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Vanishing coccolith vital effects with alleviated CO₂ limitation

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BGD

12, 15835–15866, 2015

Vanishing coccolith vital effects with alleviated CO₂ limitation

M. Hermoso et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

⏪

⏩

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Abstract

By recreating a range of geologically relevant concentrations of dissolved inorganic carbon (DIC) in the laboratory, we demonstrate that the magnitude of the vital effects in both carbon and oxygen isotopes of coccolith calcite of multiple species relates to ambient DIC concentration. Under high DIC levels, all the examined coccoliths lacked any offset from inorganic calcite, whereas in low (present-day) DIC concentrations, these vital effects and interspecies differences become substantial. These laboratory observations support the recent hypothesis from field observations that the appearance of interspecific vital effect in coccolithophores coincides with the long-term Neogene decline of atmospheric CO₂ concentrations. The present study brings further valuable constraints on coccolith isotopic compositions by demonstrating the threshold for the absence of vital effects under high DIC regimes. From a mechanistic viewpoint, we show that the vital effect is determined by physiology; growth rate, cell size and relative rates of photosynthesis and calcification, and a modulation of these parameters with ambient carbon availability. This study provides palaeoceanographers with a biogeochemical framework that can be utilised to further develop the use of calcareous nanofossils in palaeoceanography to derive sea surface temperature and $p\text{CO}_2$ levels.

1 Introduction

The quest to generate reliable and accurate palaeoenvironmental reconstructions is hindered by uncertainties in our current proxies of the sedimentary archive. One prominent caveat owes to the biological origin of sedimentary calcareous particles in marine and oceanic realms. As a consequence of the biological controls on chemical signals in algae (vital effects), geochemical data from ancient biomineralising organisms must be corrected in order to derive the primary signals from palaeoseawater. In the case of the foraminifera, corals and coccoliths, the foremost carbonate producers in the marine

Vanishing coccolith vital effects with alleviated CO₂ limitation

M. Hermoso et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Vanishing coccolith vital effects with alleviated CO₂ limitation

M. Hermoso et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



5 realm, there has been a considerable number of studies during which living organisms were cultured in strictly controlled environmental conditions and their biominerals measured for a range of isotopic systems ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$, $\delta^{11}\text{B}$, Δ_{47}) to generate empirical proxy calibrations (Erez and Boas, 1982; Dudley et al., 1986; Spero et al., 1997; Bemis et al., 1998; Ziveri et al., 2003; Tripathi et al., 2010; Rickaby et al., 2010; Rollion-Bard et al., 2011; Grauel et al., 2013; Hermoso et al., 2014; Minoletti et al., 2014; Hermoso, 2015).

10 Another important aim in palaeoceanography is to determine whether the physiology-induced fractionation for a given taxon was constant through time from an evolutionary perspective, and over shorter time intervals comprising large climatic fluctuations, in turn inducing environmentally-driven modulation of the vital effect (Hermoso, 2014). In the absence of more reliable information, the Uniformitarianism principle – by which, the processes that were operating in the geological past still exist today, and vice-versa – is commonly applied for elucidating vital effects and reconstructing primary oceanographic signals.

15 Although coccoliths are relatively challenging to extract at the species-specific level from sediments compared to foraminifera, coccolith-based studies represent a growing field since the pioneering work by Anderson and Steinmetz (1981). To better interpret coccolith isotope signals and generate more reliable palaeoenvironmental estimates from these cosmopolitan organisms, we need to gain a broader picture of their vital effects, and more specifically determine how environmental parameters govern their magnitude. Several studies have specifically measured coccolith $\delta^{18}\text{O}$ with changing temperature in laboratory cultures in order to determine and calibrate the temperature/ $\delta^{18}\text{O}$ relationship for a wide range of species (Dudley et al., 1986; Ziveri et al., 2003; Candelier et al., 2013; Stevenson et al., 2014). Meanwhile, other culture studies have kept temperature constant but have manipulated the carbonate chemistry of the culture medium and the irradiance level (Ziveri et al., 2003; Rickaby et al., 2010; Hermoso, 2015) and found substantial modulation of the oxygen isotope vital effect with these parameters at constant temperature. In most cases, only one parameter was con-

trolled at a time, and we are lacking cross-parameter investigations that are required for the effective application of palaeoproxies, as in nature, environmental parameters generally co-vary, such as sea surface temperatures and $p\text{CO}_2$ concentrations. This is illustrated by the recent natural environment study by Hermoso et al. (2015) analysing coccoliths microseparated from core top sediments, which further illustrates the intricate (multi-parameter) control of coccolith oxygen and carbon isotope compositions ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$, respectively).

These biogeochemical proxies raise questions regarding what vital effect coefficients should be applied to ancient coccolith species extracted from Meso–Cenozoic sediments as temperature and $p\text{CO}_2$ significantly evolved before reaching the present-day levels. In the present study, we document a multi-species control of stable carbon and oxygen isotope composition under a wide range of DIC (hence $p\text{CO}_2$) levels recreated in the laboratory. As varying the availability of ambient DIC (primarily CO_2) substrate may modulate the degree of carbon limitation for algal growth (cell division rate and size) and influence the dynamics of the internal carbon pool (Sekino and Shiraiwa, 1994; Laws et al., 2002; Rickaby et al., 2010; Aloisi, 2015; Hermoso, 2015), this culture approach will allow us to determine the static vs. dynamic nature of the vital effect in coccolith calcite in response to CO_2 with a geological perspective.

2 Material and methods

2.1 Coccolithophore strains studied

Emiliania huxleyi has attracted most recent attention on coccolithophore research due to its dominance in present-day oceans and the consecutive relevance to study it with ongoing concerns about our Anthropocene ocean chemistry and climate (e.g., Bidigare et al., 1997; Riebesell et al., 2000; Iglesias-Rodriguez et al., 2008; De Bodt et al., 2010; Suffrian et al., 2011; Müller et al., 2012; Bach et al., 2013; Sett et al., 2014; Tchernov et al., 2014; Young et al., 2014; Aloisi, 2015; Holtz et al., 2015). The strain

BGD

12, 15835–15866, 2015

Vanishing coccolith vital effects with alleviated CO_2 limitation

M. Hermoso et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



2.2 Culture medium preparation

A raw batch of natural seawater collected from the English Channel (Station L4; 50°15.00' N–4°13.02' W) was supplied by MBA, Plymouth (UK). The batch of seawater was first acidified using concentrated HCl to reach pH ~ 2, conditions under which most of the dissolved inorganic carbon was present in form of aqueous CO₂. The batch was bubbled overnight with pure N₂ to remove DIC. Subsequently, pH was brought back to a value around 8 by addition of NaOH. Still under N₂ purge, we amended the medium in nitrate, phosphate, EDTA and vitamins according to the *K/2* recipe (see Hermoso et al., 2014 for further details). To obtain the desired DIC level (2, 4, 6, 8, 10 and 12 mM k_{sw}⁻¹), we proceeded to add calculated amounts of NaHCO₃ powder (Sigma – Batch CAS 144-55-8) in different aliquots with immediate pH adjustment to 8.2, after which each DIC batch then was promptly filtered-sterilised and kept in Teflon-sealed flasks without headspace. Prior to inoculation, each medium was measured for its total alkalinity using a 916 Ti Touch automatic titrator (Metrohm). Successive alterations of the carbonate chemistry did not induce change in total alkalinity compared to the original seawater batch, and there was a very good agreement between target and measured DIC concentrations for each batch (within a range of 5 %).

2.3 Cell density, size and growth

During the acclimation and culture phases, cells were maintained at 15 °C and illuminated under a daily 14 h/10 h light/dark cycle in Sanyo MLR-351 plant growth chambers. The irradiance was measured as 150 μmol photons m⁻² s⁻¹. Cultures were performed semi-continuously to allow DIC to remain stable with cell growth and preferential CO₂ assimilation and utilisation by the cells leading to increasing pH (Hermoso, 2014), which conforms to experimental guidelines (Barry et al., 2010). Unfortunately due to this experimental set-up, too low amount (mass) of harvested culture residues has prevented us to generate meaningful PIC/POC ratios for this study.

BGD

12, 15835–15866, 2015

Vanishing coccolith vital effects with alleviated CO₂ limitation

M. Hermoso et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



3.1.2 Carbon isotope composition of coccolith calcite

The interspecies range in coccolith $\delta^{13}\text{C}$ values grown under present-day CO_2 levels (~ 280 ppm/ ~ 2 mM DIC) is considerable, on the order of 3‰. This variation is due to the presence of very large vital effects for the carbon isotope system (Ziveri et al., 2003; Hermoso et al., 2014). Coccolith calcite carbon isotopic compositions are distributed either side of the inorganic reference value (Fig. 2a): *E. huxleyi* and *P. placolithoides* exhibit positive $\delta^{13}\text{C}$ values. Due to insufficient calcite yield at harvest for isotopic analysis for *P. placolithoides* grown at 2 mM DIC, the assignment of *P. placolithoides* to an isotopic “heavy group” (*sensu* Dudley et al., 1986) is inferred by extrapolation from the 4–12 mM range. *C. pelagicus* and *C. leptoporus* meanwhile have relatively similar $\delta^{13}\text{C}_\text{C} - \delta^{13}\text{C}_\text{DIC}$ values of -1.5 ‰ below that of inorganic calcite. These numbers are in good agreement with published literature when cultures were implemented at low cell concentration (see synthesis in Hermoso, 2014).

3.1.3 Oxygen isotope composition of coccolith calcite

The $\delta^{18}\text{O}$ of coccolith calcite grown by algae exposed to 2 mM of DIC is also comparable to values reported in literature with media aerated with laboratory air (Ziveri et al., 2003; Candelier et al., 2013; Hermoso et al., 2014; Stevenson et al., 2014). Our data are thus compatible with the assignment of coccolith species into three groups on the merit of oxygen isotope composition either from $\delta^{18}\text{O}_\text{C} - \delta^{18}\text{O}_\text{sw}$ or from $\delta^{18}\text{O}_\text{C} - \delta^{18}\text{O}_\text{inorg}$ values (the latter being used to quantify the magnitude of the “vital effect”; Eq. 2). *Emiliania huxleyi* (“heavy group”) has the most positive $\delta^{18}\text{O}_\text{C}$ values and large vital effects ($+1.5$ ‰). *Coccolithus pelagicus* (“equilibrium group”) produces calcite with oxygen isotope composition close to that of inorganic calcite (calculated using the equation by Kim and O’Neil, 1997). *Calcidiscus leptoporus* (“light group”) exhibits lighter $\delta^{18}\text{O}_\text{C}$ values than the inorganic reference. The offset from inorganic calcite is -1.2 ‰ for *C. leptoporus*, the same magnitude of the vital effect reported by Candelier et al. (2013) rather than those by Dudley et al. (1986). By extrapolation from higher DIC

levels in amended medium, it can be deduced that *P. placolithoides* would belong to the “light group”, which is consistent with the work of Dudley et al. (1986) concerning the closely related species *Pleurochrysis carterae*.

3.2 Effect of increased DIC on growth and isotopes (4–12 mM DIC)

3.2.1 Change in cell size and growth rate with increased DIC

Contrasting responses among examined species are observed in the evolution of specific growth rates and coccosphere volume with increased ambient DIC level, and as a result, in the carbon resource around the cells (Fig. 1a). The relatively fast growing *E. huxleyi* species exhibits fertilisation from 2 to 8 mM, beyond which a decrease is observed at the highest DIC levels. A similar decrease at high alkalinity was previously observed on the close relative *Gephyrocapsa oceanica* (Rickaby et al., 2010). Both *C. leptoporus* and *C. pelagicus* decreased cellular division rates over the 2 to 12 mM range of DIC concentration, but decreased growth rates are drastic for *C. pelagicus* with μ linearly changing from 0.5 down to 0.1 day⁻¹ with increasing DIC concentrations. Changing ambient DIC does not induce significant modulation of growth rate for the species *P. placolithoides*. Overall, there is no covariation between growth rates and coccosphere and cell sizes for the species examined here (Fig. 1b), as one may expect decrease μ to be accompanied by longer generation time, and hence larger cell sizes (Aloisi, 2015). Nevertheless, the data indicate that both *E. huxleyi* and *P. placolithoides* species become relatively larger with elevated DIC levels, as observed for the former in the work by Müller et al. (2012). *Calcidiscus leptoporus* exhibits no response for this parameter, whereas *C. pelagicus* shows significantly decreased coccospheres sizes at high DIC levels.

BGD

12, 15835–15866, 2015

Vanishing coccolith vital effects with alleviated CO₂ limitation

M. Hermoso et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



3.2.2 Change in carbon isotope composition of coccolith calcite

With increased DIC concentration in the culture medium, species that exhibited high $\delta^{13}\text{C}$ values at 2 mM DIC show a significant decrease in $\delta^{13}\text{C}_c - \delta^{13}\text{C}_{\text{DIC}}$ values (Fig. 3a). The observed decreases in $\delta^{13}\text{C}_c - \delta^{13}\text{C}_{\text{DIC}}$ with increasing DIC are linear ($r^2 = 0.96$ for *E. huxleyi* and 0.70 for *P. placolithoides*). At the highest DIC concentrations, it appears that coccolith calcite for these two species becomes indistinguishable to that of the inorganic reference (*sensu* Romanek et al., 1992). By contrast, species with lowest $\delta^{13}\text{C}$ at 2 mM (*C. pelagicus* and *C. leptoporus*) show increased carbon isotope compositions with addition of DIC in the medium. This positive evolution is linear for *C. leptoporus* ($r^2 = 0.83$) and *C. pelagicus* ($r^2 = 0.85$), although for the latter largest species the 2 mM datapoints departs from the 4–12 mM linear trend with substantial low $\delta^{13}\text{C}$ values. This “jump” in *C. pelagicus* $\delta^{13}\text{C}$ values between 2 and 4 mM represents most of the evolution in the $\delta^{13}\text{C}$ composition over the whole range of DIC concentration investigated here. At the highest DIC concentration, *C. pelagicus* exhibits near inorganic $\delta^{13}\text{C}$ values, whereas *C. leptoporus* remains 0.5‰ negatively shifted from this reference.

Overall, a noteworthy feature of the data is that all the vital effects ($\delta^{13}\text{C}_c - \delta^{13}\text{C}_{\text{DIC}}$ values) converge within a narrow range comprised between +0.5 and +1 ‰, representing an almost complete erasing of the original (measured under 2 mM of DIC) interspecific differences in carbon isotopes.

3.2.3 Change in oxygen isotope composition of coccolith calcite

The typology of a heavy and light isotopic group for the oxygen isotope system still exists with increased ambient DIC concentration, but the magnitude of the vital effect is considerably reduced with coccolith $\delta^{18}\text{O}_c$ tending towards inorganic values over the 2 to 12 mM DIC range. Not only are interspecies ^{18}O vital effects reduced at high DIC, but also as is the case for carbon isotopes, the absolute vital effects become significantly reduced at the highest DIC level (Fig. 2b). The large species *C. pelagicus*, assigned

Vanishing coccolith vital effects with alleviated CO₂ limitation

M. Hermoso et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



to the isotopic equilibrium group shows a constant, vital effect-free $\delta^{18}\text{O}$ regardless of changes in ambient DIC concentrations.

3.3 Estimates of the degree of utilisation of the carbon internal pool

Growth rate (μ), cell size and ambient DIC (or CO_2) concentrations can be combined to generate an index accounting for the supply and utilisation of carbon by the cells (Rau et al., 1996; Bidigare et al., 1997; Burkhardt et al., 1999; Laws et al., 2002). This widely used metric for the degree of utilisation of the internal carbon pool relies on the assumption of molecular CO_2 passive diffusion in phytoplankton (Bidigare et al., 1997; Laws et al., 2002; Hermoso, 2015). Assuming that the passive influx of CO_2 constitutes the only source of carbon to the cell, the flux of carbon is proportional to $[\text{CO}_{2\text{aq}}] \times \text{surface-area}$, and carbon usage is proportional to $\mu \times \text{volume}$ (assuming constant carbon density). The ratio of carbon supply to carbon usage (Eq. 4) reflects the index describing the degree of carbon utilisation, hereafter referred to as “DCUt”. As the increase in ambient $[\text{CO}_2]$ concentration (from low to high DIC concentrations) was the same for all species, interspecific differences in this index were driven by growth rates and coccosphere size.

$$\text{DCUt} = (\mu \cdot \text{volume}) / ([\text{CO}_{2\text{aq}}] \cdot \text{surface-area}) \quad (4)$$

In the present study, we used coccosphere size rather than naked cellular size to account for both organic and inorganic (calcite) carbon fixation. As coccosphere and cell size are linearly related (Henderiks, 2008), this slight adaptation of the original formula does not alter the validity of the calculated DCUt index. Furthermore, this proxy implicitly considers a carbon assimilation from aqueous CO_2 . The species *E. huxleyi* is the only coccolithophore algae, which has been reported with the ability to shift from CO_2 to HCO_3^- assimilation under ambient carbon limitation (Kottmeier et al., 2014; Bach et al., 2014). We are aware of no evidence for increased HCO_3^- assimilation in coccolithophores under high CO_2 environments, so we assume prominent CO_2 influx at high DIC, consistent with the work of Kottmeier et al. (2014) and Hermoso (2015).

Vanishing coccolith vital effects with alleviated CO_2 limitation

M. Hermoso et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

⏪

⏩

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



For all species, increasing DIC led to less utilisation of the carbon pool explained by alleviated carbon limitation, although the relative changes differ among species. This effect is considerable for the largest cell, namely *C. pelagicus*, especially apparent between 2 and 4 mM of DIC. At maximum DIC concentration, all species converge to similar $([\text{CO}_{2\text{aq}}] \times \text{surface-area})/(\mu \times \text{volume})$ values, possibly indicating that carbon replete internal conditions are attained, except for the coastal species *P. placolithoides* that has a significantly higher DCUt index at 12 mM compared to all other species (Fig. 1c).

3.4 Link between the magnitude of the vital effect and the degree of utilisation of the carbon internal pool

3.4.1 Coccolith carbon isotope composition

In *C. pelagicus*, there is a large relative change in the DCUt index and $\delta^{13}\text{C}_c$. These two parameters are statistically linked with a linear relationship ($r^2 = 0.98$): the greater DCUt, the more negative $\delta^{13}\text{C}_c$. The “jump” in carbon isotope composition of *C. pelagicus* calcite also corresponds to a large difference in the DCUt between 2 and 4 mM DIC.

In other species, there is a narrower spread of $\delta^{13}\text{C}_c$ and DCUt values, and coccolithophore algae show contrasting relationships between these parameters. *Emiliana huxleyi* and *Pleurochrysis placolithoides* exhibit decreased $\delta^{13}\text{C}$ values with a lower degree of internal carbon utilisation with linear fits ($r^2 = 0.82$ and 0.60 , respectively). It is worth noting that the non-linear nature of the statistical link mostly arises at the lowest DIC (2 mM) concentrations. In *C. leptoporus*, the response is somewhat comparable to that described for *C. pelagicus* with the exception of the 2 mM datapoints which are isotopically heavier than predicted by a linear fit for this species.

BGD

12, 15835–15866, 2015

Vanishing coccolith vital effects with alleviated CO_2 limitation

M. Hermoso et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

⏪

⏩

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



3.4.2 Coccolith oxygen isotope composition

The observed changes in coccolith $\delta^{18}\text{O}$ are correlated with the DCUt index with the notable exception of *C. pelagicus*. For this large species, near-equilibrium oxygen isotope composition is always expressed with no effect of $[\text{CO}_{2\text{aq}}]$, $[\text{DIC}]$ or coccosphere size. This result differs from other examined species. In *E. huxleyi* a linear fit ($r^2 = 0.68$) links the decreased DIC (hence, potentially under carbon limitation) and coccolith $\delta^{18}\text{O}$, leading to a smaller expression of the ^{18}O vital effect. Species from the light group show similar behaviour with $\delta^{18}\text{O}$ tending towards equilibrium with alleviated carbon limitation (at high DIC), albeit with linear trends ($r^2 = 0.77$ for *C. leptoporus* and 0.76 for *P. placolithoides*).

Finally, we observe that the data for *C. pelagicus* in both carbon and oxygen isotope systems are compatible with those reported by Rickaby et al. (2010), but in the present study, we extend the isotopic response of these species to a higher range of DIC, from 8 to 12 mM (equivalent to a range of 1400–2200 μCO_2 level).

4 Discussion

4.1 Nature of observed isotopic changes: inorganic or vital effect?

In biological systems, an increase in the DIC concentration of the ambient medium may not be linearly related to that of the mineralising fluid due to the effects of physiology. The observation of such contrasting responses in μ , $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ with increased DIC levels in different species points towards a biological control. That the light group increases and the heavy group decreases coccolith $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values precludes a unified thermodynamic mechanism, as the direction of isotopic changes with increased DIC are opposite (Fig. 2a and b).

Theoretical work and experiments seeking to identify the control of inorganic calcite isotopes have provided useful reference points that are valuable to understand

BGD

12, 15835–15866, 2015

Vanishing coccolith vital effects with alleviated CO_2 limitation

M. Hermoso et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



4.2 From enhanced intracellular carbon dynamics to lowered vital effects

4.2.1 Carbon isotope system

Specific to photosynthetic, or photosynthetic-associated biomineralisers such as the foraminifera, corals and coccoliths is the super-imposition of ^{12}C -DIC depletion of the internal carbon pool due to photosynthetic RuBisCO fractionation (McConnaughey, 1989; Spero et al., 1997; Hermoso et al., 2014). This interplay plays a primary role in controlling the isotopic composition of coccolith calcite (Hermoso et al., 2014).

The species *E. huxleyi* and *P. placolithoides* show particularly high calcite $\delta^{13}\text{C}$ values, whereas *C. pelagicus* and *C. leptoporus* have low carbon isotope composition at ambient (2 mM) DIC levels. In species characterised by low PIC/POC, typically *E. huxleyi*, the internal DIC pool is isotopically offset towards high $\delta^{13}\text{C}$ values due to intense ^{12}C -depletion by photosynthetic carbon fixation (Laws et al., 2002; Benthien et al., 2007; Hermoso et al., 2014; Tchernov et al., 2014). Langer et al. (2009) showed that the PIC/POC ratios in RCC 1256 *E. huxleyi* (the strain being examined here) were constant (~ 0.8) with changing carbonate chemistry. Thus, to explain lowered coccolith $\delta^{13}\text{C}$ with a lower degree of carbon utilisation (Fig. 1c), we suggest that the isotopic composition of the internal carbon pool becomes less imprinted by ^{12}C photosynthetic-driven Rayleigh fractionation because the latter process is “diluted” in a larger internal carbon pool. Hence, a similar biogeochemical control operated by the DCUt and the size of the carbon pool seems to set $\delta^{13}\text{C}$ values of both organic matter and coccolith calcite produced by *E. huxleyi*.

Species originally with low $\delta^{13}\text{C}$ values at 2 mM of DIC show a clear increase in their coccolith carbon isotopic ratios. Adopting the inverse reasoning of that made for the ^{13}C “light group”, one may expect enhanced production of POC relative to PIC if the mere isotopic control on coccolith $\delta^{13}\text{C}$ was indeed photosynthetic $^{13}\text{C}/^{12}\text{C}$ Rayleigh fractionation. The increase in *C. leptoporus* and *C. pelagicus* $\delta^{13}\text{C}$ with increased DIC is correlated with decreased DCUt values (Fig. 3a). It is surprising to observe a clear decrease of specific growth rates of *C. pelagicus* with more carbon resource in the

internal carbon pool (Hermoso, 2015). This relationship is also apparent in our dataset (Fig. 3b). Under carbon limited growth conditions, there is a fast turnover of the internal carbon pool that controls the record of isotopic disequilibrium of the DIC system due to incomplete re-equilibration of CO₂ with whole DIC and H₂O reservoir at the time of calcification in the coccolith vesicle (Hermoso et al., 2014). We note that unifying carbon and oxygen isotopes of coccolith calcite, the data do not support any shift from CO₂ to HCO₃⁻ assimilation by the cells.

In the present study, in all species except *C. pelagicus*, a causal link between DCut and δ¹⁸O values confirm that the ¹⁸O vital effect is set by the overturning rate, and via the residence time of the internal carbon pool from cell assimilation of carbon resource to calcification. This process can explain why δ¹⁸O of *E. huxleyi* significantly decreases and converges towards the composition of inorganic calcite under higher [DIC]. Comparing our isotopic data for *E. huxleyi* and those for *G. oceanica* by Rickaby et al. (2010), we observe that there seems to be an isotopic continuum between the two species on their isotopic composition/[DIC] relationship (Fig. 4).

For *C. pelagicus*, the inferred change of the residence time of the carbon pool prior to its partial mineralisation does not induce expression of an ¹⁸O vital effect. Near-equilibrium composition of *C. pelagicus* calcite was consistently found under changing temperature and pH conditions (Stevenson et al., 2014; Hermoso, 2015). This lack of an ¹⁸O vital effect is a fundamentally important observation with respect to palaeoclimate studies in deep time, due to the geological importance of this “vital effect-free” species that can be used as a reference.

With this biogeochemical control of ¹⁸O/¹⁶O fractionation in coccolith calcite in mind, it remains difficult to explain the lower magnitude of the ¹⁸O vital effect for the isotopic light group (*C. leptoporus* and *P. placolithoides*). That higher coccolith δ¹⁸O values are recorded with higher ¹⁸O rich CO₂ influx may represent an intuitive reasoning, and reconcile the data. However, their δ¹⁸O values are “capped” by equilibrium values and do not go towards the heavy group end-member as observed in *E. huxleyi* or *G. oceanica*, challenging this hypothesis. As to date we are still unable to identify the biogeochemical

BGD

12, 15835–15866, 2015

Vanishing coccolith vital effects with alleviated CO₂ limitation

M. Hermoso et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Vanishing coccolith vital effects with alleviated CO₂ limitation

M. Hermoso et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



the opposite: the smallest coccoliths are closest to the foraminifera, and the bigger coccoliths show lighter values. This is in agreement with the isotopic typology of coccolith calcite, with the notable difference that in culture, larger cells such as *C. pelagicus* exhibit near equilibrium composition. One possible explanation for this discrepancy between culture and sediment data may be the exacerbation of the vital effect in culture due to highly fertilising growth conditions of coccolithophores exposed to high light and nutrient levels (Hermoso et al., 2015).

The hypothesis by Bolton and Stoll (2013) about a possible “Late Miocene threshold” at about 575–375 ppm of atmospheric (assumed to correspond to a range between 12–19 μM of aqueous CO₂) is expressed in our dataset by a big “jump” in $\delta^{13}\text{C}$ value for *Coccolithus pelagicus* (not seen in $\delta^{18}\text{O}$ values). We observe, however, that through this range, other coccoliths show progressive isotopic trends (Fig. 2a). In our experiments, above a threshold of 10 mM of DIC in the culture medium (corresponding to atmospheric composition of 1600 ppm of CO₂ – *the inferred concentrations that prevailed during the Palaeogene*), there is unsubstantial vital effect in coccolith oxygen isotopes.

Using our empirical calibration between the magnitude of the vital effect with DIC concentration (Fig. 1c) or with equivalent $p\text{CO}_2$ (Fig. 2a and b), we suggest the use of coccolith monotaxic (providing a foraminiferal reference can be measured) to infer palaeo-DIC levels, or exploiting interspecies signals, as the large-small coccolith isotopic offset proposed by Bolton et al. (2012). A notable advantage of the interspecific approach is that other uncertainties that complicate palaeoceanographic reconstructions (salinity, temperature, seawater $\delta^{18}\text{O}$) are cancelled out, as they have, at least to first order, a similar effect on coccolith calcite composition. Considering the arguments presented in this study showing a control by the growth dynamics, the DCUt index derived from best correlated with size and growth rate, but also contains an important environmental parameter sought in palaeoceanography, namely DIC concentrations. As it appears that there is a strong coccosphere size component related to – *and possibly controlling* – the magnitude of the vital effect, especially for $\delta^{13}\text{C}$ values, a coc-

cololith size-based proxy can be used in turn to derive palaeo-DIC concentration in the geological record.

Interspecies $\Delta\delta^{18}\text{O}$ and $\Delta\delta^{13}\text{C}$ offsets with [DIC] can be calculated in the context of the investigated geological period using the data from the present work or those in Rickaby et al. (2010). Although these data can be regarded as robust, based on reproducibility of growth and isotope composition in replicated bioassays and thanks to the very dilute cultures undertaken, we should stress the importance to consider the whole set of environment parameters, as in our study case, light, nutrient and DIC conditions were likely replete with respect to the natural environment. Overall, under the assumption that in culture, growth rate reached their maxima, it would appear that in the natural environment growth rates were lower, and as a consequence the vital effect, especially for the oxygen isotopes, were also lower.

In high DIC (elevated atmospheric CO_2) regimes of ocean history, departures from the unified +1‰ in $\delta^{13}\text{C}_c - \delta^{13}\text{C}_{\text{DIC}}$ values that can be reconstruct with paired coccolith/foraminifera measurements can be used as a proxy for photosynthetic activity in coccolithophores. A “reverse” approach using the present calibration utilising the magnitude of the vital effect, appears possible to derive DCUt estimates that can be, in turn, linked to $[\text{CO}_{2\text{aq}}]$ concentrations. This approach could complement alkenone-derived palaeo- CO_2 estimates by significantly contribute constraining the so-called “*b*” coefficient (Pagani, 2002; Pagani et al., 2005). This novel approach (outlined in Hermoso, 2015; Hermoso et al., 2015) will require coupled foraminiferal data that may serve as inorganic reference (Spero et al., 2003). In addition, it appears possible to reconstruct cell geometry via morphometric measurements made on fossil coccoliths (Henderiks and Rickaby, 2007; Henderiks, 2008; Henderiks and Pagani, 2008), as this parameter is of paramount importance for inferring DCUt in the absence of preserved coccospheres in the sedimentary register, except in some peculiar settings (Gibbs et al., 2013).

Vanishing coccolith vital effects with alleviated CO_2 limitation

M. Hermoso et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



5 Conclusions

This work provides mechanistic constraints on the “*mobilis in mobilis*” nature of the vital effect in coccolith calcite (Hermoso, 2014). We show that the turnover of carbon and differences in growth rates and relative allocation of the internal pool to photosynthesis and calcification (PIC/POC) concurrently set the magnitude of the vital effect in both carbon and oxygen isotope systems. In coccolithophores, the expression of the vital effect is stronger with a small internal carbon reservoir. Several lines of evidence now point towards reduced, if not absent, vital effect under high CO₂ levels, as prevailed during the most of the Meso–Cenozoic. Therefore, the assumption that downcore coccolith $\delta^{18}\text{O}$ can be transferred into SST estimates using the equations outlined in Kim and O’Neil (1997) becomes practical when studying deep time intervals. Due to the complex physiological and environmental control on isotopes in coccolithophores, a fully quantitative modelling approach is now essential.

Since the pioneering studies on coccolith geochemistry in the 1980s (Anderson and Steinmetz, 1981; Steinmetz and Anderson, 1984; Dudley et al., 1986), a growing body of literature highlights the potential for application to palaeoceanography. Recent work shows major steps towards a complete understanding of the vital effect imprinting isotopes of coccolith calcite based on biogeochemistry and physiology, which may “rival” our quantitative understanding of foraminiferal proxies. These studies and the present work point towards the possibility to generate coccolith-derived long term SST reconstruction and/or $p\text{CO}_2$ levels during periods of abrupt climate change, such as the PETM, Cenozoic climate optima or Mesozoic OAEs.

Data availability

The data used for the present study will be made available on the Oxford Research Archive (ORA-data) website (<http://ora.ox.ac.uk>).

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Vanishing coccolith vital effects with alleviated CO₂ limitation

M. Hermoso et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

⏪

⏩

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Vanishing coccolith vital effects with alleviated CO₂ limitation

M. Hermoso et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

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Vanishing coccolith vital effects with alleviated CO₂ limitationM. Hermoso et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

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Vanishing coccolith vital effects with alleviated CO₂ limitation

M. Hermoso et al.

[Title Page](#)

[Abstract](#)

[Introduction](#)

[Conclusions](#)

[References](#)

[Tables](#)

[Figures](#)

[⏪](#)

[⏩](#)

[◀](#)

[▶](#)

[Back](#)

[Close](#)

[Full Screen / Esc](#)

[Printer-friendly Version](#)

[Interactive Discussion](#)

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Vanishing coccolith vital effects with alleviated CO₂ limitation

M. Hermoso et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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Vanishing coccolith vital effects with alleviated CO₂ limitation

M. Hermoso et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

⏪

⏩

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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Vanishing coccolith vital effects with alleviated CO₂ limitation

M. Hermoso et al.

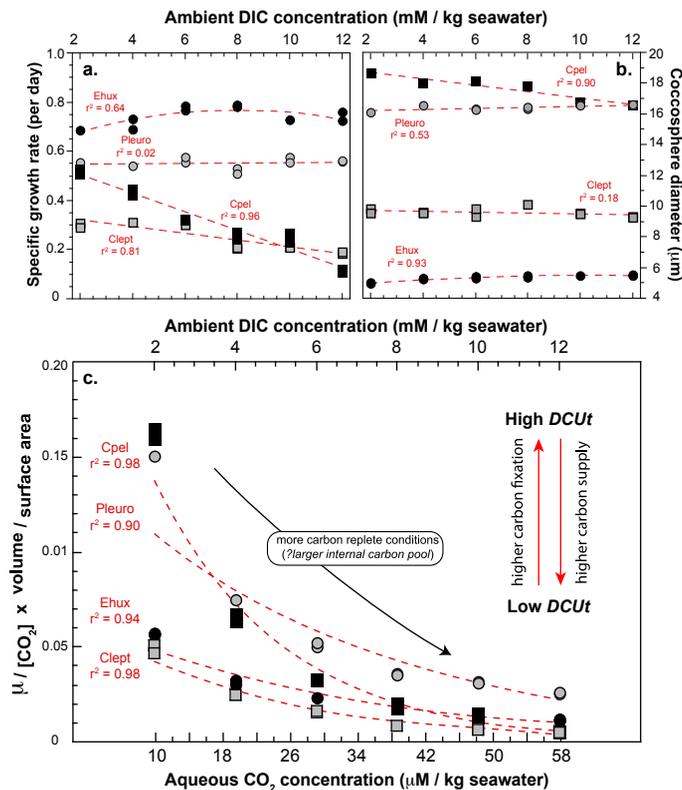


Figure 1. Changes in algae specific growth rates (a), coccosphere diameter (b) and the degree of utilisation of the internal carbon pool (c) on a range of 2 to 12 mM of DIC in the culture medium. Equivalent aqueous CO₂ concentrations in each batch are given for reference at the bottom of panel (c). Key for species is inset at the top of the figure.

Vanishing coccolith vital effects with alleviated CO₂ limitation

M. Hermoso et al.

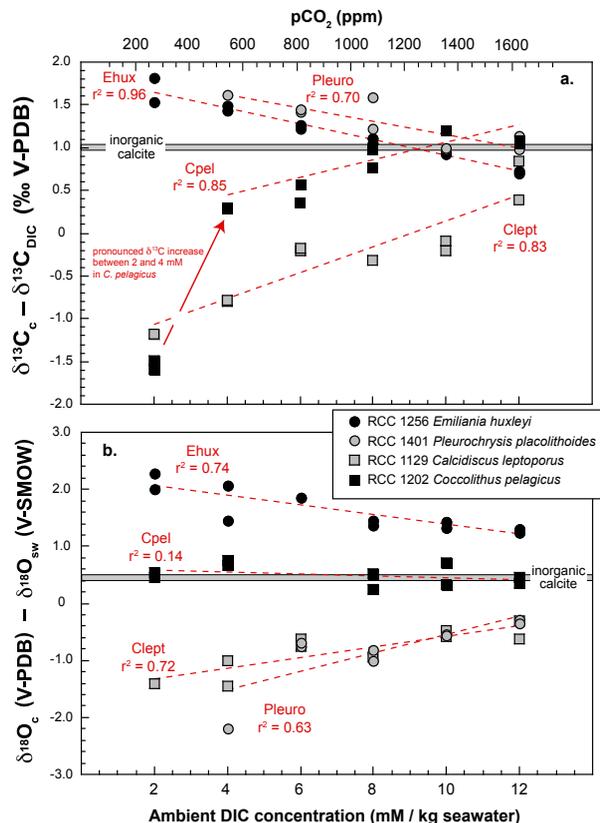


Figure 2. Changes in coccolith carbon and oxygen isotopes with DIC addition in the culture medium. The results are expressed by isotopic offset of coccolith composition from $\delta^{13}\text{C}_{\text{DIC}}$ for carbon (a) and from medium $\delta^{18}\text{O}_{\text{sw}}$ for oxygen (b). Inorganic calcite references as materialised by the grey horizontal bars on the graphs are calculated according to the equation given by Romanek et al. (1992) and Kim and O’Neil et al. (1997).

Vanishing coccolith vital effects with alleviated CO₂ limitation

M. Hermoso et al.

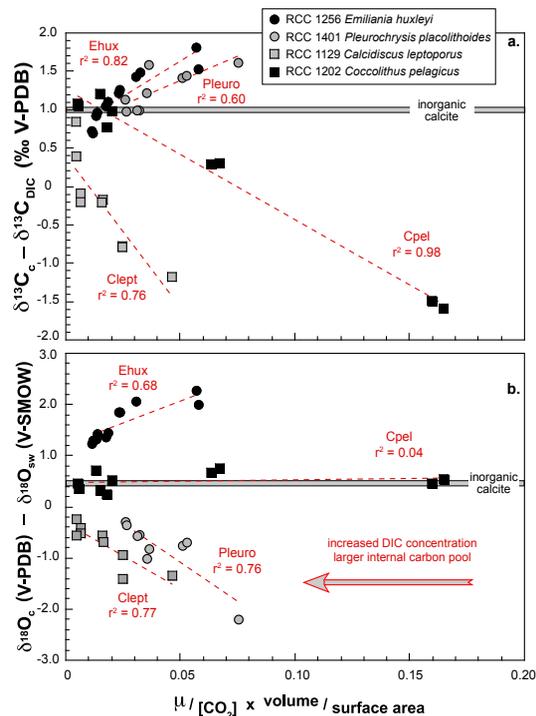


Figure 3. Changes in coccolith carbon and oxygen isotopes with the degree of utilisation of the internal carbon pool (referred to as DCUt index in text – see Eq. 4). We observe relatively good relationship in the evolution of the vital effects via the degree of utilisation of the internal carbon pool in various coccolithophore species. These statistical link are much greater than the simple correlation between “ $\delta - \delta$ ” values and $[\text{CO}_{2\text{aq}}]$, confirming a preponderant role of cell dynamics in the expression of the vital effect.

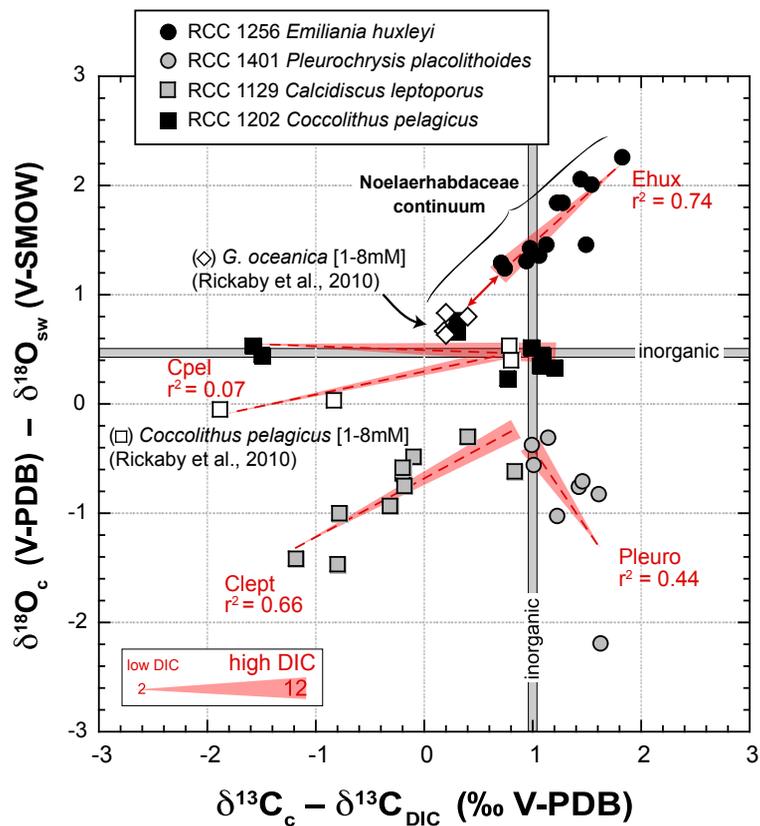


Figure 4. Scatter plot of carbon and oxygen isotopic offsets with increased DIC concentration. Superimposed on the linear regression lines, the wider side of the red triangles denotes higher DIC level. With increased DIC and aqueous CO₂ concentration in the medium, we observe a clear decrease in the magnitude of isotopic disequilibria in both carbon and oxygen systems, with coccolith isotope compositions converging towards inorganic (equilibrium) composition.