

Isotopic record of Pleistocene glacial / interglacial cycles in pelagic carbonates: Revisiting historical data from the Caribbean Sea

Michaël Hermoso

University of Oxford – Department of Earth Sciences. South Parks Road, Oxford OX1 3AN, United Kingdom.

Correspondence to Michael.Hermoso@earth.ox.ac.uk.

Abstract

The glacial / interglacial cycles of the Pleistocene were first recognised by variations in the oxygen isotopic composition of planktonic foraminifera from cores in the Caribbean Sea. Since this pioneering work by Emiliani, this proxy has been extensively applied to a variety of carbonate biominerals over the entirety of the Meso-Cenozoic. However, palaeoceanographic studies have overwhelmingly focused on foraminifera compared to other calcifying microorganism fossils, such as the coccoliths. In this study, I revisit coccolith stable isotopic data obtained from the classic P6304-4 core in light of recent developments in the biogeochemistry of coccolithophores. In particular, I show that the coccolith stable isotope record of the last 13 Marine Isotope Stages (~ 480 kyrs) is significantly biased by large vital effects. The magnitude of coccolith carbon and oxygen isotope vital effects is not uniform, but shows remarkable co-variance with the Vostok CO₂ ice record. During periods of relatively elevated CO₂ (interstadials), the expression of the vital effect is relatively small, whereas it can as high as +3 ‰ for the oxygen isotopes during glacial stadials, which I argue is a result of enhanced CO₂ limitation of coccolithophores. Using this paradigm, I propose that coccolithophore vital effects are not a complicating factor, but rather the signal of interest. As the magnitude of the coccolith vital effect is shown to scale with pCO₂, coccolith carbon and oxygen isotopes may be used in conjunction with foraminifera data to reconstruct and refine aqueous CO₂ concentrations in the past.

Highlights

- Reinterpretation of historical coccolith data with a biogeochemical perspective
- Varying C and O vital effects in *Gephyrocapsa* coccoliths correlate with pCO₂ curve
- Coccolith vital effects stronger during glacials relative to interglacials
- Carbon (aqueous CO₂) limitation confirmed as a cause for coccolith vital effect
- A potential inorganic proxy that may complement alkenone-based pCO₂ reconstructions

Keywords

Coccoliths; Foraminifera; Stable isotopes; Vital effect; Pleistocene; Glacials / Interglacials; Surface water chemistry; Low latitude realm

1. Introduction

Sixty years after Harold Urey and Cesare Emiliani demonstrated the potential of oxygen isotope composition of marine carbonate as a proxy for ocean temperatures (Urey, 1947; Emiliani, 1955), we are still working on refining this geochemical tool. Aside from the phenomenal gain of knowledge of the palaeoclimates that this proxy has permitted, it is laudable that we are continuously developing this isotopic tool to generate more reliable SST estimates (Zeebe et al., 2008; Arbuszewski et al., 2010; Watkins et al., 2013; Minoletti et al., 2014; Prentice et al., 2014; Reghellin et al., 2015; Tripati et al., 2015). It is now possible to isotopically measure individual foraminifera specimens thanks to analytical progress in mass spectrometry, but the interpretation of such high-precision results and how they can be translated into robust SST estimates remains a much larger source of uncertainty. Marine sediments formed in the pelagic environment predominantly originate from the accumulation of foraminifera shells and coccoliths, and their biogenic nature suggests that they are compositionally distinct from theoretical inorganic calcite (vital effect).

Multidisciplinary approaches bridging traditional geosciences, and biological techniques and concepts have greatly contributed to increasing our understanding of stable isotope fractionation in biologically-precipitated calcite. In particular, laboratory cultures of living calcifying organisms have enabled a mechanistic understanding of the vital effect, as well as the development of the alkenone temperature proxy (Dudley et al., 1986; Spero et al., 1997; Grimalt et al., 2000; Ziveri et al., 2003; Hermoso et al., 2014; Hermoso, 2016). On one hand, planktonic foraminifera are relatively easy to separate from the sediment by handpicking. Towed plankton net and core top studies both enabled calibration of the oxygen isotopic composition with the environment (not only temperature) in which the foraminifera are thought to have lived (Bouvier-Soumagnac and Duplessy, 1985; Mulitza et al., 1998). On the other hand, the coccolithophores are relatively easy to culture, but it is more complicated to isolate their fossil remains, the coccoliths, from other carbonate constituents of the sediments due to their microscopic size. Relatively recent techniques based on decanting, settling, microsieving and microseparation of sediment fine fractions allow obtention of near-monotaxic coccolith assemblages (Stoll and Ziveri, 2002; Minoletti et al., 2009).

A series of piston cores (P6304) from the Caribbean Sea have particular historical significance in the development of the $\delta^{18}\text{O}$ proxy since it is from this core material that the pioneering work by Emiliani was conducted (Emiliani, 1966, 1972). Species-specific isotopic analyses of the foraminifera *Globigerinoides sacculifer* led Emiliani to recognise glacial and interglacial periods in the Late Pleistocene. Subsequent work on isotopic analyses performed on the fine fraction in the P6304-4 site investigated the response of another prominent calcium carbonate, perhaps dominant, fraction of pelagic sediments, the coccoliths (Steinmetz and Anderson, 1984).

The present study follows on from the historical work in the Caribbean Sea, and aims to establish how the coccoliths have isotopically recorded a suite of environmental change (temperature, pCO_2 , pH, alkalinity) through the last 13 glacial stages in the Pleistocene. If coccolith $\delta^{18}\text{O}$ strictly follows the trends seen in foraminiferal record in the P6304-4 site, the reason(s) for which the magnitude of the isotopic cycles is much larger and more highly variable in coccolith calcite than in foraminifera remain unexplained thirty years on.

2. Material and Methods

2. 1. The classic P6304-4 core and available isotopic measurements

The P6304-4 site (15°27'N; 70°43'W) is part of a series of piston cores from the Caribbean Sea in 1963 (Fig. 1). Correlations with the other P6304 cores and various other historical sites (A172-6 and V12-122) were obtained by oxygen stratigraphy, but to date, the age model for P6304-4 has not been attempted.

The isotopic ($\delta^{18}\text{O}$ only) data from the foraminifera *Globigerinoides sacculifer* during the last 450 kyrs used in the present study originate from the work by Emiliani (1972). *G. sacculifer* is a key foraminifera species used to retrieve surface water conditions in low latitude realms. The depth habitat of *G. sacculifer* is very shallow, commonly assigned to a range comprised between 20 and 30 m (Multiza et al., 1998; Farmer et al., 2007), hence reflecting the physico-chemical evolution of surface waters.

Coccolith assemblages from the P6304-4 core sediments were purified from the same samples used by Emiliani (Anderson and Steinmetz, 1981). A settling/centrifuging technique has allowed concentrating assemblages consisting “almost exclusively of well-preserved coccoliths” after Anderson and Steinmetz (1991). These microfractions were taxonomically and isotopically analysed (Anderson and Steinmetz, 1981; Steinmetz and Anderson, 1984). Over the entire range of the studied interval, the coccolith assemblages have revealed to be largely dominated by *Gephyrocapsa* spp. (Fig. A.1). Notably, it was argued that the applied microseparation technique has excluded *Emiliania huxleyi* from the microfractions owing to the small size ($< 3\ \mu\text{m}$) of this coccolith species. The preservation of coccoliths was very good, albeit with some evidence of dissolution in samples predating MIS 10 (Fig. 2), as originally reported by Emiliani (1972) and Steinmetz and Anderson (1984).

2.2. Age model and the ODP 999A site

ODP Site 999A (12°45'N, 78°44'W) was drilled during the Leg 165 (Fig. 1). The Pleistocene portion of the core has been extensively studied in recent years (Schmidt et al., 2006; Foster, 2008; Bolton et al., 2012). Available data are abundant, and especially those from *G. ruber* analyses ($\delta^{18}\text{O}$; $\delta^{13}\text{C}$; $\delta^{11}\text{B}$, and B/Ca and Mg/Ca elemental ratios), offering a well-constrained chemostratigraphic and palaeoenvironmental framework in the Caribbean region (Schmidt et al., 2006; Foster, 2008). It is thus possible to utilise the current age model of the Late Pleistocene of the core ODP 999A and the LR04 $\delta^{18}\text{O}$ stack record (Lisiecki and Raymo, 2005; Schmidt et al., 2006) to constrain the age model for site P6304-4, applying classic foraminiferal oxygen stratigraphy methods. Ages of site P6304-4 were assigned from glacial and interglacial $\delta^{18}\text{O}$ extremes, and ages and data interpolated using the AnalySeries 2.0.8 software (Paillard et al., 1996). Present-day differences in mean annual SSTs and oxygen isotopic composition of seawater in the two sites are minor ($< 0.5^\circ\text{C}$ and 0.10 ‰ V-SWOW, respectively), as documented in the last release of the World Ocean Atlas (Locarnini et al., 2013).

2.3. Quantification of the magnitude of coccolith vital effect

The magnitude of the vital effect imprinting biologically-synthesised calcite requires an inorganic reference. Computing such “equilibrium” isotopic values can be easily done in culture experiments, as ambient temperature and $\delta^{18}\text{O}_\text{w}$ are imposed. It is, however, much

more complicated to achieve in a downcore approach. Compelling experimental evidence indicate that *G. ruber* and *G. sacculifer* both precipitate their shell at near-equilibrium oxygen isotope conditions (Bemis et al., 1998). This is a contrasting situation with the coccoliths, which potentially exhibit substantial (up to 3 ‰) departure on both sides of inorganic values (see review by Hermoso, 2014). All culture data indicate ^{18}O enrichment in the species *Gephyrocapsa oceanica* isotope composition (Dudley et al., 1986; Ziveri et al., 2003; Rickaby et al., 2010; Hermoso et al., 2014; Stevenson et al., 2014; Hermoso, 2015).

Exploiting the isotopic offset between coccolith and foraminiferal composition ($\delta^{18}\text{O}_{\text{coccos}} - \delta^{18}\text{O}_{\text{foram}}$) has the advantage to be independent of a number of changing environmental parameters, under the hypothesis that their effects are the same on organisms that live in more or less comparable water masses. It has to be acknowledged that the present study does not account for any substantial contrast in the ecologies of the surface dwelling foraminifera and *Gephyrocapsa* coccolithophores. Noelaerhabdaceae coccolithophores predominantly thrive in upper/intermediate photic zone, whilst other larger species are reported to calcify deeper (Hagino and Young, 2015). Overall, it can be assumed both *Gephyrocapsa* and *Globigerinoides* foraminifera reflect a mixed layer signal, which is characterised by homogenous temperature regime and relatively constant seawater carbonate chemistry. Further, there are no sufficiently detailed data that would enable a correction for a seasonality effect on stable isotope composition of calcite between the two biological groups. It is, however, likely that sediment bioturbation would erase this possible ecological bias from downcore sediments. In culture, it was shown that planktonic foraminifera and *Gephyrocapsa oceanica* coccoliths have similar response on their $\delta^{18}\text{O}$ values with changing carbonate chemistry of the medium (Spero et al., 1997; Hermoso, 2015). In the expression $\delta^{18}\text{O}_{\text{coccos}} - \delta^{18}\text{O}_{\text{foram}}$, changes in temperature, $\text{pH}/[\text{CO}_3^{2-}]$ and $\delta^{18}\text{O}_w$ (salinity and ice volume) characterising glacial-interglacial fluctuations would therefore tend to cancel out each other, forming the basis of a coccolith palaeo-vital effect proxy being presented here and elsewhere (Bolton et al., 2012).

There is an average 0.65 ‰ $\delta^{18}\text{O}$ offset between site P6304-4 *G. sacculifer* and site ODP 999A *G. ruber* compositions over the stratigraphic range examined here, with *G. sacculifer* being more positive than *G. ruber* (Fig. 2). The co-variation of trends is relatively good between the two species ($r^2 = 0.56$; $p\text{-value} \ll 0.00005$). There is no significant difference between glacial and interglacial periods in the evolution of this isotopic offset. Both sites have very similar present-day SSTs and surface water $\delta^{18}\text{O}_w$ values. A temperature control (between sites or by a vertical gradient within the mixed layer) is therefore unlikely to explain the $\delta^{18}\text{O}$ offset between the two species. One difference is that the species *G. ruber* precipitates calcite at equilibrium conditions for the oxygen isotope values, whereas *G. sacculifer* can be 0.8 ‰ offset towards negative $\delta^{18}\text{O}$ values (Spero and Lea, 1993; Bemis et al., 2008). A differential vital effect may thus explain this offset in the foraminiferal record and needs to be considered to express coccolith vital effect.

With this biogeochemical point in mind, the expression of the vital effect in coccolith calcite would require a sedimentary component isotopically close to an inorganic reference. Hence, using *G. ruber* $\delta^{18}\text{O}$ data would appear to be more appropriate. As an alternative, we could use the P6304-4 *G. sacculifer* $\delta^{18}\text{O}$ data from the same site, with a foraminiferal vital effect correction of +0.65 ‰. The same expression of coccolith vital effect, albeit without the 0.65 ‰ coefficient, was implicitly applied by Bolton et al. (2012) on the Pliocene – Pleistocene Transition in site ODP 999A. Whichever means to express the oxygen isotope vital effect is chosen, differences on the trends and magnitude of coccolith oxygen isotope vital effects are

only minor (see Fig. A.2 for a comparison in the expression of the oxygen vital effect alternatively using *G. sacculifer* or *G. ruber* $\delta^{18}\text{O}$ data).

Unfortunately, there are no published carbon isotope data from *G. sacculifer* on the core P6304-4. Expressing foraminifera and coccolith carbon isotope vital effect is usually done using the offset of their carbon isotope compositions and that of dissolved inorganic carbon (DIC) in seawater. In this study, we can only compare *Gephyrocapsa* spp. and *G. ruber* $\delta^{13}\text{C}$ values from site ODP 999A (Schmidt, unpublished data), bearing in mind that coccoliths and foraminifera originate from nearby sites (Fig. 1). Before we can express a coccolith carbon isotope vital effect, we must first convert $\delta^{13}\text{C}_{G. ruber}$ into $\delta^{13}\text{C}_{\text{DIC}}$ estimates following the calibration provided by Spero et al. (2003). The $\delta^{13}\text{C}$ normalisation of *G. ruber* to DIC is obtained by adding a coefficient of +0.94 ‰ to the foraminiferal carbon isotope composition.

3. Results

3.1. Key observations reported by Anderson and Steinmetz (1981)

The authors clearly identified saw-toothed oxygen isotope fluctuations in *Gephyrocapsa* spp. assemblages following the cycles from the *G. sacculifer* oxygen stratigraphy by Emiliani (1972) (Fig. 2). Coccolith $\delta^{18}\text{O}$ systematically exhibit more positive values with respect to the *G. sacculifer* curve. The amplitude of glacial / interglacial $\delta^{18}\text{O}$ variations were reportedly larger in coccoliths ($\Delta\delta^{18}\text{O}_{\text{coccos}} \sim 2.4$ ‰) than in foraminifera ($\Delta\delta^{18}\text{O}_{\text{foram}} \sim 1.2$ ‰). This latter point led the authors to argue that the coccolith record was more suitable to track glacial / interglacial shifts in temperature and $\delta^{18}\text{O}_w$. The reason put forward to explain a greater isotopic variation in coccoliths compared to the foraminifera was a probable dissolution effect of *G. sacculifer* shells analysed by Emiliani. No correlation between coccolith and foraminifera carbon isotopes was found, with the exception of the upper part of the core in which a concomitant $\delta^{13}\text{C}$ decrease of 0.8 ‰ with the $\delta^{18}\text{O}$ decrease of the last deglaciation were registered. This observation implies the lack of a co-variation between raw coccolith $\delta^{13}\text{C}$ / $\delta^{18}\text{O}$ values over the entire interval.

The subsequent companion study by the authors (Steinmetz and Anderson, 1984) refined the taxonomy and the composition of the purified assemblages. This work led to an important finding that there was no change in the species composition between samples corresponding to glacial and interglacial times. They further noted that there was no correlation between measured $\delta^{18}\text{O}$ and the relative abundance of *Gephyrocapsa* in the assemblage, nor with any other species. The preservational state of the coccolith seems to be remarkable in the samples, albeit with some discrete dissolution intervals deeper than 800 cm in the core (MIS 11). Lowered preservation state of foraminifera shells was also observed in coeval sediments spanning the MIS 11 in site ODP 999, hence compromising the validity of Mg/Ca-derived SSTs (Schmidt et al., 2006).

3.2. How did the magnitude of coccolith vital effect evolve over the last 450 kyrs?

3.2.1. Oxygen isotope vital effect

As *Gephyrocapsa* spp. $\delta^{18}\text{O}$ composition is greater than that of *G. sacculifer* and *G. ruber*, the direction of the coccolith vital effect is always positive (relative ^{18}O enrichment), compatible with previously-reported culture data (Dudley et al., 1986). The range of oxygen isotope vital effect over the entire interval is comprised between 1.25 and 3.50 ‰, when expressed with

the ' $\delta^{18}\text{O}_{\text{Gephyrocapsa}} - \delta^{18}\text{O}_{\text{G. sacculifer}} + 0.65 \text{‰}$ ' offset (Fig. 3). The magnitude of these offsets is not constant through time, and the coccolith vital effect seems to follow glacial/interglacial fluctuations, and to be more pronounced during glacials.

3.2.2. Carbon isotope vital effect

The attempt to correlate and interpret coccolith $\delta^{13}\text{C}$ values by Anderson and Steinmetz (1981) lacked an inorganic reference (in the case of the carbon isotope system, $\delta^{13}\text{C}_{\text{DIC}}$). This has been made possible subsequently, thanks to palaeoceanographic and biogeochemical work in the Caribbean Sea (Schmidt et al., 2006; M. Schmidt, unpublished data).

Figure 3 shows large variations in the vital effect imprinting the carbon isotopes in coccoliths. The range of the carbon isotope vital effect is about 2 ‰ throughout the studied interval. Coccolith $\delta^{13}\text{C}$ values are systematically offset towards negative isotopic values (hence a CO_2_{aq} source) compared to foraminiferal calcite, or computed inorganic values (sensu Romanek et al., 1992). Regardless the reasons for which seawater (DIC) $\delta^{13}\text{C}$ changed from glacial to interglacial periods that are outlined in Charles et al. (2010), these variations in the vital effect reflect a coccolith signal more than $\delta^{13}\text{C}_{\text{DIC}}$ variations registered in the foraminiferal isotopic record (standard deviation of 0.36 ‰ and 0.23 ‰, respectively). As a matter of fact, it is noticeable that the carbon isotope vital effect curve follows the oxygen isotope vital effect cycles, especially over the last 250 kyrs. From a more quantitative point of view, the linear correlation is only significant ($r^2 = 0.62$; p -value $\ll 0.00005$) in the more recent interval of the core, namely over the last 130 kyrs. In oldest samples (predating MIS 5), comparable trends between coccolith oxygen and carbon isotope vital effects can be followed on Fig. 3, but the statistical correlation becomes insignificant (it is of 0.12 over the entire interval, yet with a p -value less than 0.005).

4. Discussion

4.1. Biogeochemistry constraints on *Gephyrocapsa* $\delta^{18}\text{O}$ composition (culture calibrations)

In cultures, *Gephyrocapsa oceanica* $\delta^{18}\text{O}$ composition was consistently found to be enriched in ^{18}O relative to inorganic calcite (Dudley et al., 1986; Ziveri et al., 2003; Stevenson et al., 2014). Over a wide range of examined temperatures (10 to 26 °C), coccolith and inorganic curves parallel, implying that the magnitude of the ^{18}O vital effect (not the actual $^{18}\text{O}/^{16}\text{O}$ fractionation) is not temperature dependent with a constant +1 to +1.5 ‰ offset. In the present study, the magnitude of the coccolith oxygen isotope vital effect does not show the trends seen in Mg/Ca-derived SSTs (Fig. 2; Fig. 3), confirming the *a priori* non-dependence of temperature on the vital effect. It is worth pointing out that in this low latitude region, variations in reconstructed SSTs are relatively modest, less than 3 °C between glacial / interglacial terminations (Schmidt et al., 2006) (Fig. 2).

Rickaby et al. (2010) performed a series of laboratory cultures on *Gephyrocapsa oceanica* exposed to changing pCO_2 concentrations. The magnitude of oxygen isotope vital effect decreased with increased CO_2 concentrations ($r^2 = 0.91$ on averaged values), although over a very large carbon concentration range (200 to 1400 ppm). The same biogeochemical control was more recently reported by Hermoso et al. (2016) on the close relative taxon *Emiliania huxleyi*. On a narrower range, more compatible with Late Pleistocene pCO_2 fluctuations, this decrease would be negligible between 200 and 380 ppm CO_2 ($\sim -0.1 \text{‰}$ in $\delta^{18}\text{O}$ values). This ambient CO_2 effect is however stronger on $\delta^{13}\text{C}$ composition, $\sim +0.32 \text{‰}$. It remains difficult

to properly compare monoclonal laboratory culture and sedimentary data due to complexity of parameters operating in the natural environment, whilst in most culture studies, one parameter was manipulated at a time. In the work by Rickaby et al. (2010) or Hermoso et al. (2016), the modulation of the vital effect in coccolith calcite was the result of changing aqueous CO₂ concentrations, with no consideration of other key parameters such as temperature, pH, salinity that typify glacial / interglacial climate contrasts, and perhaps more critically the ecological competition between functional groups and species that also differs between glacial / interglacial periods (Raven and Falkowski, 1999).

Lastly, the so-called “carbonate ion effect” has been reported with similar influence on $\delta^{18}\text{O}$ signatures of planktonic foraminifera shells and *G. oceanica* coccoliths (Spero et al., 1997; Hermoso, 2015). As this concept is relevant for a comparative foraminifera / coccolith study over glacial and interglacial periods, this point provides evidence that the ‘ $\delta^{18}\text{O}_{\text{Gephyrocapsa}} - \delta^{18}\text{O}_{\text{G. sacculifer}}$ ’ offset, hence the magnitude of oxygen isotope vital effect in coccolith calcite is not sensitive to seawater pH / [CO₃²⁻] in the context of the present study, ruling out a at least direct control of carbonate ion concentration on the changing nature of the coccolith vital effects.

4.2. Geological evidence for a strong vital effect component in *Gephyrocapsa* coccoliths

Recent core top investigation of stable isotope fractionation of coccoliths belonging to the Noelaerhabdaceae family (consisting > 80 wt% of *Gephyrocapsa* spp.) has shown the lack of a correlation between oceanic temperatures and $\delta^{18}\text{O}$ values (Hermoso et al., 2015). The results of this natural environment study conflict with aforementioned laboratory data. In the studied core top locations, the range of temperature was relatively large (~ 10 °C), but the range of other key environmental parameters such as [DIC], pH and pCO₂, was much narrower than reproduced in culture experiments. Computed light irradiances in the mixed layer with a strong latitudinal gradient have been shown to scale with the magnitude of oxygen isotope vital effect in *Gephyrocapsa* coccoliths. Hence, a modulation of the biological fractionation by algal growth rate was suggested. In the same core top study, a positive co-variation between the oxygen isotope vital effect and $\delta^{13}\text{C}$ was also reported. Besides demonstrating a growth rate-mediated control on coccolith vital effects, this study has somehow challenged the applicability of culture data to the natural environment, a bias likely resulting from the highly fertilising conditions applied in the laboratory and leading to saturating algal growth rates (see discussion in Hermoso et al., 2015).

Downcore study of the coccolith vital effect conducted by Bolton et al. (2012) suggested a causal link between pCO₂ and coccolith carbon isotope composition with respect to foraminifera on a longer time scale. Relatively good constraints have been provided on the carbon isotope system in coccoliths, but oxygen isotope offsets from inorganic (foraminifera) values remain elusive. For reference, an “isolated” LGM samples has been microseparated in the work by Bolton et al. (2012) on site ODP 999A and a reticulofenestrid (~ *Gephyrocapsa* spp.) assemblage was measured with a ‘ $\delta^{18}\text{O}_{\text{Gephyrocapsa}} - \delta^{18}\text{O}_{\text{G. sacculifer}}$ ’ offset of ~ +2 ‰, a figure that compares well with calculated vital effect in coeval sample of site P6304-4 (Fig. 3).

4.3. Seeking a control on the modulation of the coccolith oxygen isotope vital effect

From our current knowledge on conjugated $\delta^{18}\text{O}_w$ / temperature fluctuations in the Late Pleistocene, it can be established with confidence that the very large (> 2.5 ‰ change) in

coccolith $\delta^{18}\text{O}$ cannot merely reflect compositional changes of surface waters between glacial and interglacials cycles, as postulated by Anderson and Steinmetz (1981). Furthermore, the amplitude in *G. sacculifer* $\delta^{18}\text{O}$ signals produced by Emiliani (1972) and challenged by the authors was in fact pristine. This foraminiferal record is in good agreement with regional reconstructed $\delta^{18}\text{O}_w$ / SSTs, and is also corroborated by comparable variations in diagenetically-screened *G. ruber* and numerous other records (Schmidt et al., 2006).

When Anderson and Steinmetz (1981) produced and interpreted their data, little was known about the control of glacial / interglacials climates, and even less on the biogeochemistry of coccoliths. Only the *avant la lettre* culture work by Dudley and Goodney (1979) existed. The existence of the vital effect in coccoliths was identified, but the modulation thereof as a function of the environment was not. Rather, the vital effect was conceived as an immutable species-specific component imprinting $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ signatures.

The tight correlation between fluctuations in SST, $\delta^{18}\text{O}$ and atmospheric pCO_2 is permitted by the revolutionary work on the Vostok ice core by Petit et al. (1999), amongst a number of subsequent studies. Hence, combining these two aspects of significant research development in palaeoceanography and biogeochemistry, clear similar patterns in the oxygen isotope vital effect and the pCO_2 curve over the last 450 kyr become evident and can be rationalised (Fig. 3).

Least-squares coefficients (r^2) of linear regression between oxygen isotope vital effect and pCO_2 is 0.42 taken the whole dataset (p -value $\ll 0.00005$). The correlation becomes slightly better ($r^2 = 0.51$) when focusing on the interval postdating MIS 11, hence excluding discrete coccolith dissolution intervals (Fig. 2). A number of uncertainties inherent to natural environmental data are prone to blur a primary biogeochemical correlation between ambient CO_2 concentration around coccolithophore algae and the magnitude of oxygen isotope vital effect in coccolith calcite. First, although the purity of the assemblages produced by Anderson and Steinmetz (1981) convincingly indicates *Gephyrocapsa* spp.-dominated signals (Fig. A.1), other coccoliths cohabiting in the obtained microfractions, as *Syracosphaera* spp. may jeopardise the validity of measured signals. In the case of *Gephyrocapsa* spp. assemblages, the presence of other coccoliths would lead to decrease $\delta^{18}\text{O}$ values and hence lower the magnitude of oxygen isotope vital effect (with the exception of *E. huxleyi*, which abundance is insubstantial in the fractions). With the micropalaeontological data available from site P6304-4, it is not possible to propagate the errors inherent to each parameter (primarily the purity of the microfractions and associated isotopic arrays) and determine a cumulative uncertainty in the relationship between the magnitude of the vital effect and atmospheric pCO_2 . Second, the Vostok record reflects high latitude atmospheric levels that may not necessarily be the same than in aqueous CO_2 concentrations in the equatorial realm owing to a temperature and alkalinity control, along with other local / regional oceanographic forcing. On this note, I stress that this correlation is made with atmospheric CO_2 and not with aqueous CO_2 that the coccolithophores utilise. Contrasting seawater pH, salinity and temperature variations during glacial and interglacial periods would need to be combined to reconstruct $[\text{CO}_2]_{\text{aq}}$ via the Henri's law. This would however lead to a circular reasoning, as these parameters have similar effect on both coccolith and foraminifera isotopic compositions, and as such, would appear twice in the ' $[\text{CO}_2]_{\text{aq}}$ / vital effect' correlation. Third, differences in the water depth and seasonality in foraminifera and coccolith calcification may challenge the robustness of ' $\delta_{\text{coccos}} - \delta_{\text{foram}}$ ' as a proxy for the palaeo-vital effects. Altogether, these potential complicating factors may explain that the statistical robustness of our analyses is lower than calibrations produced in the laboratory. But, overall least-square coefficients

obtained in the present work can be regarded as good considering a natural environment study.

It further appears that the Glacial Maxima datapoints stand as outliers in the overall $p\text{CO}_2$ / vital effect correlation (Fig. 4a). Coccoliths formed during these relatively cold periods exhibit higher oxygen isotope vital with a relative ^{18}O -enrichment on the order of 1 ‰ compared to expected values from the global correlation. All other correlations tested between oxygen isotope vital effect and ancillary environmental parameters (temperature, $\delta^{18}\text{O}_w$, $\delta^{18}\text{O}$ of foraminifera and coccoliths themselves) are insignificant. The biogeochemical causes underlying this correlation and the outliers are discussed in Section 4.4.

In the future, it would be extremely valuable to examine a possible correlation between the degree of calcification of the coccoliths, $p\text{CO}_2$ ($\text{CO}_{2\text{aq}}$) concentrations and the magnitude of the vital effect. A clear relationship between the two former parameters was shown by Beaufort et al. (2011) in recent sediments with less calcified coccoliths found during periods of relative high $p\text{CO}_2$ levels. It thus seems plausible that a modulation in the production of calcite by the cells, and at the scale of an individual coccolith may influence the geochemical composition of these biominerals (McClelland, 2015). The nature of this possible control remains to be fully understood, especially given the dual “actor and recorder” function of the coccolithophores and coccoliths through glacial / interglacials dynamics (see recent hypothesis by Omta et al., 2013).

4.4. Biogeochemical forcing of CO_2 on coccolith isotopic composition

4.4.1. Oxygen isotope composition of coccoliths

From a biogeochemical perspective, aqueous CO_2 is the main carbon substrate for marine phytoplankton, and its availability sets organic matter build-up and limits algal growth rate (Riebesell, 2004; Giordano et al., 2005; Reinfelder, 2011). This feature is a prominent difference with the foraminifera predominantly acquiring HCO_3^- and CO_3^{2-} (de Nooijer et al, 2014). The ^{18}O enrichment of the internal carbon pool of coccolithophores relative to ambient seawater due to predominant CO_2 assimilation likely causes oxygen isotopic departure from inorganic and foraminiferal calcite towards positive values in Noelaerhabdaceae coccoliths (Hermoso et al., 2014). The more ambient CO_2 is available around the cell, the less intense the degree of expression of the vital effect (high $\delta^{18}\text{O}$), although this simple link is modulated by cell geometry and growth dynamics (Rickaby et al., 2010; Bolton and Stoll, 2013; Hermoso, 2015; Hermoso et al., 2016). Palaeoecological work indicates lower nutrient supply in surface waters during glacial periods in the Caribbean Sea (Kameo et al., 2004). Along with slight cooling ($\sim -2^\circ\text{C}$ on average) and lowered CO_2 concentrations during glacials, the environment may have led to diminished coccolithophore growth rates compared to interglacials, with possible effect on the dynamics of the internal carbon pool in the cell, and in turn, on the expression of the vital effect. In the present study, the aqueous CO_2 concentration forcing (inferred from variations in atmospheric $p\text{CO}_2$) on coccolith $\delta^{18}\text{O}$ is substantial. Even though the range of Late Pleistocene atmospheric CO_2 is relatively limited (± 100 ppm), with even lower $[\text{CO}_{2\text{aq}}]$ variations, compared to Neogene fluctuations, the modulation of coccolith oxygen isotope composition assigned to this driver appears to be very large (> 2 ‰).

Other key parameters of carbonate chemistry in seawater were contrasting during climatic oscillations of the Pleistocene. This is notably of the case for pH and CO_3^{2-} concentration

(Sanyal et al., 1995; Foster, 2008). The relatively high pH and increased seawater $[\text{CO}_3^{2-}]$ during glacial periods have probably strengthened CO_2 limitation of phytoplanktonic algae as the coccolithophores (see modelling work by Omta et al., 2013). Hence, during the Glacial Maxima, the correlation between aqueous CO_2 and atmospheric CO_2 may have been shifted towards even lower ambient CO_2 levels. This biogeochemical feature is thus isotopically expressed by more pronounced oxygen isotope vital effect (Fig. 4a).

4.4.2. Carbon isotope composition of coccoliths

As noted above, the ambient CO_2 concentrations in seawater to which the coccolithophore cells are exposed to do not correspond to atmospheric pCO_2 levels, as alkalinity, pH and temperature concurrently set the relationship between atmospheric and oceanic (dissolved) pCO_2 . Nevertheless, it can be postulated with confidence that these two parameters co-varied over glacial / interglacial periods (e.g., Foster et al., 2008). The observation of concomitant larger carbon isotope and oxygen isotope vital effects during glacial periods (corresponding to lowered CO_2 concentrations) supports a common control by growth dynamics for the two isotopic systems. A growth rate control in coccolith calcite is more established for the carbon isotopes than it is for the oxygen isotopes (Ziveri et al., 2003; Ennyu et al., 2002; Rickaby et al., 2007). This link also forms the basis of the ‘*b*’ coefficient used in alkenone pCO_2 barometry (Pagani, 2002).

Several lines of evidence indicate a tight coupling between intracellular carbon fixation by photosynthesis and calcification in coccolithophores with large isotopic consequences (Rickaby et al., 2010; Bolton and Stoll, 2013; Hermoso et al., 2014, 2016). It is likely that the large isotope fractionation of carbon partitioned into the organic matter (-25‰ with respect to aqueous CO_2 ; Bidigare et al., 1997) affects the apparent carbon fractionation in coccoliths (Hermoso et al., 2014). This isotopic linkage between photosynthesis and calcification explains the large departure (towards positive values) of coccolith $\delta^{13}\text{C}$ values compared to that of sourced CO_2 .

Measured coccolith $\delta^{13}\text{C}$ signals at site P6304-4 are likely confounded by a strong modulation of carbon isotope fractionation in the organic matter produced by the coccolithophores, as in the study by Bolton et al. (2012) and Reghellin et al. (2015) on older sediments. The magnitude of the photosynthetic ^{12}C carbon depletion of the internal DIC pool sets the apparent ‘ $\delta^{13}\text{C}_{\text{cocco}} - \delta^{13}\text{C}_{\text{DIC}}$ ’ fractionation; the more photosynthesis (\sim primary productivity), the highest $\delta^{13}\text{C}_{\text{cocco}}$ values, mimicking a yet possible isotopic HCO_3^- sourcing. The work by Rau et al. (1991) showed lowered organic $\delta^{13}\text{C}$ values with increased pCO_2 in the Pleistocene. This relationship determined from the last deglaciation matches the observations on the geophyrocapsid record by Anderson and Steinmetz (1981) with the Vostok ice record (Fig. 3; Fig. 4b). To date, we have no experimental or modelling data supporting the same control of ambient CO_2 on the magnitude of fractionation in organic (alkenone) and inorganic (coccolith) carbon. In the future, it would be also particularly interesting to explore the link between alkenone $\delta^{13}\text{C}$ and coccolith $\delta^{13}\text{C}$ over a range of ambient CO_2 concentrations, both in laboratory cultures and in downcore analyses.

4.5. Geological implications: A palaeo- CO_2 proxy?

Bulk calcium carbonate or sediment fine fraction $\delta^{18}\text{O}$ measurements provide biased SST reconstructions with a systematic exaggeration of cooling during glacial periods – a result from the “*mobilis in mobili*” nature of coccolith vital effect that illustrates the fact that the

intensity of this biological phenomenon itself changes under changing environments (Hermoso, 2014). More broadly, the present work strongly confirms that it becomes fundamental to acquire a biogeochemical perspective on interpretations made from the biogenic climatic archive.

Compositional divergences between foraminifera and coccoliths owe to distinct mode of DIC incorporation by the cells ($\text{HCO}_3^- / \text{CO}_3^{2-}$ versus CO_2 , respectively). The relative limited availability of CO_2 in marine settings is a foremost reason for the vital effects, although the biogeochemical causes on isotope systematics are complex and outside the scope of this paper. Nevertheless, from an empirical point of view, the present study documents a natural environment calibration between the magnitude of the carbon and oxygen isotope vital effects, and atmospheric CO_2 concentrations (Fig. 4a; Fig. 4b). This suggests that paired coccolith and foraminifera measurements appear relevant to quantify past CO_2 concentrations in the mixed-layer.

The utilisation of the calibrations presented in Fig. 4a and Fig. 4b with a paleoceanographic aim requires a number of constraints, as those presented in this study and beyond, particularly from ecological contrasts between foraminifera and coccolithophores. Extrapolation of the relationship between *Gephyrocapsa* vital effects and pCO_2 levels (or variations therein) has to be rationalised through a much needed modelling effort including biogeochemical (size of coccoliths) and chemical aspects (ultimately refining the link between atmospheric carbon dioxide concentrations and surface water $[\text{CO}_{2\text{aq}}]$).

Ongoing effort to generate more reliable pCO_2 reconstruction through alkenone carbon isotope analyses (Pagani, 2002) should ideally include coccolith size-separated $\delta^{13}\text{C}$ analyses from Noelaerhabdaceae (*Gephyrocapsa*) assemblages. Under the assumption that coccolith $\delta^{13}\text{C}$ contain a palaeo-growth rate information, as suggested by the present study and others (Rickaby et al., 2007; Hermoso et al., 2015), it will be possible to significantly reduce the uncertainties introduced in the use of a rather “arbitrary” b coefficient to translate ϵ_p coefficient into pCO_2 estimates (Zhang et al., 2013).

5. Conclusions

Sediment fine or size-restricted fractions bear valuable palaeoceanographic (climatic) information. A number of recently-published studies indicate that carbon (CO_2) limitation in marine algae is the main cause of coccolith vital effects. The present study provides compelling evidence for such a control in relatively recent settings, and on both carbon and oxygen isotopic systems. This biogeochemical feature has ramifications in palaeoceanography, as a link between carbon isotopes in coccolithophores and growth rate is also behind the alkenone pCO_2 proxy. The range of aqueous CO_2 fluctuation over the last 480 kyrs, either due to atmospheric CO_2 changes and/or to seawater pH, is accompanied by a substantial modulation of the magnitude of carbon and oxygen vital effects in *Gephyrocapsa* coccoliths. As found in core tops recovered from various latitudes, the oxygen isotope vital effect in *Gephyrocapsa* spp. coccoliths ranges from 1.2 to 3 ‰, confirming the assignment of this species to an isotopic “heavy group” (Dudley et al., 1986), yet highlighting discrepancies between the culture and the sedimentary approaches. Changes in the oxygen isotope vital effect with fluctuations in atmospheric CO_2 concentrations from 180 ppm (low coccolith $\delta^{18}\text{O}$) to 280 ppm (high coccolith $\delta^{18}\text{O}$) seem much more pronounced than reported in the work by Bolton et al. (2012), although in the present study I report genus-specific, also disregarding probable purity artefacts, rather than interspecific vital effects.

Overall, the present study points towards the possibility to use the underexplored geochemistry of the coccoliths as a source of palaeoceanographic information, and especially to reconstruct more reliably ancient CO₂ concentrations in surface oceanic waters. This can be done in the future by exploiting the CO₂ dependence of the magnitude of the vital effect in coccolith calcite *per se*, and transcending existing caveats in the alkenone pCO₂ proxy using $\delta^{13}\text{C}$ composition of the coccoliths.

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Figures

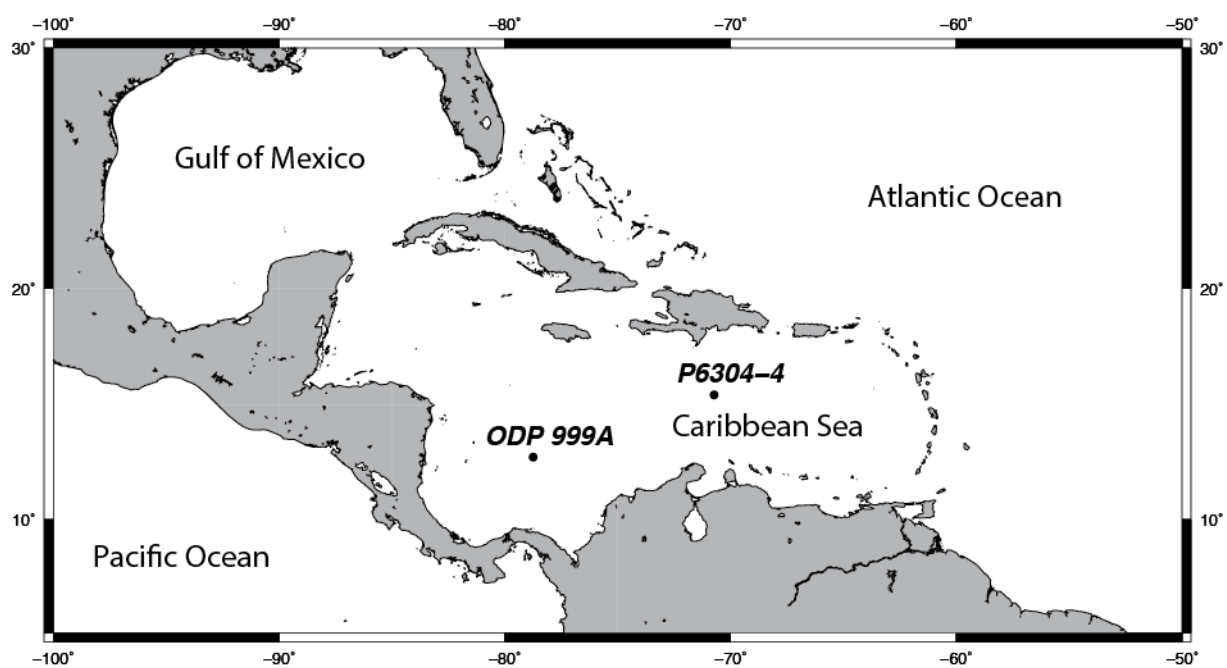


Figure 1: Map showing the location of the P6304-4 and ODP 999A sites in the Caribbean Sea. The map layout was generated using the GMT software (Wessel et al., 2013).

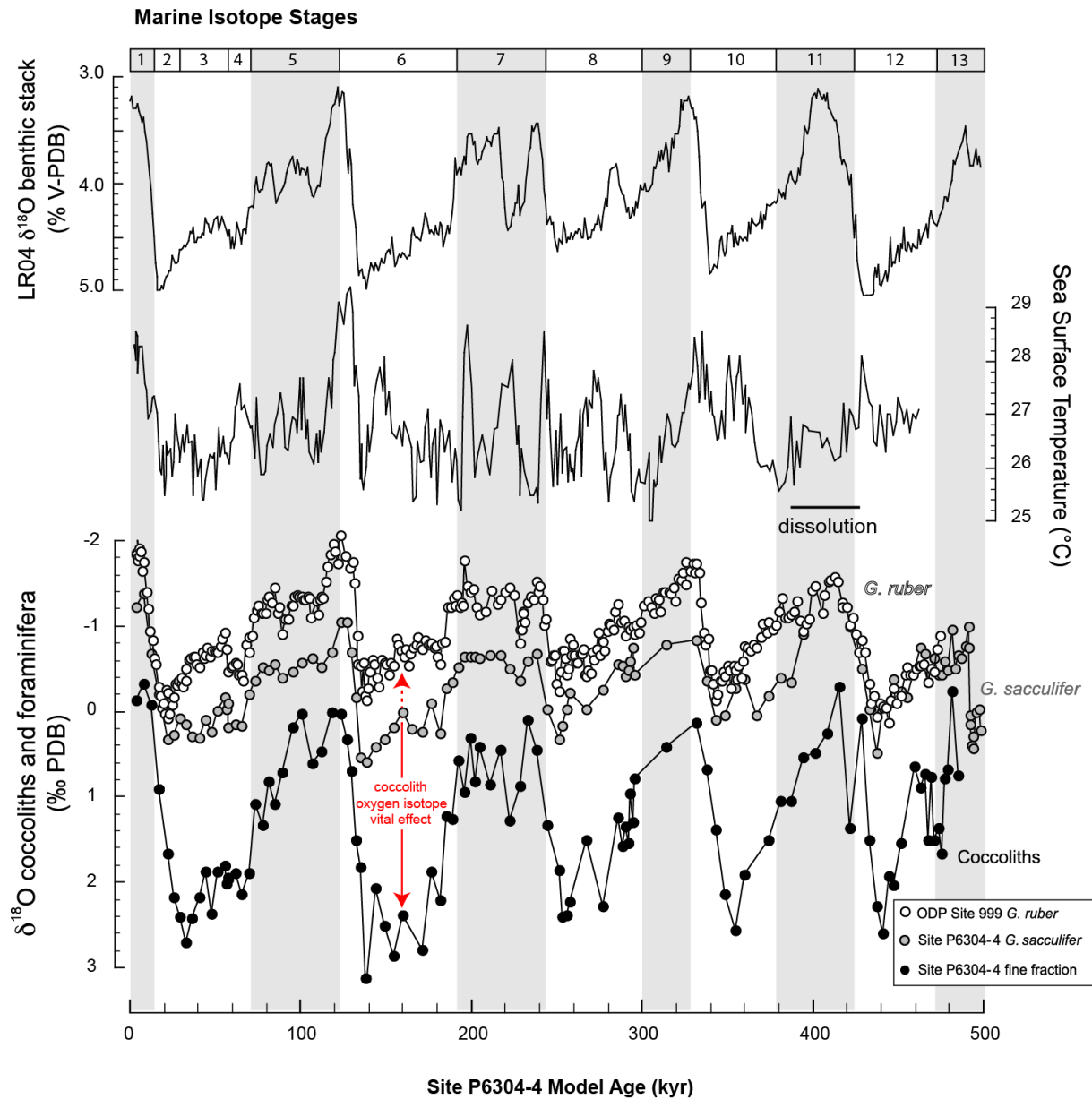


Figure 2: Raw oxygen isotopic data ($\delta^{18}\text{O}_{\text{benthic foraminifera}}$; $\delta^{18}\text{O}_{\text{planktonic foraminifera}}$; and $\delta^{18}\text{O}_{\text{coccoliths}}$) and Mg/Ca-derived SST in the Caribbean Sea over the last 480 000 years. The Marine Isotopic Stages (MIS), defined after the LR04 $\delta^{18}\text{O}$ stack record (panel a), denote glacial and interglacial periods. The grey shaded are represent the interglacials. Data source from top to bottom of the figure: $\delta^{18}\text{O}_{\text{benthic foraminifera}}$ from Lisiecki and Raymo (2005); Sea Surface temperature estimates from Site ODP 999A *Globigerinoides ruber* Mg/Ca ratios (panel b) from Schmidt et al. (2006). Panel c shows from top to bottom: $\delta^{18}\text{O}$ of *G. ruber* from Site ODP 999A from Schmidt et al. (2006); $\delta^{18}\text{O}$ of *G. sacculifer* from Site P6304-4 from Emiliani (1972); $\delta^{18}\text{O}$ of coccoliths from Site P6304-4 from Anderson and Steinmetz (1981). The $\delta^{18}\text{O}$ offset between coccolith and the foraminifera *G. sacculifer* composition (indicated by the vertical red arrow) is used to quantify the magnitude of coccolith oxygen isotope vital effect, as shown in Fig. 3.

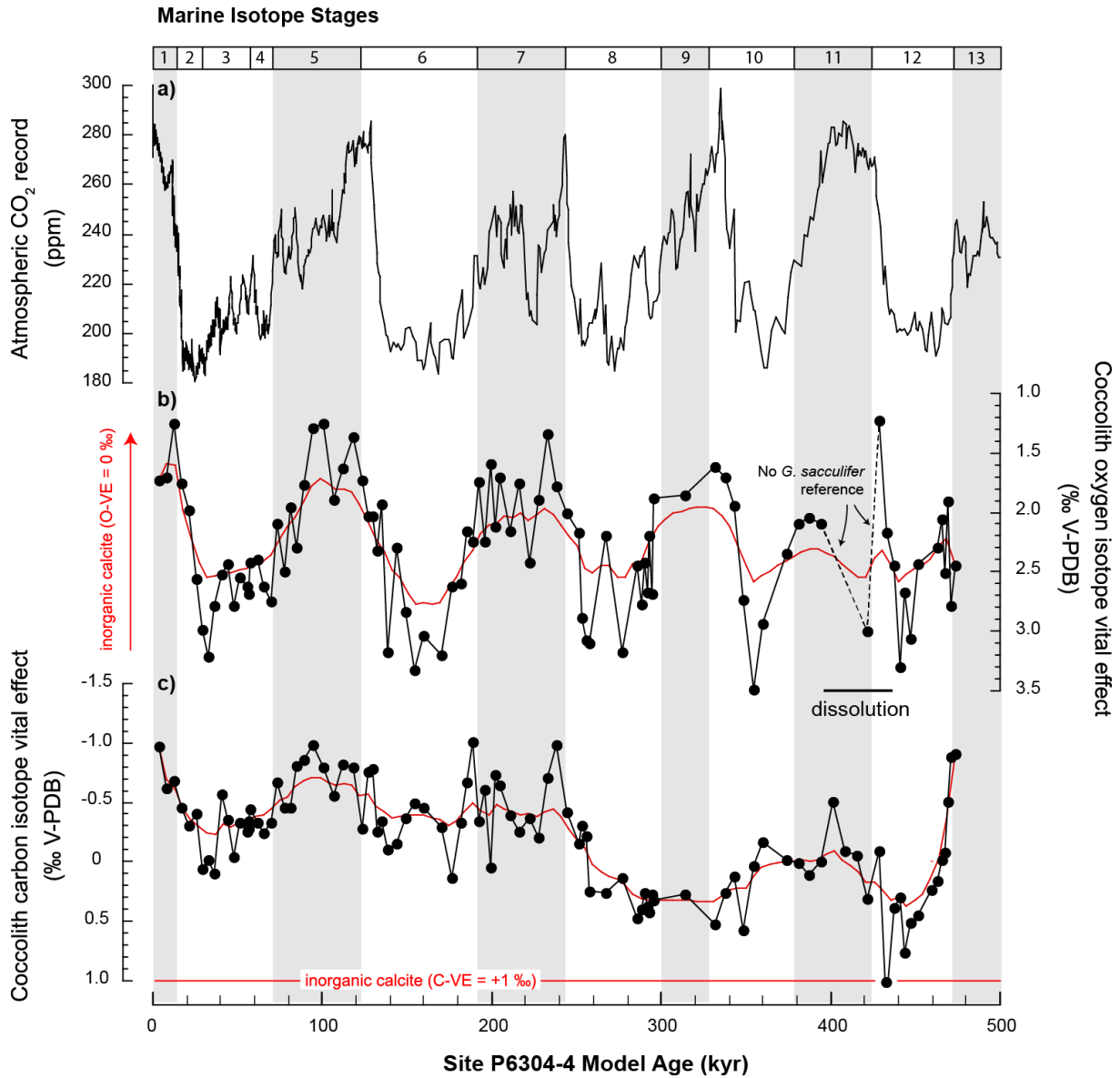


Figure 3: Comparison of the Vostok pCO₂ record (panel a) with the magnitude of the coccolith vital effects (panel b for oxygen and c for carbon) over the last 480 kyr. We see a good correlation between pCO₂ and oxygen isotope vital effect in *Gephyrocapsa* spp. coccoliths, especially over the last 10 MIS. In the present study, the magnitude of the vital effect is estimated from the isotopic offset of coccolith and *G. sacculifer* foraminifera (see text). See Figure A.2. for a comparison between the oxygen isotope vital effect derived from *G. sacculifer* $\delta^{18}\text{O}$ data with the +0.65 ‰ coefficient (shown here) and from *G. ruber* with no correction. During glacial periods, the vital effect is maximum (up to 3‰ PDB), whereas it is much more limited during periods with relatively higher CO₂ levels corresponding to the interglacials. Good correlation seems stratigraphically more restricted for the carbon isotope vital effect, i.e. over the last 5 MIS only, beyond which limit, only trends are visible (see scatter plots shown in Fig. 4). The horizontal red line on Panel c denotes the theoretical carbon isotope composition of inorganic calcite calculated as $\delta^{13}\text{C}_{\text{inorg}} = \delta^{13}\text{C}_{\text{DIC}} + 1$ (Romanek et al., 1992). Data source: Atmospheric pCO₂ composite curve from Petit et al. (1999); data used to calculate coccolith oxygen isotope vital effect from Emiliani (1972) and Anderson and Steinmetz (1981); data used to calculate carbon isotope vital effect from Schmidt (unpublished data) and Anderson and Steinmetz (1981) using the normalisation of *G. ruber* $\delta^{13}\text{C}$ to DIC by Spero et al. (2003).

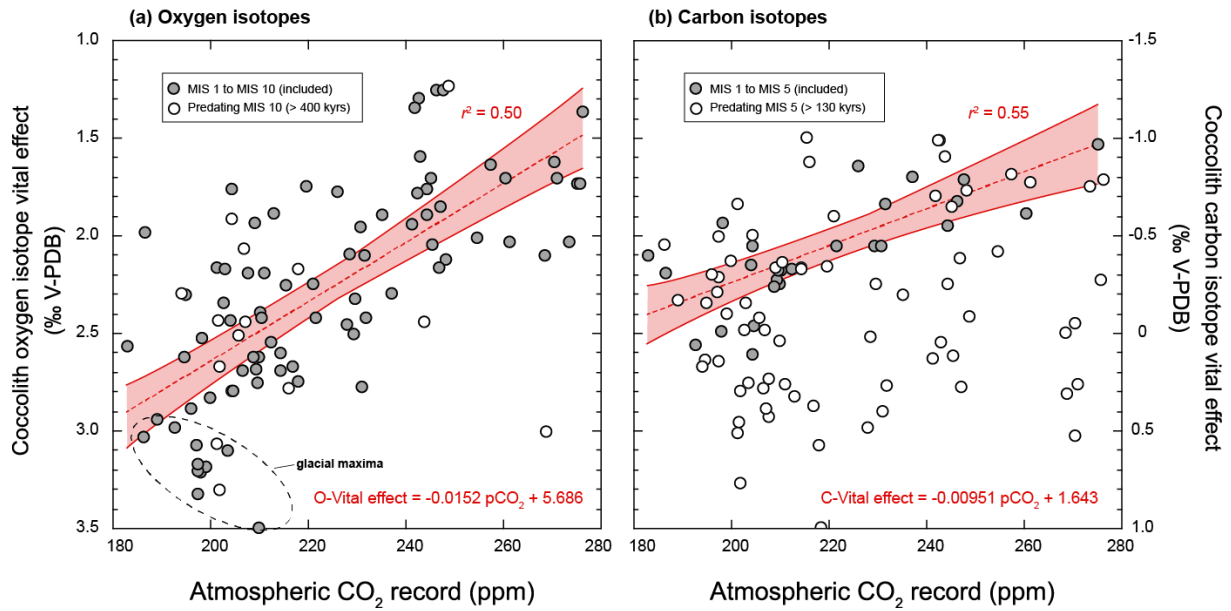


Figure 4: Scatter plots showing the relationship between atmospheric $p\text{CO}_2$ levels from the Vostok ice record (Petit et al., 1999) and (a) the magnitude of coccolith oxygen isotope vital effect and (b) carbon isotope vital effects. Note the reverse y axes. The pink areas correspond to the 95 % confidence intervals of the linear regressions. Key to datapoints is inset. Glacial Maxima are slightly offset from the overall correlation for the oxygen isotopes, as a consequence of more intense CO_2 limitation, and therefore expression of the vital effect due to increased seawater pH values.

Abundance of coccolith species in fine fractions of site P6304-4
(Reproduced from Steinmetz and Anderson, 1984)

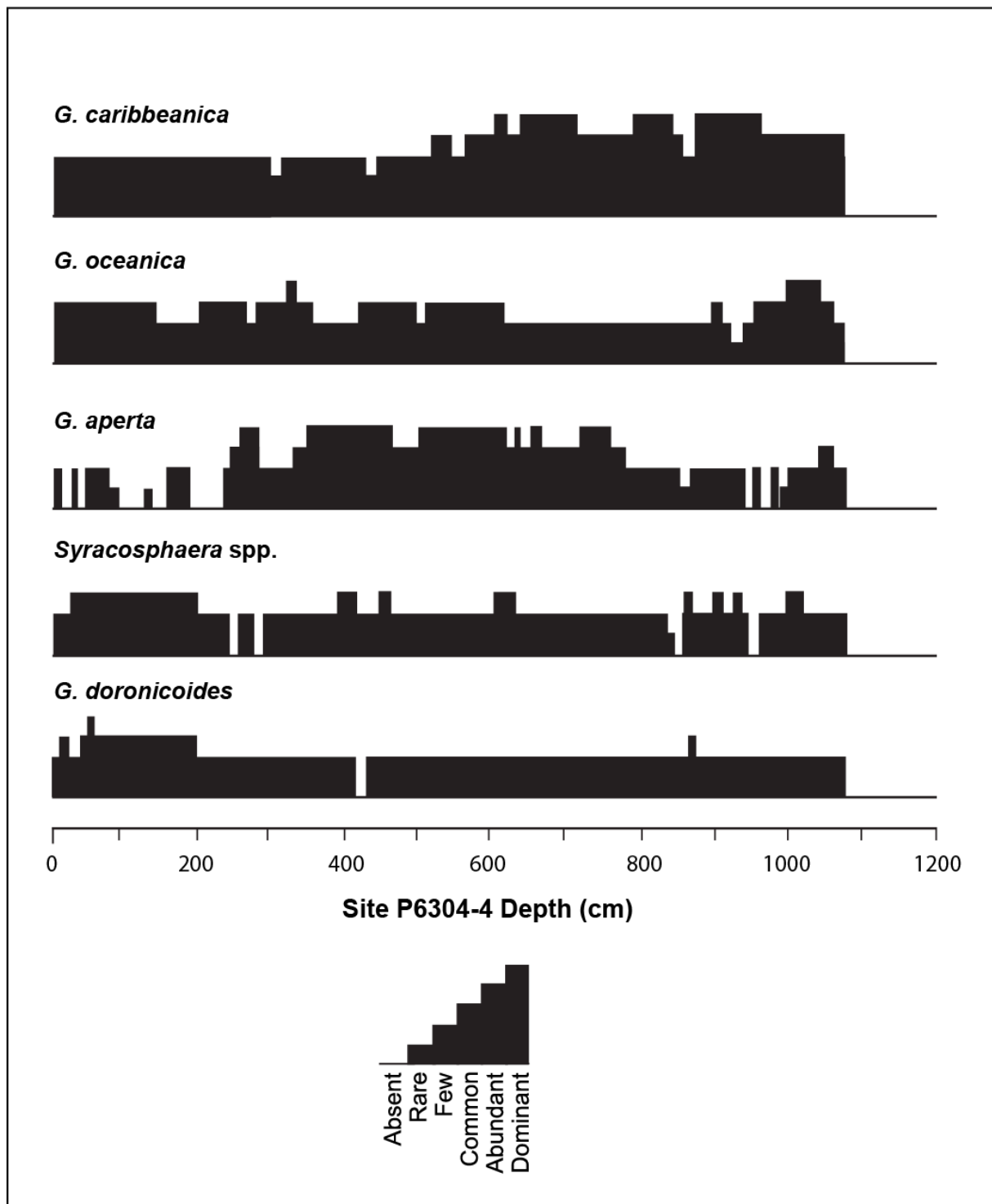


Figure A.1: Coccolith abundances in purified microfractions of site P6304-4 quantified in the work by Steinmetz and Anderson (1984). Reproduced with permission from Elsevier Ltd.

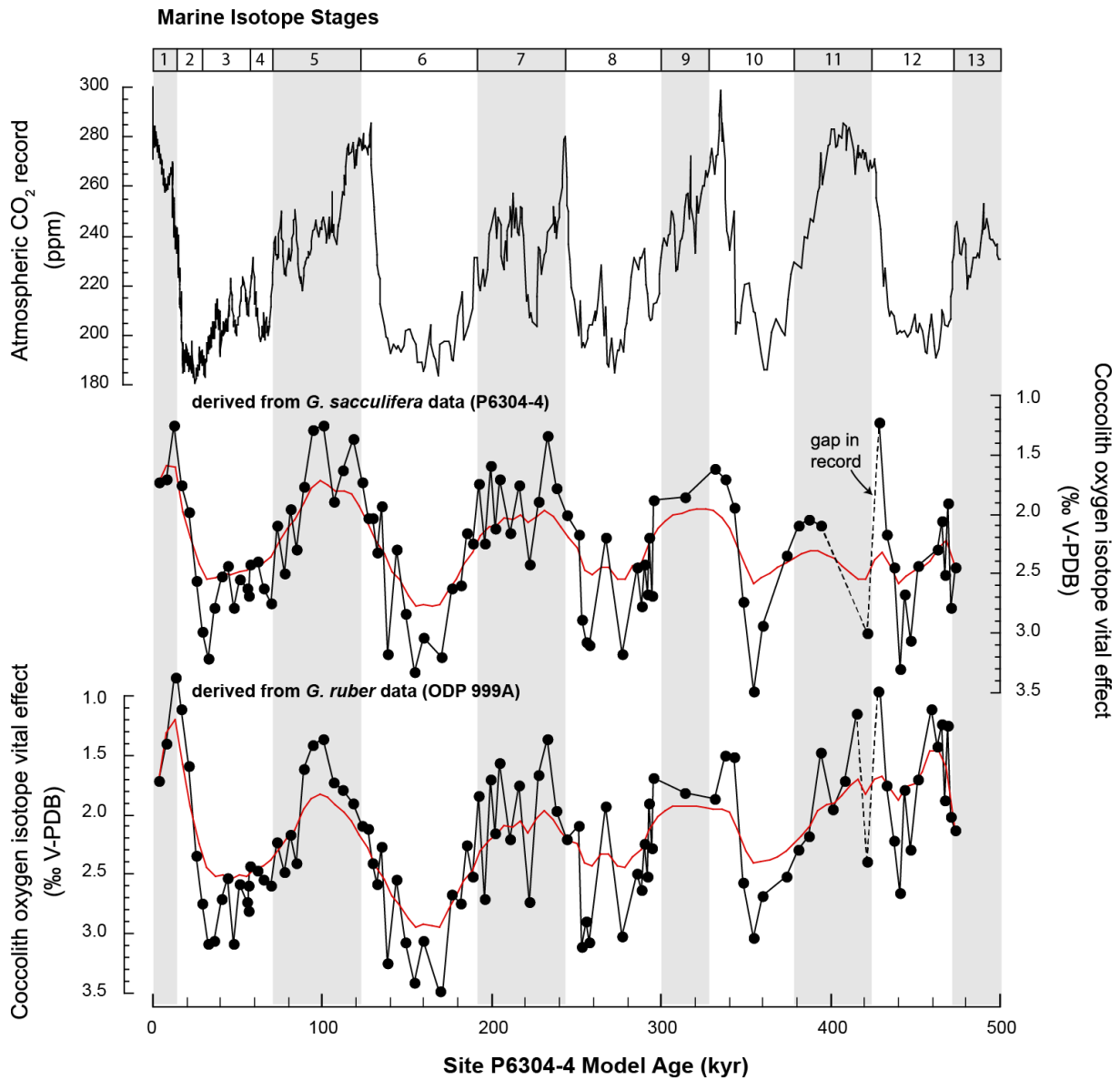


Figure A.2: Comparison of the $p\text{CO}_2$ record with the magnitude of coccolith oxygen vital effects over the last 480 kyr, either expressed using *G. sacculifera* $\delta^{18}\text{O}$ data with the $+0.65\text{‰}$ coefficient (as shown in Fig. 3), or using *G. ruber* $\delta^{18}\text{O}$ (Site ODP 999A; Schmidt et al., 2006) with no isotopic correction.