

Predicting coccolithophore rain ratio responses to calcite saturation state

Samuel R. Fielding*

School of Environmental Sciences, University of Liverpool, Liverpool, UK

ABSTRACT: The response of the coccolithophore calcite and particulate organic carbon (POC) ratio, also known as the rain ratio, to calcite saturation state (Ω_{calcite}) is increasingly being used as an input parameter for modelling ocean feedbacks to changes in atmospheric $p\text{CO}_2$. Previously, this relationship has only been determined from a small number of studies from a single genus of coccolithophore. However, there is an increasing abundance of literature calcite:POC – Ω_{calcite} data for several coccolithophore genera. Here, Ω_{calcite} and calcite:POC data were collated from literature studies of coccolithophore responses to changes in ocean carbonate chemistry. These Ω_{calcite} data were recalculated using a standardized pH scale and constants. Calcite:POC responses to Ω_{calcite} were then determined using quantile regression for 2 major orders of coccolithophore: Isochrysidales (*Emiliana*, *Gephyrocapsa*) and Coccolithales (*Coccolithus*, *Calcidiscus*). These 2 coccolithophore groups display qualitatively and quantitatively different responses to Ω_{calcite} . A general combined expression was calculated to describe the response of the calcite:POC ratio to Ω_{calcite} for coccolithophores as a single functional group for use when the relative contributions of each order to the coccolithophore community are unknown.

KEY WORDS: Calcification · Phytoplankton · Carbon cycling · Modelling · Quantile regression · *Emiliana*

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Production of calcite and organic carbon by marine coccolithophores is sensitive to changes in the carbonate system caused by oceanic uptake of CO_2 from the atmosphere (Rost et al. 2008). However, a reduction in the ratio of calcite to particulate organic carbon (POC) production (the rain ratio) will in turn enhance the ability of the oceans to take up CO_2 due to the different impacts that these 2 biogenic processes have on alkalinity and dissolved inorganic carbon (Zeebe & Wolf-Gladrow 2001). Conversely, an increase in the coccolithophore calcite:POC ratio will reduce the potential for ocean CO_2 uptake.

Recently, coccolithophore calcite:POC responses to changes in carbonate chemistry (e.g. due to ocean acidification) have started to be used as input parameters for global carbon cycle models (Gehlen et al.

2007, Gangsto et al. 2011, Pinsonneault et al. 2012). Input parameters relating calcite:POC ratios to seawater carbonate chemistry, specifically to calcite saturation state (Ω_{calcite}), have until now only been based on a small dataset of experimental observations for the coccolithophore *Emiliana* (Gehlen et al. 2007, Gangsto et al. 2011).

In the last few years, there has been some attempt to analyse the response of *Emiliana* to changes in seawater carbonate system parameters (Findlay et al. 2011). There is an ever-growing number of studies in which calcite:POC ratios for both *Emiliana* and other coccolithophores have been measured as a function of carbonate chemistry. However, there are several problems involved in extracting a calcite:POC versus Ω_{calcite} relationship from these studies before it can be applied to a coccolithophore component in a biogeochemical model.

First, not all studies provide Ω_{calcite} data. Studies which do, use different methods to calculate Ω_{calcite} from measured carbonate system parameters such as total alkalinity and dissolved inorganic carbon, making the accuracy of comparisons between experiments uncertain. Second, experiments are carried out under a diverse range of environmental conditions. Culture studies are made under varying light, temperature and nutrient regimes and during different growth phases which have all been shown to affect calcite:POC ratios (Paasche 1998, 2001, Zondervan et al. 2002). Additionally, measurements derived from *in situ* ocean microbial communities include non-calcifying organisms which skew calcite:POC values downwards. Third, intraspecific variation of the calcite:POC response of *Emiliana* to ocean acidification has been observed (Langer et al. 2009). Further, other species of coccolithophore have displayed calcite:POC responses to ocean acidification that are significantly different from those of *E. huxleyi* (Langer et al. 2006).

Therefore, calcite:POC values from the literature are a result of a wide range of factors (e.g. light, intra-

specific variation) other than the change in the carbonate system, making comparison between individual studies problematic.

In an attempt to synthesize these coccolithophore ocean acidification studies, I (1) collated data from *Emiliana* and from all other species of coccolithophore for which calcite:POC ratios have been measured as a function of carbonate chemistry, (2) recalculated literature Ω_{calcite} values from carbonate system measurements using a standardized set of constants, and (3) used quantile regression (Koenker & Bassett 1978) to determine genus- and order-level response parameters for calcite:POC versus Ω_{calcite} .

METHODS

Data collection

Data were obtained from studies where coccolithophore calcite and POC were measured in experiments mimicking future or past changes in atmospheric $p\text{CO}_2$ and subsequent changes in ocean CO_2 ,

Table 1. Coccolithophore strains used in this study with their contribution to the dataset (n = number of Ω_{calcite} – calcite:POC data points) and the latitude (Lat.) and longitude (Lon.) of the location of their isolation (shown in Fig. 1); (–) indicates °S and °W for latitude and longitude respectively. Ω_{calcite} : calcite saturation state; POC: particulate organic carbon

Species	Strain	Lat.	Lon.	n	Source
Isochrysidales					
<i>Emiliana huxleyi</i>	CCMP371	32	–62	12	Feng et al. (2008), Lefebvre et al. (2012)
<i>E. huxleyi</i>	PeECE III 2005	60	5	42	Müller et al. (2010)
<i>E. huxleyi</i>	PLYB92/11	60	5	63	Zondervan et al. (2002), Bach et al. (2011), Borchard et al. (2011)
<i>E. huxleyi</i>	PLYM219	–47	168	48	Iglesias-Rodriguez et al. (2008), Shi et al. (2009)
<i>E. huxleyi</i>	Raunefjorden 2009 monoclonal	60	5	12	Lohbeck et al. (2012), Müller et al. (2012)
<i>E. huxleyi</i>	Raunefjorden 2009 multiclonal	60	5	7	Lohbeck et al. (2012)
<i>E. huxleyi</i>	RCC1212	–34	17	4	Langer et al. (2009)
<i>E. huxleyi</i>	RCC1216	–42	170	12	Langer et al. (2009), Fiorini et al. (2011b), Richier et al. (2011), Rokitta & Rost (2012)
<i>E. huxleyi</i>	RCC1238	34	140	4	Langer et al. (2009)
<i>E. huxleyi</i>	RCC1256	63	–20	12	Langer et al. (2009), Hoppe et al. (2011)
<i>E. huxleyi</i>	TW1	38	2	2	Sciandra et al. (2003)
<i>E. huxleyi</i>	CS369	–43	148	9	Gao et al. (2009)
<i>Gephyrocapsa oceanica</i>	PC7/1	38	–9	5	Zondervan et al. (2001)
Coccolithales					
<i>Coccolithus braarudii</i>	RCC1200	–25	12	73	Langer et al. (2006), Müller et al. (2010), Krug et al. (2011)
<i>Calcidiscus leptoporus</i>	AC370	–34	17	2	Fiorini et al. (2011b)
<i>Calcidiscus quadriperforatus</i>	RCC1135	–36	16	12	Langer et al. (2006), Langer (2011)
<i>Pleurochrysis carterae</i>	Japan	40	142	2	Casareto et al. (2009)
Syracosphaerales					
<i>Syracosphaera pulchra</i>	AC418	41	14	4	Fiorini et al. (2011a)

Table 2. Pre-selection quantile regression models. k represents the number of independently varied parameters (a, b, c, d) in the model. Models are plotted in Fig. 2

Model	Equation	k	Deviance
Isochrysidales			
Logarithmic	$a + b \ln(x)$	2	2.62
Rectangular hyperbola	$c + (ax)/(b + x)$	3	2.63
Power	$c + a(x^b)$	3	2.64
Exponential	$c + a(1 - e^{-bx})$	3	2.66
Quadratic	$c + (ax) + (bx^2)$	3	2.67
Linear	$a + (bx)$	2	2.78
Coccolithales			
Log normal	$d + (a/x)e^{-0.5[(\ln(x/c)/b)^2]}$	4	3.97
Gaussian	$a e^{-0.5[(x-c)/b]^2}$	3	4.48
Lorentzian	$a / \{1 + [(x - c)/b]^2\}$	3	4.49
Linear	$a + (bx)$	2	4.69

pH and Ω_{calcite} (Table 1). Only calcite and POC data from monospecific cultures in exponential growth phase were included, as measurements which were taken in stationary phase cultures (e.g. De Bodt et al. 2010) or from multispecies assemblages such as mesocosms (e.g. Delille et al. 2005) may either under- or overestimate POC, respectively. Similarly, data from studies where pH was kept constant while other carbonate system parameters were varied (e.g. Rickaby et al. 2010) were not included. Two studies did not include sufficient data to accurately calculate Ω_{calcite} (Nimer & Merrett 1993, Buitenhuis et al. 1999). When tabular data were not available, graphical data were extracted using Engauge Digitizer 4.1 (<http://digitizer.sourceforge.net/>).

Carbonate system standardization

The literature studies analysed here used varying methods and constants for calculating Ω_{calcite} from carbonate system parameters. Therefore, to allow inter-comparability between studies, carbonate system parameters including Ω_{calcite} were recalculated from the original data using CO2Sys 2.1 (Pierrot et al. 2006) with dissociation constants for carbonic acid of Roy et al. (1993), the total pH scale, dissociation constants for KSO_4 from Dickson (1990), the total boron formulation of Lee et al. (2010) and culture temperature, salinity and phosphate concentrations from each study. Where possible, experimentally measured total alkalinity (A_T) and dissolved inorganic carbon (DIC) were used as input parameters for calculating Ω_{calcite} (Riebesell et al. 2010). Where 1 or more of these parameters were not available, they were back-calculated from pH and either $p\text{CO}_2$ or

the fugacity of CO_2 ($f\text{CO}_2$) using the appropriate pH scales and constants (see 'Results' and 'Discussion' for caveats of this method) before being used to calculate Ω_{calcite} . Where salinity data were not available but the provenance of the seawater used in cultures was known, salinity was approximated from local annual mean sea-surface salinity following Antonov et al. (2010). Where neither salinity nor seawater provenance data were available, a salinity of 35 was used. All studies provided details of culture temperature and initial phosphate concentrations.

Modelling and statistical analysis

Quantile regression can be used to extract information about the relationship between 2 parameters (e.g. calcite:POC ratio and Ω_{calcite}) from the upper edge of a scatterplot for a dataset when other limiting factors (e.g. light) contribute to a large proportion of the variation (Cade et al. 1999, Cade & Noon 2003).

Quantile regression models with non-zero intercepts (Table 2) were used to approximate the upper edge (ideally the 99th quantile) of the dataset using R 2.15.3 with the quantreg 4.98 package. Rogers (1992) suggested that the number of data points (n) should be greater than $[5/(1 - \tau)]$, where τ is the quantile, with 0.99 representing the 99th quantile. Therefore, for each dataset modelled in this study, regressions were calculated for the largest possible quantile without violating the upper limit constrained by n .

RESULTS AND DISCUSSION

Data overview and partitioning

Data were derived from 38 individual datasets from 21 separate studies on coccolithophores from diverse locations (Table 1, Fig. 1). Culture experiments were conducted on 6 genera of coccolithophore. The genus *Emiliana* contributed the largest number of datasets, with 28 out of the 38. A smaller number of datasets was also available for *Gephyrocapsa* ($n = 1$), *Coccolithus* ($n = 3$), *Calcidiscus* ($n = 3$), *Syracosphaera* ($n = 2$) and *Pleurochrysis* ($n = 1$).

The calcite:POC ratio from the 2 studies that used *Emiliana* but did not include sufficient data for cal-

culating Ω_{calcite} (Nimer & Merrett 1993, Buitenhuis et al. 1999) fell well below the upper edge of the other *Emiliana* data (Fig. 2).

Data for *Pleurochrysis* and *Syracosphaera* were limited, and at present there is no evidence to suggest that the calcite:POC ratio of either genus responds to carbonate system parameters at all. These data were omitted from subsequent analysis. The remaining data were partitioned at the order level, which broadly corresponds with ecotypic differentiation within the coccolithophores: Isochrysidales (*Emiliana*, *Gephyrocapsa*) are bloom-forming (Rhodes

et al. 1995, Paasche 2001), relatively small and fast growing, whereas the Coccolithales (*Coccolithus*, *Calcidiscus*) are generally larger, slower growing and do not readily form blooms.

The Isochrysidales dataset (232 Ω_{calcite} – calcite:POC data points) almost exclusively comprised *Emiliana* (~98%), while *Gephyrocapsa* was represented by only 5 data from 1 study (~2%). The Coccolithales dataset (87 Ω_{calcite} – calcite:POC data points) was largely comprised of *Coccolithus* (~84%) with a lesser contribution from *Calcidiscus* (~16%). The use of this order-level binning approach therefore provides a useful starting point from which to model coccolithophore calcite:POC response to ocean acidification in the absence of further data better describing responses at the genus level for e.g. *Calcidiscus* or *Gephyrocapsa*.

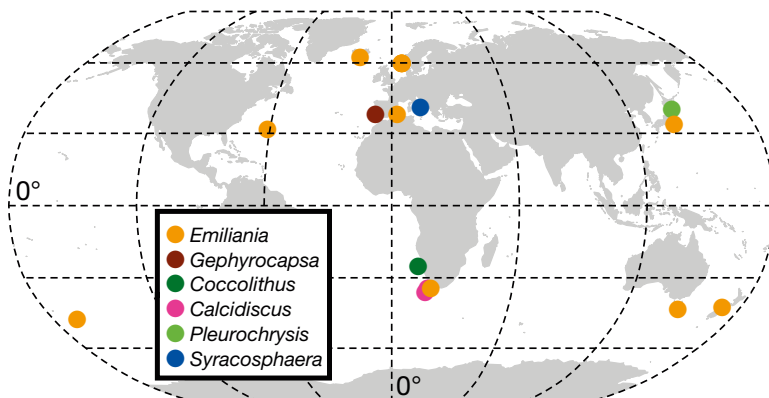


Fig. 1. Geographical locations of the coccolithophore strains used in the literature compilation in this study

Model fitting and selection

The upper edge of the Isochrysidales data was best described by a logarithmic curve closely followed by a rectangular hyperbola with a <1% higher deviance (where deviance is inversely proportional to the quality of fit; Table 2). Addi-

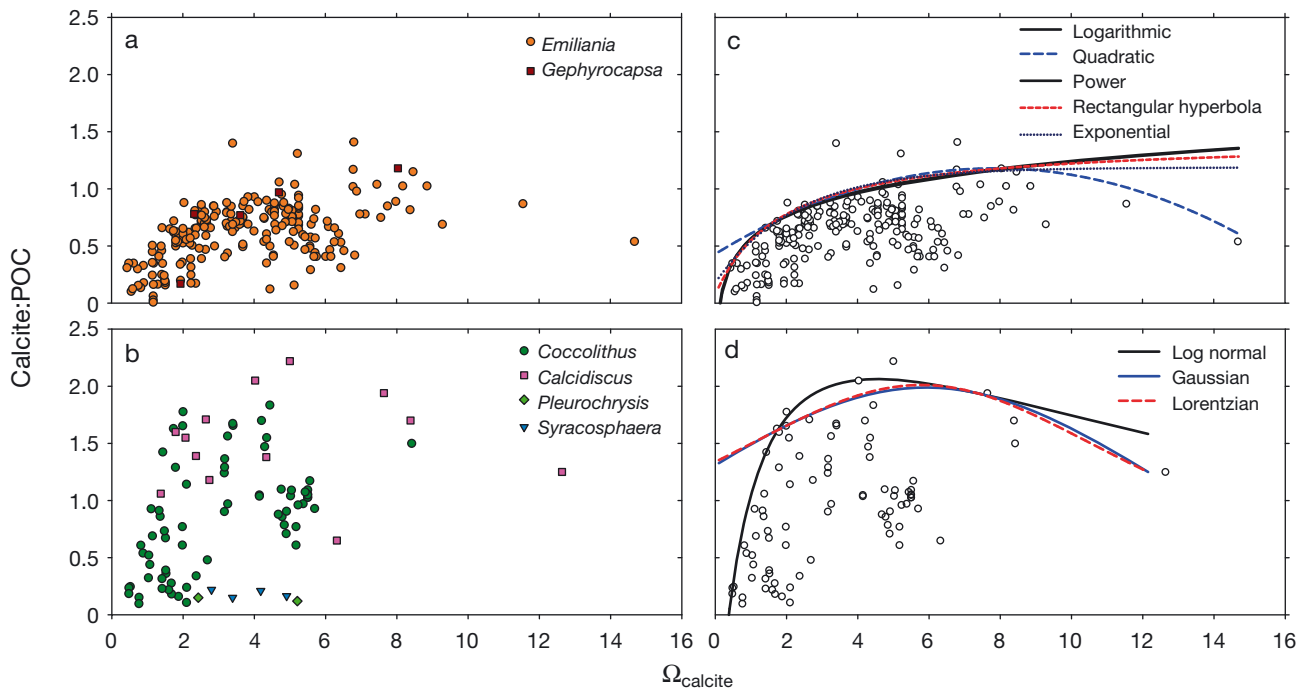


Fig. 2. Ratio of coccolithophore calcite:particulate organic carbon (POC) as a function of calcite saturation state (Ω_{calcite}) for (a) Isochrysidales and (b) Coccolithales and Syracosphaerales together with quantile regression models describing the upper edge of datasets for (c) Isochrysidales (97th quantile) and (d) Coccolithales (94th quantile). See Table 2 for model deviances

tionally, power, exponential and quadratic models had deviances which were only <2% higher than that of the logarithmic model. Despite the quadratic model predicting decreased calcite:POC ratios at high Ω_{calcite} (Fig. 2), there is currently no evidence to suggest that either *Emiliana* or *Gephyrocapsa* display optimal relationships as a function of ocean acidification. Further, there are only 2 data with higher Ω_{calcite} than at the visual calcite:POC maximum at Ω_{calcite} of ~ 8.5 , making prediction of calcite:POC ratios uncertain in this range. Therefore, no attempt was made to fit other peak models (e.g. Gaussian, log normal) to the Isochrysidales dataset.

To select the most parsimonious model from the group of models with similar deviances fit to the Isochrysidales data, they were compared using Akaike's information criterion (AIC; Akaike 1974): $\text{AIC} = -2L_m + 2k$, where k is the number of independently varied parameters in the model, and L_m is the maximized log likelihood ($-2L_m$ is equivalent to the deviance of the model fit). Smaller AIC values therefore indicate models with better relative fits to the data. The logarithmic model not only had the lowest deviance but also had only 2 parameters against the 3 parameters of the other models. As a result, AIC selected the logarithmic model as the most parsimonious descriptor of the upper edge of the Isochrysidales data with an AIC score of 6.62 compared to 8.63 for the rectangular hyperbolic model. A logarithmic response has previously been found to be a good fit for a smaller dataset of the *Emiliana* calcite:POC ratio as a function of Ω_{calcite} (Findlay et al. 2011).

Conversely, strains of both *Coccolithus* and *Calcidiscus* have been shown to have calcite:POC optima as a function of ocean acidification (Langer et al. 2006). Therefore, peak models were fit to the Coccolithales data, with a log normal curve having the best fit for the upper edge of the dataset with a deviance >5% lower than the next best model (Table 2). Gaussian and Lorentzian models did not fit the data as closely as a result of their forced symmetry and subsequent overestimation of calcite:POC ratios at lower values of Ω_{calcite} .

As the simplest mathematical model, and having previously been used to approximate calcite:POC ratios as a function of Ω_{calcite} (Gangsto et al. 2011, Pinsonneault et al. 2012), a linear equation was also fit to the upper edge of each dataset. However, these models had higher deviances than non-linear models (Table 2), reflecting the non-linearity of the relationship between Ω_{calcite} and calcite:POC ratios apparent in the data presented here.

Genus, geographical and environmental bias

Culture strains of *Emiliana* were derived from a diverse range of ocean basins (Fig. 1). However, there were only 2 strains of *Calcidiscus* which were both isolated from a geographically restricted area in the south Atlantic, while other genera were each represented by only a single strain. This sampling bias towards *Emiliana* is common in studies of coccolithophores due to the ease of isolating and maintaining this genus in laboratory culture. The results of this study should therefore be interpreted with this experimental and geographical bias in mind.

Additionally, there is a lack of data derived from strains isolated far from continental land masses (Fig. 1). Considerable intraspecific variation in physiology and morphology between near-shore and oceanic genotypes has been shown for *Emiliana* (Conte et al. 1995, Paasche 2001, Fielding et al. 2009), although this distinction has not yet been observed for other species of coccolithophore. Further, this distinction has not yet been observed for the calcite:POC response to changes in the seawater carbonate system. However, this bias away from oceanic strains should be taken into account when interpreting the results of this study.

Previously, light intensity and day length (Paasche 1999, Zondervan et al. 2002), nutrient limitation (Paasche 1998) and salinity (Paasche et al. 1996) have been shown to impact coccolithophore calcite:POC ratios, and it may be hypothesized that other environmental factors such as temperature also exhibit an influence. Both the Isochrysidales and the Coccolithales datasets were derived from cultures grown across a wide range of environmental conditions. Culture conditions ranged from 14 to 24°C, salinities of 29.7 to 38, day lengths from 12 to 24 h and photon flux densities between 15 and 580 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Variation in calcite:POC ratios due to these factors can be accounted for by scatter in the data below the upper edge of the dataset (Cade et al. 1999).

While ~96% of the data in this study were from cultures grown under nutrient-replete conditions, ~4% were from *Emiliana* growing in N- and P-limited chemostats. However, there is currently no consensus in the literature as to whether nutrient limitation increases or decreases coccolithophore calcite:POC ratios.

Data from Borchard et al. (2011) show that the calcite:POC ratio of a Norwegian coastal strain of *Emiliana* decreased negligibly from ~ 0.16 in already P-limited cultures to ~ 0.14 in increasingly P-limited cultures over a range of Ω_{calcite} . Müller et al. (2012)

showed that in a different Norwegian coastal strain of *Emiliana*, the calcite:POC ratio was reduced from >1 in nutrient-replete cultures to between 0.75 and 0.50 under N limitation, also over a range of Ω_{calcite} . These N- and P-limited calcite:POC data (included in this study) lie well below the upper edge of the dataset, and are therefore unlikely to have influenced quantile regression estimates.

Similarly, Fe limitation has been shown to decrease calcite:POC ratios from 1.29 (Fe-replete) to ~0.04 in cultures of an *Emiliana* strain from the north-east Pacific, with a reduction in calcite:POC ratios also observed when the N source was changed from ammonium to nitrate (Muggli & Harrison 1996).

Contrastingly, data from a North Atlantic strain of *Emiliana*, not included in this study, show calcite:POC ratios rising from ~1 in nutrient-replete batch cultures to as high as 1.5 in both N- and P-limited chemostats (Paasche 1998). Similarly, Riegman et al. (2000) showed an increase in calcite:POC ratios in a Norwegian coastal strain of *Emiliana* in both N- and P-limited chemostats from 0.23 to 0.50 and from 0.4 to 0.5, respectively. Kaffes et al. (2010) also showed a slight increase in calcite:POC ratios from 0.62 to 0.70 with a reduction in nitrate concentration. However, carbonate system parameters for some of these studies (Muggli & Harrison 1996, Paasche 1998, Riegman et al. 2000, Kaffes et al. 2010) are not detailed enough to relate these data to Ω_{calcite} .

Nutrient limitation does not play a large part in the calcite:POC variation observed in this study due to the small percentage of nutrient limitation studies included. However, nutrient limitation is likely to have some influence on the coccolithophore calcite:POC ratio and its response to changes in the carbonate system in the ocean, although at present we lack culture data with which to quantify this phenomenon. Future experiments using both chemostat and batch cultures over a wide range of Ω_{calcite} will be necessary to elicit how macro- and micronutrient limitation controls coccolithophore calcite:POC ratios in relation to this, and other, carbonate system variables.

Order-level partitioning

Although strains of both *Coccolithus* and *Calcidiscus* display calcite:POC optima as a function of ocean acidification, the applicability of combining both of these genera is open to debate. Each genus contributes more to different parts of the response curve: *Coccolithus* data delineate the calcite:POC response at lower (<2) Ω_{calcite} , whereas *Calcidiscus* data repre-

sent the upper edge of the data at higher (>5) Ω_{calcite} . However, there is no evidence to suggest that *Coccolithus* follows the response curve of *Calcidiscus* and vice versa. Therefore, additional calcite:POC data from *Coccolithus* at higher Ω_{calcite} and from *Calcidiscus* at lower Ω_{calcite} are required to support the use of the combined relationship presented in this study. Nonetheless, the combination of these 2 datasets may still provide a useful simplification of the calcite:POC response of the Coccolithales for incorporation into carbon cycle models.

Conversely, the inclusion of the small number of *Gephyrocapsa* data in the Isochrysidales dataset has only minimal influence on quantile regression model selection or parameter estimates. Therefore, the regression results for the Isochrysidales dataset essentially describe *Emiliana*. However, the *Gephyrocapsa* calcite:POC ratio has been shown to react to ocean acidification in a similar way to *Emiliana* (Zondervan et al. 2001), while there is currently no evidence to suggest that *Gephyrocapsa* might exhibit a calcite:POC optimum as a function of ocean acidification. Therefore, for the purposes of using these results as input parameters for biogeochemical modelling, it is suggested that they are used to encompass both *Emiliana* and *Gephyrocapsa*. Nonetheless, further data for *Gephyrocapsa* are required to better support this conclusion.

Problems with carbonate system standardization

Due to the use of varying carbonic acid dissociation constants and pH scales in the literature studies collated here, a standardization approach was used to attempt to minimize errors introduced while calculating Ω_{calcite} .

It has been shown that calculated values of $p\text{CO}_2$, Ω_{calcite} and other carbonate system parameters from measured A_T and DIC can differ from parameters calculated from pH and either A_T or DIC by up to 30% (Hoppe et al. 2012). Therefore, in this study, as the most common carbonate system parameters available were A_T and DIC, where other carbonate system data were given they were converted back to these 2 initial parameters. This calculation step is likely to have introduced a certain amount of error inherent in the constants and in the propagation of the error from the initial measurements. However, the error introduced by conversion from one set of constants to another is small compared with errors from comparing values derived using different sets of

constants and pH scales (Zeebe & Wolf-Gladrow 2001). The benefits of the method used here of recalculating the carbonate system using a standardized set of constants and from a standardized starting point (i.e. A_T and DIC) are therefore likely to outweigh any error derived from back-calculation.

Additional error in calculating Ω_{calcite} may derive from estimates of culture salinity where measured salinity values were not given. For the purposes of calculating calcite saturation state (using carbonate and calcium ion concentrations), seawater calcium concentration is assumed to be proportional to salinity. Therefore, estimated salinity values could potentially result in incorrect Ω_{calcite} . However, salinity only had to be approximated for <3% of the dataset used in this study, and where it was estimated, it would have caused an error in Ω_{calcite} of ~ 0.05 per unit salinity error. Further, all data where salinity was estimated are for *Emiliana* and fall well below the upper edge of the data. Therefore, minimal impact on the overall trends in calcite:POC ratios observed here is expected.

Calcite sub-saturation

Theoretically, abiogenic calcium carbonate should dissolve completely below an Ω_{calcite} of 1, as seawater would be sub-saturated with respect to calcite. Indeed, models incorporating coccolithophore calcite:POC ratios force values to 0 below an Ω_{calcite} of 1 (Gehlen et al. 2007, Gangsto et al. 2011, Pinsonneault et al. 2012). However, the present study highlights that coccolithophore genera belonging to both the Isochrysidales and the Coccolithales can produce calcite in seawater sub-saturated with respect to calcite (Fig. 2). Below an Ω_{calcite} of 1, the calcite:POC ratio for both clades is reduced to less than 50% of the maximum calcite:POC ratio. Persistent calcite production under these conditions is likely due to coccolithophore calcite having an organic coating which partially protects it from dissolution (Henriksen et al. 2004, Godoi et al. 2009, Hassenkam et al. 2011).

Parameter estimates for modelling

The present study uses quantile regression to determine the response of the calcite:POC ratio to Ω_{calcite} by modelling the upper edge of the scat-

terplot which represents the response of the calcite:POC ratio to Ω_{calcite} when environmental variables other than the investigated response parameter (i.e. Ω_{calcite}) are not limiting (Cade et al. 1999). Once the shape (e.g. linear, logarithmic, exponential) of the response has been determined, an average regression model can be calculated through the data using the 50th quantile. These 50th quantile estimates may be useful for approximating an averaged response of the calcite:POC ratio to Ω_{calcite} under a range of unknown environmental conditions which can then be used for biogeochemical model parameterization. A logarithmic 50th quantile regression was made through the Isochrysidales data and a log normal 50th quantile regression was made through the Coccolithales data (Fig. 3). For modelling coccolithophore calcite:POC response to Ω_{calcite} where the relative contributions of

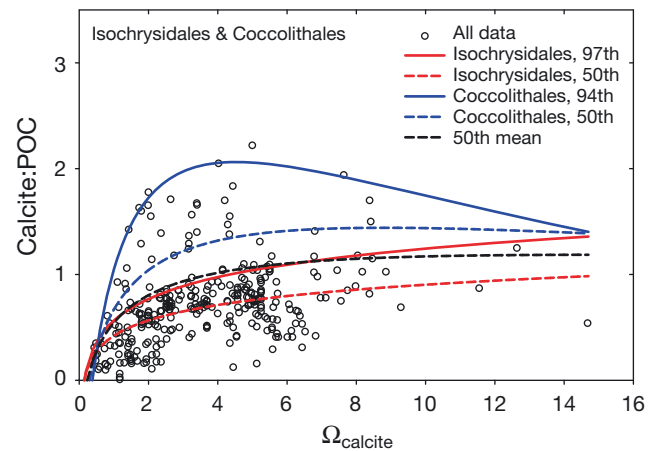


Fig. 3. Quantile regression models describing the upper edges and median (50th quantile) estimates for the ratios of calcite:particulate organic carbon (POC) of both Coccolithales and Isochrysidales as a function of calcite saturation state, Ω_{calcite} . The model describing the mean of the two 50th quantile estimates is also shown. See Table 3 for model parameters

Table 3. Post-selection quantile regression models. Models are plotted in Fig. 3. Ω_{calcite} is the calcite saturation state

Model	Quantile	Equation
Isochrysidales		
Logarithmic – upper edge	97th	$0.5650 + 0.2951 \ln(\Omega_{\text{calcite}})$
Logarithmic – median	50th	$0.4210 + 0.2094 \ln(\Omega_{\text{calcite}})$
Coccolithales		
Log normal – upper edge	94th	$-1.1700 + (66.712/\Omega_{\text{calcite}})e^{-0.5\{[\ln(\Omega_{\text{calcite}}/93.990)]/1.7413\}^2}$
Log normal – median	50th	$-0.7304 + (281.93/\Omega_{\text{calcite}})e^{-0.5\{[\ln(\Omega_{\text{calcite}}/1919.1)]/2.3209\}^2}$
All data		
Log normal – mean of medians	–	$-0.5904 + (1159.1/\Omega_{\text{calcite}})e^{-0.5\{[\ln(\Omega_{\text{calcite}}/30949)]/2.7777\}^2}$

different coccolithophore genera, orders or ecotypes is unknown, the mean of the two 50th quantile regressions may be taken (Fig. 3). This mean curve can be described by a log normal model (Table 3).

Aside from the non-zero calcite:POC ratio below Ω_{calcite} of 1, the mean 50th quantile response presented here (Fig. 3) is both qualitatively and quantitatively similar to those used previously (Gehlen et al. 2007, Gangsto et al. 2011) and therefore partially supports the results of these modelling studies. However, the inclusion of non-zero calcite:POC ratios for $\Omega_{\text{calcite}} < 1$ in biogeochemical models may more closely represent coccolithophore calcite production in the ocean.

CONCLUSION

The relationships presented here represent the first attempt to objectively quantify the relationship between coccolithophore calcite:POC ratios and Ω_{calcite} for both Isochrysidales (*Emiliana*, *Gephyrocapsa*) and Coccolithales (*Coccolithus*, *Calcidiscus*). The 50th quantile regression estimates for both Isochrysidales and Coccolithales are recommended for use in biogeochemical models incorporating either of these 2 distinct groups. Alternatively, the mean 50th quantile response is recommended for use where coccolithophores are modelled as a single functional group. However, the inclusion of further data from under-sampled genera, from nutrient-limited experiments and from open-ocean locations will allow for a more accurate reappraisal of these relationships.

Acknowledgements. Thanks to G. Langer and K. Lohbeck for providing data.

LITERATURE CITED

- Akaike H (1974) A new look at statistical-model identification. *IEEE Trans Automat Contr* 19:716–723
- Antonov JI, Seidov D, Boyer TP, Locarnini RA and others (2010) World ocean atlas 2009, Vol 2: Salinity. In: Levitus S (ed) NOAA Atlas NESDIS 69. US Government Printing Office, Washington, DC
- Bach LT, Riebesell U, Schulz KG (2011) Distinguishing between the effects of ocean acidification and ocean carbonation in the coccolithophore *Emiliana huxleyi*. *Limnol Oceanogr* 56:2040–2050
- Borchard C, Borges AV, Handel N, Engel A (2011) Biogeochemical response of *Emiliana huxleyi* (PML B92/11) to elevated CO₂ and temperature under phosphorous limitation: a chemostat study. *J Exp Mar Biol Ecol* 410:61–71
- Buitenhuis ET, de Baar HJW, Veldhuis MJW (1999) Photosynthesis and calcification by *Emiliana huxleyi* (Prymnesiophyceae) as a function of inorganic carbon species. *J Phycol* 35:949–959
- Cade BS, Noon BR (2003) A gentle introduction to quantile regression for ecologists. *Front Ecol Environ* 1:412–420
- Cade BS, Terrell JW, Schroeder RL (1999) Estimating effects of limiting factors with regression quantiles. *Ecology* 80:311–323
- Casareto BE, Niraula MP, Fujimura H, Suzuki Y (2009) Effects of carbon dioxide on the coccolithophorid *Pleurochrysis carterae* in incubation experiments. *Aquat Biol* 7:59–70
- Conte MH, Thompson A, Eglinton G, Green JC (1995) Lipid biomarker diversity in the coccolithophorid *Emiliana huxleyi* (Prymnesiophyceae) and the related species *Gephyrocapsa oceanica*. *J Phycol* 31:272–282
- De Bodt C, Van Oostende N, Harlay J, Sabbe K, Chou L (2010) Individual and interacting effects of pCO₂ and temperature on *Emiliana huxleyi* calcification: study of the calcite production, the coccolith morphology and the coccosphere size. *Biogeosciences* 7:1401–1412
- Delille B, Harlay J, Zondervan I, Jacquet S and others (2005) Response of primary production and calcification to changes of pCO₂ during experimental blooms of the coccolithophorid *Emiliana huxleyi*. *Global Biogeochem Cycles* 19:GB2023, doi:10.1029/2004GB002318
- Dickson AG (1990) Standard potential of the reaction: AgCl(s) + 12H₂(g) = Ag(s) + HCl(aq), and the standard acidity constant of the ion HSO₄⁻ in synthetic sea water from 273.15 to 318.15 K. *J Chem Thermodyn* 22:113–127
- Feng Y, Warner ME, Zhang Y, Sun J, Fu FX, Rose JM, Hutchins DA (2008) Interactive effects of increased pCO₂, temperature and irradiance on the marine coccolithophore *Emiliana huxleyi* (Prymnesiophyceae). *Eur J Phycol* 43:87–98
- Fielding SR, Herrle JO, Bollmann J, Worden RH, Montagnes DJS (2009) Assessing the applicability of *Emiliana huxleyi* coccolith morphology as a sea-surface salinity proxy. *Limnol Oceanogr* 54:1475–1480
- Findlay HS, Calosi P, Crawford KJ (2011) Determinants of the PIC:POC response in the coccolithophore *Emiliana huxleyi* under future ocean acidification scenarios. *Limnol Oceanogr* 56:1168–1178
- Fiorini S, Middelburg JJ, Gattuso JP (2011a) Effects of elevated CO₂ partial pressure and temperature on the coccolithophore *Syracosphaera pulchra*. *Aquat Microb Ecol* 64:221–232
- Fiorini S, Middelburg JJ, Gattuso JP (2011b) Testing the effects of elevated pCO₂ on coccolithophores (Prymnesiophyceae): comparison between haploid and diploid life stages. *J Phycol* 47:1281–1291
- Gangsto R, Joos F, Gehlen M (2011) Sensitivity of pelagic calcification to ocean acidification. *Biogeosciences* 8:433–458
- Gao KS, Ruan ZX, Villafane VE, Gattuso JP, Helbling EW (2009) Ocean acidification exacerbates the effect of UV radiation on the calcifying phytoplankter *Emiliana huxleyi*. *Limnol Oceanogr* 54:1855–1862
- Gehlen M, Gangsto R, Schneider B, Bopp L, Aumont O, Ethe C (2007) The fate of pelagic CaCO₃ production in a high CO₂ ocean: a model study. *Biogeosciences* 4:505–519
- Godoi RHM, Aerts K, Harlay J, Kaegi R, Ro CU, Chou L, Van Grieken R (2009) Organic surface coating on coccolithophores — *Emiliana huxleyi*: its determination and implication in the marine carbon cycle. *Microchem J* 91:266–271
- Hassenkam T, Johnsson A, Bechgaard K, Stipp SLS (2011) Tracking single coccolith dissolution with picogram resolution and implications for CO₂ sequestration and ocean acidification. *Proc Natl Acad Sci USA* 108:8571–8576
- Henriksen K, Young JR, Bown PR, Stipp SLS (2004) Coccolith biomineralisation studied with atomic force microscopy.

- Palaeontology 47:725–743
- Hoppe CJM, Langer G, Rost B (2011) *Emiliana huxleyi* shows identical responses to elevated pCO₂ in TA and DIC manipulations. *J Exp Mar Biol Ecol* 406:54–62
- Hoppe CJM, Langer G, Rokitta SD, Wolf-Gladrow DA, Rost B (2012) Implications of observed inconsistencies in carbonate chemistry measurements for ocean acidification studies. *Biogeosciences* 9:2401–2405
- Iglesias-Rodriguez MD, Halloran PR, Rickaby REM, Hall IR and others (2008) Phytoplankton calcification in a high-CO₂ world. *Science* 320:336–340
- Kaffes A, Thoms S, Trimborn S, Rost B and others (2010) Carbon and nitrogen fluxes in the marine coccolithophore *Emiliana huxleyi* grown under different nitrate concentrations. *J Exp Mar Biol Ecol* 393:1–8
- Koenker R, Bassett G (1978) Regression quantiles. *Econometrica* 46:33–50
- Krug SA, Schulz KG, Riebesell U (2011) Effects of changes in carbonate chemistry speciation on *Coccolithus braarudii*: a discussion of coccolithophorid sensitivities. *Biogeosciences* 8:771–777
- Langer G (2011) CO₂ mediation of adverse effects of seawater acidification in *Calcidiscus leptoporus*. *Geochem Geophys Geosyst* 12:Q05001, doi:10.1029/2010GC003393
- Langer G, Geisen M, Baumann KH, Kläs J, Riebesell U, Thoms S, Young JR (2006) Species-specific responses of calcifying algae to changing seawater carbonate chemistry. *Geochem Geophys Geosyst* 7:Q09006, doi:10.1029/2005GC001227
- Langer G, Nehrke G, Probert I, Ly J, Ziveri P (2009) Strain-specific responses of *Emiliana huxleyi* to changing seawater carbonate chemistry. *Biogeosciences* 6:2637–2646
- Lee K, Kim TW, Byrne RH, Millero FJ, Feely RA, Liu YM (2010) The universal ratio of boron to chlorinity for the North Pacific and North Atlantic oceans. *Geochim Cosmochim Acta* 74:1801–1811
- Lefebvre SC, Benner I, Stillman JH, Parker AE and others (2012) Nitrogen source and pCO₂ synergistically affect carbon allocation, growth and morphology of the coccolithophore *Emiliana huxleyi*: potential implications of ocean acidification for the carbon cycle. *Glob Change Biol* 18:493–503
- Lohbeck KT, Riebesell U, Reusch TBH (2012) Adaptive evolution of a key phytoplankton species to ocean acidification. *Nat Geosci* 5:346–351
- Muggli DL, Harrison PJ (1996) Effects of nitrogen source on the physiology and metal nutrition of *Emiliana huxleyi* grown under different iron and light conditions. *Mar Ecol Prog Ser* 130:255–267
- Müller MN, Schulz KG, Riebesell U (2010) Effects of long-term high CO₂ exposure on two species of coccolithophores. *Biogeosciences* 7:1109–1116
- Müller MN, Beaufort L, Bernard O, Pedrotti ML, Talec A, Sciandra A (2012) Influence of CO₂ and nitrogen limitation on the coccolith volume of *Emiliana huxleyi* (Haptophyta). *Biogeosciences* 9:4155–4167
- Nimer NA, Merrett MJ (1993) Calcification rate in *Emiliana huxleyi* Lohmann in response to light, nitrate and availability of inorganic carbon. *New Phytol* 123:673–677
- Paasche E (1998) Roles of nitrogen and phosphorus in coccolith formation in *Emiliana huxleyi* (Prymnesiophyceae). *Eur J Phycol* 33:33–42
- Paasche E (1999) Reduced coccolith calcite production under light-limited growth: a comparative study of three clones of *Emiliana huxleyi* (Prymnesiophyceae). *Phycologia* 38:508–516
- Paasche E (2001) A review of the coccolithophorid *Emiliana huxleyi* (Prymnesiophyceae), with particular reference to growth, coccolith formation, and calcification-photosynthesis interactions. *Phycologia* 40:503–529
- Paasche E, Brubak S, Skattebøl S, Young JR, Green JC (1996) Growth and calcification in the coccolithophorid *Emiliana huxleyi* (Haptophyceae) at low salinities. *Phycologia* 35:394–403
- Pierrot D, Lewis E, Wallace DWR (2006) MS Excel program developed for CO₂ system calculations. ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, TN
- Pinsonneault AJ, Matthews HD, Galbraith ED, Schmittner A (2012) Calcium carbonate production response to future ocean warming and acidification. *Biogeosciences* 9:2351–2364
- Rhodes LL, Peake BM, Mackenzie AL, Marwick S (1995) Coccolithophores *Gephyrocapsa oceanica* and *Emiliana huxleyi* (Prymnesiophyceae = Haptophyceae) in New Zealand coastal waters—characteristics of blooms and growth in laboratory culture. *NZ J Mar Freshw Res* 29:345–357
- Richier S, Fiorini S, Kerros ME, von Dassow P, Gattuso JP (2011) Response of the calcifying coccolithophore *Emiliana huxleyi* to low pH/high pCO₂: from physiology to molecular level. *Mar Biol* 158:551–560
- Rickaby REM, Henderiks J, Young JN (2010) Perturbing phytoplankton: response and isotopic fractionation with changing carbonate chemistry in two coccolithophore species. *Clim Past* 6:771–785
- Riebesell U, Fabry VJ, Hansson L, Gattuso JP (eds) (2010) Guide to best practices for ocean acidification research and data reporting. Publications Office of the European Union, Luxembourg
- Riegman R, Stolte W, Noordeloos AAM, Slezak D (2000) Nutrient uptake, and alkaline phosphate (EC3:1:3:1) activity of *Emiliana huxleyi* (Prymnesiophyceae) during growth under N and P limitation in continuous cultures. *J Phycol* 36:87–96
- Rogers WH (1992) Quantile regression standard errors. *Stata Tech Bull* 9:16–19
- Rokitta SD, Rost B (2012) Effects of CO₂ and their modulation by light in the life-cycle stages of the coccolithophore *Emiliana huxleyi*. *Limnol Oceanogr* 57:607–618
- Rost B, Zondervan I, Wolf-Gladrow D (2008) Sensitivity of phytoplankton to future changes in ocean carbonate chemistry: current knowledge, contradictions and research directions. *Mar Ecol Prog Ser* 373:227–237
- Roy RN, Roy LN, Vogel KM, Porter-Moore C and others (1993) The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45°C. *Mar Chem* 44:249–267
- Sciandra A, Harlay J, Lefèvre D, Lemée R, Rimmelin P, Denis M, Gattuso JP (2003) Response of coccolithophorid *Emiliana huxleyi* to elevated partial pressure of CO₂ under nitrogen limitation. *Mar Ecol Prog Ser* 261:111–122
- Shi D, Xu Y, Morel FMM (2009) Effects of the pH/pCO₂ control method on medium chemistry and phytoplankton growth. *Biogeosciences* 6:1199–1207
- Zeebe RE, Wolf-Gladrow DA (2001) CO₂ in seawater: equilibrium, kinetics, isotopes. Elsevier, Amsterdam
- Zondervan I, Zeebe RE, Rost B, Riebesell U (2001) Decreasing marine biogenic calcification: a negative feedback on rising atmospheric pCO₂. *Global Biogeochem Cycles* 15:507–516
- Zondervan I, Rost B, Riebesell U (2002) Effect of CO₂ concentration on the PIC/POC ratio in the coccolithophore *Emiliana huxleyi* grown under light-limiting conditions and different daylengths. *J Exp Mar Biol Ecol* 272:55–70