherwise similar organism with diffusive CO_2 entry (Giordano *et al.* 2005; Raven 2010; 2011; Raven *et al.* 2011; Reinfelder 2011). Some organisms with CCMs, e.g. many cyanobacteria, might have difficulties with growing in the absence of a CCM: while they have the capacity to recycle phosphoglycolate to sugars and have a high CO_2 permeability, they do not have a ribulose bisphosphate carboxylase–oxygenase with high enough CO_2 affinity and CO_2/O_2 selectivity to permit net CO_2 assimilation over the diel cycle (Giordano *et al.* 2005; Raven 2010; 2011; Raven *et al.* 2011; Reinfelder 2011).

Many genotypes of phytoplankton with CCMs are saturated for growth and photosynthesis under current dissolved inorganic carbon concentrations, although a significant number show an increased rate of photosynthesis and growth when grown with additional CO_2.(Giordano *et al.* 2005; Iglesias-Rodriguez *et al.* 2008a; Hurd *et al.* 2009; Berge *et al.* 2010; Neilsen *et al.* 2010; Raven 2010; 2011; Raven *et al.* 2011; Reinfelder 2011; Tyrrell 2011). These results, however, relate only to an increase in CO_2 as the only variable in cultures that are saturated with respect to other resources. Variations in the availability of other resources (photosynthetically active radiation, nitrogen, phosphorus and iron) and damaging UVB radiation have varying effects on the growth and photosynthesis of algal cultures when tested one at a time with varying inorganic carbon availability (Giordano *et al.* 2005; Raven 2010; 2011; Raven *et al.* 2011). The relevance of these studies on other environmental factors that interact with the effects of increased CO_2 to models of future CO_2 levels is that in the resulting warmer ocean, shoaling of the upper mixed layer will occur alongside changes in availability of nutrients and in mean photon flux densities of photosynthetically active radiation and ultraviolet B (Steinacher *et al.* 2010). In analyses that do not

include a CO_2 increase, phytoplankton growth is predicted to increased at high latitudes as a result of decreased mixing depth, which decreases light limitation of growth without substantially reducing nutrient supply (Steinacher *et al.* 2010). At mid- and low latitudes, although the already high mean irradiance is increased, the decreased nutrient supply dominates the effect on primary productivity, which is decreased (Steinacher *et al.* 2010). Boyce *et al.* (2010) suggest that a significant decrease in marine planktonic primary productivity has already occurred over the last century as a result of this shoaling. Further complications are added by the effects of increased atmospheric CO_2 in decreasing the rate of nitrification (Beman *et al.* 2011) and altering iron speciation (Breitbarth *et al.* 2010; Shi *et al.* 2010). These observations and analyses suggest that the major effect of atmospheric CO_2 increase on marine phytoplankton productivity will be mainly indirect, through warming and hence shoaling of the upper mixed layer with changes in the supply of resources, of which inorganic carbon is not a major determinant.

Benthic marine macroalgae are limited to coastal waters with a maximum depth of less than 300m (Littler *et al.* 1985; Raven *et al.* 2000). While a majority of species of benthic macroalgae have CCMs, there are a significant number, albeit a minority of organisms dependent on diffusive CO_2 entry (Maberly 1990; Johnston *et al.* 1992; Maberly *et al.* 1992; Kübler *et al.* 1999; Raven *et al.* 2002a; 2002b; 2005; Giordano *et al.* 2005; Kevekordes *et al.* 2006; Raven 2010). The organisms with CO_2 diffusion are generally found in subtidal or other shaded habitats, i.e. habitats where photosynthetically active radiation rather than inorganic carbon is more likely to be growth-limiting (Raven *et al.* 2002a; 2002b; 2005; Raven 2010). However, some algae lacking CCMs occur in the intralittoral fringe, e.g. *Bostrychia scorpioides* in Ireland on muddy shores and on salt marsh plants (Raven *et al.* 2002a; 2002b; 2005; Raven 2010). Here nutrients other than inorganic carbon are only available during the brief periods of submersion at high tide; inorganic carbon is available as atmospheric CO_2 during atmospheric exposure as long as the organisms stay hydrated, while atmospheric supply of other nutrients is generally very small. For these algae, nutrients other than carbon are likely to be limiting for growth. The occurrence of inorganic carbon entry by CO_2 diffusion in benthic macroalgae can thus be rationalised, although further evidence is needed, and it must be remembered that there are also macroalgae with CCMs in all of the habitats in which macroalgae relying on CO_2 diffusion occur (Raven 2002a; 2002b). An extreme example is the deepest known marine benthic macroalga, a coralline red alga from a seamount off the Bahamas, where the photosynthetically active radiation input is little more than that found at the ocean surface at night under a full moon (Littler *et al.* 1985; Raven *et al.* 2000; Raven and Cockell 2006). While there is no direct evidence on the occurrence of a CCM in this alga, coralline red algae (like other calcified macroalgae) have CCMs. An example found in Ireland is the ornithocoprophilous *Prasiola stipitata* in the infralittoral fringe of rocky shores (Raven and Johnston 1991), where the reduced availability of nutrients other than inorganic carbon from atmospheric CO_2 during emersion is offset by the nutrients in bird excreta and faeces.

In the context of anthropogenic CO_2 inputs to the ocean, it is expected that benthic algae lacking CCMs will be favoured. The situation is complicated by the predicted decrease in nutrient availability with shoaling of the upper mixed layer and its effects on inorganic carbon assimilation by CO_2 diffusion or by CCMs, although the influence on light availability to phytoplankton of this shoaling is not seen for the benthic algae (Raven *et al.* 2011). Thus the influence of environmental change on the acquisition of inorganic carbon in marine benthic macroalga differs from that in phytoplankton, with further complications due to localised nutrient inputs from rivers and the atmosphere.

CALCIFICATION BY MARINE BENTHIC MACROALGAE

Calcified red, green and, to a smaller extent, brown seaweeds are common in warmer marine waters. Ireland has no calcified green marine algae, and *Padina pavonica* is the only calcified brown alga. By contrast, there are a number of calcified red algae (Rhodophyta: Floridiophyceae) on the Irish coast (Table 2). Examples of these coralline algae, with taxa found in Ireland, are the erect articulate *Corallina officinalis*, which also has a crustose phase, and the crustose *Lithophyllum* and *Lithothamnion*, which occur on rocks (*Lithophyllum* and *Lithothamnion*) and free-living as rhodoliths (maerl)—mainly *Phymatolithon* with some *Lithothamnion* (Bosence 1976; Irvine and Chamberlain 1994; de Grave and Whitaker 1999; Blake and Maggs 2003; Wilson *et al.* 2004; Francis *et al.* 2010). The attached forms are haptophytes in the terminology of Luther (1947), while the unattached maerl forms are pleustophytes (Luther 1947) (Table 2).

Table 2—Comparison of coralline red algae and coccolithophores.

Property	*Coralline red algae*	*Coccolithophores*
Size	Macroalga	Microalga; most are nanophytoplankton (2–20μm diameter)
Life form	Haptophytic encrusting and/or erect Pleustophytic (rhodoliths/maerl)	Planktophytes; some have benthic phases in the lifecycle
Site of $CaCO_3$ deposition	Extracellular	Intracellular
Final location of $CaCO_3$	Extracellular	Extracellular

Although the earliest known fossil red alga is that of the non-calcified bangiophycean *Bangiomorpha pubescens* from deposits more than 1.2 billion years old (Butterfield 2000), the earliest known coralline red is from the Cambrian (542–488 million years ago). There is a good fossil record of coralline red alga through the Phanerozoic, and the evolution of crustose coralline red algae can be related to herbivory (Steneck 1983; 1986). These algae can live for years to decades or centuries (Bosence 1976; Steneck 1983; 1986; Littler *et al.* 1991; de Grave and Whitaker 1999; Blake and Maggs 2003; Bosence and Wilson 2003; Wilson *et al.* 2004; Mantone 2010). Maerl deposits are under threat from mining, for example for use in agriculture, although the analyses that are available do not show any additional value in maerl that is not also found in limestone (Blunden *et al.* 1997). The global annual $CaCO_3$ deposition in the coastal zone is some 23Tmol carbon; it is not clear how much of this is contributed by maerl (Gattuso *et al.* 1998).

$CaCO_3$ as calcite is sedimented in intercellular spaces in coralline red algae according to the generalised equation $Ca^{2+} + 2HCO_3^- = CaCO_3 + CO_2 + H_2O$. Lack of spontaneous $CaCO_3$ deposition in the present surface seawater, despite supersaturation with respect to calcite (and aragonite) means that one or more of removal of inhibitors of nucleation and crystal growth, and facilitation of nucleation and crystal growth using organic templates, must be missing globally (Davis *et al.* 2000). The coralline red algae have CCMs (Blinks 1963; Borowitzka 1981; Gao *et al.* 1993), which means that localised pH increases can occur beyond pH 9.0 in intercellular spaces and on the surface of the organism, facilitating $CaCO_3$ sedimentation in the light. The calcification rate is greater in the light than the dark (Borowitzka 1981; Gao *et al.* 1993). The calcification rate is proportional to the CO_3^{2-} concentration in the dark, but there is an additional 'vital effect' via photosynthesis, when the algae are illuminated presumably as a result of the net removal of (effectively) CO_2, with the associated decrease in CO_3^{2-} and increased pH in and near the cell walls (Borowitzka 1981). In the dark there is sometimes a net loss of $CaCO_3$, presumably as a result of CO_2 production in respiration with the corresponding decrease in CO_3^{2-} and pH in and near the cell wall (Chisholm 2000). Despite the evidence of a 'vital effect' on the rate of crystallisation, the Mg:Ca ratio in calcite sedimented by coralline algae follows the Mg and Ca content in the seawater they grew in (Stanley *et al.* 2002; Ries 2006).

With the CO_2 concentrations expected in 2100 the rate of calcification and the growth rate of coralline algae have been shown to decrease in laboratory cultures (Gao *et al.* 1993; Doney *et al.* 2009; Ries *et al.* 2009; Semesi *et al.* 2009; Hofmann *et al.* 2010; Tyrrell 2011). Longer-term experiments (seven weeks) in mesocosms based on natural seawater showed a decrease in growth and recruitment of crustose coralline algae (Kuffner *et al.* 2008). Similar conclusions can be drawn from field observations at marine CO_2 vents at Ischia in the Mediterranean (Hall-Spencer *et al.* 2008). Here the distance of biota from the vent determines the mean CO_2 concentration to which they are exposed. The seagrass *Posidonia* showed the greatest biomass at the lowest pH (7.6) examined, while the biomass of coralline algae is greatly decreased. A pH of 7.6 is well below what corresponds to the atmospheric CO_2 concentration expected in 2100, so the results of Hall-Spencer *et al.* (2008) show that some coralline algae can survive at CO_2 concentrations corresponding to pH values 1.5 units below the present level. Although the vents have a short life (decades) in evolutionary terms for coralline algae, which can live for years, and even decades or centuries (Blake and Maggs 2003; Wilson *et al.* 2004; Marbà *et al.* 2007; Francis *et al.* 2010), the possibility of genotypic adaptation of the coralline algae to increased CO_2 at vents should be investigated. It is important to realise that, while the vents have increased CO_2, mimicking what will happen decades hence, they are not subject to the

predicted changes in the availability of nitrogen and phosphorus (see Steinacher *et al.* 2010).

Regardless of the effects on the rate of calcification as a function of decreasing external CO_3^{2-} concentration as CO_2 increases, calcite dissolved in surface seawater is undersaturated with respect to the relevant (high-Mg or low-Mg) calcite form. The rate of dissolution is proportional to the degree of undersaturation. The 'vital effects' of photosynthesis and respiration will decrease the rate of dissolution in the light, and increase the rate in the dark (Borowitzka 1981; Chisholm 2000). This dissolution will be more significant for the coralline red algae than for the coccolithophores (see below) because of the smaller mean lifetime of coccolithophores than of the coralline red algae under natural conditions (Bosence 1976; Steneck 1983; 1986; Littler *et al.* 1991; de Grave and Whitaker 1999; Blake and Maggs 2003; Bosence and Wilson 2003; Marbà *et al.* 2007; Mantone 2010).

CALCIFICATION BY COCCOLITHOPHORES

Coccolithophores are calcified phytoplankton algae (planktophytes in the terminology of Luther 1947): some have a benthic phase in the life cycle (Green and Leadbeater 1994; Thierstein and Young 2004) (Table 2). The coccolithophores are members of the class Prymnesiophyceae in the phylum Haptophyta of the kingdom Chromista (Green and Leadbeater 1994; Thierstein and Young 2004). The calcitic coccoliths that the algae produce are major contributors to marine pelagic $CaCO_3$ precipitation: they are responsible for at least half of the annual $CaCO_3$ deposition of about 110Tmol carbon per year, the rest being attributed to foraminiferans (Balch *et al.* 2007; Broecker and Clark 2009). In comparison, annual production of only 30Tmol carbon per year has been cited for the marine pelagial (Gattuso *et al.* 1998). Poulton *et al.* (2007) suggested that the production of global organic carbon due to coccolithophores is similar to their production of particulate inorganic carbon. Other members of this phylum (some of the Prymnesiophyceae and all of the Pavlovophyceae) are non-calcified (Green and Leadbeater 1994; Thierstein and Young 2004). The non-calcified picoplanktonic and small nanoplanktonic haptophytes are significant contributors to global marine primary productivity (Liu *et al.* 2009; Cuvellier *et al.* 2010; Jardiller *et al.* 2010; Uitz *et al.* 2010). Coccolithophores, and especially *Emiliania huxleyi*, form blooms in temperate waters, including those near Ireland: the blooms can be readily imaged by satellites as a result of light scattering from attached and detached coccoliths (Harris 1994; Thierstein and Young 2004; Tyrrell and Merico 2004; O'Boyle and Silke 2010).

Calcite formation in coccolithophores occurs inside the cells in coccolith-forming vesicles (Green and Leadbeater 1994; Thierstein and Young 2004; Mackinder *et al.* 2010) (Table 2). This gives the organism more control over the environment in which calcification occurs. While coccoliths generally have low-Mg calcite in the present ocean with its high Mg/Ca ratio, the lower Mg/Ca of Cretaceous seawater does not change the Mg/Ca of species with low-Mg calcite, but decreases the Mg content of calcite in those forms that today have high-Mg calcite (Stanley *et al.* 2005). These data show that the control over Mg/Ca in coccoliths is not uniform across coccolithophores, despite calcification being intracellular. The completed coccoliths are then exocytosed (Green and Leadbeater 1994; Thierstein and Young 2004; Mackinder *et al.* 2010) (Table 2). The functions of coccoliths in the ecology of coccolithophores have been much debated (Sikes and Wilbur 1982; Green and Leadbeater 1994; Young 1994; Raven and Waite 2004; Harris *et al.* 2005; Guan and Gao 2010a; 2010b). It is likely that there are multiple functions that can be acted on by natural selection, increasing the difficulty of modelling the influence of variations in the extent of calcification with environmental change (Irie *et al.* 2010). In addition to the effects of calcification on the ecophysiology of living coccolithophores, there can be post-mortem (emergent) properties of coccospheres and coccoliths that may act as ballast on organic particles and increase the rate of sinking (Engel *et al.* 2009a; 2009b; Bierman and Engel 2010).

The overall equation for calcite precipitation with dissolved substrates from seawater by coccolithophores can be approximated by $Ca^{2+} + 2HCO_3^- = CaCO_3 + CO_2 + H_2O$, just as for the extracellular calcification by the coralline red algae. However, this refers to the reaction observed in the bulk seawater in which the coccolithophores are growing. As will be seen, what happens inside the cells where calcification occurs depends on the species of inorganic carbon entering the cells and the degree of coupling between calcification and photosynthesis.

There are still several unsolved questions about the mechanism of transport of the components of coccolithophore calcification from the medium to the lumen of the coccolith-forming vesicle. Raven (1980) pointed out that the very

low free Ca^{2+} concentration in the eukaryote cytosol caused problems for supply of Ca at the rate required for the rate of coccolith formation if the flux was by Ca diffusion through the cytosol from the plasmalemma to the coccolith-forming vesicles. This question has not yet been resolved (Mackinder *et al.* 2010).

The inorganic carbon component used to produce the calcite crosses the plasmalemma as HCO_3^-. Sikes *et al.* (1980), for instance, used the isotope disequilibrium technique and showed that HCO_3^- or CO_3^{2-} rather than CO_2 was the inorganic carbon species entering the cell. While such results are usually interpreted as showing HCO_3^- influx, the technique is based on the slow equilibration between CO_2 and H_2CO_3, HCO_3^- and CO_3^{2-}: the equilibration among the latter three molecular species is too rapid to be captured by the technique (Zeebe and Wolf-Gladrow 2001). The other technique used is based on studying calcification rates in multifactorial experiments with changed pH and inorganic carbon concentration (Paasche 1964; Buitenhuis *et al.* 1999). Plots of the rate of calcification as a function of the concentration of one of CO_2, HCO_3^- and CO_3^{2-} show that the rate–HCO_3^- relationship is the one that agrees with a saturating, rectangular hyperbola-like, relationship expected for a transporter (Paasche 1964; Buitenhuis *et al.* 1999).

It is clear from the work of Paasche (1964) and Buitenhuis *et al.* (1999), as well as from measurements of calcification as a function of inorganic carbon concentration by other workers using 'normal' seawater pH, that calcification occurs even when calcite is undersaturated in the medium, so that coccolith formation in coccolith-forming vesicles continues even when the externalised coccoliths are in a medium leading to net dissolution of calcite. While Paasche (1964) found that the relationship between calcification and the external inorganic carbon concentration showed a near-zero intercept on both axes, i.e. zero calcification rate at zero inorganic carbon, Buitenhuis *et al.* (1999) found that calcification did not occur below a certain finite concentration of total inorganic C ($0.5 mol\ m^{-3}$).

The significance of the influx of HCO_3^- rather than of CO_3^{2-} when the intracellular precipitation of calcite consumes CO_3^{2-} is one related to acid–base and charge balance. Based on Ca^{2+} and HCO_3^- entry, intracellular calcification can be represented as $Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H^+$. There is the possibility of consumption of the excess H^+ in photosynthesis if HCO_3^- is the form of inorganic carbon entering the cells via CO_2 production from H^+ and HCO_3^-, since CO_2 is the species of inorganic carbon consumed by RuBisCO, assuming a 1:1 stoichiometry of carbon use in calcite production and in photosynthesis, as follows: $Ca^{2+} + 2HCO_3^- + >8$ photons $\rightarrow CaCO_3 + (CH_2O) + O_2$. This cannot be the whole story, as there is growing evidence that the CO_2 generated during intracellular calcification in coccolithophores is not stoichiometrically consumed in photosynthesis, or otherwise necessarily involved in supplying CO_2 to Rubisco, including (in some cases) calcification continuing in the dark (Paasche 1964; 1965; 1966a; 1966b; 2001; Balch *et al.* 1992; 1996; Fernández *et al.* 1993; Herfort *et al.* 2004; Trimborn *et al.* 2007; Leonardos *et al.* 2009; Barcelos e Ramos 2010). When less H^+ is consumed in photosynthesis than produced in calcification there is (i) a need to remove H^+ from the cell to the medium if acid–base and charge balance are to be maintained when the rate of carbon incorporation in calcification exceeds that in photosynthesis (Sikes *et al.* 1980), and (ii) for H^+ to enter when the rate of carbon assimilation in photosynthesis exceeds that in calcification. The limiting case for photosynthesis exceeding calcification is zero calcification, bringing non-calcifying coccolithophores into the same category with respect to acid–base balance and charge balance as other phytoplankton with a CCM based on HCO_3^- entry with a 1:1 stoichiometry of H^+ influx or OH^- efflux. It is important to remember that the overall reaction in seawater in the light is, for a given stoichiometry of photosynthesis and calcification, independent of the degree of intracellular coupling of photosynthesis and calcification.

A further point about acid–base regulation is that the uptake and reductive assimilation of NO_3^- and SO_4^{2-} generate OH^- in excess of what is required in intracellular acid–base balance, and OH^- is lost from the cell (equivalent to H^+ uptake) (Raven and Smith 1974; 1976; Smith and Raven 1979; Goldman and Brewer 1980; Raven and Farquhar 1990; Raven 1993). Raven (1993) suggested that the high concentration of the compatible solute dimethylsulphoniopropionate in coccolithophores would increase the cellular sulphur quota and thus the OH^- efflux, but the measured sulphur content of the two coccolithophores examined is lower than that of the mean sulphur content of the fifteen marine phytoplankton species examined by Ho *et al.* (2003). When the nitrogen sources is NH_4^+ the net effect of nitrogen and sulphur assimilation on intracellular acid–base balance is an excess of H^+, which is excreted (Raven and Smith 1974; 1976; Smith and Raven 1979; Goldman and Brewer 1980). The use of organic nitrogen (urea, amino acids) gives acid–base perturbations within the range

known for NH_4^+ for NO_3^- (Raven and Smith 1974; 1976; Smith and Raven 1979). Beman *et al.* (2011) suggest that nitrification could be inhibited in higher CO_2 concentrations, so reduced forms of nitrogen may become more important relative to NO_3^- under these conditions.

Imbalance of acid–base relations in the cytosol of cells is indicated by changes in the cytosolic pH, while charge imbalance is indicated by changes in the electrical potential difference across the plasmalemma. Walker (1976, 47–48) elegantly shows that the values of the membrane capacitance (relating potential difference to charge imbalance across a given area of membrane) and of the cytosol H^+ buffer capacity (relating H^+ addition or removal to the pH of a given volume) are such, when related to the cytosol volume per unit area of plasmalemma that a (notional) given rate of addition of H^+ to the cytosol takes the electrical potential difference outside the tolerable range much more quickly than it changes the pH to an intolerable value. What are these intolerable values? Measurement of the pH of the intracellular compartments of plant cells is difficult (Small 1946; 1954; 1955; Raven and Smith 1974; 1976; Smith and Raven 1979), and there are also problems with measuring the electrical potential difference across the plasmalemma in small cells such as those of most coccolithophores (Raven and Smith 1974; 1976; Smith and Raven 1979). There are many estimates of the potential difference across the plasmalemma of marine algae, these are mainly for large cells (Raven 1976), though there are estimates for coccolithophores (Sikes and Wilbur 1982; Anning *et al.* 1996). There have been relatively few estimates of the pH of the cytosol of marine algal cells since the early work of Raven and Smith (1980), though there are values for coccolithophores (Anning *et al.* 1996). It is known from work on marine and other photosynthetic organisms that the pH of the cytosol can only vary by about 0.5 units and the electrical potential difference across the plasmalemma can only vary by about 0.2 V (Raven and Smith 1974; 1976; Smith and Raven 1979). Within the tolerable cytosolic pH range there is typically a 0.1 unit decrease in cytosolic pH with each unit decrease in external pH, and vice versa for an external pH increase (Smith and Raven 1979). In the context of calcification in coccolithophores, such a scaling factor of 0.1 between internal and external pH would mean that the cytosolic CO_3^{2-} concentration only decreases by ~10% of the decrease in external CO_3^{2-} concentration with a given decrease in external pH.

The means by which H^+ moves across the plasmalemma of coccolithophores is not yet clear. An interesting possibility for those cells with very small electrochemical potential differences across the plasmalemma (Anning *et al.* 1996) is the voltage-gated H^+ channel predicted from genomic analysis (Mackinder *et al.* 2010), and now functionally demonstrated by Suffrian *et al.* (2011) and Taylor *et al.* (2011). While such a transport mechanism is passive, i.e. no exergonic reaction is stoichiometrically coupled to the transport of H^+ through the transporter protein, energy must be applied to the movement of other ions in maintaining the electrical potential difference across the plasmalemma that results in an inward- or outward-directed H^+ gradient. A more direct application of metabolic energy occurs if the flux of H^+ occurs against the H^+ free energy gradient. The overall energy requirement for calcification in seawater in equilibrium with the present atmosphere was estimated by Anning *et al.* (1996) to be 30% of that required to assimilate an equal quantity of inorganic carbon in photosynthesis.

For the less likely option of CO_3^{2-} entry (Paasche 1964; Sikes *et al.* 1980; Buitenhuis *et al.* 1999), the pH of the cytosol of coccolithophores ranges from at least one, and sometimes more than two, units lower than the pK_{a2} of the inorganic carbon system at the ionic strength of the cytosol, so that the equilibrium concentration of CO_3^{2-} is always less than 10% of the total inorganic carbon in the cytosol (Anning *et al.* 1996). The very high rate constants for the interconversion of HCO_3^- and CO_3^{2-} mean that the equilibrium is reached more rapidly than either species can diffuse from the plasmalemma to the coccolith-forming vesicle (or the plastids). The reasoning here is that the half-time of equilibration of the two ionised inorganic carbon species under cytosolic conditions is about 10^{-7}s (Zeebe and Wolf-Gladrow (2001, 104–105, 109–110)), while the half-time for diffusive equilibration of either ionised inorganic carbon species over an assumed 1μm of cytosol between the plasmalemma and the coccolith-forming vesicle is of the order of 10^{-4}s (Nobel 2005, 19). If CO_3^{2-} was the species taken up by coccolith-forming vesicles, there would be no H^+ production during calcification, so that there would be no production of CO_2 from HCO_3^- using H^+ from calcification as required by hypotheses that envisage an internal mechanistic link between photosynthesis and calcification. Similarly, there would be no need for H^+ excretion from cells during coccolithogenesis as is the case with HCO_3^- entry if there is no mechanistic link between the supply of inorganic carbon substrates for photosynthesis and for coccolithogenesis. The only H^+ cycling needed in relation to coccolithogenesis when CO_3^{2-} enters the cells

is that of (i) buffered H^+ in the cytosol from the coccolith vesicle back to the plasmalemma in charge and (ii) pH balance if HCO_3^- is the main inorganic carbon species diffusing through the cytosol, as would be expected in view of its dominance in terms of concentration. For photosynthesis, CO_3^{2-} entry to the cytosol would double the need for mechanisms (H^+ influx or OH^- efflux) dealing with acid–base and charge balance attendant on entry of an ionised inorganic carbon species and the consumption of CO_2 that are seen when HCO_3^- enters the cell. As for intracellular calcification, the evidence does not favour CO_3^{2-} as the inorganic carbon species entering the cells for photosynthesis in with coccolithophores (Paasche 1964; Sikes *et al.* 1980; Buitenhuis *et al.* 1999) or other cells with CCMs (Maberly 1992).

Turning to the impact of anthropogenic CO_2 inputs on calcification by coccolithophores, there has been a wide range of reported effects that had been attributed to differences in experimental methods and genetic variations among the coccolithophores, or to both effects (Iglesias-Rodriguez *et al.* 2008a; 2008b; Riebesell *et al.* 2008; Doney *et al.* 2009; Hurd *et al.* 2009; Ridgwell *et al.* 2009; Schulz *et al.* 2009; Hofmann *et al.* 2010; Barcelos e Ramos *et al.* 2010; Müller *et al.* 2010). Only recently has it been shown that, using the same techniques in all cases, there are significant interspecific (Langer *et al.* 2006) and intraspecific (Langer *et al.* 2009) variations in the response among coccolithophores, and that the use of different techniques results in only small differences between calcification as a function of CO_2 concentration in a single coccolithophore strain (Shi *et al.* 2009) and the length of time for which the cells are cultured at the experimental CO_2 concentrations (Barcelos e Ramos 2010; Müller *et al.* 2010). The outcome of this large body of work is that there are many genotypes for which the 'expected' decrease in calcification rate with increased CO_2 occurs, but there are some strains for which there is an increase and yet others for which there is some intermediate response.

Based on the frequently observed decrease in calcification with increasing CO_2 (Ridgwell *et al.* 2009), it has been suggested that there is a greater energy cost for coccolith formation in seawater equilibrated with higher than current atmospheric CO_2 concentrations (e.g. Raven *et al.* 2005; Irie *et al.* 2010). Considering first the more likely case of the entry of HCO_3^-, the mechanism of such an increased cost presumably does not involve HCO_3^- influx alone since there is a slight increase in external HCO_3^- with increased CO_2. If coccolithophores have a scaling factor of 0.1 between cytosolic and external pH similar to that found for other organisms (Smith and Raven 1979), the cytosolic CO_3^{2-} concentration would only decrease by 10% of the decrease in external CO_3^{2-} concentration with a given decrease in external pH. Regulation of cytosolic pH with this scaling factor involves a larger minimum energy input as external pH decreases, since the driving force for downhill H^+ entry is greater, provided the electrical potential difference across the plasmalemma is constant. Any increased energy cost for calcification in higher CO_2 environments would then have a significant H^+ transport component related to the efflux of H^+ from calcification, provided that the H^+ had not been used in converting HCO_3^- to CO_2 in photosynthesis.

Before going further it is necessary to point out the constraints on an energetic comparison of cells grown under different conditions, e.g. today's CO_2 concentration and the CO_2 expected in 2100 CE. One point is that there is not necessarily a linear relationship between the energetic driving force for a reversible biological transmembrane transport (or biochemical, or mechanical) process, or an essentially irreversible process, and the net rate of the process. While the rate of the forward and the back reactions can be linearly related to the driving force for reversible reactions (Odum and Pinkerton 1955; Katchalsky and Curran 1965; Beard and Qian 2007), there are many biological situations that complicate such analyses (e.g. Michaelis-Menten kinetics (Beard and Qian 2007)) and variations in the catalytic capacity of the protein involved in catalysis (e.g. from post-translational modification and other kinds of regulation of the protein) or variations in expression of the protein, between organisms grown under (for example) present-day and 2100 CE CO_2. There is also the question of when a reversible reaction that is far from thermodynamic equilibrium can be regarded as irreversible (Odum and Pinkerton 1955; Katchalsky and Curran 1965). Thus, even if the energetic stoichiometry of energy input (e.g. mol ATP converted to ADP and phosphate) and mol solute transported is unchanged, there may be other energy costs (e.g. increased expression of transporters in one growth condition relative to the other).

Based on the Nernst Equation, for a fixed number of H^+ channels to be involved in removing excess H^+, a smaller value of the inside-negative electrical potential difference is needed to maintain the outwardly directed net driving force on H^+. Maintenance of such a change in the potential difference would ultimately be dependent on metabolic energy, and the decreased electrical potential difference would mean a smaller

driving force for any electrically driven nutrient co-transport systems, e.g. those coupled to Na^+ entry down a free energy gradient. An alternative mechanism for H^+ efflux would be active efflux. For this to be required there would, for a given cytosol pH, have to be a more inside-negative electrical potential difference across the plasmalemma, giving an inwardly directed driving force on H^+ greater than the energy requirement per H^+ moved out of the cytosol. Much more experimentation is needed to examine the qualitative and quantitative plausibility of these suggestions. Making the assumption that there is an increased energy cost of calcification in higher CO_2 environment, the evolutionary model of Irie *et al.* (2010) suggests that the optimal growth strategy is to grow more slowly but with greater calcification in a higher-CO_2 environment. Irie *et al.* (2010) point out the evidence for increased calcification of coccolithophores in the fossil record over the latest 200 years of marine sediments in Iglesias-Rodriguez *et al.* (2008a). However, although Iglesias-Rodriguez *et al.* (2008a), like some other workers (see Langer *et al.* 2009; Ridgwell *et al.* 2009), found increased calcification with increased CO_2, they also found faster rather than slower growth at higher CO_2.

The only experimental evidence on the energy cost of acid–base regulation in algae is for aciophilic green algae growing in inland waters (Messerli *et al.* 2005; Langner *et al.* 2008; Bethman and Schönknecht 2009). Here a major energy requirement seems to be for a large energised H^+ efflux against a substantial free energy difference related to the maintenance of a near-neutral cytosolic pH without a completely compensating change in the electrical potential difference across the plasmalemma. The active H^+ efflux removes H^+ that had leaked into the cells down the large H^+ free energy difference across the plasmalemma (Messerli *et al.* 2005; Langner *et al.* 2008; Bethman and Schönknecht 2009). The energy costs of acid–base regulation can exceed 10% of the total cell energy budget in these cases; however, the H^+ free energy difference across the plasmalemma of coccolithophores is much lower than in acidophilic algae, and it will be even lower at the projected 2100 surface ocean pH values.

An alternative potential energy cost of calcification with increasing external CO_2 and assuming HCO_3^- entry involves a less strict homoeostatic regulation of cytosol pH (see Anning *et al.* 1996): if the cytosol pH more closely tracks external pH, then with a constant HCO_3^- accumulation ratio (cytosol:medium) the CO_3^{2-} concentration in the cytosol is lower than is the case for present day CO_2, with the consequence of an additional energy cost to achieving a CO_3^{2-} accumulation in the coccolith-forming vesicle that is sufficient to precipitate calcite.

For the less likely case of CO_3^{2-} entry, the lower external CO_3^{2-} concentration in a higher-CO_2 environment means that, to maintain a given CO_3^{2-} concentration in the cytosol, a CO_3^{2-} influx would be acting against a larger free energy difference and hence there would be a larger minimum energy input required to move a given amount of CO_3^{2-} into the cytosol. Alternatively, with a constant cytosol:medium concentration ratio for CO_3^{2-}, and hence a lower CO_3^{2-} concentration in the cytosol under high CO_2 conditions, the minimum energy input for accumulation of CO_3^{2-} in the coccolith-forming vesicle would be greater. For CO_3^{2-} entry from the medium to the cytosol and uptake from the cytosol to the coccolith-forming vesicle, the flux of inorganic carbon through the cytosol would be largely as HCO_3^- (as is the case for HCO_3^- entry at the plasmalemma), again with the buffered H^+ flux through the cytosol from plasmalemma to coccolith-forming vesicle (see discussion above).

Once formed and externalised, coccoliths on living cells dissolve in surface seawater undersaturated with respect to the relevant (high-Mg or low-Mg) calcite form. The rate of dissolution is proportional to the degree of undersaturation. There is evidence consistent with a decreased dissolution rate as a result of the presence of a surface layer of organic matter (Iglesias-Rodriguez *et al.* 2008a): this layer is 280–350nm thick in dried specimens (Godoi *et al.* 2009). Diatoms show that organisms with short mean lifespans in nature (Marbà *et al.* 2007) can maintain an internally precipitated extracellular mineral (opaline silica) skeleton in a habitat that is always very significantly undersaturated with respect to the mineral. The rate of dissolution of the silica is decreased about 100-fold by the presence of the natural organic surface layer (Natori *et al.* 2006). In the present context, it is of interest that the rate of diatom silica dissolution is, by an unknown mechanism, a linear function of the CO_2 concentration (Milligan *et al.* 2004). Of course, the differences in chemistry of calcite and opal mean that the apparent similarities of coccolithophore and diatom skeletal dissolution need further investigation.

CONCLUSIONS

Increased atmospheric CO_2 is increasing the sea surface CO_2 concentration, with a corresponding

smaller proportional increase in HCO_3^- and a decrease in CO_3^{2-} and pH. These changes will, in isolation, either have no effect on the growth of non-calcified marine algae or will increase the growth rate. Inclusion of an indirect effect of increased CO_2, i.e. shoaling of the upper mixed layer of the ocean as a consequence of warming, complicates the direct response to CO_2. One outcome of this indirect effect is that CCMs are likely to be more persistent in a higher CO_2 world than would be expected from the effects on increased CO_2 alone. Many calcified red benthic algae and planktonic coccolithophores have decreased calcification and growth rate when grown at higher CO_2, but some coccolithophores have an increased calcification and growth rate at higher CO_2 while others show an intermediate response. The decreased rate of the extracellular calcification of red algae at higher CO_2 relates to the decreased extracellular CO_3^{2-} concentration, with interactions with photosynthesis. For the intracellular calcification of coccolithophores, examples where there is a decreased rate of calcification at higher CO_2 could be related to increased energy cost of H^+ extrusion if the cytosol pH is kept essentially constant, or a decreased concentration of CO_3^{2-} in the cytosol, if the cytosol pH more closely tracks external pH.

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