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Environmental controls on coccolithophore calcification

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ABSTRACT: Coccolithophores are major contributors to global marine planktonic calcification, and in nature coccolithophores are invariably calcified through almost all of their life cycle. The response of calcification to environmental factors is essential in understanding the persistence of coccolithophores through at least 220 million years of changing global environments, and their prospects for current environmental change. So far the responses examined have been at the level of acclimation rather than adaptation in evolution. Variation in results of CO_2 manipulation experiments can be tentatively attributed to variation among genotypes rather than differences in experimental procedure. Comparisons of methods using the same genotype, and of several genotypes using a single method, suggest significant variation among genotypes. The general response is a decreased particulate inorganic carbon (PIC) to particulate organic carbon (POC) ratio in higher than present CO_2 concentrations and vice versa for lower CO_2 concentrations. Fewer studies have investigated the effect of other environmental factors. Decreased availability of phosphorus and, to a lesser extent, nitrogen, as well as decreasing photosynthetically active radiation (PAR) down to a certain low value increase PIC:POC, while variable results have been found for changes in ultraviolet radiation (UVR). Many of these results can be accommodated by considering the restriction of calcification to the G1 phase of the cell cycle and the length of this phase under different growth conditions. Fewer studies have investigated the interactions among environmental factors which change with increased CO_2 and increasing sea surface temperature; the shoaling of the thermocline will increase the mean PAR and UVR whilst decreasing nitrogen and phosphorus availability. More studies of these interactions, as well as of genetic adaptation in response to changed environmental factors, are needed.

KEY WORDS: Calcification · Coccolithophores · Carbon dioxide · Phosphorus · Nitrogen · Photosynthetically active radiation · Temperature · Ultraviolet radiation

INTRODUCTION

In order to review the literature concerning calcification in coccolithophores, it is interesting to first note that the function, or selective advantage, that coccoliths provide is still unknown. We will explore the evidence for the various hypotheses proposed. The mechanisms involved in calcification are also still under investigation, so to work towards a process-based understanding, we will summarise the current evidence. From this base we will then examine the evidence from numerous studies and attempt to draw out systematic responses to environmental variables. There is much scope for future research in this area, and we will try to expose areas of particular interest.

Coccolithophores (Prymnesiophyceae: Haptophyta) are calcified planktonic primary producers found in both coastal and oceanic regions where they often form blooms. At least 150 species are known (West-

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broek et al. 1993, Winter & Siesser 1994), and within these formal species, strains with unique physiology and morphology have been characterised. Coccolithophores have existed for at least 220 million years. This minimum age for the origin of coccolithophores comes from the fossil record (Falkowski et al. 2004), with the origin of heterococcoliths at least 215 million years ago and of holococcoliths at least 185 million years ago (Medlin et al. 2008). In the Cretaceous, they were more cosmopolitan and diverse than present-day species, which are largely dominant in warm, stratified, nutrient-poor oceanic waters (Brand 1994). In the present ocean, coccolithophores are believed to account for at least half of the 80 to 120 Tmol particulate inorganic carbon (PIC) produced each year in the marine pelagial (Degens & Ittekkot 1986, Westbroek et al. 1993, Balch et al. 2007, Berelson et al. 2007, Broecker & Clark 2009). A further 21% of the marine pelagial $CaCO_3$ is deposited by foraminiferans (Langer 2008), some of which are symbiotically photosynthetic. The other photosynthetic $CaCO_3$ producers in the marine pelagial are certain dinoflagellates (Gadd & Raven 2010) and cyanobacteria such as *Trichodesmium* (Kranz et al. 2010), but these only make very minor contributions to global pelagial $CaCO_3$ precipitation. The high density of the liths, with the present depth of the calcite lysocline and the occurrence of organic coatings, means that much of the calcite produced is exported to the deep ocean, constituting the carbonate pump (Westbroek et al. 1993, Balch et al. 2007, Berelson et al. 2007, Broecker & Clark 2009, Lebrato et al. 2010). There seem to be no coccolith-specific estimates, but the total PIC flux sinking below 2000 m may be as much as 50 Tmol C yr^{-1} (see Table 3 of Berelson et al. 2007). This consists predominantly of liths which have become associated with other particulate organic matter, e.g. faecal pellets and transparent exopolymeric particles (Pedrotti et al. 2012), and are effective ballast for export to the deep ocean, fuelling the biological pump of organic carbon (Rost & Riebesell 2004, Biermann & Engel 2010). While ballasting of particulate organic carbon (POC) by PIC increases the atmosphere-to-ocean CO_2 flux by decreasing mineralisation in the surface ocean, it also decreases PIC dissolution in the surface ocean, leaving a larger fraction of the CO_2 generated in PIC production in the surface ocean which decreases the atmosphere-to-ocean flux of CO_2. Note that calcite, the form of $CaCO_3$ in coccoliths, is only two-thirds as soluble as aragonite (Mucci 1983).

We begin by reflecting on the possible function(s) of coccoliths and the formation of hetero- and holococcoliths. The interactions of coccolithophores with environmental change are then discussed, including experimental methods for investigating calcification responses.

FUNCTIONS OF COCCOLITHS

Despite the efforts made to discover the function of calcification, none of the various hypotheses are supported by sufficient evidence to have been fully accepted. Table 1 summarises the hypotheses proposed and references providing evidence for and against them. These hypotheses will be discussed briefly (see also Table 1) but, due to insufficient evidence, for the purposes of this review we assume that coccoliths provide some benefit to the cell which outweighs the cost of production.

The addition of liths, with higher density than other cell components, results in increased sinking rates (see Raven & Waite 2004, Biermann & Engel 2010). Nutrient limitation generally increases PIC:POC and hence cell density, bringing the cells into deeper waters with higher nutrient concentrations. However, cells in conditions which restrict the growth rate and cultures containing senescent cells may shed most or all of their liths (Paasche 2001). Decreased photosynthetically active radiation (PAR) reduces ballasting, slowing the rate at which cells move into deeper, low-PAR waters (Raven & Waite 2004).

One suggested function of coccoliths is physical restriction of virus infection (Raven & Waite 2004). However, Frada et al. (2008) found that, while the giant phycodnaviruses infect the diploid (heterococcolith-bearing) phase of *Emiliania huxleyi*, where they can be significant in terminating blooms, the haploid phase, without heterococcoliths, is immune and comprises a refuge from the viruses. However, this refuge is only temporary since the diploid phase is dominant in *E. huxleyi* and probably in other coccolithophores as well. Thus, the haploid phase is not the state in which *E. huxleyi* produces large populations in nature; the haploid phase may be involved in over-wintering (von Dassow et al. 2009).

Alternatively, cells infected by viruses or parasitoids may sink out, thus protecting the uninfected population, based on kin selection (Raven & Waite 2004). Hypothetically, infection may decrease the capacity of the protoplast to maintain a low density; however, this mechanism may apply more to large, vacuolate, silicified diatoms than to smaller coccolithophores with less vacuolation (Raven & Waite 2004). No experimental evidence supports this hypo-

Table 1. Potential functions of coccoliths. See 'Functions of coccoliths' in the main text for further details. PAR: photosynthetically active radiation; UV: ultraviolet

Hypothesis	Advantage	For	Against
Ballasting	Allows vertical migration to access nutrients	Paasche (2001), Raven & Waite (2004)	
Viruses	Affords protection	Raven & Waite (2004)	Frada et al. (2008) (holococcoliths vs. heterococcoliths)
Grazers	Affords protection	Nejstgaard et al. (1994)	Harris (1994)
PAR	Photoprotective at the surface	Braarud & Nordli (1952)	Paasche (1964), Paasche & Klaveness (1970), Nanninga & Tyrrell (1996), Houdan et al. (2005), Trimborn et al. (2007)
PAR	Focuses light to chloroplasts in deep water	Young (1994)	Nanninga & Tyrrell (1996), Raven & Waite (2004), Trimborn et al. (2007),
UV	Protective	Gao et al. (2009)	Gao et al. (2009) (not unequivocally for)
H^+ (hence CO_2) production	H^+ used to convert HCO_3^- to CO_2 or other uses of H^+, e.g. neutralising OH^- produced in assimilation of NO_3^- and SO_4^{2-}		Can occur, but not an obligate means of generating CO_2 for photosynthesis. Herfort et al. (2004), Leonardos et al. (2009)
Avoiding intracellular phosphate precipitation	Ca^{2+} precipitates HPO_4^{2-} and phosphate esters; calcification may have evolved to prevent this	Degens & Ittekkot (1986), Couradeau et al. (2012) reported intracellular carbonate deposits in an early-branching cyanobacterium, containing almost as much (Mg + Sr +Ba) as Ca	Low free Ca^{2+} in the cytosol predates coccolithogenesis by at least 2 billion years; low cytosolic free Ca^{2+} a problem for Ca transport from the plasmalemma to the coccolith-forming vesicle (Raven 1980, Sanders et al. 1999, Dodd et al. 2010)

thesis. The most obvious theory is that coccoliths deter grazers (Nejstgaard et al. 1994); however, coccolithophores have been found to be the preferred prey of copepods both in the laboratory (Sikes & Wilbur 1982, Harris 1994) and in mesocosms (Nejstgaard et al. 1994). Experimental evidence actually shows preferential grazing rates on lithed rather than naked cells by the heterotrophic dinoflagellate *Oxyrrhis marina* (Hansen et al. 1996).

Coccoliths may increase radiation scattering in surface waters with high photon flux densities. This would reduce photoinhibition of photosynthesis by PAR and ultraviolet radiation (UVR) as well as the damage by UVR. However, a similar lack of photoinhibition at high PAR is seen when comparing lightly and heavily calcified strains (Israel & Gonzalez 1996) or when the degree of calcification is experimentally reduced (Paasche 1964, Paasche & Klaveness 1970, Nanninga & Tyrrell 1996, Trimborn et al. 2007). However, Nielsen (1995) found a higher light-saturated rate of photosynthesis at a given inorganic carbon concentration in highly calcified cells than in cells with little calcification. Alternative evidence suggests that increased calcification in *Emiliania huxleyi* can increase photochemical quenching of excess excitation energy following a steep increase in PAR (Barcelos e Ramos et al. 2012). Coccoliths may also be able to focus PAR to the plastids in deeper waters where photosynthesis is PAR-limited (Nanninga & Tyrrell 1996, Raven & Waite 2004). However, in deeper waters, the ratio of diffuse (scalar) as opposed to direct (vector) radiation increases, making focussing of radiation by coccoliths more difficult. It is of interest that calcite is used in radiation focussing in extant ophiuroids and in extinct trilobites but in a sensory rather that an energetic role (Aizenberg et al. 2001). The effects of UVB radiation (UVBR) on calcification are discussed in this review but here it is sufficient to say that at least *Emiliania huxleyi* is very sensitive to UVBR and coccoliths do not seem to protect it (Peletier et al. 1996).

A hypothesis which has been discussed at great length is the role of calcification as an intracellular source of CO_2, based on the entry of HCO_3^- into the cells for calcification (Sikes et al. 1980, Brownlee et al. 1995a, Anning et al. 1996, Fabry et al. 2008, von Dassow et al. 2009, Mackinder et al. 2010). Within the coccolith vesicle, HCO_3^- is converted to calcium carbonate, releasing either CO_2 or H^+, depending on the equation used. These by-products must be

removed to the cytosol to prevent acidification of the coccolith vesicle inhibiting calcite formation (Brownlee et al. 1995a). The CO_2 form of this argument is expressed as:

$$2HCO_3^- + Ca^{2+} \rightarrow CaCO_3 + CO_2 + H_2O \quad (1)$$

with the CO_2 consumed in photosynthesis. The alternative is to frame the argument in terms of the production of H^+ in calcification:

$$HCO_3^- + Ca^{2+} \rightarrow CaCO_3 + H^+ \quad (2)$$

with subsequent use of the H^+ to generate CO_2 from HCO_3^-:

$$H^+ + HCO_3^- \rightarrow CO_2 + H_2O \quad (3)$$

The sum of Eqs. (2) and (3) is identical to Eq. (1). The 2 mechanisms are experimentally indistinguishable, since intracellular carbonic anhydrase equilibrating the inorganic species with the H^+-OH^- is required when calcification and/or photosynthesis uses HCO_3^- as the form entering the cell. It must be acknowledged that the overall equation is an oversimplification, and varies with the pH of the intracellular compartment concerned.

A strict 1:1 stoichiometry of calcification and photosynthesis is clearly not applicable in the large number of cases in which the PIC:POC ratio is significantly different from 1:1 (Raven 2011a). These cases cannot be rescued by considering loss of photosynthate as (photo-) respired CO_2 or as dissolved organic matter or transparent exopolymeric particles (Raven 2011a, Pedrotti et al. 2012). This correction gives PIC per integrated net photosynthetic C accumulation which is lower than PIC:POC (Raven 2011a). This adjustment still yields cases in which generation of PIC produces more CO_2 than is consumed in photosynthesis as well as cases in which generation of PIC produces less CO_2 than is used in photosynthesis, or even produces no CO_2 (Raven 2011a). An interesting line of evidence for the lack of an obligatory coupling of photosynthesis to calcification comes from the growth of *Emiliania huxleyi* at low external [Ca^{2+}] which abolishes calcification yet leaves photosynthesis unaffected (Herfort et al. 2004, Trimborn et al. 2007). When the calcification rate exceeds the rate of photosynthesis, excess CO_2 or its equivalent as H^+ is excreted (Suffrian et al. 2011, Taylor et al. 2011). Conversely, when the photosynthetic rate exceeds that of calcification, additional CO_2 (Sikes et al. 1980) or HCO_3^- influx to the cell is required in addition to the CO_2 produced in calcification. These arguments speak against a widespread causal linkage of photosynthesis to calcification.

When the rate of calcification exceeds the rate of organic carbon production, there is an excess of CO_2 (Eq. 1) or H^+ (Eq. 2) relative to that needed to maintain intracellular acid–base balance.

This excess of H^+ could be used in converting HCO_3^- into CO_2 which is consumed in photosynthesis. A variety of methods have been used to clarify the carbonate species taken up and used in coccolithophore photosynthesis. Data from the membrane inlet mass spectrometer method suggest uptake of both CO_2 and HCO_3^- to supply photosynthesis in coccolithophores (Rost et al. 2003, 2007, Tchernov et al. 2003, Schulz et al. 2007), while isotope disequilibrium experiments suggest a predominant role for CO_2 entry (Sikes et al. 1980, Sikes & Wheeler 1982, Sekino & Shiraiwa 1994, but see Rost et al. 2007), and inhibitor studies indicate a significant role for HCO_3^- (Herfort et al. 2002). Examination of the rate of photosynthesis as a function of the concentration of inorganic carbon and of extracellular pH also suggests a predominant role for HCO_3^- entry (Paasche 1964, Buitenhuis et al. 1999). In the absence of this sink, H^+ could be lost from cells across the plasmalemma using the recently discovered plasmalemma H^+ channel (Suffrian et al. 2011, Taylor et al. 2011). The passive (energetically downhill) nature of this flux means that there is no direct energy input from metabolism to the H^+ transport. However, Raven (2011a) pointed out that energy input is needed to maintain the appropriate transplasmalemma electrical potential difference to maintain the H^+ efflux, and that this input is likely to be greater per unit calcification in a higher-CO_2 ocean. A similar H^+ channel has been found in a non-calcifying dinoflagellate (Smith et al. 2011). A complication in analysing the quantitative requirement for H^+ efflux is that H^+ is consumed when HCO_3^-, rather than CO_2, is the form in which inorganic carbon destined for photosynthesis enters the cell, and in acid–base balance following assimilation of NO_3^- and SO_4^{2-} (Raven 2011a). Ries (2011) also considered energetic constraints on calcification in a high-CO_2 environment.

HETEROCOCCOLITHS AND HOLOCOCCOLITHS

Coccolithophores produce characteristic coccoliths, comprising an organic template with crystalline calcite deposited on it in species-specific patterns (Braarud & Nordli 1952, Young 1994, Young & Henriksen 2003, Young et al. 2005) according to Eqs. (2) and (3). The process obviously requires calcium and bicarbonate ions; it also requires energy and, to con-

struct the synthetic and exocytotic apparatus, macro- and micronutrients. Once exocytosed, the calcite liths are exposed to ambient seawater, and the tendency for dissolution will be determined by the calcite saturation state of the seawater, which is determined by Eq. (4):

$$\Omega_{cal} = [Ca^{2+}]\,[CO_3^{2-}]/K_{sp} \quad (4)$$

where K_{sp} is the stoichiometric solubility product of calcite, which varies as a function of temperature, salinity and pressure (Mucci 1983, Zeebe & Wolf Gladrow 2001). In the modern ocean, $[Ca^{2+}]$ is considered as being constant (varies only with salinity), thus the carbonate ion concentration is the only real variable in this equation.

Coccolithophores typically have a diploid, heterococcolith-bearing phase, with calcified plates made up of complex crystal units in radial arrays. For many species, a haploid phase has been observed with either non-calcified organic scales, as in *Emiliania huxleyi,* or with a different calcification mode. The formation of holococcoliths, calcareous scales composed of many small identical euhedral crystallites (Frada et al. 2009), is the most common form of haploid biomineralisation in extant coccolithophores (Young et al. 2005). Studies have investigated gene expression by the 2 phases (von Dassow et al. 2009, Rokitta et al. 2011) and effects of CO_2 and their modulation by light (Rokitta & Rost 2012).

Heterococcoliths are formed internally in coccolith-forming vesicles which are part of the endomembrane system (Marsh 2003, Young & Henriksen 2003, Brownlee & Taylor 2004). Coupling to exergonic cell activities provides the energy which makes the depositional environment supersaturated with respect to calcite, and provides the organic template on which deposition occurs (Anning et al. 1996, Marsh 2003, Young & Henriksen 2003, Brownlee & Taylor 2004). The process of deposition is under more control by the organism than is the case for those organisms, e.g. coralline algae, which deposit $CaCO_3$ extracellularly (von Dassow et al. 2009, Mackinder et al. 2010, 2011, Raven 2011a).

The finished coccoliths are then externalised. The tendency for them to then dissolve is dependent on the saturation state in the boundary layer and may be reduced by the occurrence of a surface coating of organic material. Although holococcolith formation has not yet been well characterised, it is suggested that calcification still occurs within a delicate envelope or, less likely on grounds of comparative cell biology (Raven 1980), occurs rapidly just below the cell membrane (Young & Henriksen 2003). Von Dassow et al. (2009) showed for *Emiliania huxleyi* that transcript levels of genes whose products are involved in coccolithogenesis (Ca^{2+}, H^+ and HCO_3^- transporters) are expressed much more in the diploid (heterococcolith) than in the haploid (holococcolith) phase which has non-calcified coccoliths. The few studies concerning environmental effects on holococcoliths (Quintero-Torres et al. 2007, Fiorini et al. 2011a, Pedrotti et al. 2012) will be discussed in the relevant sections.

COCCOLITHOPHORES IN PAST AND PRESENT ENVIRONMENTS

Coccolithophores have experienced and thrived with significant variations in surface ocean chemistry, temperature and, through variations in the mixing depth, exposure to solar radiation. They are found throughout the world ocean, in both open ocean and coastal regions where they are more likely to be exposed to larger and faster changes in environmental factors. A number of studies have investigated changes in coccolithophore morphology related to environmental changes over times ranging from tens of millions of years (Henderiks & Rickaby 2007, Henderiks 2008, Henderiks & Pagani 2008) to tens or hundreds of years (Iglesias-Rodriguez et al. 2008a, Rickaby et al. 2010a, Beaufort et al. 2011). There have also been modelling studies (e.g. Young 1994, Merico et al. 2006) and molecular clock investigations of changes in coccolithophore biochemistry (Young et al. 2012).

During the course of coccolithophore history, CO_2 (with implications for the rest of the inorganic carbon system and pH) and also Ca^{2+} and Mg^{2+} have varied dramatically (Orr 2011, Müller et al. 2011, Zeebe & Ridgwell 2011). The extent of stratification which controls solute transfer between deeper waters and the upper mixed layer, and therefore nitrogen and phosphorus availability, has also varied (Steinacher et al. 2010).

CO_2 concentrations over the last 220 million years have, with the exception of the last 10 to 20 million years, been higher than the present level. However, the calcite saturation index has probably varied little over the past 100 million years as documented in deep-sea sediments, due to the halving of the $[Ca^{2+}]$ and increase in $[CO_3^{2-}]$ (Tyrrell & Zeebe 2004). The decrease in $[Ca^{2+}]$ to around 10 mM in present-day seawater (Tyrrell & Zeebe 2004) and concomitant doubling of $[Mg^{2+}]$ to around 50 mM (Dickson & Goyet 1994), possibly modulated by changes in sulphate

concentration, resulted in the alternating 'aragonite ocean' and 'calcite ocean' (Bots et al. 2011, Müller et al. 2011, Orr 2011, Zeebe & Ridgwell 2011). Over shorter time scales, Rickaby et al. (2010a) found that the glacial Southern Ocean had a higher alkalinity than occurs today, with implications for calcification.

Past environments have also, with the exception of glacial episodes, been warmer than today, probably with a more stratified ocean, shoaling of the thermocline and the associated increase in the mean PAR and UVR incident on photosynthetic organisms, as well as decreased fluxes of phosphate and of combined nitrogen (i.e. nitrogen other than N_2) from the deep ocean to the surface (Rost & Riebesell 2004, Steinacher et al. 2010, Raven et al. 2011, 2012, Zeebe & Ridgwell 2011).

With the increase of CO_2 and temperature over the past 2 centuries since the start of the industrial revolution, and the predicted (inevitable) continuation of these trends, the coccolithophores will return to an approximation of what they have experienced over most of their existence, although the rate of change is probably higher than has generally occurred in the past. There will be some increases in dissolved inorganic carbon (DIC), as a result of dissolution of anthropogenic atmospheric CO_2 and of upwelling of waters in which sedimentary $CaCO_3$ has dissolved following interaction with the increased CO_2 in downwelled water. This latter process will also slightly increase surface water alkalinity and Ca^{2+} (Doney et al. 2009, Orr 2011). Anthropogenic combined nitrogen and sulphur inputs from the atmosphere reduce surface water alkalinity, but this accounts for only a few percent of the increase caused by CO_2 dissolution, although in localised coastal regions the effect may be 10 to 50% (Doney et al. 2007). The increased dissolved CO_2 and H^+, and reduced [CO_3^{2-}] in the oceans will result in the shoaling of the calcite saturation horizon over the coming centuries (Caldeira & Wickett 2003, Orr et al. 2005, Fabry et al. 2008). This may result in reduced calcification (see Merico et al. 2006) and increased dissolution of biogenic calcite. This would decrease the ballasting of organic particles and the carbonate transfer to deeper waters, resulting in a reduction of the CO_2 sink. Simultaneously, this outcome would decrease the CO_2 source in surface waters, owing to reduced calcification (Eq. 1) and increased dissolution (the reverse of Eq. 1).

Warming is increasing stratification with shoaling of the thermocline, so increasing mean fluxes of PAR and UVR, and decreasing fluxes of nutrients (phosphorus and combined nitrogen) to the upper mixed layer, resulting in decreased primary productivity (Steinacher et al. 2010, Boyd 2011, Joint et al. 2011, Raven et al. 2011, 2012). Further influences on nutrient availability come from anthropogenic inputs of atmospheric combined nitrogen (Doney et al. 2007), decreased nitrification (Beman et al. 2011) and iron availability (Shi et al. 2010) in an acidified surface ocean, as well as subsurface deoxygenation which increases denitrification (Oschlies et al. 2008, Keeling et al. 2010, Boyd 2011). Overall, the predicted future scenario is an increase in the low-productivity mid-ocean regions due to reduced nutrient fluxes caused by stratification (Behrenfeld et al. 2006, Cermeño et al. 2008, Doney et al. 2009, Steinacher et al. 2010, Tyrrell 2011). These highly stratified surface waters with high PAR and UVR are the very regions where coccolithophores are dominant due to their tolerance of strong light and high affinity for nutrients (Paasche 2001). However, the simultaneous increase in CO_2 concentrations and decrease in Ω_{cal} may have adverse effects on their ability to calcify and potentially increase dissolution of liths.

The experiments on coccolithophores were too short-term and were otherwise inappropriately designed to address evolutionary issues in the ways that were used in work on non-calcified microalgae (Collins & Bell 2004, Bell & Collins 2008, Collins & de Meaux 2009, Huertas et al. 2011) until the work of Lohbeck et al. (2012) on 500 generations of freshly isolated clones of *Emiliania huxleyi* which provided evidence of increased evolutionary fitness in higher CO_2 through genetic change in the cultures growing at high CO_2.

In order to compare the data presented in the literature, it is necessary to note that many different parameters pertaining to calcification are reported. In much of the recent literature examining the carbonate system, PIC production rates, or cellular PIC are reported. This is often accompanied by POC production rates and a PIC:POC ratio. This is informative as to the relative photosynthesis and calcification occurring within a cell. In studies with a different focus, coccolith mass, other dimensions or degree of malformation may be analysed. Although not directly comparable, the effect of environmental variables may still be extracted from the data sets.

CALCIFICATION AS A FUNCTION OF THE INORGANIC CARBON SYSTEM

Methodology

Much consideration has been given to the most appropriate methods to use in mimicking the contin-

uing increase in atmospheric, and hence surface ocean, CO_2 (e.g. Dickson & Goyet 1994, Hurd et al. 2009, Schulz et al. 2009, Shi et al. 2009, Riebesell et al. 2010, Gattuso & Hansson 2011, Hoppe et al. 2011, 2012). While the case for common methodology in future experiments is well made in the volume edited by Riebesell et al. (2010), earlier experiments can still be used to draw useful conclusions (see discussion by Shi et al. 2009). To briefly summarise, the most common methods of DIC manipulation are the addition of acid/base to the medium, or bubbling with either a CO_2/air combination or pure CO_2 to equilibrate the medium to the desired pCO_2. The addition of acid/base or bubbling alters the composite parameters of total alkalinity (TA) and DIC, respectively, whilst the other parameter remains constant. However, the effects of the 2 methods on the individual parameters of the carbonate system, i.e. pH, $[CO_2]$, $[CO_3^{2-}]$ and Ω_{cal}, are very similar (Schulz et al. 2009). Gas bubbling has generally been preferred because it more accurately reflects what will occur in the future; however, the mechanical effects of bubbling may adversely affect the study organisms (Shi et al. 2009, Hoppe et al. 2011). Alternative or complementary methods include the pre-equilibration of the medium and then growth of very dilute cultures in a closed system (Hoppe et al. 2011, 2012) or dilution with the CO_2-equilibrated medium (Riebesell et al. 2010). The use of $NaHCO_3$ or Na_2CO_3 followed by HCl is also possible (Schulz et al. 2009). The use of pH buffers has been found to introduce additional problems such as effects on growth (Blanchemain et al. 1994, Hurd et al. 2009) and trace metal speciation (Hurd et al. 2009, Shi et al. 2009). Due to apparently conflicting results of experiments on *Emiliania huxleyi* using different manipulative techniques (Riebesell et al. 2000a, Iglesias-Rodriguez et al. 2008a,b) several investigators set out to test the importance of the technique used on the outcomes for the same strain(s) (Shi et al. 2009, Bach et al. 2011, Hoppe et al. 2011), as well as comparing strains using a single technique (Langer et al. 2009). Hoppe et al. (2011) found no difference in the response of 2 strains of *E. huxleyi*, NZEH (as examined by Iglesias-Rodriguez et al. 2008a) and PLY M219, to closed-system TA or closed-system DIC manipulation. In these experiments, they did not see the large increase in PIC and POC seen by Iglesias-Rodriguez et al. (2008a). They also tested the more usual open system DIC manipulative technique of bubbling and found slightly different results. Shi et al. (2009) also compared the effects of closed TA and open DIC manipulations on strain NZEH, and they reported no significant differences between the treatments apart from a small decrease in growth rate in bubbled cultures which may be due to mechanical effects of bubbling or to real differences in the carbonate chemistry. Presumably all cultures were subject to similar mechanical stresses, so there is some additional reason for the apparent effect of increased pCO_2 when supplied by aeration.

Some measurements require quite a large biomass. As they grow, cells inevitably take up CO_2, so although the target gas may be added, this may not be what is seen in the experimental vessels. This leaves a philosophical question as to whether they are experiencing the pCO_2 that is added or the net pCO_2 that remains after carbon acquisition by the culture. The evidence suggests that manipulation by acid/base addition may mimic the future scenario sufficiently well to provide useful data. It is probably prudent to test this for individual species if dramatic results are seen. A thorough description of the carbonate system parameters is essential.

Strain differences

The alternative explanation proposed for the differences in response seen in *Emiliania huxleyi* cultures is that there are intra-specific responses. As indicated above, Langer et al. (2009) tested 4 different strains of *Emiliania huxleyi* and found different responses for all of them. Hoppe et al. (2011) tested the strain previously examined by Iglesias-Rodriguez et al. (2008a) and Shi et al. (2009) and, as indicated above, found somewhat different results. More experiments of this type are required.

Ω_{cal} and calcification

The intracellular calcification by coccolithophores (Mackinder et al. 2010, 2011) involves the supply of inorganic carbon to the coccolith-forming vesicle involving influx of HCO_3^- at the plasmalemma (Paasche 1964, Buitenhuis et al. 1999; cf. Maberly 1992). There is evidence that calcification can frequently still occur when the external Ca^{2+} and/or CO_3^{2-} are so low as to cause undersaturation of the medium with respect to calcite. This section will use evidence on the effects of Ω_{cal} on calcification from laboratory studies, field observations of modern day coccolithophores and from the sedimentary record.

In *Emiliania huxleyi* (as *Coccolithus huxleyi*), Paasche (1964) found that the calcification rate be-

came 0 at an external [HCO_3^-] of 0, with constant Ca^{2+}. Buitenhuis et al. (1999) found that calcification in *E. huxleyi* strain Ch 24-90 ceased when [HCO_3^-] was decreased to 0.5 mM or lower. Similar results were found for experiments at constant inorganic carbon with variable external Ca^{2+} concentration (Paasche 1964, Herfort et al. 2004, Trimborn et al. 2007, Leonardos et al. 2009, Xu et al. 2011). The intracellular precipitation of calcite by coccolithophores when the bulk medium is undersaturated has parallels in the intracellular deposition of celestite in acantharians (Raven & Knoll 2010) and of silica (opal) by diatoms (Raven & Waite 2004). However, in the case of celestite and silica, the present surface ocean is well below the saturation value for these 2 minerals, and for silica this has been the case since (at least) soon after the appearance of diatoms in the fossil record (Raven & Waite 2004, Raven & Knoll 2010). By contrast, the present surface ocean is supersaturated with respect to calcite, although this is forecast to change due to increases in CO_2 without compensatory parallel increases in ocean surface total inorganic carbon and alkalinity and/or Ca^{2+}, at least over timescales less than the ocean mixing time (Orr 2011, Tyrrell 2011, Zeebe & Ridgwell 2011).

A major recent preoccupation of those working on coccolithophores has, not unexpectedly, been the examination of the effects of increased CO_2 on coccolith formation. The problem of the use of different methodologies in comparing data sets has already been mentioned. Rather than deal in detail with the primary data, we refer mainly to review articles in presenting the main outcomes of the work. The analyses by Doney et al. (2009), Hurd et al. (2009), Ridgwell et al. (2009), Kroeker et al. (2010) and Moolna & Rickaby (2012) relate to work with coccolithophores grown with saturating concentrations of nutrients and at saturating fluxes of PAR. These show that the predominant response is a decreased rate of calcification when cells are grown at CO_2 levels higher than those found today (390 ppm) or at least a decrease in PIC:POC and a corresponding increase in calcification in low CO_2 concentrations such as the 190 ppm or so seen at the last glacial maximum 18 000 yr ago (e.g. Riebesell et al. 2000a,b, Zondervan et al. 2001, Casareto et al. 2009). However, different strains of *Emiliania huxleyi* and *Calcidiscus leptoporus* showed different responses, which are summarised in Table 2. The response patterns include no effect of changing CO_2 in the range examined (Langer et al. 2006, Rickaby et al. 2010b); a decreased calcification rate in both higher and lower CO_2 concentrations than the present values (Langer et al. 2006); an increased calcification rate, but not PIC:POC, with higher CO_2 concentrations (Iglesias-Rodriguez et al. 2008a,b, Riebesell et al. 2008); and both increased photosynthesis and calcification but usually greater photosynthesis leading to reduced PIC:POC (e.g. Rickaby et al. 2010b; see also Iglesias-Rodriguez et al. 2008a). However, a different response was seen by Hoppe et al. (2011) using the same strain of *E. huxleyi*. Reduced growth rates leading to increased PIC and POC were seen by Rickaby et al. (2010b) and Langer et al. (2009). Feng et al. (2009) incubated a natural population from the North Atlantic and saw much more abundant lightly calcified coccolithophores in their combined high temperature and CO_2 treatment.

A meta-analysis of the available laboratory studies by Findlay et al. (2011) suggests that for *Emiliania huxleyi*, the PIC:POC ratio can be predicted by the dissolved CO_2 concentration, TA and phosphate concentration. From a biogeochemical point of view, PIC production and growth rate must be examined together to determine whether there will be an overall increase or decrease in calcite production. This still cannot be directly related to calcite export without knowledge of the dissolution, aggregation and other variables affecting sinking rates of organic material. Bach et al. (2011) examined *E. huxleyi* grown either at constant alkalinity with CO_2 fugacity ranging from 2 to 600 Pa at sea level (20 to 6000 ppm), or at a constant pH (pH 8) with CO_2 fugacity of 4 to 370 Pa (40 to 3700 ppm). The constant alkalinity experiments showed optimal CO_2 fugacities for growth of ~20 Pa (200 ppm), for calcification of ~40 Pa (400 ppm) and for organic carbon production of ~80 Pa (800 ppm). Comparison with the constant-pH approach showed that the growth rates and organic carbon production were closely similar at the low and intermediate CO_2 values. However, at high CO_2, growth rates and organic carbon production were higher at constant pH than when pH decreased, suggesting an inhibitory effect of lower pH or allocation of resources to maintaining pH. pH dependence was also seen for calcification, though it was not clear which carbonate system parameter determined calcification at low CO_2 fugacities. These optima explain to some extent the general pattern of increased POC (optimum 80 Pa), decreased PIC (optimum 400 µatm) and sometimes decreased growth (optimum 20 Pa) seen in many of the studies performed. These optima can only be applied to this strain, and it would be interesting to test whether other strains, particularly CAWP-06, differ in these optimal values.

Table 2. Influence of species, strain and experimental methods on the outcome of experiments investigating the effects of ocean acidification on coccolithophore growth rate (μ), particulate inorganic carbon (PIC) production, particulate organic carbon (POC) production and PIC:POC ratio. ↓(↑): decrease (increase); ↔: no significant effect; ∩(∪): optima (minima). DIC: dissolved inorganic carbon; TA: total alkalinity; na: not applicable

Species/strain	Method	Light (μmol m^{-2} s^{-1})	PO_4^{3-} (μM)	NO_3^- (μM)	Temp. (°C)	pH_T range	μ	POC	PIC	PIC:POC	Mal-formation	CO_2sys	Source
Emiliania huxleyi													
RCC1256	Acid–base	400	6.25	100	17	8.33–7.69	↓	↑	↑	↔	–	DIC; TA	Langer et al. (2009)
RCC1256	CO_2	170	6	100	15	7.88–8.44	↓	↔	↓	↓	–	DIC; pH	Hoppe et al. (2011)
RCC1256	Acid–base	170	6	100	15	7.72–8.32	↓	↔	↓	↓	–	DIC; pH	Hoppe et al. (2011)
NZEH[b]	CO_2	150	6.25	100	19	8.15–7.79	↓	↑	↑	↔	N		Iglesias-Rodriguez et al. (2008a)
NZEH	CO_2	150	6.25	100	20	8.10–7.80	↔	–	–	↔	–	DIC; pH	Shi et al. (2009)
NZEH	Acid–base	150	6.25	100	20	8.10–7.80	↑	↑	↑	↓	–	pH; TA	Shi et al. (2009)
NZEH	CO_2 open	170	6	100	15	7.86–8.51	↔	↔	↓	↓	–	DIC; pH	Hoppe et al. (2011)
NZEH	CO_2 closed	170	6	100	15	7.79–8.14	↔	↔	↓	↓	–	DIC; pH	Hoppe et al. (2011)
NZEH	Acid–base	170	6	100	15	7.73–8.4	↔	↔	↓	↓	–	DIC; pH	Hoppe et al. (2011)
PML B92/11A	Acid–base	150	6.25	100	15	8.45–7.80	↔	↑	↓	↓	Y	DIC; TA	Riebesell et al. (2000a)
PML B92/11A	Acid–base	15, 30, 80	6.25	100	15	8.39–7.81	↔	↑	↓	↓	–	DIC; TA	Zondervan et al. (2002)
Bergen	Acid–base	140	3.6	88	16	8.21–7.60	↓	↑	↓	↓	N	DIC; pH	Müller et al. (2010)
AC481	CO_2	150	1	32	13	8.3–7.60	↓	↔	↔	↔	Y + size↓	pH; TA	De Bodt et al. (2010)
AC481	CO_2	150	1	32	18	8.0–7.50	↔	↔	↔	↔	Y + size↓	pH; TA	De Bodt et al. (2010)
AC472 diploid	CO_2 closed	160	10	160	19	7.80–8.04	↑	↔	↑	↑	Size ↔	pH; TA	Fiorini et al. (2011a)
AC472 haploid	CO_2 closed	160	10	160	19	7.80–8.04	↑	↓	na	na	Size↓	pH; TA	Fiorini et al. (2011a)
RCC1212	Acid–base	400	6.25	100	20	8.33–7.69	↓	↔	↓	↓	–	DIC; TA	Langer et al. (2009)
RCC1238	Acid–base	400	6.25	100	20	8.33–7.69	↑	↓	↔	↔	–	DIC; TA	Langer et al. (2009)
Calcidiscus leptoporus													
AC370 diploid	CO_2 closed	160	10	160	19	7.80–8.04	↑	↔	↔	↔	Size ↔	pH; TA	Fiorini et al. (2011a)
AC370 haploid	CO_2 closed	160	10	160	19	7.80–8.04	↑	↑[a]	–	–	Size↑	pH; TA	Fiorini et al. (2011a)
AC365 diploid	Acid–base closed	350	6.25	100	20	7.86–8.74	↔	↔	∩	∩	Y	TA; DIC	Langer et al. (2006)
Syracosphaera pulchra													
AC418 diploid	CO_2 closed	160	10	160	19	7.80–8.04	↑	↓	↔	↔	Size↓	pH; TA	Fiorini et al. (2011a)
AC418 haploid	CO_2 closed	160	10	160	19	7.80–8.04	↑	↓	–	–	Size↓	pH; TA	Fiorini et al. (2011a)
Gephyrocapsa oceanica													
PC7/1	Acid–base	150	6.25	100	15	8.45–7.80		↑	↓	↓	Y	DIC; TA	Riebesell et al. (2000a)
Pz 3.1	Acid–base	200	6.25	100	18	8.13 DIC altered	↑	∩	↔	∪	N	DIC; TA; pH	Rickaby et al. (2010b)
***Coccolithus pelagicus* (spp. *braarudii*)**													
RCC 1200	Acid–base	140	3.6	88	16	7.6–8.21	↓	↔	↓	↓	Y	DIC; pH	Müller et al. (2010)
4762	Acid–base	200	6.25	100	18	8.13 DIC altered	↓	↑	↑	↔	Y + size↓	DIC; TA; pH	Rickaby et al. (2010b)
RCC 1200	Acid–base closed	350	6.25	100	17	7.81–8.56	↔	↔	↔	↔	N	TA; DIC	Langer et al. (2006)

[a]POC production but not POC content significant; [b]also known as PLY M219

Recent work has shown that experiments over much longer (~150 generations; Müller et al. 2010), or shorter (Barcelos e Ramos et al. 2010) than the normal several days of acclimation before measurements are made do not alter the response. The short-term experiments (Barcelos e Ramos et al. 2010) using net (rather than tracer) changes in calcite showed that changes occur over periods of hours, so all 3 experimental time scales permit changes to the proteome (acclimation). There is no evidence of the effects of carbonate system chemistry on holococcolith-bearing haploid cells. This was examined by Fiorini et al. (2011a) for *Calcidiscus leptoporus* and *Syracosphera pulchra*; however, the PIC of the haploid cells was below the detection limits. This may be of interest to pursue because holococcoliths persisted in the fossil record through variations in atmospheric and ocean chemistry (Medlin et al. 2008).

A recent study (Lohbeck et al. 2012) addressing the potential of coccolithophores to adapt to future CO_2 concentrations showed that the responses described above may be short term. Even within 1 yr, *Emiliania huxleyi* adapted to CO_2 partial pressure of 220 Pa so that PIC production and growth rate at this high level of CO_2 were significantly greater than that of cultures adapted to 40 Pa CO_2 when grown at 220 Pa CO_2. This adaptation also translated into increased PIC production when cells were returned from 220 Pa to 40 Pa CO_2. Cell diameter, growth rate, PIC production and PIC:POC were all reduced but POC cell^{-1} was increased in cells taken from 40 Pa directly into 220 Pa CO_2 conditions. Cells acclimated to 220 Pa were of the same size as those at 40 Pa with slightly reduced PIC cell^{-1}, PIC production and growth rate but increased POC cell^{-1}. This study also neatly demonstrated the emerging dominance of different genotypes selected from a mixed-genotype founding population at the different CO_2 concentrations.

In the modern ocean, Merico et al. (2006) found that *Emiliania huxleyi* blooms in the Bering Sea shelf over 7 yr correlated with high [CO_3^{2-}], with less calcification and production of malformed liths at lower [CO_3^{2-}]. However, Merico et al. (2006) did not claim that a high [CO_3^{2-}] was a critical factor in the success of *E. huxleyi.* Beaufort et al. (2011) found a clear pattern of distribution of differentially calcified species and morphotypes according to the carbonate chemistry. A decrease in coccolith mass, largely due to change in species composition, was strongly correlated with Ω_{cal} and [CO_3^{2-}], with other environmental variables only having a geographically localised influence. In this study, mixed-layer irradiance was not recorded, but was found by Charalampopoulou et al. (2011) to be a major determinant of species composition of the coccolith communities in the North Atlantic. pH or some related variable(s) of the inorganic carbon system was also a determining factor in the study by Charalampopoulou et al. (2011). Effects on community calcification were seen to vary with position on a transect between the North Sea and the Arctic Ocean, largely as a result of differences in calcification at the species level. Despite a generally consistent response of calcification to seawater inorganic carbon chemistry, Beaufort et al. (2011) found a heavily calcified R-like morphotype of *E. huxleyi* in the low-pH Patagonian shelf and Chilean upwelling waters. Another exception to the rule was found by Smith et al. (2012) during winter in the Bay of Biscay. Despite the low $CaCO_3$ saturation state, heavily calcified *E. huxleyi* type A predominated over the less calcified type A cells. However, the pH did not fall below 8.05, and cells were much reduced in number in winter. This seems to point to reduced growth rates, which may account for overcalcification. Hagino et al. (2011) also saw morphotypes associated with water masses with distinct temperature and nutrient conditions.

A recent analysis of calcite mass of dominant coccoliths in the sedimentary record over the last 40 000 yr (Beaufort et al. 2011; see Henderiks & Rickaby 2007, Henderiks & Pagani 2008) showed a clear pattern of decreasing calcification with increasing CO_2 and decreasing carbonate concentration in seawater. Both *Gephyrocapsa* and *Emiliania* showed a ~25% decrease in coccolith mass from the last glacial maximum to near-present in cores from the North Atlantic and South Indian Oceans. However, Iglesias-Rodriguez et al. (2008a) reported a 40% increase in coccolith mass over the past 220 yr, in the 0.65 to 10 µm fraction of a box core from a region of the subpolar North Atlantic with exceptional open-ocean sedimentation rates. This was not related to changes in species composition, although further analysis of the same core suggested that it was not consistent for all species (Halloran et al. 2008). The larger coccolithophores, including *Calcidiscus leptoporus* and *Coccolithus pelagicus*, showed increased lith sizes, but smaller species (*E. huxleyi*, *G. oceanica* and *G. mullerae*) showed more lightly calcified liths, or possibly dissolution of liths during or after sinking (Halloran et al. 2008). This increase in coccolith mass over the recent past was partly supported by Grelaud et al. (2009) in the Santa Barbara Basin (California, USA). They reported a 33% increase in coccolithophore shell carbonate mass for the order Isochrysi-

dales, comprising *E. huxleyi, G. oceanica* and *G. mullerae*, in response to increasing pCO_2 and sea surface temperature between 1917 and 2004. Obviously, these are the smaller species found to have the opposite response by Halloran et al. (2008). The coccolith mass enhancement was found only when the water mass was influenced by the Californian counter current flowing from the south, originating on the Chilean coast, and not when dominated by the south-flowing Californian current. Therefore, this morphotype may be the same as that seen by Beaufort et al. (2011) as an exception to the rule in their data set. The data sets of Beaufort et al. (2011) and Iglesias-Rodriguez et al. (2008a) do not span the same time period, so although they show an opposite response in coccolith mass to increased CO_2 concentrations, this may be due to other environmental variables. It appears that there are species- or strain-specific differences and influences of other variables so that broad generalisations cannot be made from individual studies.

Upwelling regions are characteristically low in pH and act as sources of CO_2 to the atmosphere, so species thriving here will be adapted to these conditions and may respond favourably whilst other species do not. At the level of the organism, modelling by Irie et al. (2010) suggested that an increase in coccolith mass (as reported by several but not all investigators: see above and Fukuda et al. 2011, Krug et al. 2011, Langer 2011, Richier et al. 2011) was, counter-intuitively, the optimal evolutionary response to decreasing ocean CO_3^{2-}. It must be emphasised that this conclusion rests on the untested assumption that increased mass decreases mortality. Where studies provide reports of malformations, or normally formed coccoliths, this is included in Table 2. Although it is normal for there to be some malformed coccoliths even under present-day conditions (Paasche 2001), there is certainly evidence that changes in carbonate chemistry can cause increasing malformation of coccoliths (Riebesell et al. 2000a, Langer et al. 2006, De Bodt et al. 2010, Müller et al. 2010, Rickaby et al. 2010b) but this is not always the case (Iglesias-Rodriguez et al. 2008a, Crawfurd 2010, Müller et al. 2010, Rickaby et al. 2010b). Rickaby et al. (2010b) suggested that there is a change in the interaction between the polysaccharide template and the calcite being laid down, due to a pH change within the coccolith-forming vesicle. Table 2 gives the outcome of experiments on the effect of Ω_{cal} on calcification, PIC, POC and PIC:POC ratio in coccolithophores as a function of strain and experimental method.

Ω_{cal} and dissolution

The calcified parts of coccoliths would be expected to dissolve in seawater undersaturated with calcite, with the rate increasing with the degree of undersaturation. This rate of dissolution might be slowed by the organic layer found around newly exocytosed coccoliths (Iglesias-Rodriguez et al. 2008a, Godoi et al. 2009, Hassenkam et al. 2011). Tyrrell et al. (2008) attributed the absence of coccolithophores from the brackish Baltic Sea to winter dissolution of the coccoliths in water undersaturated with calcite, coupled with low winter rates of metabolism and coccolithogenesis due to undersaturation. Dissolution of the externalised mineral skeleton in seawater undersaturated with respect to the mineral phase does not seem to be a major problem for acantharians (celestite) or diatoms (silica). At least for the diatoms, there is evidence that the organic layer surrounding the silicified frustules can decrease the dissolution rate of silica by 2 orders of magnitude (Natori et al. 2006) so that the first-order rate constant for dissolution (d^{-1}) falls from a value similar to the maximum specific growth rate (d^{-1}) to a value of 0.01× the maximum specific growth rate (Raven & Giordano 2009, Raven 2011a). Milligan et al. (2004) showed a small increase in the rate of dissolution of silica from diatom frustules with increasing CO_2 concentration in seawater; the mechanism of this effect is unknown. A further probable similarity between the dissolution of coccolith calcite and diatom silica is that, presumably, the organic layer restricting dissolution is gradually removed by the activity of heterotrophic microbes. A recent study by Hassenkam et al. (2011) showed that neither fossil nor modern coccoliths dissolved in Ca^{2+}-free artificial seawater at pH 8.2 despite $\Omega_{cal} = 0$, whereas inorganic $CaCO_3$ did dissolve. At pH 7.8 in Ca^{2+}-free artificial seawater, coccoliths dissolved completely. Biogenic calcite is more robust than inorganic calcite; it is thought that the organic coating protecting extant coccolithophores may also protect the liths during diagenesis, resulting in smaller crystals which are less prone to dissolution than inorganic crystals (Hassenkam et al. 2011). In comparing fossil and extant coccoliths, it must be acknowledged that any organic layer around fossil coccoliths might not reflect the original state but is a diagenetic effect. More work is needed on the extent to which organic coating on coccoliths restricts their dissolution.

EFFECTS OF PHOTOSYNTHETICALLY ACTIVE RADIATION ON CALCIFICATION

Calcification is an energy-dependent process (Raven 1980, 2011a, Brownlee et al. 1995a,b, Anning et al. 1996) and so is ultimately dependent on photosynthesis in the obligately photolithotrophic (Paasche 1965, 1966a,b, 2001) coccolithophores. Before considering the effect of PAR on calcification, we explore some aspects of the energetics of coccolith formation and photosynthesis, assuming that there is no close coupling of the carbon assimilation processes in calcification and in photosynthesis. From Falkowski & Raven (2007), the absolute minimum energy cost for the conversion of 1 mol CO_2 to carbohydrate is 8.43 mol of absorbed photons (400–700 nm), assuming that the additional ATP needed in addition to that produced in non-cyclic photophosphorylation comes from cyclic photophosphorylation; see also Tsuji et al. (2009) for the CO_2 assimilation mechanisms in coccolithophores. The implicit assumption is that the CO_2 concentration at the site of fixation by ribulose bisphosphate carboxylase-oxygenase (Rubisco) requires, in the present air-equilibrium surface ocean, a CO_2 concentrating mechanism (CCM). The minimum stoichiometry of a CCM is 1 mol ATP per mol CO_2 which, with cyclic photophosphorylation as the ATP source, with 1.14 mol photons needed to generate 1 mol ATP (Falkowki & Raven 2007), means a total of 8.43 + 1.14 = 9.57 mol photons per mol CO_2. Considerations of reductive assimilation of nitrate and sulphate, and ATP use in nutrient transport, biosynthesis and maintenance (Falkowski & Raven 2007) gives a total cost of photosynthetic growth of at least 15.5 mol photons per mol CO_2.

Turning to the energetics of coccolithogenesis, coccolithophores have the typical inside-negative electrical potential across the plasmalemma. The electrical potential of the cytosol relative to the medium has been estimated for *Emiliania* (as *Coccolithus*) *huxleyi* at −145 ± 8 mV (SD) (calcified strain) and −146 ± 18 mV (uncalcified strain), and for *Hymonomonas carterae* at −92 ± 11 mV, using the lipophilic singly-charged cationic dye 3,3′-di-propylthiocarbocyanine (Sikes & Wilbur 1982). Using the lipophilipic singly-charged cation tetra[^{3}H]phenylphosphonium, Nimer et al. (1992) found a value of −60 mV for *E. huxleyi.* Anning et al. (1996) found rather less negative values for *E. huxleyi* using the cationic fluorescent probe tetramethylrhodamine ethyl ester.

The process(es) maintaining the electrical potential difference across the plasmalemma in coccolithophores are unclear. An active electrogenic mechanism is needed to explain the electrical potential difference in the work of Sikes & Wilbur (1982) where the potential is more negative (–92.3 to –146 mV) than the K^+ diffusion potential (–65.5 to –86.5 mV). While there are reservations about the use of lipophilic cations to measure electrical potential differences across the plasmalemma (Ritchie 1984), it is very unlikely that the cytosol is not electrically negative by tens of mV relative to the medium.

The inside-negative electrical potential at the plasmalemma means that the entry of the Ca^{2+} used in calcite formation is only energized by maintaining the electrical potential difference in the face of positive charge entry which decreases the inside-negative value of the potential difference. However, the accumulation of HCO_3^- (as with the CCM involved in photosynthesis) requires direct or indirect energization (Raven, 1980, 1984, Berry et al. 2002). It is likely that 1 ATP is the minimum energy cost of moving 1 Ca^{2+} and 1 HCO_3^- in, and 1 H^+ out, at the plasmalemma. The argument for HCO_3^- is the same as for photosynthesis in the CCM considered above, although the H^+ flux is in the opposite direction.

Half as much ATP is probably needed for the transport of these 3 ions across the coccolith vesicle membrane. In this case, the directly energized process is likely to be Ca^{2+} entry using the Ca^{2+} ATPase (stoichiometry 2 Ca^{2+} influx and probably 2 H^+ efflux for 1 ATP converted to ADP + P_i using a P-ATPase: Evans et al. 1991, Anning et al. 1996, Araki & González 1998) from the very low free Ca^{2+} concentration in the cytosol. HCO_3^- entry could be driven with a relatively small, 10 to 20 mV electrical potential difference (lumen positive with respect to cytosol) produced by the active Ca^{2+}/H^+ antiport (but see below: Anning et al. 1996). However, with free Ca^{2+} in the cytosol of not more than 0.1 mmol m^{-3}, the 55 kJ mol^{-1} available from the conversion of 1 mol ATP to ADP and P_i could not give a concentration of free Ca^{2+} in the coccolith vesicle of more than about 1 mol m^{-3}, compared to 10.6 mol m^{-3} in seawater. To keep the product of free Ca^{2+} and CO_3^{2-} concentrations above the value equivalent to the saturation of calcite, this would require a relatively high pH and concentrations of inorganic C and of Ca^{2+}. Measurements of the pH in cytosol, coccolith vesicle and chloroplast of *Emiliania huxleyi* and *Coccolithus pelagicus* by Anning et al. (1996) showed that the values increase in that order, with the coccolith vesicle 0.2 units higher than the cytosol, but 0.6 to 0.8 units lower than the chloroplast, and 1.1 to 1.2 units lower than the seawater value of pH 8.3. The higher, even by only by a mean of 0.2 units, value of coccolith vesicle than of cytosol pH

is difficult to reconcile with the involvement of a V-type H^+ ATPase pumping H^+ into the coccolith vesicle (Araki & González 1998, Corstjens et al. 2001, Corstjens & González 2004). It must be borne in mind that the mean coccolith vesicle pH in *C. pelagicus* is relatively higher (7.6–8.3) when cytosol pH is higher than 7.2. It is lower (6.9–7.2), and not significantly different from cytosol pH, when the cytosol pH is lower than 7.2 (Anning et al. 1996). Another data set which does not favour the 'usual' direction of action of the H^+ V-ATPase, i.e. pumping H^+ from the cytosol to the endomembrane lumen, is that of the electrical potential of the coccolith vesicle lumen relative to the cytosol. The mean value is −6.2 mV, lumen negative relative to the cytosol (Anning et al. 1996), with a wide range from −30 mV for the highest coccolith-forming vesicle pH values and +13 mV for the lowest pH in the coccolith-forming vesicle. While a higher coccolith vesicle lumen pH than cytosol pH is expected if the dominant energization of the membrane is by the $2Ca^{2+}$:$2H^+$:1 ATP P-ATPase, this does not explain the inside-negative electrical potential of the coccolith vesicle relative to the cytosol (Anning et al. 1996).

A final twist is that, although the 'normal' direction of action of the H^+ V-ATPase does not agree with the mean values of pH and electrical potential differences across the coccolith vesicle membrane, the expression of the H^+ V-ATPase parallels that of calcification (Corstjens & González 2004). Although Ziegler et al. (2004) referred to 'polarity reversal' in a plasmalemma-located V-type H^+-ATPase in an epithelium in calcification–decalcification during moulting cycles in the terrestrial isopod *Porcellio scaber*, the 'polarity reversal' refers to the side of the epithelium in which the ATPase is expressed, not the direction of active H^+ flux relative to the side of the membrane which interacts with adenine nucleotides. Here deposition of $CaCO_3$ using soluble ions derived from the 'old' mineralised cuticle occurs on the side of the epithelium lacking the V-ATPase; when the deposited $CaCO_3$ is resorbed prior to deposition of the new, larger, mineralised cuticle, the V-ATPase relocates to the side from which $CaCO_3$ is resorbed.

Wieczorek (1992) and Wieczorek et al. (2000) showed how a plasmalemma V-ATPase in the luminal membrane in insect (*Manduca sexta*) midgut can account for alkalization to pH 11 of the gut lumen, with H^+ secretion from the goblet cells, and 2 H^+ taken up into these cells in exchange for 1 K^+, with a 200 mV potential difference (gut lumen positive) and a haemolymph pH of 6.8 (see also Raven 1994). This structurally complex arrangement is not readily envisaged with the coccolith-forming vesicle equivalent to the lumen of the goblet cell connected to the gut lumen, the coccolithophore cytosol equivalent to the goblet cell cytosol and the seawater medium equivalent to the haemolymph. The role, if any, of the endomembrane-located H^+-translocating pyrophosphatase in calcification is unclear.

Some energetic savings could be achieved by the movement of Ca^{2+} from a hypothesised relatively high free concentration just inside the plasmalemma to the coccolith vesicles via other components of the endomembrane system (Berry et al. 2002, Brownlee & Taylor 2004, cf. Corstjens et al. 1998). It seems doubtful that any such flux of Ca^{2+} to the endomembrane lumen is passive through Ca^{2+} channels yet would still allow the free Ca^{2+} in the coccolith-forming vesicle to be adequate to precipitate calcite. If there is still an involvement of the $1Ca^{2+}$:$2H^+$ ATPase, then any energy saving relative to uptake into the coccolith-forming vesicle from a cytosolic free Ca^{2+} of less than 100 µmol per m^3 would require variable stoichiometry of the ATPase. This suggestion could involve problems with HCO_3^- entry to provide a high enough concentration of CO_3^{2-} for calcite precipitation if HCO_3^- entry involves an electrical potential difference generated by the Ca^{2+} ATPase.

Such a mechanism was suggested in part (Berry et al. 2002) because of problems with maintaining a large flux of Ca^{2+} in the cytosol from the plasmalemma to the coccolith-forming vesicle, as a consequence of the low concentration of free and chelated Ca^{2+} in the cytosol (Raven 1980; see also Gussone et al. 2006). However, the endomembrane pathway presents a problem of charge balance if movement of only Ca^{2+} is considered. One solution would be for each Ca^{2+} destined for coccolith production that moves through the endomembrane system to move with a CO_3^{2-}. This would not necessarily involve CO_3^{2-} influx to the endoplasmic reticulum with Ca^{2+}; it could be achieved by entry of 1 Ca^{2+} and 1 HCO_3^- with the efflux of 1 H^+. If Ca^{2+} entry involves the $1Ca^{2+}$:$2H^+$ antiporter ATPase, then the required stoichiometry would involve the parallel entry of 1 H^+ and 1 HCO_3^-, or of 1 CO_2. If the endoplasmic reticulum lumen is, as usual, more acidic than the cytosol (by contrast with the coccolith vesicle, which is more alkaline: see Table 2 of Anning et al. 1996), then the CO_3^{2-} concentration in the lumen at passive equilibrium through a hypothetical CO_3^{2-} channel would be lower than that in the cytosol, although this would be counteracted by any inside-positive electrical potential difference. Another possible charge-balancing mechanism which does not involve the inorganic C fluxes (from just inside the plasmalemma to

the coccolith-forming vesicle) for coccolithogenesis occurring through the endomembrane system is for some other charge-compensating ion fluxes to occur upon entry of Ca^{2+} into the endoplasmic reticulum, with a corresponding reverse flux when the Ca^{2+} is consumed in coccolithogenesis. The circuit would be completed by cation flux through the cytosol from near the coccolith-forming vesicle to the plasmalemma, or anion flux in the opposite direction.

Despite the perceived problems with large Ca^{2+} fluxes through the cytosol, Allemand et al. (2004) suggested that diffusible Ca-binding proteins are involved in the calicoblastic layer outside the aboral endoderm adjacent to the aragonitic skeleton of scleractinian corals. Alternatives, such as the pinocytotic uptake of external Ca^{2+} (and other solutes), or in endomembrane vesicles loaded in the cytosol using a Ca^{2+}-ATPase, with movement across the epithelium in vesicles, have been ruled out by treatments with selective inhibitors (Tambutté et al. 1996). It is likely that Ca^{2+} entry for calcification in foraminifera involves fluid-phase endocytosis (pinocytosis) (Erez 2003, Bentov et al. 2009). Fluid-phase endocytosis could decrease the energy costs of Ca^{2+} transport associated with coccolithogenesis, and overcome problems with Ca^{2+} diffusion through the cytosol by endocytotic transport from the medium to the golgi and hence the endomembrane system (Berry et al. 2002). However, a search for the required fluid-phase endocytosis in coccolithophores did not yield positive results (Berry et al. 2002, Brownlee & Taylor 2004).

There seems to be a minimum energy cost of 1.5 mol ATP per mol $CaCO_3$ deposited. This involves a photon cost of 1.71 photons per $CaCO_3$ with cyclic electron flow generating ATP, i.e. 11% of the cost of photosynthetic growth. If it is assumed that the Mehler-peroxidase reaction is used to supply additional ATP in photosynthesis, and the ATP used in calcification, the photon costs are 24 mol photons and 4.6 mol photons, respectively, so the calcification cost is 19% that of photosynthetic growth. These values are rather lower than the 30% computed by Anning et al. (1996) which do not include all the cost of growth in the cost of photosynthesis.

This analysis suggests that calcification is unlikely to be a major energy sink for excess (to photosynthesis) excitation energy in photosynthesis. Coccolithophores are generally characterised as not being very susceptible to photoinhibition (with the haploid phase of *Emiliania huxleyi* being more sensitive to high PAR fluxes than the diploid phase: Houdan et al. 2005), but low-calcification cells are not generally more susceptible to photoinhibition (Nanninga & Tyrrell 1996, Harris et al. 2005; cf. Juneau & Harrison 2005, van de Poll et al. 2007). Nielsen (1995) had previously shown that high-calcification cells of *E. huxleyi* had higher values of α (the increase in rate of photosynthesis on a chlorophyll *a* basis per increment of incident PAR) than do low-calcification cells at all 3 concentrations of inorganic C tested. Another possible influence of the energy cost of calcification concerns the observed decrease in calcification in *E. huxleyi* as the seawater CO_3^{2-} concentration decreases (and external pH decreases) but HCO_3^- and, even more, CO_2 concentrations increase with increasing atmospheric CO_2 (Raven 2011a). The increased energy cost of calcification could, perhaps, explain the decreased calcification rate. However, as mentioned above, the changes in concentration of these solutes in seawater would not greatly alter the energetic cost unless leakage was increased as downhill energy gradients increase, or the increased energy requirement involved a doubling in the ratio of energy source consumed (e.g. ATP) to calcification substrate (or waste product) transported.

While the calcification rate increases with increasing irradiance from the very low dark rate, there is also an increase in the rate of photosynthesis, although calcification saturates at lower PAR values than does photosynthesis (Paasche 1964, Balch et al. 1992, Zondervan et al. 2002, Zondervan 2007). Even allowing for POC loss by respiration and loss of soluble organic compounds, it would be expected that PIC:POC would increase with decreasing irradiance below that required to saturate growth. As pointed out by Zondervan (2007), this was found in short-term experiments (Paasche 1964, Balch et al. 1992 Nimer & Merrett 1993, Müller et al. 2008). However, in more ecologically relevant growth experiments, this increase in PIC:POC with decreasing PAR only occurs from saturation down to below 30 µmol photons m^{-2} s^{-1} (Zondervan 2007). There is then a decrease in PIC:POC as a result of smaller liths with less calcite per lith and/or fewer coccoliths per unit POC (Zondervan 2007).

The available evidence (Paasche 1965, 1966a,b) is consistent with the light dependence of coccolithogenesis involving the photochemical reactions of photosynthesis more directly than use of the stored carbohydrate or lipid products of photosynthetic CO_2 assimilation. The action spectrum of calcification is similar to that of the action spectrum of photosynthesis and the absorption spectrum of the photosynthetic pigments apart from a greater activity of calcification in the blue region of the spectrum (Paasche 1966b). Calcification is less sensitive to the photosystem II (PSII) inhibitor 3-(p-chlorophenyl)-1,1-dimethyl urea

than is photosynthesis (Paasche 1965). Paasche (1965, 1966b) suggested an involvement of ATP from cyclic photophosphorylation which is energized by photosystem I (PSI) alone. Paasche (1965, 1966b) further suggested that part of the blue light stimulation of calcification is catalytic rather than energetic since light absorbed by carotenoids in the blue part of the PAR is not used significantly to energize PSI. This suggestion needs further investigation. If the higher rate of calcification than of photosynthesis in limiting irradiances of blue wavelengths, as compared to longer wavelengths, is confirmed, it could increase the PIC:POC ratio of coccolithophores living deep in the photic zone in clear oceanic waters where blue wavelengths predominate.

Low PAR may reduce both calcite content of the liths by around 35% (Paasche 1999) and the cell size (van Bleijswijk et al. 1994, Paasche 1999). This is seen especially when the light period is shorter (Paasche 1999), perhaps as a result of lower energy availability with a preferential allocation to processes other than calcification, or interaction between the photoperiod and the G1 phase of the cell cycle, as discussed below.

In conclusion, PAR indirectly affects calcification by regulating the energy supplied by photosynthesis. Protons released during calcification may be used to convert HCO_3^- to CO_2 to supply Rubisco with substrate. However, there is no obligate coupling of calcification and photosynthesis. There are indications that the energy for calcification may be supplied, at least partially, from cyclic phosphorylation involving PSI rather than PSII and/or that calcification may be stimulated by catalytic activity rather than energetic effects of light (Paasche 1966b).

A problem with discussing the relationship of calcification rate to PAR and to ocean acidification is uncertainty about the energy cost of calcification and the extent to which the energy cost increases with ocean acidification (Raven 2011a). The additional energy requirement for intracellular pH regulation is probably not more than 1% or so of respiratory energy output; intracellular pH regulation in an acidophilic *Chlamydomonas* species growing at an external pH of 2 uses less than 7% of the respiratory energy output (Messerli et al. 2005, Raven 2011a).

INTERACTIONS BETWEEN PAR AND OTHER ENVIRONMENTAL FACTORS

Zondervan et al. (2002) grew *Emiliania huxleyi* strain PML B92/11 at a range of CO_2 concentrations from 5 to 34 µmol l^{-1} (280 to 750 ppm) combined with a range of photon flux densities of 15, 30, 80 and 150 µmol m^{-2} s^{-1}. Their PAR values were based on a statement by Tyrrell et al. (1999) that the light attenuation by coccoliths in a bloom would reduce PAR to <35 µmol m^{-2} s^{-1} in the photic zone for >50% of the time, apart from in the top few meters. PIC and POC were both highly light dependent at subsaturating irradiance. An increase in photon flux density (PFD) from 15 to 80 µmol m^{-2} s^{-1} resulted in a 32% increase in PIC $cell^{-1}$ and 56% increase in POC $cell^{-1}$. The specific growth rate was doubled at 150 µmol m^{-2} s^{-1} compared to 15 µmol m^{-2} s^{-1}, but CO_2 concentration was found to have no effect. With increasing CO_2 concentration, PIC $cell^{-1}$ decreased only under saturating light intensity (80–150 µmol m^{-2} s^{-1}), whilst POC $cell^{-1}$ increased at intermediate light intensities (30–80 µmol m^{-2} s^{-1}). When adjusted for growth rate, POC l^{-1} increased at all irradiances with increasing CO_2 concentration, whilst PIC decreased only at the highest PFD giving an overall decrease in PIC:POC. It is important to realise that the decrease in PIC:POC is predominantly caused by increased photosynthesis here; however, when light is saturating, a decrease in calcification is seen as CO_2 concentration rises. For situations where Tyrrell et al.'s (1999) calculation is correct, this may mean that in a bloom situation, PIC is less likely to be affected by CO_2 concentration.

Feng et al. (2008) grew *Emiliania huxleyi* strain CCMP 371 at 2 levels of PAR (50 and 400 µmol m^{-2} s^{-1}) and 2 levels of CO_2 (376 and 750 ppm). PIC:POC at low light was independent of the CO_2 availability, while at high irradiances, PIC:POC was decreased, driven by decreased PIC, relative to low irradiances and was further decreased by high CO_2. This agrees with the findings of Zondervan et al. (2002). Further studies are needed of CO_2–PAR interactions in relation to the energetics of calcification, and the various mechanistic (Anning et al. 1996, Raven 2011a, Ries 2011) and evolutionary (Irie et al. 2010) implications.

INFLUENCE OF ULTRAVIOLET RADIATION ON CALCIFICATION

UVR can reduce phytoplankton productivity and growth by disturbing photosynthesis, nutrient uptake, amino acid synthesis and pigment production as well as damaging DNA (Buma et al. 2000). Due to the dominance of coccolithophores in stratified high-irradiance waters, it has been suggested that coccoliths play a protective role by scattering electromagnetic radiation, including UVR. Gao et al. (2009)

measured the transmission of radiation through naked and coccolith-covered cells and found a 20 to 25% decrease in UVR and 10 to 22% decrease in PAR due to coccoliths. While such experiments are technically very demanding, these findings suggest that coccolithophores may be at an advantage compared to other phytoplankton in this respect (Raven & Waite 2004). However, some results show that *Emiliania huxleyi* is in fact more sensitive to ultraviolet B radiation (UVBR; 280–320 nm) than other phytoplankton species, showing a 50% growth reduction at 150 J m^{-2} d^{-1} whilst the 5 other pelagic species tested, i.e. 3 diatoms and 2 (uncalcified) dinoflagellates, did not show 50% reduction in growth rates until at least 600 J m^{-2} d^{-1} (see Table 1 of Peletier et al. 1996). Incident doses of UVBR in excess of 1000 J m^{-2} d^{-1} are common in temperate waters (Buma et al. 2000 and references therein). With prolonged doses of UVBR, for 3 h d^{-1} for several days, *E. huxleyi* showed greatly reduced growth rates and increases in cell volume at 300 J m^{-2} d^{-1}. At 400 J m^{-2} d^{-1}, growth ceased, very high levels of cyclobutane pyrimidine dimers were evident, and the cell cycle was arrested in the G1 phase. It appeared that cells were unable to repair the DNA damage and so did not enter the S phase. This finding was corroborated by further field and laboratory studies showing increased cell size with UVBR exposure (Buma et al. 2000).

In some shorter-term experiments, Xu et al. (2011) examined the effect of UVR and temperatures of 20 and 25°C on calcification in an Australian *Emiliania huxleyi* strain CS-369, usually grown at 20°C. Cultures were grown for at least 148 generations (100 d) in normal present-day seawater calcium concentration (10 mM), and at low calcium concentration (0.1 mM) to restrict calcification. The treatment involved cultures which presumably had not been exposed to significant UVR since they were isolated from the ocean. The cells were exposed for 2 h to a range of wavelengths from >280 to >395 nm. This work showed that, for the cells in 10 mM calcium, UVBR, especially at 280 to 295 nm, inhibited photosynthesis by around 50% and calcification by around 65%. The resulting decrease in PIC:POC was not seen with ultraviolet A radiation (UVAR). PIC:POC of cells acclimated to 0.1 mM calcium was about a third that of the 10 mM calcium acclimated cells. UVBR inhibited photosynthesis by around 65% and calcification around 50%, but this was more variable so PIC:POC was not significantly different. There was no interactive effect of temperature and UVR. The decreased calcification of normal cells in the presence of UVBR does not support the hypothesis that cells are unable to divide and become more heavily calcified. It is also counter-intuitive, as cells become less calcified and potentially more susceptible to damage by UVBR, assuming the coccoliths play some protective role. As the lightly calcified cells show no further decrease in PIC:POC with UVBR, this may suggest that UVBR is attacking the energy supply for coccolithogenesis rather than the mechanism itself. Increased temperature would amplify this effect, and the combination of all these factors leads to an overall decrease in the calcification:photosynthesis ratio in a future scenario (Xu et al. 2011). Interesting though these results are, longer-term experiments using less extreme UVR fluxes would be more ecologically and evolutionarily significant.

Gao et al. (2009) found decreased rates of photosynthesis and calcification in response to UVAR and UVBR in combination with reduced pH in *Emiliania huxleyi* strain CS369. Cells cultured for 11 d at pH 7.6 were on average 7% smaller and the mean thickness of the coccolith layer was reduced by 31% compared to controls at pH 8.2; however, this was not significant. Exposure to UVBR almost totally inhibited calcification whilst reducing photosynthesis by ~10% in the lightly calcified cells at pH 7.6.

Although the experiments described above were conducted with the same strain and by the same research team, the controls showed much greater inhibition by both UVAR and UVBR, particularly of photosynthesis, in the study by Xu et al. (2011). This may be due to the PAR supplied being 150 µmol photons m^{-2} s^{-1} and then PAR of 290 µmol photons m^{-2} s^{-1} being used in the experiment (Xu et al. 2011) whilst cells of Gao et al. (2009) were acclimated to 425 µmol photons m^{-2} s^{-1}. With more available PAR, cells may be more able to repair damage to cellular machinery (Raven 2011b, 2012). However, the trend towards greater inhibition of photosynthesis than calcification in the low-calcium treatment and the opposite trend at low pH suggests that different mechanisms are at work. That energy is still being supplied to calcification at low calcium concentration in the presence of UVB but not at low pH may be to avoid the additional pH imbalance caused by calcification (Gao et al. 2009).

Guan & Gao (2010a,b) found that the shorter UVBR wavelengths damaged photosynthetic machinery and the longer UVAR damaged calcification machinery more in the same strain of *Emiliania huxleyi*. Damage was repaired and the overall specific growth rate was reduced by 25%, resulting in increased size and coccoliths per cell. After prolonged exposure to UVR, cells were more able to repair damage, had higher concentrations of UVR-absorbing compounds

and were increasingly calcified. These were interpreted as protective strategies in response to UVR. Gao et al. (2009) found reduced growth by 12% at pH 7.9 with UVBR. Guan & Gao (2010a) saw 52% and additional 10% reduction in POC fixation, with UVAR and UVA+BR respectively. PIC fixation was inhibited by 68 and 8% with UVA and UVA+BR respectively; these results were similar to those of Xu et al. (2011) but again much higher than those reported by Gao et al. (2009). In all of these studies, cells grown under normal conditions showed calcification to be more inhibited than photosynthesis by UVA and UVBR. Both UVA and UVB inhibited both photosynthesis and calcification, with UVA having had a greater effect than UVBR. Guan & Gao (2010b) suggested that the inhibition of photosynthesis may be caused by damage to the D1 protein of PSII. Growth rate reduction may be caused by damage or by allocation of resources to photoprotective compounds (Raven 1991, Garcia-Pichel 1994).

In summary, UVAR and UVBR reduce calcification and photosynthetic carbon fixation, increase photoprotective pigments but may reduce growth rates sufficiently to result in more heavily calcified cells. When combined with a stressor which reduces calcification, such as pH or low calcium concentration, different responses are seen. However, coccolithophores in nature are unlikely to experience low [Ca^{2+}], and pH as low as 7.6 is unlikely in the near future.

Holococcoliths have been much less studied; an exception is Quintero Torres et al. (2007), who modelled the probable scattering of radiation by their structure. They found that this would cause greater scattering of radiation in the 200 to 300 and 700 to 900 nm ranges. Thus holococcoliths could protect cells from UVR damage without attenuating light in the 400 to 700 nm range.

EFFECTS OF TEMPERATURE ON CALCIFICATION

Reduced temperature leads to increased dissolution of CO_2 in seawater, reduced carbonate and thus reduced Ω_{cal}. Temperature affects metabolic rates of both the phytoplankton and heterotrophic bacteria, giving optimal growth and dissolution rates, respectively.

Temperature alone

Early experiments on *Emiliania huxleyi* (as *Coccolithus huxleyi*) strain BT-6 (Watabe & Wilbur 1966) showed temperature effects on coccolith morphology, with increased malformations above and below the optimal temperature of 18°C. Growth rates were maximal at 18 to 24°C for this strain. Watabe & Wilbur (1966) found very low percentages of calcified cells when these cultures were grown at 7 or 27°C, the limits of their temperature range. Increases in width and decreases in length of coccolith elements were seen with increasing temperature between 12 and 27°C. The authors suggested that malformations were due to growth of crystals at different rates causing asymmetry and also suggested that local differences in calcium carbonate and inhibitory substances would also have the same effect. This work was followed up, again with *E. huxleyi*, by Paasche (1968) using a different technique. Maximum growth occurred at 17.5 to 26.5°C; at 12.5 and 26.5°C, growth was greatly decreased, but calcification (on a cell volume basis) was still 70 to 80% of the value at maximum growth rate. The cells had a complete covering of coccoliths at 12.5 to 23°C, but 30% of cells had an incomplete covering at 26.5°C. Paasche (1968) did not mention malformed coccoliths, although Langer et al. (2009) saw increased numbers of malformed coccoliths in *E. huxleyi* RCC1238 at 25°C compared to those grown at 10 to 20°C.

Interactions of temperature with other variables

The effect of the interaction of temperature and pCO_2 on calcification has been investigated by a number of authors (Feng et al. 2008, 2009, De Bodt et al. 2010, Borchard et al. 2011, Fiorini et al. 2011b, Xu et al. 2011). Xu et al. (2011) found that elevated temperature, within the range studied, increased photosynthesis and calcification of *Emiliania huxleyi* strain CS-369 at present-day seawater [Ca^{2+}], but reduced both if [Ca^{2+}] was reduced to 0.1 mM. Increased photosynthetic rate (but not POC) and growth rate of *E. huxleyi* strain CCMP 371 was also seen by Feng et al. (2008) under a higher temperature. Unlike Xu et al. (2011), Feng et al. (2008) found no effect of temperature (20 versus 24°C) on PIC, POC or PIC:POC at any of the light and CO_2 combinations used for their *E. huxleyi* CCMP 371 cultures. De Bodt et al. (2010) found a trend towards decreased PIC production with increasing CO_2 concentration (180, 375, 750 µatm) at both 13 and 18°C in *E. huxleyi* strain AC481. PIC:POC ratio decreased with increased growth temperature only at present-day CO_2 concentration; this was driven by greatly increased POC and slightly reduced PIC. At 18°C, both PIC and POC were

higher at present-day rather than future CO_2 concentrations. POC increased with increased CO_2 concentration only at 13°C between present and future treatments. There was also a reduction in cell size, but no difference in growth rate, with both increasing CO_2 concentration and increasing temperature. An increased number of malformed coccoliths was seen with increasing CO_2 concentration, but no effect of temperature.

Feng et al. (2009) examined the interactive effects of increased temperature and CO_2 concentration on a natural community from the North Atlantic, using ship-board continuous cultures. Addition of nitrate and phosphate but no silicic acid to the natural seawater caused a coccolithophore bloom. Increased temperature stimulated POC production rates per unit chlorophyll, with no difference caused by CO_2 concentration. Neither high temperature nor CO_2 concentration alone affected PIC, but despite a much higher abundance of coccolithophores in the high CO_2 concentration and temperature treatment, overall PIC was significantly reduced.

Fiorini et al. (2011b) grew *Syracosphaera pulchra* at 19 and 22°C and with 400 and 740 ppm CO_2; no significant differences in the PIC:POC ratio were found among the treatments. These experiments used realistic temperature and CO_2 values for today and later this century, and generally showed no effect of increased temperature on PIC:POC, or a decrease with increasing temperature in present, but not future, CO_2. This suggests that any alteration in [CO_3^{2-}] due to temperature does not alter net calcification.

Satoh et al. (2009) examined the interaction of HPO_4^{2-} limitation and low temperature on growth and calcification (as ^{45}Ca incorporation and microscopic observation) in batch cultures of *Emiliania huxleyi* NIES 837. They showed that temperature reduction from 20 to 12°C caused a much greater increase in calcification in HPO_4^{2-}-limited cultures than in HPO_4^{2-}-sufficient cultures. The different responses may be due to strain and species differences and/or differences in the sensitivities of calcification and photosynthesis to temperature (Xu. et al. 2011).

A possible mechanistic interpretation for the differential effects of temperature on the calcification and growth, at least below the optimal temperature for growth, is a lower activation energy for calcification than for growth. This 'explanation' could, of course, be regarded as a restatement of the observations in terms of physical chemistry. The relationship of calcification to the length of the G1 phase (Paasche 1998, Müller et al. 2008) of the cell cycle could relate to the temperature effects by the hypothesis that the fraction of the cell cycle time taken up by the G1 phase decreases up to the temperature optimum for growth, and decreases at higher temperatures (but see de Bodt et al. 2010). These suggestions could be followed up experimentally.

EFFECTS OF THE MACRONUTRIENTS NO_3^- AND PO_4^{3-} ON CALCIFICATION

Paasche & Bruback (1994) and Paasche (1998) were the first to investigate the effects of variations in nitrogen (as nitrate) and phosphorus (as phosphate) supply on calcification in a coccolithophore, in this case *Emiliania* (as *Coccolithus*) *huxleyi*; these, and later, experiments were reviewed by Zondervan (2007), while Langer et al. (2012) discussed more recent publications as well as providing original data. The general finding is an increasing PIC:POC ratio with decreasing NO_3^- and HPO_4^{2-} in the range which restricts growth rate (as POC increases). The increase in PIC:POC is often greater for decreasing HPO_4^{2-} than for decreasing NO_3^- (Zondervan 2007). Paasche (1998) noted that NO_3^- limitation reduces POC per cell, calcite per coccolith and coccolith size, but increases the number of coccoliths per cell, resulting in higher Ca:POC (on a cell basis) at reduced growth rates. However, Fritz (1999) found no change in coccolith size with a 3.3-fold change in growth rate of *E. huxleyi* 88E (CCMP 378) in NO_3^--limited chemostats at high irradiance. In that study, increased calcite per lith was seen as growth became more NO_3^--limited. Müller et al. (2008) found greatly decreased cell diameter with moderately increased calcite per cell when growth became NO_3^--limited. Coccolith calcite content was found to increase by 15% in PO_4^{3-}-limited chemostat cultures, whilst decreasing by 20% with a similar NO_3^--limitation (Paasche 1998).

Riegman et al. (2000) showed that *Emiliania huxleyi* has the highest affinity for PO_4^{3-} ever recorded in a phytoplankton species. At a specific growth rate of 0.14 d^{-1} (16% μ_{max}), the affinity of the PO_4^{3-} uptake system (defined as the initial slope of the plot of the rate of PO_4^{3-} uptake on a cell phosphorus basis against the external P concentration) was 19.8 l μmol^{-1} cell PO_4^{3-} h^{-1}. They found that NO_3^--limited cells were smaller and contained 50% less organic and inorganic carbon than PO_4^{3-}-limited cells. Both calcification and induction of the PO_4^{3-} uptake system were inversely correlated with growth rate in PO_4^{3-}-limited cultures. At the lowest growth rate (0.13 d^{-1}), the cells were 37% larger than in faster-growing cul-

tures and had more than 3 times greater PIC cell^{-1} due to increased lith coverage. Under NO_3^- limitation, no correlation of PIC or POC cell^{-1} was seen with variation in growth rate.

When PO_4^{3-}-limited, cells can continue to produce biomass and calcite but are unable to divide due to lack of PO_4^{3-} for nucleic acid synthesis. When NO_3^--limited, they cannot synthesise proteins, but calcification does continue and also results in higher calcite per cell. The cells are smaller due to reduced biomass, not calcite. Overall, nutrient limitation increases the PIC:POC ratio.

INTERACTIONS BETWEEN MACRONUTRIENTS AND OTHER VARIABLES

NO_3^--limited chemostat cultures of *Emiliania huxleyi* strain TW1 showed no change in PIC:POC ratio when grown at 700 rather than 400 ppm CO_2 (Sciandra et al. 2003); no data are given for (non-chemostat) NO_3^--replete cultures of the strain used. As indicated above when considering PAR, Müller et al. (2008) confirmed the speculation of Paasche (2001) that calcification is restricted to the G1 phase of the cell cycle, and showed in *E. huxleyi* that the length of the G1 phase increased under NO_3^- and PO_4^{3-} limitation and may be related to the increased calcite cell^{-1} in the nutrient-, particularly PO_4^{3-}-, limited cultures. Lefebvre et al. (2012) studied the interaction of CO_2 (166 to 194 ppm compared to 308 to 367 ppm) with nitrogen source (NH_4^+ plus NO_3^- compared to NO_3^- alone, both treatments with 200 µM nitrogen) in *E. huxleyi* strain CCMP371, and found that PIC:POC decreased with increasing CO_2 with NO_3^- as the nitrogen source while PIC:POC was lower and invariant with CO_2 when NH_4^+ plus NO_3^- was the nitrogen source. Lefebvre et al. (2012) pointed out that environmental change is increasing the availability of NH_4^+ relative to NO_3^-, with increasing cyanobacterial nitrogen fixation in the surface ocean and inhibition of nitrification by increased CO_2, so the results of their work have implications for future PIC:POC of coccolithophores. The nitrogen source for growth alters the Fe requirement: diazotrophy needs more Fe per unit nitrogen assimilation rate than NO_3^- or, particularly, NH_4^+ assimilation (Kustka et al. 2003). How the analysis of Lefebvre et al. (2012) is altered by consideration of the effects of increasing CO_2 on Fe availability awaits resolution of conflicting evidence as to the effects of ocean acidification on Fe availability (Millero et al. 2009, Breitbarth et al. 2010, Shi et al. 2010). Also, with high light and low PO_4^{3-} (i.e. usual bloom conditions for the diazotrophic cyanobacterium *Trichodesmium* and for many coccolithophores), *Trichodesmium* precipitates $CaCO_3$ as fibres of aragonite (Kranz et al. 2010). Marine pelagic cyanobacterial calcification is of relatively little quantitative importance in the present oceans. In the past, however, very large carbonate sediments have been produced by filamentous marine cyanobacteria on different occasions between 750 and about 50 million years ago (Riding 2006). Work on the coccolithophore *E. huxleyi* in phosphorus-limited chemostats investigated interactive effects of changes in CO_2, temperature and phosphorus: there were no significant trends with variation in the 3 factors (Borchard et al. 2011). The interaction between macronutrient supply and other factors is complex and needs further investigation.

EFFECTS OF MICRONUTRIENTS ON CALCIFICATION

Zondervan (2007) reviewed the limited information available up to 2007 on the effects of micronutrient availability on calcification of coccolithophores. Variations in Fe concentration in the medium which yielded a 6-fold range of growth rates had no significant effect on PIC:particulate organic nitrogen (PON), i.e. accumulation of PIC decreased in parallel with decreasing PON as Fe became more growth-limiting (Schulz et al. 2004, 2007). What will happen to Fe availability under increasing CO_2 is not clear. Millero et al. (2009) modelled Fe speciation under increased CO_2 and showed an increased fraction of Fe(II) and a slower oxidation of Fe(II) to Fe(III). Breitbarth et al. (2010) showed increased soluble Fe concentrations, Fe(II) concentration and Fe(II) half-life in a coastal mesocosm experiment with CO_2 enrichment. By contrast, Shi et al. (2010) found a decrease in the Fe uptake rate under increased CO_2 in the coccolithophore and 2 diatoms examined, although the cellular Fe requirement for growth is not changed with ocean acidification.

Limitation of growth rate by decreased Zn concentration led to a PIC:PON increase by over 2-fold (Schulz et al. 2004). There was no change in the rate of calcification, and cells with many layers of coccoliths were seen. Müller et al. (2008) pointed out that Zn is necessary for Zn finger proteins which play a central role in DNA replication and transcription, hence Zn deficiency may inhibit cell division. In the North Pacific, coccolithophore growth is limited by Zn (Crawford et al. 2003). On addition of Zn to natu-

ral samples, coccolithophore abundance increased 20-fold with a significant increase in total ^{14}C uptake into PIC. This suggests that although Zn limitation may cause increased cellular calcification, with the reduction in growth rate this does not translate to increased total PIC production. Zn concentrations similar to those in the study area of Crawford et al. (2003) are common in many oceanic regions (see Schulz et al. 2004). Increased cellular, but not total calcification, may also apply to phosphate limitation and UVB cell cycle arrest. Zn is also required for alkaline phosphatase needed to acquire phosphate from organic phosphate esters when phosphate is limiting, and for carbonic anhydrase required for carbon acquisition (Steele et al. 2009), noting that *Emiliania huxleyi* has low activity of extracellular carbonic anhydrase (Nimer et al. 1994). Buitenhuis et al. (2003) showed a co-limitation of growth of *E. huxleyi* by Zn and HCO_3^-, but the PIC:POC ratio was not addressed. Schulz et al. (2004) found that the effects of variation in the carbonate system parameters over a range of pH from 7.75 to 8.35 were not discernible due to the massive Zn response and variation amongst the CO_2 treatment results. These co-limitations will require further investigation.

EFFECTS OF CALCIUM, MAGNESIUM AND SULPHATE ON CALCIFICATION

Lower than present-day seawater [Ca^{2+}] (10 mM) have been used experimentally to decrease, or eliminate, calcification (Paasche 1964, Herfort et al. 2004, Trimborn et al. 2007, Leonardos et al. 2009, Xu et al. 2011). Xu et al. (2011) found that acclimation to 0.1 mM compared to 10 mM [Ca^{2+}] (present-day concentrations) reduced photosynthesis by 81.3% and calcification by 55.4% at 20°C. However, no effect on photosynthesis was suggested by the work of Herfort et al. (2002, 2004), Trimborn et al. (2007) and Leonardos et al. (2009). Trimborn et al. (2007) found only naked cells at 0.1 mM [Ca^{2+}].

Experiments with [Ca^{2+}] higher than present-day seawater concentrations (up to 50 mM) and varying Mg^{2+}, and hence Ca:Mg ratios (Herfort et al. 2004, Stanley et al. 2005, Katagiri et al. 2010, Müller et al. 2011), are relevant to understanding the effects on calcification of the changes in ocean chemistry over the last 220 million years of the fossil record of coccolithophores (Zeebe & Ridgwell 2011). Doubling the Ca^{2+} concentration from the present seawater concentration of 10 mM has no significant effects on the PIC:POC ratio, but 50 mM Ca^{2+} decreased the PIC:POC ratio and the rate of POC accumulation in *Emiliania huxleyi* (Herfort et al. 2004). High Mg^{2+} (87, 116 mM) and low Mg^{2+} (0, 14 mM) both caused malformation of coccoliths relative to Mg^{2+} at 29 and 58 mM (the present-day concentration); the extent of calcification was inhibited less by low than by high Mg^{2+} concentrations (Herfort et al. 2004). Stanley et al. (2005) examined the effect of Ca^{2+} (20–30 mM) and Mg^{2+} (≤20–30 mM) concentrations believed to have occurred in seawater on *Coccolithus neohelis*, *Ochrosphaera neopolitana* and *Pleurochrysis carterae*, with higher growth rates in the Cretaceous than in the modern seawater. In the only organism tested (*P. carterae*) calcite production was higher in Cretaceous than recent seawater, apparently giving more calcite per cell in the Cretaceous seawater (see Fig. 2 of Stanley et al. 2005). Calcification was not quantified, although Katagiri et al. (2010) examined the effects on calcification in *P. haptonemofera* of calcium in the concentration range of 0, 0.5, 5, 10 and 50 mM and Mg^{2+} at concentrations of 5, 50 and 140 mM. Calcification (measured as Ca^{2+} and Mg^{2+}) on a per cell basis was highest at 10 mM external Ca^{2+} when Mg^{2+} was constant at 50 mM, and at 50 mM Mg^{2+} when Ca^{2+} was varied (Katagiri et al. 2010). Müller et al. (2011) found no significant effect on PIC:POC in *E. huxleyi* of variations in Mg^{2+} from 5.5 to 92 mM with present Ca^{2+} (9.6 to 9.9 mM); for present or half the present Mg^{2+} and 2.6 to 51 mM Ca^{2+}, PIC:POC is essentially constant except for a decrease at the lowest Ca^{2+} concentrations. For *C. braarudii* with present or half the present concentrations of Mg^{2+} and 2.6 to 46.8 mM Ca^{2+}, PIC:POC decreases at the lowest Ca^{2+} concentration (Müller et al. 2011).

Herfort et al. (2004) and Katagiri et al. (2010) both showed that PIC:POC is greatest at [Ca^{2+}] and [Mg^{2+}] similar to present ocean concentrations at the present inorganic carbon concentration, which was the only one examined. Müller et al. (2011) found essentially constant PIC:POC with varying Ca^{2+} and Mg^{2+}, apart from a decrease at the lowest [Ca^{2+}]. With a rather different experimental design (constant divalent cation concentration with varying Ca:Mg ratio), Stanley et al. (2005) found that calcification was greatest with the Ca:Mg ratio of 1 found in the Cretaceous (see Stanley 2008). Further experimentation is needed to resolve these differences among the experiments. It is also desirable to examine the interaction between Ca:Mg and the absolute [Ca^{2+}] and increased CO_2. The high [Ca^{2+}] in the Cretaceous would partly offset the effect of the higher CO_2 (and lower carbonate) in decreasing the saturation state of calcite (see Fig. 1 of Stanley 2008, and Fig. 2.3 of Zeebe & Ridgwell 2011);

this could maintain the rate of calcification and prevent calcite dissolution.

A final aspect of the effect of $[Ca^{2+}]$ and $[Mg^{2+}]$ on calcification is the effect of $[SO_4^{2-}]$. Increased $[SO_4^{2-}]$ decreases the Mg:Ca ratio at which calcite is destabilised and aragonite becomes the commonest polymorph (Bots et al. 2011). Ocean $[SO_4^{2-}]$ has doubled over the last 65 million years (Kurtz et al. 2003), so variation in $[SO_4^{2-}]$ has been an important factor in marine calcification in the time for which coccolithophores have existed. However, the intracellular calcification by coccolithophores permits the organism to control the $[Ca^{2+}]$, $[Mg^{2+}]$ and $[SO_4^{2-}]$ in the coccolith-forming vesicle to at least some degree independently of the external concentrations. More widely, ocean $[SO_4^{2-}]$ changes could have been related to the evolutionary expansion of the alveolates and chromists, the latter containing the coccolithophores (Ratti et al. 2011).

The arguments of Ratti et al. (2011) are based on culture experiments in which 5 marine phytoplankton organisms, namely the cyanobacterium *Synechococcus* sp., a green alga (the prasinophycean *Tetraselmis suesica*), and 3 algae containing chlorophylls *a* + *c* derived from secondary endosymbiosis in which the plastids arose from a red algal endosymbiont, viz. the coccolithophorid *Emiliania huxleyi*, the dinoflagellate *Protoceratium reticulatom* and the diatom *Thalassiosira weissflogii*, were cultured with SO_4^{2-}, a nitrogen source and trace element concentrations reflecting modern seawater, Palaeozoic and Proterozoic seawater. In monospecific culture, all 5 species grew fastest in the Proterozoic-like seawater. In mixed cultures, *Thalassiosira weissflogii* outgrew the others in modern seawater, while *Tetraselmis suecica* outgrew the others in Palaeozoic seawater. These data are interpreted as suggesting that increases over time in the $[SO_4^{2-}]$ in seawater (Ratti et al. 2011, Halevy et al. 2012, Wortmann & Paytan 2012) could have been a factor in the rise of chlorophyll *a* + *c* phytoplankton relative to green algae and cyanobacteria in the Mesozoic (Ratti et al. 2011).

While the reported effects of changes in Ca^{2+}, Mg^{2+} and SO_4^{2-} on calcification and the PIC:POC ratio have relevance for the palaeoecology of coccolithophores, there will be no significant changes in these 3 ions in the ocean over the next few centuries.

EFFECTS OF SALINITY ON CALCIFICATION

Increased salinity increases Ω_{cal}, as does increased temperature (Green et al. 1998, Marion et al. 2009). These changes in Ω_{cal} are complicated, in terms of $CaCO_3$ precipitation, in the ocean by the general correlation of salinity with carbonate alkalinity, and the temperature and salinity effects on the speciation of inorganic carbon and the solubility of CO_2. These interactions lead, for example, to effects of low temperatures such as in winter (Tyrrell et al. 2008) and with ice melt input (Chierici & Fransson 2009). Coccolithophores as exemplified by *Emiliania huxleyi* are restricted to natural waters with salinity above 11 (Winter & Siesser 1994, Tyrrell et al. 2008). Beaufort et al. (2011) saw no strong correlation between calcification and salinity. After considering the various possible reasons for the absence of coccolithophores from the brackish Baltic Sea but abundance in the brackish Black Sea, Tyrrell et al. (2008) concluded that the most likely cause is not low salinity per se, but rather the decalcification of coccolithophores in the winter when calcite is close to being, or often is, undersaturated. This dissolution of coccoliths cannot be countered by replacement in a time of little or no growth. Chierici & Fransson (2009) are among those who have commented on the undersaturation with $CaCO_3$ of coastal arctic waters as a result of freshwater input from ice melt and temperature. Bollmann et al. (2009) noted that coccolith morphology changed with varied salinity.

INTERPRETING EARLIER CALCIFICATION BY COCCOLITHOPHORES

Over much of the 220 million years for which coccolithophores are known to have existed, the CO_2 concentration has been higher than at present, especially in the Pliocene and Pleistocene. At that time, the high CO_2 concentration with corresponding lower carbonate concentration was accompanied by a higher $[Ca^{2+}]$ and Ca:Mg ratio. The ocean surface calcite saturation value was apparently lower back to 220 million years ago than it was in the Pleistocene, but not so much lower as would have been the case had the $[Ca^{2+}]$ not been higher. With a warmer, more stratified ocean with shoaling of the thermocline there would have been a decreased input of nutrients (combined N, P, Zn) to the upper mixed layer; this may have increased the PIC:POC ratio, but probably reduced cell growth rates. Increased mean UVBR incident on the cells with less deep mixing may have had similar effects. However, the increased PAR incident on cells with less deep mixing would decrease the PIC:POC ratio. Overall, the sum of these effects could have helped explain the continuity of coccoliths in the fossil record up to the Miocene, with lower

CO_2 taking over in the Pleistocene and Pliocene outweighing the influence of a general increase in mixing depth.

PREDICTING CALCIFICATION BY COCCOLITHOPHORES IN THE NEXT CENTURY

Much emphasis has been placed on the effects of the continuing increase in CO_2 in decreasing the saturation state of calcite, and the related general decrease in PIC:POC in coccolithophores. As discussed above, increased stratification in a warmer ocean, with associated shoaling of the thermocline, will decrease the input of nutrients to the upper mixed layer, increasing the PIC:POC ratio in individual cells but reducing total PIC production if there is a more than compensating decrease in POC production rate per unit area. Increased mean UVBR incident on the cells with less deep mixing may also reduce growth rates but increase PIC:POC. However, the increased mean PAR incident on cells with less deep mixing would decrease the PIC:POC ratio, assuming that the initial mean PAR with deep mixing was high enough to give a decrease in PIC:POC with increased PAR. Overall, it is very likely that calcification will decrease in the future. However, with increased stratification, larger areas of the ocean may become dominated by coccolithophores. At increased temperature and irradiance, growth rates may increase, so, although PIC:POC per cell may be reduced, cell numbers may increase provided there are enough nutrients.

However, it is very likely that shoaling of the thermocline will mean decreased productivity as a result of nutrient limitation (Steinacher et al. 2010). If there is nutrient limitation, particularly if PO_4^{3-} limits cell growth, then these cells may become more heavily calcified, increasing PIC:POC but reducing growth rates. Increased UVR exposure in stratified waters could also potentially reduce growth rates and increase calcification. There are still many uncertainties and seemingly contradictory results despite the intense research effort targeting the responses of coccolithophores to environmental change. It is clear that changes in the carbonate chemistry, pH and PAR associated with environmental change will affect phytoplankton calcification. The balance between the effects on individual cells, population growth rates and species representation in the community will determine the global effects on PIC production from calcification by coccolithophores in the future, assuming no genetic changes (summarised in Table 3). Experimental evolution studies on coccolithophores are now in progress, with Lohbeck et al. (2012) having grown 500 generations of *Emiliania huxleyi* in high or ambient CO_2 concentrations and shown adaptive evolution to high CO_2. These data may alter predictions based solely on acclimatory changes in response to environmental changes.

MECHANISTIC UNDERSTANDING OF ENVIRONMENTAL EFFECTS ON CALCIFICATION

From the research presented it can be seen that very distinct mechanisms are at play (Table 3). There is evidence that factors affecting growth rates, and particularly those which halt cell division, seem to result in continued calcite production. Overall, this results in heavily calcified cells, both in terms of lith number and Ca^{2+} content. It has long been known that calcification is highly dependent on PAR, and to our knowledge, this is solely due to the energy supplied through photosynthesis. Direct effects on the calcification process are either related to Ω_{cal} or temperature. When these factors are outside the optimal range, they may cause malformation of liths. Coccolith production depends upon the physical process of crystal growth regulated by the cell and based on the organic template. Extreme changes in the physical environment are liable to disrupt crystal formation. The coccolith vesicle allows for a highly regulated environment, but temperature would be outside the control of the cell, and extreme ionic changes may be beyond its capabilities to control. Changes in cellular calcite production expressed as PIC $cell^{-1}$ may also reflect a range of different responses. Reduced PIC may occur if there are fewer liths, they are smaller or thinner, or incompletely formed (Zondervan et al. 2002).

CONCLUSIONS

Predicted future environmental changes are increased temperature, stratification leading to increased PAR, UVR and decreased nutrients in the photic zone accompanied by increased CO_2 concentrations resulting in decreased pH and $\Omega_{cal.}$ The interaction studies which have been reviewed here reveal some consistent trends (Table 3). At high light levels, increased CO_2 concentration either reduces calcification or does not affect it except in a few exceptional cases. Simultaneously, photosynthesis is often stimulated by increased CO_2 concentrations with high

Table 3. Summary of effects of environmental factors on the particulate inorganic carbon to particulate organic carbon (PIC:POC) ratio in coccolithophores. See discussions in the main text for more details and sources. PAR: photosynthetically active radiation; UVR: ultraviolet radiation; PON: particulate organic nitrogen

Environmental factor(s)	Effect on PIC:POC
CO_2	Usually a decrease with increasing CO_2 above the present level, but sometimes no effect. Usually an increase with decrease in CO_2.
PAR	Decreasing PAR below saturating level increases PIC:POC down to a low PAR below which PIC:POC decreases.
CO_2–PAR interaction	Relative to saturating PAR and present CO_2, decreased PIC:POC with increasing CO_2, limiting PAR increases PIC:POC with no effect of increased CO_2.
UVR	In short-term (2 h) experiments, no effect of UVAR added to PAR, decrease when UVBR is added to PAR and UVAR. In the longer term, UVAR had a greater relative effect on calcification but UVBR had a greater relative effect on photosynthesis, or continued calcification in UVBR after cell division had ceased.
Temperature	Generally no effect at saturating PAR and present CO_2.
Temperature–PAR–CO_2 interaction	Either no effect of any CO_2 and PAR, or temperature sensitive at higher but not at present CO_2.
NO_3^-, PO_4^{3-}	PIC:POC increases at limiting relative to saturating CO_2, especially for limiting PO_4^{3-}.
Temperature–PO_4^{3-} interactions	Larger temperature effect at limiting than at saturating PO_4^{3-}.
NO_3^-–CO_2 interactions	No effect of increased CO_2 on PIC:POC in nitrate-limited cultures.
Fe, Zn	Measured as PIC:PON; no effect of Fe deficiency, PIC:PON increased at limiting Zn.

light. For some species of coccolithophore, there is a general trend towards increased growth rates, and for others decreased rates; for *Emiliania huxleyi*, the 2 responses are found equally. Likewise in natural waters, *E. huxleyi* was found in greater numbers by Feng et al. (2009) and in lower numbers by Engel et al. (2005), but in both cases, PIC l^{-1} was reduced. With increased temperature and CO_2 concentration, PIC is also seen to decrease, while POC, abundance, size and malformation show varying responses. Nutrient limitation with both macro and micronutrients limits growth and so ultimately reduces PIC l^{-1}. In individual cells, PIC may continue to accumulate in extra liths, but these cells will ultimately be grazed or sink out and the population will decrease. These nutrient limitations are highly influential and mask any small effects of CO_2 concentration. UVR reduces photosynthesis and calcification and coupled with increased CO_2 concentration, but not due to the more lightly calcified liths, may almost completely halt calcification and/or cell division. This may also result in cells with extra liths but ultimately again PIC production per unit area or volume of culture (or habitat) will be reduced. The increased light available in a deeper mixed layer, with lower nutrients and thus less shading, may stimulate calcification and photosynthesis at depths where light is limiting if sufficient nutrients are available. Taken as a whole, the data and models suggest decreased oceanic calcification in the future, with possible exceptions in upwelling regions. The rate of change in the environment expected in the foreseeable future is greater than those commonly seen in the past. Migration is one possible solution, if appropriate habitat exists elsewhere. Adjustment to a changed environment at a given location poses problems when acclimation to the new environment using the existing genome is not possible or is too costly in resources, and genetic adaptation is too slow. However, the work of Lohbeck et al. (2012) on *E. huxleyi* suggests that such problems may not be insuperable.

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