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Environmental control of the isotopic composition of subfossil coccolith calcite: Are laboratory culture data transferable to the natural environment?

Michaël Hermoso [a,*], Yaël Candelier [b,c], Thomas J. Browning [a,1], Fabrice Minoletti [b,c]

[a] University of Oxford – Department of Earth Sciences, South Parks Road, Oxford OX1 3AN, United Kingdom
[b] Université Pierre et Marie Curie – UMR 7193 Institut des Sciences de la Terre de Paris, 4 Place Jussieu, 75005 Paris, France
[c] CNRS – UMR 7193 Institut des Sciences de la Terre de Paris, 4 Place Jussieu, 75005 Paris, France

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ABSTRACT

Coccoliths contribute significantly to pelagic sediments formed over the last 200 million years, yet their geochemistry has been largely overlooked as a potential record of palaeoenvironmental information. Recently developed techniques have enabled successful extraction of coccolith-dominated sediment fractions. However, the reliability of palaeoenvironmental interpretations that can be drawn from coccolith analyses is still confounded by a poor understanding of the "vital effect" – the physiological component of the isotopic composition of biominerals. Here we demonstrate that oxygen isotope composition in core-top coccoliths is not only set by the temperature and isotopic composition of seawater, but appears to be controlled to first order by the environmental factors regulating algal growth rate. Partial registration of the isotopic signature of assimilated CO_2 (with a heavy isotopic composition relative to other dissolved inorganic carbon species) is confirmed to be the dominant mechanism behind the vital effect recorded in the Noelaerhabdaceae coccoliths. Our data point towards a strong role of growth irradiance on expression of the ^{18}O and ^{13}C vital effects, ranging from limited (near equilibrium composition) in low light regimes to 3‰ offset in oxygen isotopes at higher growth irradiances, such as those found under light-saturated conditions typically imposed in laboratory cultures. This highlights the importance of considering environmental controls when translating oxygen isotope composition of coccoliths into temperature estimates. Furthermore, our calibration suggests that the alkenone-based CO_2 palaeobarometer proxy may be refined by combining paired organic/calcite measurements during the Cenozoic.

1. Introduction

Studies in the 1980s demonstrated substantial isotopic offset of fine sediment fraction (assigned to coccolith signals) from species-specific foraminifera analyses during the Late Pleistocene [1,2]. This discrepancy, and the difficulty in attaining species-specific assemblages of coccoliths, have led to very few attempts to utilise coccolith signals in palaeoceanography relative to foraminifera-based studies. New separation methods mean it is now possible to extract coccolith-dominated assemblages from pelagic sediments [3,4]; thus it is now necessary to determine whether their geochemistry, and in particular their oxygen isotope composition can be used in palaeoceanographic studies or if primary (seawater) signals, such as sea surface temperatures (SSTs), are partially or completely overwritten by the vital effect.

Culture studies of coccolithophores indicate that the magnitude of the interspecific vital effect for the oxygen isotopes ($\delta^{18}O$) can reach 5‰[5–10] (Fig. 1). In contrast with other marine biomineralisers such as the foraminifera, coccolithophores exhibit a particularly large vital effect because biomineralisation occurs intracellularly [11]. Recent work by Bolton and Stoll [12] has provided insightful constraints on the vital effect recorded in coccoliths for the carbon isotope system. They suggested an evolutionary control of the vital effect in coccolithophores resulting from decreased pCO_2 over the Cenozoic and the consequent emergence of CO_2-concentrating mechanisms (CCMs) operating to maintain a sufficient intracellular carbon pool [13]. The behaviour of oxygen isotopes within coccolith calcite remains, however, largely unexplained. Only empirical calibrations between external (environmental) forcing and the oxygen isotopic composition of phytoplanktonic calcite have been attempted [8,14]. A quantitative comprehension of the ^{18}O vital

* Corresponding author. Tel.: +44 (0) 1865 272003.
E-mail address: Michael.Hermoso@earth.ox.ac.uk (M. Hermoso).
[1] Present address: GEOMAR Helmholtz Centre for Ocean Research Kiel, Wischhofstraße 1-3, D-24148 Kiel, Germany.

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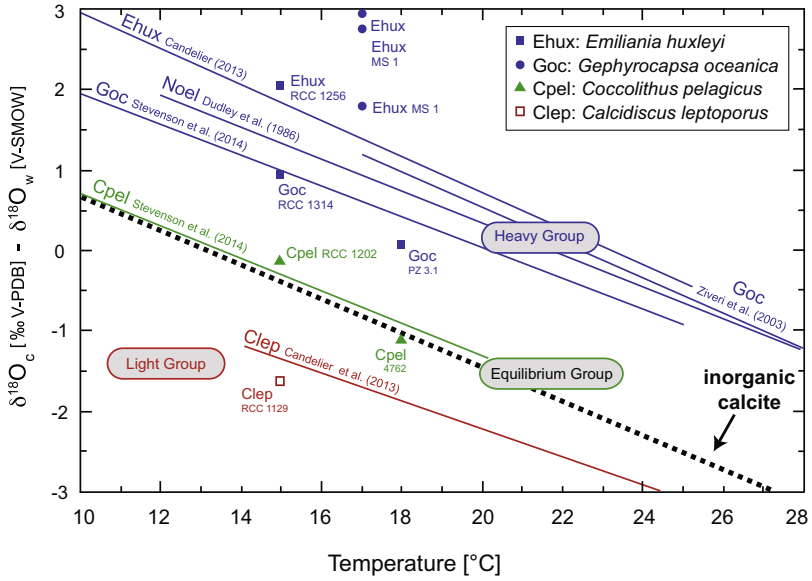

Fig. 1. Compilation of published oxygen isotope composition of coccolith calcite ($\delta^{18}O_c$ in ‰ V-PDB) grown in the laboratory at different temperatures. Lines represent the ordinary least squares fit for calibrations. The composition of the inorganic calcite (*sensu* [25]) was calculated using Eq. (3) for an isotopic composition of seawater ($\delta^{18}O_w$) of +0.5‰ in ‰ V-SMOW, and is denoted by the bold dotted line. Coccoliths studied in this work, with oxygen isotope composition above the inorganic line, are assigned to an isotopic "heavy group" [5]. The offset from inorganic calcite (≈ equilibrium) towards positive $\delta^{18}O_c$ values can be considerable (up to 3‰). Source of data are specified in the legend inset; otherwise for individual temperature measurements: 15 °C: [9]; 17 °C: [6]; 18 °C: [7]. Figure simplified from [24].

effect has not been addressed in the recent cellular models developed by Bolton and Stoll [12] and Holtz et al. [15]. A geochemical characteristic of the coccoliths of the Noelaerhabdaceae family (including the present-day dominant species *Emiliania huxleyi* and *Gephyrocapsa oceanica*) in culture is that they precipitate calcite with positive $\delta^{18}O$ with respect to inorganic calcite (up to +3‰), and as such, have been attributed to an isotopically "heavy group" [5] (Fig. 1). In contrast, downcore investigation of oxygen isotope signatures measured from Noelaerhabdaceae-dominated coccoliths indicates that the representatives of the family in the Neogene (the reticulofenestrids) are relatively close to equilibrium [16]. This divergence between culture and field approaches represents a potential 3‰ offset in $\delta^{18}O$ (equating to ~12 °C), and therefore justifies the need to better constrain the vital effect in coccoliths and the underlying cause of this significant discrepancy.

2. Methods

The present study used Noelaerhabdaceae coccolith assemblages comprising *G. oceanica* and *E. huxleyi* microseparated from core top sediments to attempt a calibration of oxygen isotope composition in coccoliths with oceanic temperatures and ancillary oceanic parameters that may influence $\delta^{18}O$ of calcite.

2.1. Core top sediments and microseparation

We attempted to process 23 core top sediments from the Atlantic and Indian Oceans (Fig. 2). Sediment samples were processed using the protocol described in [4] based on cascade microseparation steps through screen membranes with well-calibrated apertures. Noelaerhabdaceae coccoliths were concentrated in fractions smaller than 3 µm (Figs. 3 and 4). However, the finest fraction of sediments also contained fragments of larger coccoliths, specimens of *Florisphaera profunda* and the so-called "micarbs" [4]. As none of the originally obtained assemblages were sufficiently concentrated in Noelaerhabdaceae coccoliths, we added a purification step based on short centrifugation runs (4000 rpm for 45 s). The supernatant containing the micarbs and fragments were discarded and the pelleted fraction was repeatedly processed in the same way until sufficient Noelaerhabdaceae purity (>90 *wt%*) was obtained (Fig. 4). Pellets were eventually re-filtered onto a 2 µm screen membrane. Final fractions were quantified for their calcite particle content under a Zeiss AxioImager M1 light microscope (1575× magnification) using circular polarisation of light [17] (Table S1).

2.2. Physical and chemical parameters of the mixed-layer

Annual and monthly-averaged modern day temperatures, oxygen composition of seawater ($\delta^{18}O_w$), and pH for each core top site were extracted from the World Ocean Atlas 2009 gridded 1° × 1° dataset (http://odv.awi.de/en/data/ocean/world_ocean_atlas_2009/) [18,19] (Table S2). Annual average mixed-layer depth at each site was extracted from the gridded 2° resolution mixed-layer depth climatology of [20]. The mixed layer depth criterion in this data product is the depth of the 0.03 kg m^{-3} density increase relative to that at a 10 m reference depth (derived from ARGO float profiles). To assess for any seasonal bias, we calculated "productivity-weighted" sea surface temperatures using the approach of Müller et al. [21], where the productivity proxy used was either monthly-averaged surface chlorophyll (SST_{PROD_ORG}) or calcite (SST_{PROD_PIC}) retrieved from remote sensing data products (Eq. (1)):

$$SST_{PROD} = \frac{\sum_{i=1}^{i=12} P(i)T(i)}{\sum_{i=1}^{i=12} P(i)} \quad (1)$$

where P is either chlorophyll a concentration (mg m^{-3}), or particulate inorganic carbon concentration (mol m^{-3}), T is temperature (°C), and i is calendar month.

Annual average above sea surface photosynthetically available radiation (PAR, moles photons m^{-2} day^{-1}) and the vertical diffuse attenuation coefficient at 490 nm ($K_{d(490)}$, m^{-1}) at each site were retrieved from the respective MODIS annual average climatologies available from NASA (http://oceancolor.gsfc.nasa.gov/). MODIS satellite remote sensing data products were averages for 2003–2013

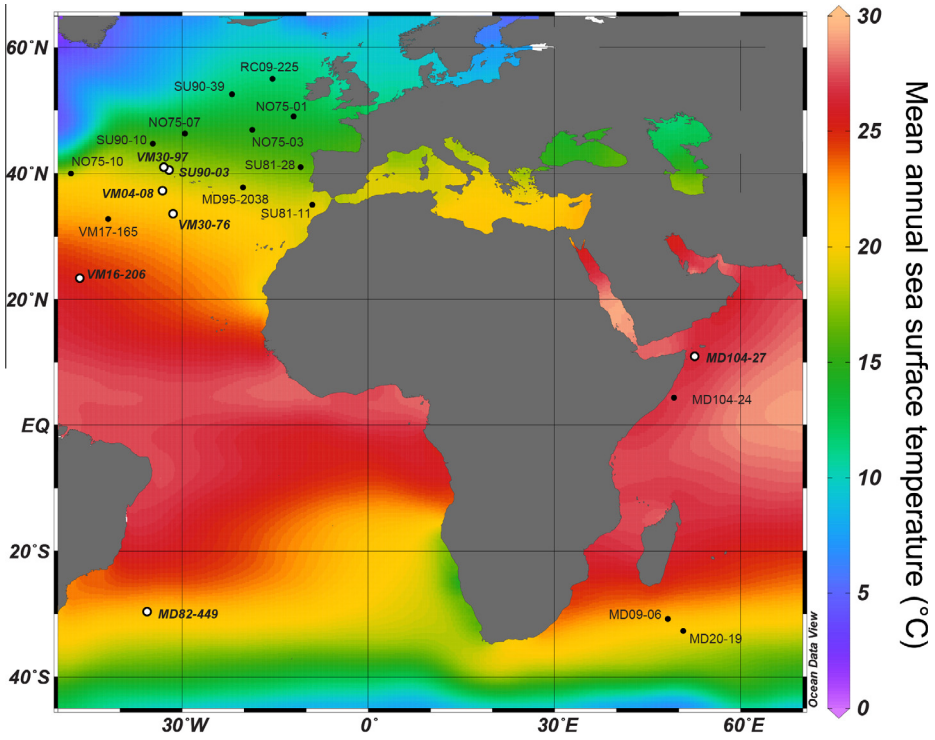

Fig. 2. Map showing mean annual sea surface temperatures and the locations of attempted core top sediments. From the 23 sites processed with the microseparation, only seven (denoted by the larger and white circles and bold text) have successfully provided near (>90 *wt%*) monotaxic Noelaerhabdaceae fractions (see Table S1 for purities). The map was generated using Ocean Data View (http://odv.awi.de).

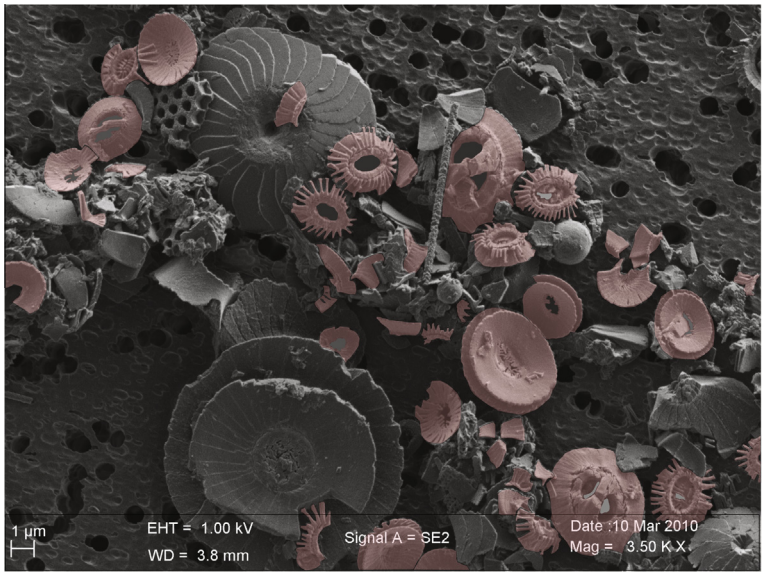

Fig. 3. Scanning electron micrograph of core top MD95-2038 (bulk sediment) in the North Atlantic Ocean. Coccoliths (and fragments) of the Noelaerhabdaceae family are coloured in pink with *Gephyrocapsa oceanica* characterised by a bridge over the central area, and the relatively small *Emiliania huxleyi* with slits between elements of the distal shield. Other calcareous nannofossils comprise *Calcidiscus leptoporus* coccoliths and laths of *Florisphaera profunda*. Scale bar is inset. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(MODIS lifetime), downloaded from the NASA ocean color website. A mean average mixed-layer PAR ($\overline{\mathrm{PAR}}_{\mathrm{mixed\text{-}layer}}$ in Eq. (2)) was estimated using these estimates of PAR and $K_{\mathrm{d}(490)}$, alongside the modelled mixed-layer depths described previously (*e.g.*, [22]):

$$\overline{\mathrm{PAR}}_{\mathrm{mixed\text{-}layer}} = \frac{\mathrm{PAR}}{K_{\mathrm{d}(490)\times\mathrm{MLD}}}\left(1 - e^{-K_{\mathrm{d}(490)}\times\mathrm{MLD}}\right) \quad (2)$$

2.3. Isotopic measurements, inorganic reference and quantification of the vital effect

Bulk sample and Noelaerhabdaceae coccoliths fractions were measured for their carbon and oxygen isotope ratios using a Delta V Advantage isotope mass spectrometer fitted with a Kiel IV carbonate device at UPMC, Paris. Around 20 µg of homogenised

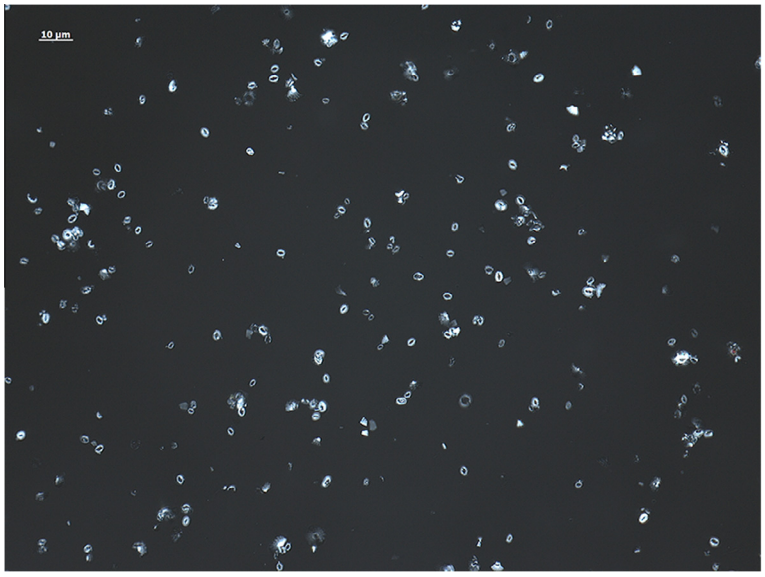

Fig. 4. Micrograph of smear slide showing purified Noelaerhabdaceae coccoliths site VM04-08 in the North Atlantic. The image was taken using a circular polariser [17] at a magnification of 1575×. Scale bar is inset.

samples were purified with orthophosphoric acid at 70 °C. Calibration to V-PDB standard via NBS-19 was made using the in-house Carrara marble standard (Marceau). Reproducibility of replicated standards was better than 0.1‰ for $\delta^{13}C$ and $\delta^{18}O$.

The offset between oxygen isotope composition of Noelaerhabdaceae coccoliths ($\delta^{18}O_{Noel}$) and seawater ($\delta^{18}O_w$) is commonly used in biogeochemical and palaeoceanographic studies to estimate the magnitude of fractionation between the fluid and the mineral [5,23], but this offset does not integrate the temperature control on the magnitude of oxygen isotope fractionation. Conversely, the isotopic departure of coccolith calcite from inorganic calcite can be used to quantify the vital effect [6,24]. The composition of the inorganic calcite (expressed in ‰ V-PDB) was calculated using the equation given in [25] accounting for a pH effect of 1.1‰ in $\delta^{18}O_c$ per pH unit [26]:

$$\delta^{18}O_{inorganic} = (0.0009 \times T^2) - (0.2468 \times T) + 3.7434 - (1.1 \times [pH - 7.8]) + (\delta^{18}O_w - 0.27) \quad (3)$$

where T is the sea surface temperature (°C) and $\delta^{18}O_w$ the isotopic composition of seawater (in ‰ V-SMOW), both retrieved from oceanic databases. The $\delta^{18}O_{inorganic}$ is expressed as ‰ in the V-PDB scale via the −0.27 coefficient to account for the V-SMOW into the V-PDB scale conversion [27].

Subsequently, the magnitude of the vital effect for the oxygen isotopes (V-PDB) was calculated as:

$$^{18}O \text{ Vital effect} = \delta^{18}O_{Noel} - \delta^{18}O_{inorganic} \quad (4)$$

where all parameters are expressed in ‰ V-PDB. The composition of $\delta^{18}O_{inorganic}$ is given in Eq. (3). The oxygen isotope composition of Noelaerhabdaceae coccoliths ($\delta^{18}O_{Noel}$) was measured from purified coccolith core top assemblages.

3. Core-top calibration of oxygen isotope composition in coccolith calcite

3.1. Oxygen isotope composition and sea surface temperature

Only 7 assemblages were successfully processed to final *Gephyrocapsa* spp. and *E. huxleyi* contents of >90 *wt%* of total calcite particles (see composition of fractions in Table S1). This result may appear surprising given the substantial abundance of these species, yet was a result of the presence of other calcite components in the finest sediment fractions.

The raw isotopic composition of Noelaerhabdaceae assemblages ($\delta^{18}O_{Noel}$) was compared to anticipated physical and chemical seawater parameters retrieved from modern-day records (Table S2). Using these data, we first estimated the magnitude of fractionation by considering the isotopic offset between coccolith and seawater oxygen composition ($\delta^{18}O_{Noel}-\delta^{18}O_w$). Using mean annual mixed-layer temperatures above each core site we found that the "$\delta^{18}O_{Noel}-\delta^{18}O_w$" values did not appear to record any temperature dependence (Fig. 5a). In fact, the oxygen isotope compositions of Noelaerhabdaceae from core tops are actually more negative and overall closer to the inorganic reference relative to culture data.

3.2. Sensitivity study: Effect of seasonality and calcification depth on the calibration

We investigated for possible bias arising from the use of mean annual temperatures, which as a result of seasonality in coccolithophore productivity, may not accurately reflect the temperature the phytoplankton grew and calcified at. Seasonal variability in the production of *E. huxleyi* has been reported for *in situ* studies in the North Atlantic (*e.g.*, [28]). However, this limited number of studies did not report the same seasonal patterns over the course of a year, meaning there was no clear indication of exactly which season (if any) calcite production was dominated in. As an alternative means to account for seasonality, we calculated a mean annual temperature weighted towards months where either remotely sensed chlorophyll or particulate inorganic carbon (PIC) was more dominant ("SST_{PROD}", Eq. (1); Table S3). Neither correcting for seasonality in coccolith production using PIC- and ORG-SST_{PROD} estimates reconciles the isotopic fractionation found in our core top calibration (all p-values > 0.65) (Fig. 5a). Similarly choosing parameters for either surface waters, the entire integrated mixed-layer depth, or the base of the mixed-layer for the calibration did not improve the correlation of $\delta^{18}O_{Noel}$–$\delta^{18}O_w$ values with temperature (Fig. S1).

This lack of correlation challenges the use of the $\delta^{18}O$ proxy to record past sea surface temperatures using Noelaerhabdaceae coccoliths. Successful culture and core top calibration has been achieved for the cosmopolitan coccolithophore species *Calcidiscus*

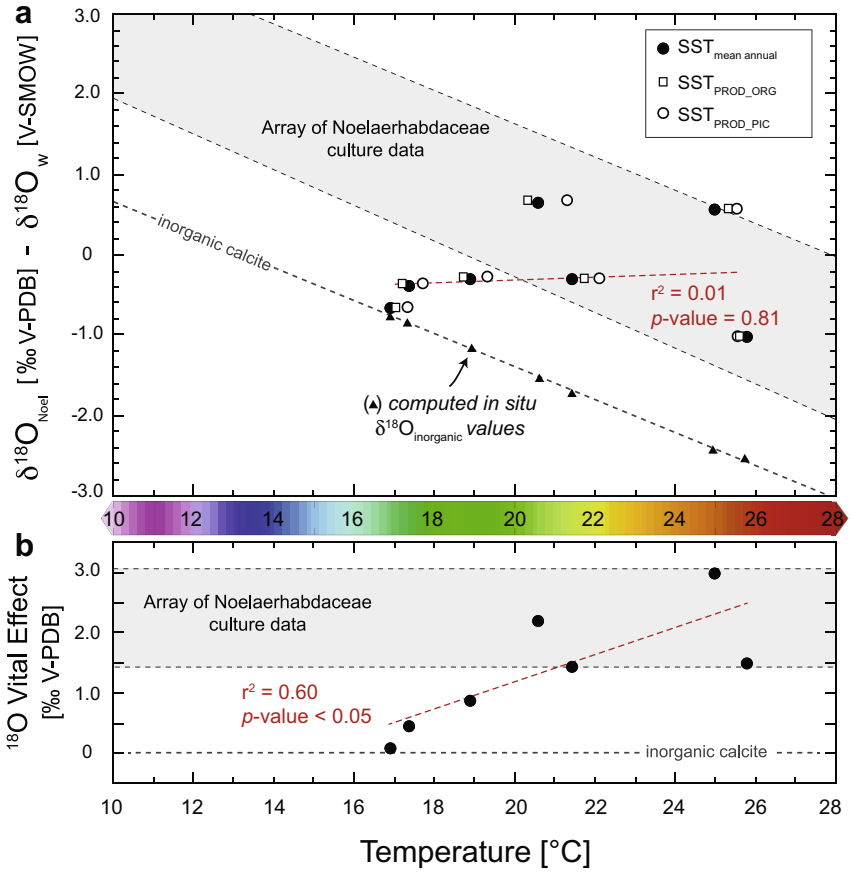

Fig. 5. Oxygen isotope fractionation factor and the magnitude of the vital effect of Noelaerhabdaceae core top assemblages with temperature. Panel (a): Magnitude of ^{18}O fractionation estimated by the offset between coccolith calcite (V-PDB) and seawater (V-SMOW) isotopic compositions for mean average annual mixed-layer temperature; and examining the effect of the seasonality in production either via monthly chlorophyll or calcite contents; termed $PROD_{ORG}$ and $PROD_{PIC}$, respectively (legend inset upright). The red dash line represents the linear fit on mean annual temperatures. The grey shaded area represents the range of ^{18}O fractionation from published culture experiments (see Fig. 1). *In situ* composition of equilibrium calcite is denoted by the black triangles. We found no systematic link between the oxygen isotope composition of the assemblages and their relative abundance in *E. huxleyi* and *Gephyrocapsa* spp. (Table S1). Panel (b): Magnitude of the vital effect (as defined in Eq. (4)) obtained from the isotopic difference between coccolith calcite (V-PDB) and inorganic calcite (V-PDB) calculated using Eq. (3) for mean annual mixed-layer temperature. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

leptoporus with a good agreement between temperature and coccolith $\delta^{18}O$ [14]. This would suggest that, in our study case, an additional component of the vital effect other than temperature is present in the coccoliths of the isotopic "heavy group" being examined here.

3.3. Seeking a connection between the vital effect and environmental parameters

The offset between $\delta^{18}O_{Noel}$ and $\delta^{18}O_{inorganic}$ represents the physiological imprint (vital effect) on the oxygen isotopes in coccolith calcite (Eq. (4)) that may obscure the temperature control on oxygen isotope fractionation. Calculating and removing the thermodynamic effect from core top coccoliths, we observe a positive correlation between temperature and the residual $\delta^{18}O$ that corresponds to the vital effect (p-value < 0.05) (Fig. 5b). This correlation suggests that the magnitude of the vital effect itself is correlated with temperature, with more positive $\delta^{18}O$ values at higher temperatures. All culture data generated in previous studies conflict with this observation, with each individual calibration line paralleling that of inorganic calcite, implying a constant magnitude of the ^{18}O vital effect across the examined temperature ranges (Fig. 1). This leads us to suggest that the vital effect is perhaps not strongly temperature dependent, being regulated instead by another environmental parameter. We found no significant correlation between $\delta^{18}O_{Noel}$ and $\delta^{18}O_w$ values and salinity, alkalinity, dissolved inorganic carbon (DIC) concentration, pH or macronutrients concentrations (Table S2). The "carbonate ion effect" found in culture on *C. leptoporus* [29] can be ruled out as a primary driving mechanism for oxygen isotope composition of coccolith calcite in the natural environment because $[CO_3^{2-}]$ and the magnitude of the ^{18}O vital effect are not correlated. However, we do see a striking correlation between the magnitude of ^{18}O vital effect and (i) the mean annual mixed-layer light intensity ($r^2 = 0.91$; Fig. 6a), and (ii) raw $\delta^{13}C_{Noel}$ values ($r^2 = 0.62$; Fig. 6b).

4. Divergence from laboratory culture data: the effect of growth rate

One may argue whether or not culture data can be confidently transferred to sedimentary coccolith assemblages (*e.g.*, [24,30]). Previously reported magnitudes of the vital effect have typically originated from laboratory experiments conducted under irradiance-saturated, nutrient-replete conditions, with no ecological competition with other species or phytoplanktonic groups for DIC and light resources. Hence, culture-based isotopic fractionation factors likely correspond to measurements obtained under maximum growth rates. In these culture studies oxygen isotope compositions consistently have a substantial offset towards positive $\delta^{18}O$ values (Fig. 1). The few culture studies that have been

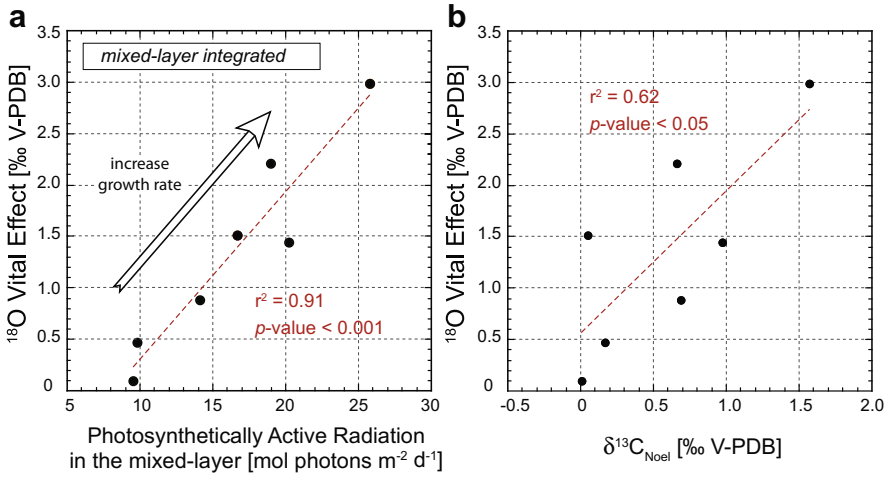

Fig. 6. Relationship between the vital effect imprinting the oxygen isotope composition of coccoliths and light availability in the mixed-layer and carbon isotope composition of calcite. Panel (a): The correlation between ^{18}O vital effect ($\delta^{18}O_{Noel}$–$\delta^{18}O_{inorganic}$) and light availability (annual average mixed layer irradiance) is interpreted to result from a modulation of the growth dynamics that governs the residence time of the DIC prior to calcification. With relatively low light intensity the growth rate is low and calcite exhibits near-equilibrium isotopic composition for the oxygen isotopes. With higher light irradiance and consecutively higher growth rates, the residence time of CO_2 in the cell is shorter and a memory effect of its ^{18}O-rich isotopic composition is registered into calcite as mineralisation in the coccolith vesicle occurs prior to hydration/hydroxylation of CO_2 aqueous/HCO_3^-/CO_3^{2-} and isotopic re-equilibration with the oxygen atoms of water molecules. Panel (b): There is a correlation between the ^{18}O vital effect and $\delta^{13}C_{Noel}$ that provides compelling evidence for an effect of phytoplankton physiology (via growth rate) on the magnitude of stable isotope fractionation in coccolith calcite. Under culture conditions where growth rates reach maximum values due to nutrient, DIC and light saturation, calcite is significantly offset from equilibrium leading to more positive $\delta^{18}O$ and $\delta^{13}C_{Noel}$ values (see Fig. 1).

conducted below light saturation levels have shown the significant influence of light availability on the growth rate of *E. huxleyi* [31,32]. A similar observation has been made for *G. oceanica*, where a ~1‰ modulation of $\delta^{18}O$ was reported [6].

We therefore suggest that the large array of oxygen isotope compositions of Noelaerhabdaceae coccoliths in culture work may originate from maximum growth rates promoted by light saturation at a given temperature. Unfortunately, the scarcity of reported growth rates and light levels along with published isotopic data, together with inter-study differences in experimental set-up prevents us from testing a relationship from existing culture data. In the natural environment, ecological competition for nutrients, DIC and light probably leads to relatively lower coccolithophore growth rates (*e.g.*, [33]), producing coccolith $\delta^{18}O$ that are close to inorganic (equilibrium) conditions. This hypothesis can potentially reconcile the Neogene record [16], core top and culture data. However, the underlying mechanistic link between light-modulated cell division rate and oxygen isotope fractionation has not yet been identified.

5. Vital effect in coccolith: A palaeo-growth proxy?

5.1. Coccolith carbon isotope composition and implication for alkenone interpretations

A link between growth rate and carbon isotope composition in coccolith calcite and phytoplanktonic organic matter has been demonstrated by numerous studies [7,9,34–36]. At the cellular level, high temperatures and light levels lead to elevated photosynthetic rates in *E. huxleyi* [35,37]. An isotopic consequence of this is that carbon isotope fractionation by the enzyme ribulose-1,5-bisphosphate carboxylase (RuBisCO) induces more ^{13}C-enrichement of the remaining intracellular carbon pool and subsequently an isotopically heavier calcite [9,12,15,24]. In the present study, the significant correlation between ^{18}O vital effect and $\delta^{13}C_{Noel}$ (Fig. 6b) provides compelling evidence for an imprint of phytoplankton physiology (*i.e.*, growth rate) on the magnitude of stable carbon and oxygen isotope fractionation in coccolith calcite. Since the coverage in seawater $\delta^{13}C_{DIC}$ in oceanic databases is too sparse to extract data for our sites [38], we were however unable to calculate $\delta^{13}C_{Noel}$–$\delta^{13}C_{DIC}$ values. Furthermore, it has proved impossible to obtain pre-industrial $\delta^{13}C$ of the DIC averaged over the mixed-layer depth for our core top locations, nor to account for seasonality. Considering the wide range of $\delta^{13}C_{Noel}$ that we have measured (~3‰), differential expression of the "Suess effect" between the studied sites is unable to explain the relatively strong co-variation between ^{18}O vital effect and $\delta^{13}C_{Noel}$ [39,40]. In addition, there is a temperature effect on the $\delta^{13}C$ of the DIC that needs to be considered [41,42]. However, over the range of investigated temperatures in the present study (~9 °C), this thermodynamic equilibrium effect is less than 1‰ on $\delta^{13}C$ values [41]. For these reasons we have not exploited the raw $\delta^{13}C_{Noel}$ values in further calculations, but only their co-variation with oxygen isotope data, making the assumption that the effect of the aforementioned factors are all similar, at least for subtropical gyre conditions.

A positive correlation between phosphate concentrations and $\delta^{13}C$ values has been empirically determined in previous studies for relatively nutrient rich waters, typically >0.2 μM PO_4^{3-} [34]. This relationship represents the basis of the "b" coefficient widely used for reconstructing palaeo-growth rate and deriving $\delta^{13}C_{alkenone}$-based pCO_2 estimates [43,44]. In our study, most of the core top sites originate from oligotrophic gyre environments (<0.15 μM PO_4^{3-}; Table S2), representing a possible explanation for the lack of a phosphate/$\delta^{13}C$/growth rate correlation. This was also found for the calibration of Bidigare et al. [34] for the relatively low [PO_4^{3-}] Bahamas time series (*i.e.*, these data points fall off the correlation found at higher PO_4^{3-} concentrations).

Using combined $\delta^{13}C$ and Sr/Ca evidence from the sedimentary record, Rickaby et al. [45] have demonstrated variable coccolithophore productivity between glacial and interglacial cycles in the Pleistocene. A mechanism suggested by Rickaby et al. [45] as a possible explanation for this was variability in insolation driven by eccentricity cycles. If confirmed, this study would represent a potential source of downcore evidence for a modulation of growth rate on stable isotope composition of coccolith calcite. Unfortunately, $\delta^{18}O$ values were not available alongside $\delta^{13}C$ and

Sr/Ca data in this work preventing further investigation of a link between inferred palaeoproductivity and the magnitude of the vital effect.

Finally, we note that in cultured symbiont-bearing foraminifera and corals, the effect of irradiance on the oxygen isotopic composition of calcite has been reported; however the change in $\delta^{18}O$ values is rather small (less than 0.5‰), and the cause elusive [23,46].

5.2. Mechanism for the modulation of the ^{18}O vital effect by growth rate

Intracellular calcification, as occurs in coccolithophores, is accompanied by the formation of protons due to the conversion of bicarbonate to carbonate ions prior to mineralisation [11]. The consequence of this is that higher growth and calcification rates may decrease intracellular pH [7]. As a mechanism for increased $\delta^{18}O$ at higher growth rates, we first hypothesise the expression of a pH-dependence on ^{18}O fractionation at higher calcification rates (Eq. (3)). However, in order to explain a 3‰ change in $\delta^{18}O_{Noel}$, a reduction of 3.5 pH units would be necessary. Such an acidic environment is very unlikely to be occurring in a calcifying biological system thus we rule this mechanism out as a potential control.

A recent hypothesis has been put forward to explain the isotopic "heavy group" characterising the Noelaerhabdaceae coccoliths [9]. For slow growing coccolithophore algae, regardless of which carbon substrate is acquired by the cell (*i.e.*, CO_2 or HCO_3^-) and subsequently used for calcification, complete isotopic re-equilibration of the DIC pool and ambient water molecules is achieved. This is illustrated by the slow growing (*i.e.*, low cellular division rate rather than PIC production) species *Coccolithus pelagicus*, which precipitates calcite with $\delta^{18}O$ values near to inorganic conditions ([10]; see Fig. 1). In contrast fast dividing CO_2-utilising species [47,48], such as *E. huxleyi*, calcite formation may take place before complete oxygen isotopic re-equilibration between DIC and H_2O (a thermodynamic process that occurs within a matter of hours [49]). If calcification occurs prior to loss of the heavy $\delta^{18}O$ signature of the internal DIC pool, coccolith calcite thus becomes enriched in ^{18}O isotopes compared to inorganic calcite. Recent work has demonstrated that in some strains of *E. huxleyi*, a small and variable proportion of the cell's internal carbon pool may be derived from assimilation of bicarbonate ions [11,50]. As HCO_3^- assimilation would not induce substantial isotopic disequilibrium (transient ^{18}O enrichment) of the DIC/H_2O system inside the cell, this effect has to be considered to assess the degree of disequilibrium effect imparted to the vital effect, particularly in modelling studies [15]. We examined a possible link between CO_2/HCO_3^- (hence pH) and the magnitude of the vital effect in our samples, but found no correlation (Tables S1 and S2).

In summary, we suggest that the modulation of the vital effect observed in sedimentary Noelaerhabdaceae coccoliths operates via a tight relationship between growth rate and the residence time of the DIC in the cell. The environmental driver for growth rate, and thus intracellular DIC residence time, appears from our analyses to be the light availability in the mixed layer (Fig. 6a), at least for our core-top sites that mainly originate from subtropical gyre environments.

6. Geological (downcore) implications and conclusions

The geological implication of the physiological control on the magnitude of ^{18}O fractionation in coccoliths is that the $\delta^{18}O$ of preserved coccoliths represents some combination of past seawater temperature and potentially coccolithophore growth rates. Applying an invariant ^{18}O fractionation coefficient for *E. huxleyi* and *G. oceanica* from cultures (as summarised in [24,51]) to all sedimentary Noelaerhabdaceae coccoliths may therefore lead to temperature estimates that are biased significantly low. Using additional evidence, such as that introduced by Rickaby et al. [45] concerning the modulation of growth rate via eccentricity-derived irradiance changes, our hypothesis postulating that $\delta^{18}O$ of coccolith calcite increases during period of more intense productivity could potentially reconcile the discrepancy between the foraminiferal and coccolith records.

The present study develops our understanding of the $\delta^{18}O$ and $\delta^{13}C$ proxies that have been used extensively over the last 6 decades. Our findings have implications for palaeoenvironmental interpretations derived not only from coccolith microseparated assemblages, but more broadly, from bulk carbonate sample analyses that represent the vast majority of published literature in this field. The vital effect contains ecological information that, by adopting a species-specific approach to geochemical analysis of sediments, could potentially be used to refine our knowledge of palaeoenvironments. For example as the Noelaerhabdaceae synthesise alkenones [43,44,52], coccoliths of this family represent an ideal archive for undertaking multiproxy (organic and inorganic) analyses that would fully exploit the predominant Noelaerhabdaceae record of the Neogene, especially with insight into a better characterisation of palaeo-$\delta^{13}C$ of CO_2 and cell growth dynamics.

Future implementation of a comprehensive culture campaign is needed to develop a quantitative link between the magnitude of the vital effect for oxygen isotopes and growth rates and to decouple the combined effect of temperature and growth rate on the modulation of ^{18}O apparent fractionation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.grj.2015.05.002. These data include Google maps of the core top locations presented in this article.

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