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Amandine Katz, Magali Bonifacie, Michaël Hermoso, Pierre Cartigny, Damien Calmels. Laboratory-grown coccoliths exhibit no vital effect in clumped isotope ($\Delta 47$) composition on a range of geologically relevant temperatures. *Geochimica et Cosmochimica Acta*, 2017, 208, pp.335 - 353. 10.1016/j.gca.2017.02.025 . hal-01513013

HAL Id: hal-01513013

<https://hal.science/hal-01513013>

Submitted on 24 Apr 2017

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Laboratory-grown coccoliths exhibit no vital effect in clumped isotope (Δ_{47}) composition on a range of geologically relevant temperatures

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ABSTRACT

The carbonate clumped isotope (or Δ_{47}) thermometer relies on the temperature dependence of the abundance of $^{13}\text{C}^{18}\text{O}^{16}\text{O}_2^{2-}$ ion groups within the mineral lattice. This proxy shows tremendous promise to reconstruct past sea surface temperatures (SSTs), but requires calibration of the relationship between Δ_{47} and calcification temperatures. Specifically, it is important to determine whether biologically-driven fractionation (the so-called “vital effect”) overprints Δ_{47} values, as reported in some biominerals such as the foraminifera and the coccoliths for the carbon and oxygen isotope systems. Despite their abundance in the pelagic environment, coccolithophores have not been comprehensively investigated to test the reliability of coccolith Δ_{47} -inferred temperatures. In this study, we cultured three geologically-relevant coccolith species (*Emiliania huxleyi*, *Coccolithus pelagicus*, and *Calcidiscus leptoporus*) at controlled temperatures between 7 and 25 ± 0.2 °C. Other variables such as pCO₂, pH, alkalinity, nutrient concentrations and salinity were kept constant at mean present-day oceanic conditions. Although cultured coccoliths exhibit substantial species-specific oxygen and carbon isotope vital effects, we found that their Δ_{47} composition follows a statistically indistinguishable relationship with $1/T^2$ for all three species, indicating a lack of interspecific vital effects in coccoliths. Further, the Δ_{47} composition of coccolith calcite is identical to inorganic calcite precipitated at the same temperature, indicating an overall absence of clumped isotope vital effect in coccolith biominerals. From a paleoceanographic perspective, this study indicates that the Δ_{47} values of sedimentary coccoliths – even from highly diverse / mixed assemblages – can be analyzed to reconstruct SSTs with confidence, as such temperature estimates are not biased by taxonomic content or changing interspecies vital effects through time.

Keywords:

Carbonate clumped isotopes; Laboratory culture; Coccolithophores; Sea Surface Temperature proxy; Vital effects.

1. INTRODUCTION

One of the most important paleoenvironmental parameter used to characterize Earth climate is temperature. Reconstructing accurate sea surface temperatures (SSTs) is a central goal in paleoceanography, as temperature at the interface between the ocean and the atmosphere has implications for constraining the sensitivity of Earth's climate system in concert with the evolution of atmospheric pCO₂ (e.g., Pagani, 2014). Rock-forming marine biocarbonates are often measured applying a variety of geochemical proxies to reconstruct SSTs and unravel broader environmental conditions (e.g., Henderson, 2002; Lea, 2014). Some biocarbonate minerals, such as those produced by foraminifera have been overwhelmingly exploited to estimate SSTs, while the coccoliths remain far less used in geochemically-derived reconstruction of paleoenvironments, mainly due to the minute size of these calcareous nannofossils and the challenge in obtaining coccolith assemblages from the sediments. Coccoliths are calcite platelets produced intracellularly by coccolithophore algae that thrive in the oceans since the Late Triassic, about 200 million years ago. As these calcifying organisms are primary (photosynthetic) producers, they only calcify in sunlit oceanic waters, and thus are ideally placed to record temperature of the uppermost part of the water column that is in contact with the atmosphere. Furthermore, the coccoliths have a compact ultrastructure made of imbricated monocrystalline elements without any internal porosity, and as such, are less prone to dissolution and recrystallization compared to the foraminifera (McIntyre et McIntyre, 1971; Schneidermann, 1977; Bouvier-Soumagnac et Duplessy, 1985; Caron et al., 1990; Wefer et Berger, 1991; Kucera, 2007; Broecker et Clark, 2009; Prentice et al., 2014). This differential preservational state makes the coccoliths a valuable paleoclimatic archive, especially when planktonic foraminifera are badly preserved or even absent (e.g., during high temperature regime of the Paleogene, Pearson et al., 2001). Recent methodological developments and subsequent refinements allow separating coccoliths into different size fractions with reduced species diversity, and importantly can provide microfactions barren of foraminiferal fragments or the so-called micarbs (Minoletti, 2001; Stoll et Ziveri, 2002; Minoletti et al., 2009; Halloran et al., 2009). These techniques enable new paleoenvironmental information to be specifically derived from the geochemistry of these microfossils (see review by Hermoso, 2014). Yet, one of the most prominent caveat in the use of biominerals in paleoceanography arises from the biological origin of calcite, which potentially leads to prominent isotopic offsets from inorganic (abiogenic) conditions. This phenomenon, referred to as the vital effect, is particularly expressed in the case of the coccoliths (Dudley et al., 1986; Ziveri et al., 2003; Bolton et al., 2012; Candelier et al., 2013; Hermoso, 2014; Stevenson et al., 2014; Hermoso, 2015; Hermoso et al., 2015; McClelland et al., 2017).

Various proxies have been used to generate SST estimates, such as the oxygen isotope composition of calcareous fossils ($\delta^{18}\text{O}$), the undersaturation index of coccolithophore-produced alkenones (U^{k}_{37}), TEX₈₆, or elemental Mg/Ca ratios of microfossil shells (Epstein et al., 1951; Emiliani, 1966; Brassell et al., 1986; Prahl et al., 1988; Nürnberg et al., 1996; Kim et O'Neil, 1997; Conte et al., 1998; Lea et al., 1999; Elderfield et Ganssen, 2000; Minoletti et al., 2014). These approaches are, however, still confounded by a number of uncertainties inherent to all proxy reconstruction (Herbert, 2014; Lea, 2014; Zhang et al., 2016). The quest to generate reliable and more accurate SSTs can now potentially benefit from the most recent developed paleothermometer, namely the carbonate clumped isotope (or Δ_{47}) thermometry (Eiler, 2007; Eiler, 2011). The Δ_{47} thermometry is based on the temperature-dependent degree of bonding between ¹³C and ¹⁸O in the carbonate

mineral lattice. The equilibrium constant of the reaction $\text{Ca}^{13}\text{C}^{16}\text{O}_3 + \text{Ca}^{12}\text{C}^{18}\text{O}^{16}\text{O}_2 \leftrightarrow \text{Ca}^{13}\text{C}^{18}\text{O}^{16}\text{O}_2 + \text{Ca}^{12}\text{C}^{16}\text{O}_3$ solely depends on temperature (Schauble et al., 2006). Ab-initio calculations have provided theoretical constraints on the relative abundance of the $^{13}\text{C}^{18}\text{O}^{16}\text{O}_2$ isotopologue (Δ_{63} values) in the carbonate lattice with respect to temperature (Schauble et al., 2006). Carbonate clumped isotope compositions (Δ_{47} values) reflects the over-abundance of bonds between ^{18}O and ^{13}C isotopes inside the carbonate lattice compared to stochastic distribution. At low temperatures, the bonds between the two heavy isotopes $^{13}\text{C}-^{18}\text{O}$ are favored, thus carbonate Δ_{47} values decrease with increasing temperature (Ghosh et al., 2006; Eiler, 2007). Since 2006, several experimental studies aiming at calibrating the temperature dependence of Δ_{47} values in inorganic carbonate minerals have been conducted (Ghosh et al., 2006; Dennis et Schrag, 2010; Zaarur et al., 2013; Fernandez et al., 2014; Tang et al., 2014; Defliese et al., 2015; Kele et al., 2015; Kluge et al., 2015; Tripathi et al., 2015; Bonifacie et al., 2017; Kelson et al., 2017). Altogether, these works have confirmed that the Δ_{47} thermometer is independent of the composition of the fluid from which the mineral grew, contrasting with the $\delta^{18}\text{O}$ or Mg/Ca temperature proxies. Similar empirical calibrations on biogenic carbonates have been previously made on biomineralizing systems such as: the foraminifera, corals, brachiopod and mollusk shells (Ghosh et al., 2006; Tripathi et al., 2010; Thiagarajan et al., 2011; Eagle et al., 2013; Grauel et al., 2013; Henkes et al., 2013; Came et al., 2014; Douglas et al., 2014; Wacker et al., 2014; Saenger et al., 2012; Spooner et al., 2016). With the exception of shallow-water and cold-water corals (Saenger et al., 2012; Spooner et al., 2016), overall, these studies have established the lack of vital effect imprinting the Δ_{47} composition of most biologically-precipitated calcite. Of particular relevance to the present study, Tripathi et al. (2010) investigated cultured coccoliths, among other calcifiers, for their Δ_{47} composition. No vital effects were identified on coccoliths, although it should be noted that such a conclusion arises from only two species (*Emiliania huxleyi* and *Coccolithus pelagicus*) grown at one single temperature each, and analyzed only once, resulting in large uncertainty in Δ_{47} composition. It remains therefore important to examine in more details the response of coccolith Δ_{47} values on a wider range of geologically relevant temperatures before it can be concluded that these nannofossils are indeed barren of vital effects, and ultimately be in position to use them in paleoceanographic research. Here, we present the first comprehensive laboratory-based calibration of Δ_{47} with temperature of calcification of coccolith calcite, grown under controlled environmental conditions. To this end, we cultured three different coccolithophore species chosen on the merit of very large interspecific oxygen isotope vital effects. Indeed, for a given temperature, the range of coccolith $\delta^{18}\text{O}$ values for a given temperature of calcification can be as high as 5‰ (virtually leading to ± 20 °C biologically-induced biases in temperature estimates). An isotopic typology of the coccoliths was coined by Dudley et al. (1986) and refers to the relationship between coccolith calcite and inorganic calcite synthesized by Kim and O’Neil (1997) (see review by Stoll and Ziveri, 2002; Hermoso, 2014). The species investigated here comprise *Emiliania huxleyi* (representing the “isotopically heavy group”) for which carbonate is enriched in ^{18}O compared to the inorganic calcite of Kim and O’Neil (1997), *Coccolithus pelagicus* ssp. *braarudii* characterized by $\delta^{18}\text{O}$ values close to inorganic values and *Calcidiscus leptoporus* (representing the “isotopically light group”) with coccolith calcite being depleted in ^{18}O still compared to the inorganic calcite of Kim and O’Neil (1997).

2. MATERIALS AND METHODS

2.1. Laboratory cultures of coccolithophores

Laboratory cultures of monoclonal coccolithophore strains were performed at the Department of Earth Sciences of Oxford University following the same methods described previously (e.g. Hermoso et al., 2014; Rickaby et al., 2016). We grew three strains sourced from the Roscoff Culture Collection: *Emiliania huxleyi* originating from the Icelandic Coast (RCC 1256); *Coccolithus pelagicus* ssp. *braarudii* from the Portuguese Coast (RCC 1202) and *Calcidiscus leptoporus* from South Africa West Coast (RCC 1129). The experiments were conducted at five distinct temperatures (7, 10, 15, 20 and 25 °C) for *E. huxleyi* and at three temperatures (10, 15 and 20 °C) for both *C. braarudii* and *C. leptoporus*, as the two latter species have a narrower range of temperature tolerance. Before the experiment started, algae were slowly acclimated from 15 °C to the target temperatures and maintained at these temperatures during at least 10 generations (acclimation phase).

The culture medium was made from natural seawater obtained from the Marine Biologist Association, Plymouth (UK). This batch was left for at least six months in the dark and at 4 °C to ensure bio-consumption of macronutrients and residual organic compounds by bacteria prior to final preparation of the culture medium (Keller et al., 1987). This process allows better mimicking open ocean conditions. To test the effect of the $\delta^{18}\text{O}$ values of the medium ($\delta^{18}\text{O}_{\text{medium}}$) on the Δ_{47} composition of the coccoliths, the cultures of *E. huxleyi* grown at 15 °C were made in both natural seawater and artificial (ESAW; Keller et al., 1987) media ($\delta^{18}\text{O}_{\text{seawater}} = 0.65\text{‰}$ and $\delta^{18}\text{O}_{\text{medium}} = -6.14\text{‰}$ SMOW respectively; Table 1). All media were air-bubbled for 24h prior to inoculation in order to re-establish chemical and isotopic CO_2 equilibrium between the air and the medium at each target temperature. For the natural and synthetic media, nitrate, phosphate, trace metals and EDTA enrichment correspond to a *K/2* medium (Keller et al., 1987) and vitamins enrichment to the *f/2* medium (Guillard, 1975). The final pH of the medium was adjusted to 8.2 obtained by addition of 0.2 M NaOH. Prior to algae inoculation, the medium was sterilized by steri-filtration achieved with a Millipore Stericup device (nominal pore aperture of 0.22 μm).

The cultures were grown in 600 mL Nalgene polycarbonate sterile flasks with 50 mL headspace. In the incubator, the cultures were illuminated with light/dark cycles of 14/10 hours. The irradiance was $\sim 150 \mu\text{mol photon/m}^2/\text{s}$ during the illuminated period. The temperature variations in the incubator were less than ± 0.2 °C. Over the course of the experiments, the culture flasks were gently stirred several times per day to reduce algal settling and accumulation of cell clusters at the bottom of the flask. To implement the cultures at low cell density and prevent a reservoir effect (see Barry et al., 2010), we opted for a semi-continuous batch culture strategy with two to five replicated sub-batches per condition (one species / one temperature). This technique consisted of refreshing the medium with new medium every two days for *E. huxleyi* and 3 days for *C. leptoporus* and *C. braarudii*, after most of the cells have settled in the flask left upright for two hours (see Rickaby et al. (2016) for more details). The cultures were harvested by centrifugation at low cell density (10000 cells per mL for *E. huxleyi*, and between 5000 and 6000 cells per mL for *C. braarudii* and *C. leptoporus*). At these low cell densities, the algal populations were still in their exponential growth phase (Hermoso, 2014) and the composition of the medium and pH did not significantly drift from the initial conditions. Three rinsing cycles with distilled water neutralized to pH 8 were applied on the culture residues to remove traces of NaCl and other salts. Removal of organic matter was achieved following the method described in Stevenson et al. (2014) consisting of the reaction of the culture residues with hydrogen peroxide 10% neutralized at pH 8 overnight and thoroughly

rinsed again with neutralized deionized water.

2.2. Stable isotope measurements and data processing

As for traditional stable carbon and oxygen isotope compositions, the determination of clumped isotope ratios in carbonate by gas-source mass-spectrometry is made from gaseous CO₂ released from the digestion of the carbonate sample by anhydrous phosphoric acid. The methods used for carbonate digestion, CO₂ purification and stable isotope measurements at Institut de Physique du Globe de Paris (IPGP, Laboratoire de G ochimie des Isotopes Stables) are detailed in Bonifacie et al. (2017) and outlined below.

2.2.1. Carbonate digestion and CO₂ purification

Each individual measurement was obtained from about 5 mg of organic matter-free culture residue digested at 90 °C during 20 min. The reaction took place in a common acid bath containing about 10 mL of ~104% phosphoric acid H₃PO₄ (ρ = 1.914 mg/mL). The gas products from the carbonate reaction (mainly CO₂ – H₂O – phosphoric acid vapor) were continuously collected in liquid nitrogen (LN₂ trap at -196 °C) during the reaction. After complete digestion, non condensable gases were discarded. The gases were then transferred to a second LN₂ trap after passing through a LN₂-ethanol slush held at ~ -117 °C to retain water and phosphoric acid vapor. After manometric CO₂ quantification with a capacitance gauge to check the yields, the extracted CO₂ was transferred through silver wool (to remove sulfur contaminants) and through a Porapak-Q 50-80 mesh trap held at -28 °C (to remove possible organic and halocarbon contaminants). Finally, the purified CO₂ was condensed in a sample tube, and introduced to the mass spectrometer for isotopic measurements.

2.2.2. Isotopic measurements

Measurements of clumped, stable carbon and oxygen isotopic compositions of carbonates (respectively Δ₄₇, δ¹³C and δ¹⁸O) were simultaneously performed from gaseous CO₂ using a Thermo Scientific MAT 253 gas source isotope ratio mass spectrometer at the Institut de Physique du Globe de Paris. The abundance of isotopologues with m/z from 44 to 49 were analyzed. Results are reported as a deviation to a standard in per mil using the notation:

$$\Delta_{47} = \left[\left(\frac{R^{47}}{R^{47*}} - 1 \right) - \left(\frac{R^{46}}{R^{46*}} - 1 \right) - \left(\frac{R^{45}}{R^{45*}} - 1 \right) \right] \times 1000 \quad (\text{Eq. 1})$$

where R⁴⁷, R⁴⁶ and R⁴⁵ are the respective abundance ratio of gaseous CO₂ with masses 47, 46, 45 relative to the mass 44 measured in the analyzed sample. R^{47*}, R^{46*} and R^{45*} denote for ratios calculated if the sample had a stochastic abundance of all isotopologues (see Eiler and Schauble, 2004 and Wang et al., 2004 for more details). Each measurement consisted of seven acquisitions (signal of 12V on m/z = 44) of ten cycles of comparison between the CO₂ extracted from sample versus a pre-calibrated working internal reference CO₂ gas provided by Oztech Trading Corporation with δ¹³C = -3.72‰ and δ¹⁸O = -6.06‰ (VPDB, verified through measurements of the international reference material NBS-19). The signal integration time was of 26 seconds (i.e., total integration time of 1820 seconds for each CO₂ sample). Peak centering, background measurement and pressure

balancing were performed before each acquisition. As recommended by Dennis et al. (2011), the raw Δ_{47} values (i.e., the Δ_{47} values expressed relative to the working gas) were *directly* transferred into the “absolute” Carbon Dioxide Equilibrated Scale (CDES) using Empirical Transfer Functions (ETF) built with CO₂ gases driven to isotopologue equilibrium at two temperatures with absolute Δ_{47} values known from theoretical calculations (Wang et al., 2004) and ran interspersed with unknown samples. For constructing our ETF, we used CO₂ gases driven to isotopologue equilibrium both at 1000 and 25 °C and with theoretically predicted Δ_{47} values of 0.0266‰ and 0.9252‰, respectively (after Wang et al., 2004). Gaseous CO₂ equilibrated at 1000 °C were obtained by placing silica break-seals containing CO₂ in a muffle furnace at 1000 °C for three hours and rapidly quenched in air. Gaseous CO₂ equilibrated at 25 °C were obtained by placing CO₂ aliquots in a sealed flask with aqueous water maintained at 25 °C for several days. Our equilibrated CO₂ gas standards exhibit bulk isotopic compositions spanning almost the entire range of measured coccolith samples (i.e., δ^{47} values between -1‰ and +24‰, $\delta^{13}\text{C}$ values between -4‰ and -2‰, $\delta^{18}\text{O}$ values between -19‰ and +7‰) and were purified and analyzed in the same way as carbonate samples and standards. Equilibrated CO₂ gases were run every 4 or 5 coccolith samples or carbonate standards. All the Δ_{47} data of the present study were obtained in seven distinct sessions of analyses performed over the course of one year, each separated by several weeks. The duration of each session was typically 2 – 4 weeks, corresponding to about 10 – 18 equilibrated CO₂ gases used for constructing the correction frames. All the isotopic values of our equilibrated CO₂ standards can be found in Table S1 together with values of coccolith samples and carbonate standards.

2.2.3. *A posteriori data processing*

Traditional $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ data were acquired as part of each Δ_{47} analysis and ^{17}O corrections were made using the ^{17}O parameters from Santrock et al. (1985). In order to account for the temperature dependence of oxygen isotope fractionation between CO₂ gas and calcite resulting from the reaction with phosphoric acid at 90°C in common acid bath, a fractionation factor of 1.00811 was used. This fractionation factor has been constrained based on replicate $\delta^{18}\text{O}$ measurements of several calcite samples digested at both 90 °C in common acid bath (CAB) and 25 °C in sealed-vessel (SV) and comparison to the widely accepted fractionation value of 1.01025 for digestion at 25 °C in SV (Friedman et O’Neil, 1977). We analyzed our in-house carbonate reference material (IPGP-Carrara marble) routinely, as well as the international reference material NBS-19 for oxygen and carbon stable isotopic compositions. We found $\delta^{13}\text{C} = 1.95 \pm 0.01\text{‰}$ and $\delta^{18}\text{O} = -2.16 \pm 0.05\text{‰}$ for NBS-19 (VPDB, 1SD, n=5) and $\delta^{13}\text{C} = 2.26 \pm 0.02\text{‰}$ and $-1.80 \pm 0.05\text{‰}$ (VPDB, 1SD, n=26) for IPGP-Carrara.

The raw Δ_{47} data were corrected for linearity effects using a fixed Equilibrated Gas Line (EGL) slope fitted to the CO₂ standard equilibrated at both 1000 and 25 °C. The EGL slopes (typically around 0.0029 over the course of this study; Table S1) were very shallow highlighting only small linearity corrections applied to raw Δ_{47} data. In addition, the slopes of EGL only slightly drifted during our one-year-long analytical campaign (from 0.0015 to 0.0040). Subsequently, the Δ_{47} data were transferred into the CDES absolute reference frame ($\Delta_{47\text{CDES90}}$ in Table S1) using Empirical Transfer Functions (ETF, as defined by Dennis et al., 2011). For this study, both EGL and ETF were continually constructed with the CO₂ gases equilibrated at both 1000 °C and 25 °C during each of the seven discrete analytical sessions (with ETF slopes from 1.1093 to 1.1379 and intercepts

from 0.9160 to 0.9331). Finally, $\Delta_{47\text{CDES90}}$ data were projected into the 25 °C acid digestion reference frame ($\Delta_{47\text{CDES25}}$ in Table S1) for easier comparison with previously published calibration data. For this, we added the acid fractionation value of + 0.082‰ determined by Defliese et al. (2015).

To ensure accuracy of Δ_{47} data and the entire post-measurement data processing described above, we routinely analyzed two carbonate reference materials (Carrara marble and 102-GC-AZ01b) that were used in the inter-laboratory comparison work by Dennis et al. (2011). The international reference material NBS-19 for oxygen and carbon stable isotopic compositions was also analyzed. These three carbonate standards were distributed along with measurements of coccolith samples and CO₂ standards in all runs (see Table S1). One carbonate reference material (either IPGP-Carrara marble or 102-GC-AZ01b) was analyzed typically every five analyses to check for analytical stability/accuracy of the whole procedure (including carbonate digestion, CO₂ purification, stability of the conditions for analyses of CO₂ inside the mass spectrometer and/or accuracy of the correction frames constructed with standards of equilibrated CO₂ gas – namely the accuracy of the EGL and ETF lines), as well as long-term reproducibility of our Δ_{47} measurements (see Table S1). Importantly, the Δ_{47} values of the carbonate standards measured during our seven discrete analytical sessions are indistinguishable from each other as they are within analytical precision (Table S1). The average Δ_{47} values obtained for these carbonates over the course of this study are: $\Delta_{47\text{CDES25}} = 0.384 \pm 0.013\text{‰}$ (1SD, n=26) for IPGP-Carrara; $\Delta_{47\text{CDES25}} = 0.706 \pm 0.016\text{‰}$ (1SD, n = 21) for 102-GC-AZ01b; $\Delta_{47\text{CDES25}} = 0.387 \pm 0.014\text{‰}$ (1SD, n=5) for NBS19. Our $\Delta_{47\text{CDES25}}$ values are indistinguishable from those obtained in previous studies (e.g. Dennis et al. 2011; Henkes et al. 2013; Bonifacie et al., 2017). Furthermore, the ± 13 ppm and ± 16 ppm standard deviations obtained here on ~ 20 -30 replicate Δ_{47} measurements of carbonate reference materials during seven sessions of analyses are consistent with the long-term external reproducibility obtained at IPGP on more than 300 independent Δ_{47} measurements over more than three years (± 14 ppm, 1SD, n > 300).

3. RESULTS

Table 1 reports the $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and Δ_{47} compositions for each coccolith culture residue. The data are plotted in Figures 1 and 2 as a function of temperature. At least three isotopic measurements were run for semi-continuous batch cultures conducted at the same temperature, with the exception of *E. huxleyi* grown at 7 °C for which only 2 isotopic measurements were made due to insufficient mass of calcite recovered from the third culture batch. For a given species at a given temperature, the measurements ($2 < n < 5$) were made at least in two different analytical sessions (that is using EGL and ETF correction frames constructed with various sets of data on standards of equilibrated CO₂ at known temperatures) in order to eliminate possible bias from potential errors in constructing each discrete correction frame. With all replicated isotopic measurements made on the distinct batches, a total of sixteen measurements were run for *E. huxleyi*, twelve for *C. braarudii* and eleven for *C. leptoporus*. The results of each measurement of coccolith calcite, carbonate standard and standards of equilibrated gaseous CO₂ are given in the Electronic Annex Table S1.

3.1. Traditional carbon and oxygen compositions of coccolith calcite

Cultured coccolith samples show significant variability in the oxygen isotope offsets from the composition of the medium from which they grew ($\Delta^{18}\text{O} = \delta^{18}\text{O}_{\text{carb}} - \delta^{18}\text{O}_{\text{medium}}$; Table 1; Figure 1). The $\Delta^{18}\text{O}$ values obtained for the three coccoliths species are strongly species-specific, yet show statistically significant linear relations with growth temperature (Figure 1). Figure 1 further shows that coccolith calcite $\delta^{18}\text{O}$ values show variable isotopic departures from calcite of Kim and O'Neil (1997), which commonly serves as an inorganic reference in paleoceanographic research and allows quantification of the magnitude of the vital effect in biogeochemical studies (see synthesis in Hermoso, 2015). Overall, the magnitude of the vital effect is in agreement with previous studies (i.e., ca +3‰ for *E. huxleyi*, 0‰ for *C. braarudii* and -1‰ for *C. leptoporus*) (Ziveri et al., 2003; Rickaby et al., 2010; Candelier, 2013; Candelier et al., 2013; Hermoso et al., 2014; Stevenson et al., 2014).

Cultured coccoliths show $\delta^{13}\text{C}_{\text{carb}} - \delta^{13}\text{C}_{\text{DIC}}$ offsets (the isotopic difference of calcite from ambient dissolved inorganic carbon, noted $\Delta^{13}\text{C}$ values; Table 1) between -1.4 and +1.7‰ for *E. huxleyi*, between -5.3 and -2.2‰ for *C. braarudii* and between -2.9 and -2.0‰ for *C. leptoporus*. These $\Delta^{13}\text{C}$ values are similar to those previously reported for coccoliths grown in very dilute culture batches (Rickaby et al., 2010; Candelier et al., 2013; Hermoso et al., 2014).

3.2. Clumped isotope compositions of coccolith calcite

Taken individually (each species at each temperature), the $\Delta_{47\text{CDES25}}$ data range from 0.686 to 0.751‰ for *E. huxleyi* between 7 and 25 °C, from 0.709 to 0.752‰ for *C. braarudii* between 10 and 20 °C and from 0.695 to 0.741‰ for *C. leptoporus* between 10 and 20 °C (Table 1; Figure 2). It is noteworthy that the dispersion of Δ_{47} data obtained for a given species at a given growth temperature is very narrow, with 1SD varying from 6 to 16 ppm (or S.E. between 3 and 9 ppm; Table 1), and compares well with the long-term external reproducibility of Δ_{47} measurements for homogeneous carbonate reference materials run at IPGP (± 14 ppm, 1SD, $n > 300$). From a methodological viewpoint, despite the fact that the cultures originate from discrete batches conducted during a period of one year, and that isotopic measurements were realized during seven distinct sessions of analyses on the mass spectrometer, yet the Δ_{47} results are highly reproducible.

3.3. Effect of medium $\delta^{18}\text{O}_{\text{water}}$ and $\delta^{13}\text{C}_{\text{DIC}}$ values on coccolith Δ_{47} values

To seek possible isotopic source effect ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ of the medium) on the Δ_{47} compositions of coccolith calcite, two cultures for *E. huxleyi* were conducted in artificial medium with $\delta^{13}\text{C}_{\text{DIC}}$ and $\delta^{18}\text{O}_{\text{water}}$ distinct from the seawater medium used for all other cultures at 15 °C (see Table 1). We found that *E. huxleyi* grown at 15 °C either in artificial or natural media gave indistinguishable $\Delta_{47\text{CDES25}}$ values of 0.715 and 0.714‰ for the former conditions, and 0.704‰ for the latter (Table 1; Figure 2) – this offset is indeed lower than our ± 14 ppm 1SD long-term external reproducibility. This is consistent with theoretical assumption that the isotopic composition of the medium does not influence the Δ_{47} composition of the mineral, and thus may not influence the $\Delta_{47} - 1/T^2$ calibration presented here.

4. DISCUSSION

4.1. Seeking possible interspecific Δ_{47} vital effect in coccolith calcite

We first examine each species independently for the relationship between Δ_{47} and temperature ($1/T^2$) (Figure 2). We conducted statistical analyses between coccolith $\Delta_{47} - 1/T^2$ relationships based on least-squares fitting according to the methods by Minster et al. (1979) modified from York (1969). By this means, uncertainties arising from both Δ_{47} measurements and growth temperature are considered. The slopes and intercepts of the $\Delta_{47} - 1/T^2$ linear regressions calculated for the three examined species are reported in Table 2. For each of the three species, we observe statistically significant correlations between the Δ_{47} values and $1/T^2$ (r^2 between 0.77 and 0.88; all p -values < 0.0005 ; Figure 2; Table 2). Because every single Δ_{47} measurement was generated from distinct culture residues, we chose to calculate linear regressions using individual Δ_{47} values rather than their average for a given species at a given temperature. This was to ensure that each datapoint equally contributes to the final calibration. Each of this individual measurement was assigned to 14 ppm (1SD) long-term external reproducibility on Δ_{47} measurements at IPGP. This approach also takes into account the fact that varying number of Δ_{47} measurements ($2 < n < 5$) were made for each investigated temperature, and thus weights the influence of each temperature on the calibration by the number of Δ_{47} measurements analyses. We note, however, that if the Δ_{47} data were averaged by temperature and weighted with their respective standard error of the mean (1 SE) (i.e., as done in the majority of previously published $\Delta_{47} - 1/T^2$ calibrations) the calculated slopes and intercepts would be comparable to those presented in Table 2, albeit with halved errors. It is also noteworthy that regardless of the means to calculate the parameters of the $\Delta_{47} - 1/T^2$ linear regressions, the equations for the three species are statistically indistinguishable from each other when their respective uncertainties are considered (Table 2). Also, when plotted together, the coccolith data from the three investigated species are indistinguishable from each other when the assigned ± 14 ppm uncertainty on each measurement is considered (Figure 2d). Close examination of Figure 2d may suggest that the Δ_{47} data for *E. huxleyi* are slightly lower than those found for the two other species. However, this apparent offset is very small, below the mass spectrometer shot noise limit of Δ_{47} measurements (about ~ 9 ppm for our conditions of analyses), and thus unlikely to be statistically/geologically significant. We also tested if such small apparent offset could result from corrections with inappropriate ^{17}O parameters (as recently underlined by Schauer et al., 2016 and Daëron et al., 2016) by recalculating all coccolith data with parameters from Brand et al. (2010). However, this reprocessing led to only small (less than 7 ppm) and not systematic differences in Δ_{47} values (ie. leading either to slightly higher or lower Δ_{47} values) compared to those obtained with parameters from Santrock et al. (1986). [Note that $\Delta_{47} - 1/T^2$ correlations obtained here for coccoliths are also unchanged by such data reprocessing]. Collectively, these observations indicate that no interspecies Δ_{47} vital effect is apparent among the three investigated species and suggests that a single coccolith $\Delta_{47} - 1/T^2$ regression line can be calculated for describing the Δ_{47} dependence to temperature of the three coccolith species investigated here (Figure 3).

4.2. A coccolith-integrated $\Delta_{47} - 1/T^2$ calibration

We calculated a single and cross-species $\Delta_{47} - 1/T^2$ least square regression (Minster et al., 1979) fitting through all the coccolith data by taking into account each Δ_{47} measurement for each temperature and species, with an assigned error of ± 14 ppm (1SD). As each datapoint was treated separately with no consideration of the species, we thus ensure that they all contributed equally to the calibration. We obtained the following equation (Eq. 2; Figure 3):

$$\Delta_{47\text{CDES25}} = 0.0330 (\pm 0.0108) \times 10^6/T^2 + 0.3245 (\pm 0.1294) \quad (r^2 = 0.82) \quad (\text{Eq. 2}),$$

with temperature (T) reported in Kelvin, $\Delta_{47\text{CDES25}}$ reported in per mil and within the 25 °C acid digestion and CDES reference frames. The reported uncertainties on the slope and intercept are 95% confidence intervals. Note that if the Δ_{47} data were averaged and weighted with their respective standard error of the mean (1 SE), the calculated slope and intercept would be comparable to those from Eq. 2 (0.0313 and 0.3423, respectively), but reported errors would be about twice lower than those reported in Eq. 2 (with 95% confidence interval errors ± 0.0057 and ± 0.0678 on the slope and intercept, respectively).

4.3. No expression of Δ_{47} vital effects in coccolith calcite

4.3.1 Comparison of coccolith Δ_{47} values with equilibrium conditions

The Δ_{47} data of cultured coccoliths are compared to the Δ_{47} values predicted for equilibrium conditions in order to seek any vital effect imprinting the Δ_{47} values of these calcite biominerals. The Δ_{47} equilibrium values ($\Delta_{47}^{\text{equilibrium}}$) used thereafter correspond to a combination of theoretically-calculated Δ_{63} abundance of $^{13}\text{C}^{18}\text{O}^{16}\text{O}_2$ isotopologues within calcite lattice (Schauble et al., 2006) to which is added the experimental fractionation between CaCO_3 and CO_2 (during phosphoric acid reaction), which is $\Delta^* = 0.268\text{‰}$. This value was experimentally determined for calcite digested at 25 °C by Guo et al. (2009) and retrospectively transferred into the absolute reference frame using the equation from Table 4 in Dennis et al. (2011). Figure 4 shows that the averaged Δ_{47} data of coccolith calcite are statistically indistinguishable from the $\Delta_{47}^{\text{equilibrium}}$ values, as they fall within our analytical uncertainty of ± 14 ppm (ie., the long-term external reproducibility determined on large number of replicate Δ_{47} measurements of homogeneous carbonate reference materials; represented by the shaded area in Figure 4). At first glance, the coccolith Δ_{47} data may, however, appear to be slightly higher than their respective $\Delta_{47}^{\text{equilibrium}}$ values. Nevertheless, this slight positive Δ_{47} offset from $\Delta_{47}^{\text{equilibrium}}$ values are not supported, mainly because: i/ additional errors on theoretical calculations should also be considered, as those arising from approximations in the parameters used to determine it; and most importantly ii/ coccolith data are fully consistent with Δ_{47} datasets on experimental inorganic calcites (Figure 5a; Ghosh et al., 2006; Dennis et al., 2010; Zaarur et al., 2013; Defliese et al., 2015; Kele et al., 2015; Kluge et al., 2015; Tripathi et al., 2015; Kelson et al., 2017) or to the inter-laboratory universal calibration for all carbonate minerals (Eq. 3 in Bonifacie et al., 2017 that compiles 103 mean Δ_{47} data from seven different laboratories and with proper error propagation) (see section 4.3.2 for more details). We can therefore infer that coccolith calcite is not only barren of interspecies vital effects, but also that these phytoplanktonic biominerals, at least for the three investigated species, faithfully record the temperature of calcification in their Δ_{47} composition (ie., without vital effects overprint). It may remain rather speculative that *all* coccolith species lack a Δ_{47} vital effect. Nevertheless, as the three species exhibiting the largest oxygen and carbon isotope vital effects have similar (and close to equilibrium Δ_{47} composition), the hypothesis of the lack of Δ_{47} vital effect in all coccolithophores appears to be a reasonable assumption.

4.3.2 Comparison with previous Δ_{47} calibrations

In this section, we compare our coccolith data with previously-reported Δ_{47} data in literature. To this aim, we focus on the individual data rather than on the determined Δ_{47} -T calibration equations because it has been recently highlighted that either a small size of sample population and/or a narrow range of investigated temperatures could strongly influence the slopes and intercepts of the Δ_{47} -T calibration equations (Bonifacie et al., 2017; Kelson et al., 2017). This caveat has been put forward as a likely cause of discrepancies between discrete Δ_{47} -T calibration equations (as discussed in the following discussion), thus hampering rigorous comparison of the temperature estimates obtained from discrete Δ_{47} -T calibrations. To circumvent this problem, it has recently been suggested that universal Δ_{47} -T calibration based on a large dataset from different laboratories should be used to standardize Δ_{47} -derived temperature estimates determined in different studies/laboratories (Bonifacie et al., 2017; Kelson et al., 2017). As a result, in the following discussion and in Figure 5, we first underline the excellent consistency of coccolith Δ_{47} data both with previously published discrete data points and with the inter-laboratory composite calibration for all carbonates (compiling 103 mean Δ_{47} data from seven different laboratories with proper error propagation; Eq. 3 in Bonifacie et al., 2017). Then, as no vital effect on Δ_{47} values is expressed in coccolith calcites, we recommend that determination of SSTs from sedimentary coccoliths Δ_{47} data is made via application of this standardized (inter-laboratory) calibration.

Taken collectively, coccolith calcites have indeed Δ_{47} values consistent with most of previously reported data (Figure 5). It is worth pointing that our Δ_{47} data on laboratory-cultured coccolith calcite fall in the center of the spread of these previous Δ_{47} data, either for inorganic calcite (Figure 5a; Ghosh et al., 2006; Dennis et Schrag, 2010; Zaarur et al., 2013; Defliese et al., 2015; Kele et al., 2015; Kluge et al., 2015; Tripathi et al., 2015; Kelson et al., 2017) or for a larger dataset from biogenic or inorganic carbonates regardless of their mineralogies and chemistries (all data being reported in the CDES frame) (Figure 5b; Henkes et al., 2013; Came et al., 2014; Douglas et al., 2014; Fernandez et al., 2014; Wacker et al., 2014; Spooner et al., 2016; Wacker et al., 2016; Winkelstern et al., 2016; Bonifacie et al., 2017; Kelson et al., 2017). It is also apparent that our coccolith data are consistent, when the respective uncertainties are considered, with the two coccolith samples measured by Tripathi et al. (2010). Figure 5 also highlights that even if they originate from three different coccolith species, our laboratory-precipitated coccoliths show, for a given temperature, only a small dispersion of Δ_{47} values compared to most available data (Figure 5a and b). Appreciable dispersion of Δ_{47} data is apparent on the cold-water corals, the only biomineralizing organisms exhibiting Δ_{47} vital effects thus far (Spooner et al., 2016).

The reasons behind the observed dispersion of Δ_{47} calibration data in inorganic carbonates (that can reach up to 60 ppm) have been extensively discussed over the past four years and tentatively attributed to the following influences or combination thereof: i/ various methods used for mineral precipitation (e.g. Kelson et al., 2017); ii/ structure or composition of the analyzed mineral (e.g. Bonifacie et al., 2017); iii/ means of data acquisition, including the temperature of phosphoric acid used to digest the carbonates, sample size, phosphoric acid preparation, methods for CO₂ gas purification (e.g. Henkes et al., 2013; Wacker et al., 2013; Defliese et al., 2015; Bonifacie et al., 2017; Kelson et al., 2017); or iv/ *a posteriori* data processing, including the choice of appropriate parameters accounting for the ¹⁷O interferences in $\delta^{13}\text{C}$ determination (e.g., parameters from Santrock et al., 1985 or Brand et al., 2010), and/or the stability/accuracy of the frames constructed to correct raw Δ_{47} values from the effects of non-linearity and fragmentation/recombination in the mass spectrometer (e.g., when insufficient or unsuitable equilibrated CO₂ standards were run

concurrently with the samples, the retrospective, or *indirect*, projection of data into the absolute CDES with Tertiary Transfer Functions (TTF) is a source of unquantifiable errors) (e.g., Schauer et al., 2016; Daëron et al., 2016; Bonifacie et al., 2017; Kelson et al., 2017). Recent studies suggest that this observed wide dispersion of Δ_{47} calibration data is unlikely to result from mineral precipitation procedures (Kelson et al., 2017), acid digestion temperature (Defliese et al., 2015; Kelson et al., 2017) or the mineralogy/structure of the analyzed carbonate (Bonifacie et al., 2017), leaving phosphoric acid preparation, CO₂ gas purification and/or data reduction methods (i.e., the construction of the EGL and ETF correction frames and/or ¹⁷O corrections) as candidates for the discrepancies among reported calibrations. Thus, Figure 5b only shows data *directly* acquired into the absolute reference frame – that is constructed with a sufficient number of equilibrated CO₂ standards with absolute Δ_{47} values known from theoretical calculations (Wang et al., 2004) and ran concurrently to unknown samples. Importantly, it is striking that the additional recent data from Kelson et al. (2017) on inorganic calcites corrected from the ¹⁷O interferences with the Brand et al. (2010) parameters (56 mean Δ_{47} data) are falling within the 95% confidence interval envelop of the inter-laboratory universal calibration (calculated with 103 mean Δ_{47} values and proper error propagation by Bonifacie et al., 2017) and also within the uncertainties of all other data plotted in Fig. 5, while the later datasets were likely all ¹⁷O corrected using the Santrock et al. (1985) parameters. As importantly, the absolute values of the slope and intercept of the composite calibration by Bonifacie et al. (2017) are unchanged if these 56 new Δ_{47} data from Kelson et al. (2017) were added to the calculation – note that the same is true when the 11 mean Δ_{47} values obtained here on coccoliths are added to the calibration calculation. These observations reinforce our suggestion that the inter-laboratory universal Δ_{47} -T calibration defined by (Eq.3) in Bonifacie et al. (2017) can be used to alleviate possible discrepancies resulting from various methods ran for both data acquisition and treatment, and ultimately standardize temperature estimates from different laboratories. We thus recommend the use of this equation for reconstructing accurate and standardized (inter-laboratory) SSTs based on Δ_{47} data from diagenetically-screened sedimentary coccoliths.

4.4. Paleooceanographic implications

The absence of vital effects in the clumped isotope system observed in coccolith calcite for temperatures between 7 and 25 °C may stimulate further paleooceanographic studies using this proxy for oceanic paleotemperatures. Considering the inter-laboratory calibration of Bonifacie et al. (2017), for a ± 5 ppm uncertainty on the average Δ_{47} values, the 1SD uncertainty in sea surface temperature (SST) reconstruction would be less than ± 1.3 °C for the full range of coccolith relevant temperatures. Such uncertainties compare well to those of other paleotemperature proxies (Lea, 2014). It is, however, worth noting that the purity of the coccolith assemblages may represent a further complicating factor, in particular due to possible co-existence of non-coccolith or diagenetic particles in the obtained coccolith microfractions (Drury and John, 2016).

Since our data were obtained from extant species, we must discuss whether the mechanisms leading to vital effects may have operated in the ancient species encountered in the sedimentary archive. As discussed in more details in Section 4.5, carbon limitation by the coccolithophores in present-day oceans and laboratory cultures is the primary factor behind the expression of the vital effects affecting $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ coccolith compositions (Bolton and Stoll, 2013; Hermoso, 2016; Hermoso

et al., 2016a; Hermoso et al., 2016b; McClelland et al., 2017). Under this biogeochemical assumption, we can thus make the safe assumption that during the Meso-Cenozoic Eras, when ambient atmospheric CO₂ concentrations were much higher than present-day values (Pagani et al., 2005; Hönisch et al., 2012), the isotopic composition of ancient coccolith calcite was likely close to equilibrium conditions. As modern coccolith species lack a Δ_{47} vital effect, the inter-laboratory Δ_{47} – T calibration of Bonifacie et al. (2017) can therefore be applied to process Δ_{47} signatures of sedimentary coccoliths.

The geochemistry of the coccoliths in paleoceanography remains under-exploited because of the relative small sizes (2 - 10 μm across) of these nannofossils compared to foraminifera that can be more easily extracted by hand-picking. Yet, coccoliths have multiple advantages over foraminifera that relate to their ecology and preservation state. Recent works have developed a mechanistic understanding of the vital effects in coccoliths and allow new paleoceanographic information to be derived from these calcareous nannofossils (Hermoso, 2014; Tremblin et al., 2016; McClelland et al., 2017). The lack of Δ_{47} vital effect for the coccolith species being examined here further indicates that measuring species-specific assemblages from sediments is not a prerequisite to generate meaningful SSTs because changes in coccolith relative abundance (themselves potentially linked to temperature change) are not likely to alter Δ_{47} -derived SSTs, conversely to the case of $\delta^{18}\text{O}$ values.

During geological time interval characterized by greenhouse conditions (e.g. the Cretaceous and the Early Paleogene), global seawater $\delta^{18}\text{O}$ values remained relatively constant through time (Cramer et al., 2009). Thus, generating Δ_{47} analyses can complement traditional geochemical tools used in paleoceanography and increase the accuracy of reconstructed temperatures. In contrast, during geological periods characterized by fluctuations in the volume of ice-caps, the reconstruction of sea surface temperatures from either foraminifera or coccoliths may be further complicated by coeval changes in seawater $\delta^{18}\text{O}$ values. Δ_{47} measurements can contribute disentangling the combination of the two factors dictating calcite $\delta^{18}\text{O}$ values (that is temperature and $\delta^{18}\text{O}$ values of seawater), and thus, access to true sea surface temperature and seawater oxygen isotopic compositions. Paired measurements of $\delta^{18}\text{O}$ and Δ_{47} values have recently been undertaken in the geological record on various carbonates across the Eocene – Oligocene climate transition (Petersen et Schrag, 2015) to estimate the Antarctic ice growth or during the late Ordovician – early Silurian glaciation (Finnegan et al., 2011) to estimate the magnitude and the duration of the glaciation. For the coccoliths, this approach requires that the oxygen isotope vital effect in coccoliths can be constrained by another means, such as the emerging “paleo-physiological” approach (e.g., Hermoso et al., 2016a).

4.5. Constraints on the carbon and oxygen vital effects in coccolith calcite

In coccolithophores, large isotopic ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) departures of their biominerals from inorganic condition are known and are primarily attributed to intracellular calcification and the carbon limitation faced by marine microalgae (Hermoso, 2014). However, the coccoliths do not exhibit Δ_{47} vital effects. This intriguing feature has already been reported in other calcifying organisms such as brachiopods, foraminifera or some cold water corals (Tripathi et al., 2010; Thiagarajan et al., 2011; Grauel et al., 2013; Henkes et al., 2013; Came et al., 2014). Recent research has established that the vital effects affecting coccolith biominerals depends on the dynamics of the internal DIC pool, thus

the rate of consumption of the carbon resource from assimilation (mostly in the form of aqueous CO₂) to calcification (ultimately in the form of carbonate ions) in the coccolith vesicle (Hermoso et al., 2014; Hermoso et al., 2016a; Hermoso et al., 2016b; McClelland et al., 2017). As such, the origin of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ vital effects in coccolith calcite are the likely result of a variety of isotopic processes including: *i/* an isotopic disequilibrium of the DIC system in the coccolith vesicle at time of calcification (discussed in the following sections 4.5.1. and 4.5.2), *ii/* a kinetic effect related to calcification itself (section 4.5.3) or *iii/* a combination of the two (Ziveri et al., 2003; Rickaby et al., 2010; Bolton and Stoll, 2013; Hermoso et al., 2014; Hermoso et al., 2016b). These hypotheses are tested hereafter in light of our novel Δ_{47} data and current knowledge on the carbonate clumped isotope system (see recent publications of Tripathi et al., 2015 and Watkins and Hunt, 2015). The following sections of the discussion consist of a comparison between species in terms of their respective Δ_{47} , $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ signatures. Existing constraints on the inorganic (abiogenic) reference and calcification rates for the coccolith biomineralizing system remains poor and as such prevent us to strictly discuss the magnitude of the vital effects. Thus, differential vital effects can be discussed using the Kim et O'Neil (1997) inorganic framework.

4.5.1. Disequilibrium in the oxygen isotope system

It has been proposed that relatively high $\delta^{18}\text{O}$ values of *E. huxleyi* can be explained as a result of the contribution of aqueous CO₂ to the internal DIC pool (Hermoso et al., 2014; Hermoso et al., 2016b). Predominant acquisition of aqueous CO₂ relative to other DIC ionic species is a contrasting feature to other calcifying systems such as the foraminifera or bivalves that perform vacuolization of seawater, and as such, mainly source HCO₃⁻ from the external environment, as it is the dominant DIC species at oceanic pH values (McConnaughey, 1989). In coccolith calcite, non-equilibrium oxygen isotope composition may arise if calcification occurs prior to full re-equilibration of the CO₂-derived DIC pool with the oxygen atoms of the surrounding water molecules (Hermoso et al., 2014). Since cultured coccoliths do not exhibit vital effect in Δ_{47} values, this prime observation requires either that: *i/* the rate of isotopic re-equilibration between CO₂, DIC and water molecules is different for the $\delta^{18}\text{O}$ and Δ_{47} systems; or that *ii/* aqueous CO₂ has a Δ_{47} signature similar to other ionic DIC species. However, these two hypotheses seem unlikely for two reasons: Firstly, Affek (2013) and Clog et al. (2015) suggested a similar time of re-equilibration between CO₂ and water for both the oxygen and clumped isotope systems. Second, aqueous CO₂ has a Δ_{47} signature extremely different from both inorganic carbonates (offset by + 0.280‰ at 25 °C although this value is for gaseous CO₂, according to Wang et al., 2004) and also from the other ionic DIC species (Figure 6). Thus, the isotopic contribution of the CO₂ legacy to the DIC pool (and ultimately in the composition of the carbonate) should also be observed in Δ_{47} values, which is not the case in the present study. It therefore appears that the available clumped isotope data does not support the hypothesis of a CO₂ source accounting for the species of the “heavy group”.

It has also been proposed that the contribution of CO₃²⁻ present in the DIC pool (depleted in ¹⁸O) could account for relatively lighter oxygen isotope composition with respect to the inorganic reference, as observed for *Calcidiscus leptoporus* (Ziveri et al., 2012). In light of current knowledge on the Δ_{47} systematics, preferential incorporation of CO₃²⁻ ions during the carbonate formation should lead to more negative Δ_{47} signatures compared to inorganic carbonate signatures. Distinct offsets between CO₃²⁻ and inorganic reference are currently reported in literature. This figure is

either quantified at -0.023 or -0.056‰ at 25 °C, according to theoretical or experimental studies, respectively (Hill et al., 2014 and Tripathi et al., 2015; Figure 6). Current uncertainty in the Δ_{47} composition of CO_3^{2-} ions does not allow a conclusive assessment of this hypothesis accounting for the relatively low $\delta^{18}\text{O}$ composition of *C. leptoporus*. More accurate Δ_{47} values of CO_3^{2-} are required to further explore the isotopic mechanism at play in coccoliths of the “isotopic light group”.

4.5.2. Relative contribution of bicarbonate and carbonate ions to the isotopic composition of calcite

A second possible hypothesis to explain relative ^{18}O enrichment observed for *E. huxleyi* is an isotopic signature from the intracellular HCO_3^- that is actively pumped from the cytosol to the coccolith vesicle where calcification takes place (Brownlee et al., 2016). HCO_3^- ions have indeed an oxygen isotope composition higher by 6.5‰ relative to CO_3^{2-} at 25 °C (Beck et al., 2005). In the coccolith vesicle, HCO_3^- is ultimately converted into CO_3^{2-} to allow calcification (Brownlee et al., 2016). If calcification occurs before the CO_3^{2-} ions have reached full isotopic equilibrium within the DIC/ H_2O system, coccolith may still bear ^{18}O excess inherited from the heavier composition of HCO_3^- . Bicarbonate ions have a Δ_{47} isotopic signature close to inorganic calcite (difference between 0.011 and 0.017‰ between HCO_3^- and inorganic calcite at 25 °C, depending on the authors, Figure 6). Therefore, such a HCO_3^- footprint would only slightly raise calcite Δ_{47} signature and such an isotopic effect would be undetectable.

4.5.3. Kinetic effect during calcite precipitation

During the culture experiments, cell division rates can be easily measured over the course of the batch culture. In contrast, actual calcification rates of coccoliths are not constrained and only averaged values are documented in literature. Coccoliths are known to calcify in a matter of hours (Westbroeck et al., 1984; Taylor et al., 2012). We discuss whether ^{18}O depletion in calcite relative to equilibrium may be the result of a kinetic effect during the precipitation associated with such high calcification rates. Under the assumption that the DIC species are re-equilibrated with water molecules at time of calcification in the coccolith vesicle, a kinetic fractionation related to fast precipitation rate would theoretically decrease calcite $\delta^{18}\text{O}$ by favoring the light isotope (Zeebe and Wolf-Gladrow, 2001; Gabitov et al., 2012; Watkins et al., 2013) and simultaneously increase Δ_{47} values by 0.01‰ at biomineralization-relevant pH conditions (Watkins et al., 2015). If theory indeed predicts such an effect on calcite Δ_{47} values, it is however not apparent in inorganically-precipitated calcite. Indeed, recent studies aiming at establishing a link between precipitation rate and calcite Δ_{47} values have not reported any substantial effect, despite the large range of precipitation rate tested (Kluge et al., 2014; Tang et al., 2014; Kele et al., 2015; Tripathi et al., 2015; Kelson et al., 2017). This ambiguity in the expression of a kinetic effect in the clumped isotope system prevents us to draw a parallel between clumped and traditional oxygen isotopes for coccolith calcite. Once again, further analytical refinement would enhance existing constraints on these aspects and potentially alleviate this uncertainty.

5. CONCLUSIONS AND OUTLOOK

We have established a calibration of Δ_{47} compositions of coccolith materials cultured in laboratory under strict temperature control ranging from 7 to 25 °C. The calibration is based on the study of three common and geologically relevant coccolith species (*Emiliania huxleyi*, *Coccolithus pelagicus* spp. *braarudii* and *Calcidiscus leptoporus*) capturing the maximum spread of oxygen isotope vital effects reported in literature. We found that:

- The three coccolith species exhibit indistinguishable Δ_{47} values for a given growth temperature. The three Δ_{47} – T calibrations are thus indistinguishable from each other. We conclude that there is no apparent vital effect in the clumped isotope system between the different coccolith species, or that, if existing, their expression is lower than resolvable by the typical precision of Δ_{47} measurements.
- The Δ_{47} coccolith data are consistent with previously-reported inorganic and most biogenic data. We therefore further conclude that the coccoliths record temperature without any vital effects.
- With current knowledge of the clumped isotope systematics (i.e., processes leading to equilibrium and disequilibrium, Δ_{47} signature of the ionic DIC species, or on clumping models during the precipitation), it still remains difficult to determine the origin of the oxygen isotope vital effects with confidence and to identify at which step of DIC acquisition and mineralization the vital effects are at play. The data, however, seem to favor some hypotheses to explain oxygen and carbon vital effects in coccoliths, as a kinetic effect behind the “light group”. In contrast, other biogeochemical possibilities, as the isotopic imprint of the CO₂ source as an explanation for the oxygen “heavy group”, are not well supported by a comparison between Δ_{47} and $\delta^{18}\text{O}$ values.

The lack of vital effects in coccolith calcite confirms this micropaleontological archive as a valuable tool in geochemistry and paleoceanography for the Meso-Cenozoic to infer SSTs. The isotopic transfer function presented in the present study is not sensitive to the coccolith assemblage or change thereof over the geological record. Temperatures derived from the oxygen isotope composition of coccoliths accounting for seawater $\delta^{18}\text{O}$ values and suitable treatment of the vital effect requires species-specific assemblages, usually obtained from very narrow size splits typically obtained applying the technique by Minoletti et al. (2009). In contrast, temperatures from coccolith Δ_{47} measurements can be performed from mixed assemblages, thus shortening the duration of the microseparation process. It remains, however, essential to remove the finest fraction (< 2 μm) of sediments and as such, exclude the microcrystalline calcite particles of unknown nature and origin (micarbs) from the analyses, as they may not convey a surface water signal.

Acknowledgements

We thank Pierre Agrinier for help for the statistical treatment of our data and Fabrice Minoletti for discussions on the paleoceanographic implications of our study. We also thank Claire Rollion-Bard, François Guyot and Emmanuelle Pucéat for discussions. The coccolithophore species used in this study were kindly provided by the Roscoff Culture Collection and we are grateful to Ian Probert for providing access to these strains. MH acknowledges funding by the Natural Environment Research Council (NERC) under grant agreement NE/H015523/1. MB thanks the Emergences Ville de Paris Program, which funded the installation of the clumped isotope laboratory at IPGP and the CNRS-INSU SYSTER program, which provided additional financial support. This publication arises from

research funded by the John Fell Oxford University Press (OUP) Research Fund to MH and MB under a joint CNRS/Oxford collaborative scheme (Project Ref: 112/871). AK was supported through Université Paris Diderot PhD scholarship. All authors thank Hagit Affek and three anonymous reviewers for their constructive comments that helped improving this manuscript. This is IPGP contribution 3824.

Author contribution

MB and MH designed the study. MH performed the culture experiments at Oxford University. AK ran isotopic analyses at IPGP. All authors discussed the data and commented the manuscript. AK, MB and MH wrote the manuscript.

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Tables and Figures

Species	T _{growth} °C	$\delta^{13}\text{C}_{\text{carb}}$ (‰ VPDB)	$\delta^{13}\text{C}_{\text{DIC}}$ (‰ VPDB)	$\Delta^{13}\text{C}$ (‰ VPDB)	$\delta^{18}\text{O}_{\text{carb}}$ (‰ VPDB)	$\delta^{18}\text{O}_{\text{medium}}$ (‰ VSMOW)	$\Delta^{18}\text{O}$ (‰ VPDB - VSMOW)	$\Delta_{47\text{CDES25}}$ (‰ CDES)	$\Delta_{47\text{CDES90}}$ (‰ CDES)	Av. $\Delta_{47\text{CDES25}}$ (‰ CDES)	Av. $\Delta_{47\text{CDES90}}$ (‰ CDES)	$\pm 1 \text{ SD}$ (‰)	$\pm 1 \text{ SE}$ (‰)
<i>E. huxleyi</i>	7	-4.16	-2.8	-1.4	-1.83	-5.82	3.99	0.733 \pm 0.014	0.651				
<i>E. huxleyi</i>	7	-3.85	-2.8	-1.1	-1.98	-5.82	3.84	0.741 \pm 0.014	0.659	0.737	0.655	0.006	0.004
<i>E. huxleyi</i>	10	1.53	1.0	0.5	3.71	0.65	3.06	0.718 \pm 0.014	0.636				
<i>E. huxleyi</i>	10	1.90	1.0	0.9	3.76	0.65	3.11	0.734 \pm 0.014	0.652				
<i>E. huxleyi</i>	10	2.27	1.0	1.3	3.82	0.65	3.17	0.751 \pm 0.014	0.669	0.734	0.652	0.016	0.009
<i>E. huxleyi</i>	15	-2.40	-2.8	0.4	-4.66	-6.14	1.48	0.715 \pm 0.014	0.633				
<i>E. huxleyi</i>	15	2.68	1.0	1.7	2.72	0.65	2.07	0.704 \pm 0.014	0.622				
<i>E. huxleyi</i>	15	-2.54	-2.8	0.3	-4.73	-6.14	1.41	0.714 \pm 0.014	0.632	0.711	0.629	0.006	0.003
<i>E. huxleyi</i>	20	1.47	1.0	0.5	1.54	0.65	0.89	0.695 \pm 0.014	0.613				
<i>E. huxleyi</i>	20	1.45	1.0	0.4	1.64	0.65	0.99	0.705 \pm 0.014	0.623				
<i>E. huxleyi</i>	20	1.43	1.0	0.4	1.63	0.65	0.99	0.709 \pm 0.014	0.627				
<i>E. huxleyi</i>	20	1.46	1.0	0.5	1.57	0.65	0.93	0.697 \pm 0.014	0.615				
<i>E. huxleyi</i>	20	1.43	1.0	0.4	1.70	0.65	1.05	0.712 \pm 0.014	0.630	0.703	0.621	0.007	0.003
<i>E. huxleyi</i>	25	0.78	1.0	-0.2	0.23	0.65	-0.41	0.698 \pm 0.014	0.616				
<i>E. huxleyi</i>	25	0.67	1.0	-0.3	0.29	0.65	-0.36	0.696 \pm 0.014	0.614				
<i>E. huxleyi</i>	25	0.75	1.0	-0.2	0.24	0.65	-0.41	0.686 \pm 0.014	0.604	0.693	0.611	0.007	0.004
<i>C. braarudii</i>	10	-2.08	1.0	-3.1	1.48	0.65	0.83	0.752 \pm 0.014	0.670				
<i>C. braarudii</i>	10	-1.50	1.0	-2.5	1.31	0.65	0.66	0.745 \pm 0.014	0.663				
<i>C. braarudii</i>	10	-1.20	1.0	-2.2	1.35	0.65	0.70	0.730 \pm 0.014	0.648				
<i>C. braarudii</i>	10	-1.65	1.0	-2.6	1.40	0.65	0.75	0.736 \pm 0.014	0.654	0.741	0.659	0.01	0.005
<i>C. braarudii</i>	15	-2.10	1.0	-3.1	0.18	0.65	-0.47	0.728 \pm 0.014	0.646				
<i>C. braarudii</i>	15	-2.34	1.0	-3.3	0.28	0.65	-0.37	0.720 \pm 0.014	0.638				
<i>C. braarudii</i>	15	-2.40	1.0	-3.4	0.17	0.65	-0.48	0.741 \pm 0.014	0.659				
<i>C. braarudii</i>	15	-2.37	1.0	-3.4	0.16	0.65	-0.49	0.731 \pm 0.014	0.649				
<i>C. braarudii</i>	15	-2.28	1.0	-3.3	0.43	0.65	-0.22	0.711 \pm 0.014	0.629	0.726	0.644	0.012	0.005
<i>C. braarudii</i>	20	-4.29	1.0	-5.3	-0.46	0.65	-1.11	0.709 \pm 0.014	0.627				
<i>C. braarudii</i>	20	-3.92	1.0	-4.9	-0.45	0.65	-1.09	0.709 \pm 0.014	0.627				
<i>C. braarudii</i>	20	-4.00	1.0	-5.0	-0.31	0.65	-0.96	0.721 \pm 0.014	0.639	0.713	0.631	0.007	0.004
<i>C. leptoporus</i>	10	-1.71	1.0	-2.7	0.33	0.65	-0.32	0.735 \pm 0.014	0.653				
<i>C. leptoporus</i>	10	-1.88	1.0	-2.9	0.35	0.65	-0.30	0.741 \pm 0.014	0.659				
<i>C. leptoporus</i>	10	-1.80	1.0	-2.8	0.25	0.65	-0.40	0.729 \pm 0.014	0.647	0.735	0.653	0.006	0.004
<i>C. leptoporus</i>	15	-1.80	1.0	-2.8	-0.69	0.65	-1.34	0.741 \pm 0.014	0.659				
<i>C. leptoporus</i>	15	-1.83	1.0	-2.8	-0.74	0.65	-1.39	0.711 \pm 0.014	0.629				
<i>C. leptoporus</i>	15	-1.71	1.0	-2.7	-0.37	0.65	-1.01	0.724 \pm 0.014	0.642	0.725	0.643	0.015	0.008
<i>C. leptoporus</i>	20	-1.69	1.0	-2.7	-1.89	0.65	-2.54	0.695 \pm 0.014	0.613				
<i>C. leptoporus</i>	20	-1.61	1.0	-2.6	-1.88	0.65	-2.53	0.719 \pm 0.014	0.637				
<i>C. leptoporus</i>	20	-1.66	1.0	-2.7	-1.87	0.65	-2.52	0.712 \pm 0.014	0.630				
<i>C. leptoporus</i>	20	-0.95	1.0	-2.0	-1.80	0.65	-2.45	0.710 \pm 0.014	0.628				
<i>C. leptoporus</i>	20	-1.06	1.0	-2.1	-1.80	0.65	-2.45	0.711 \pm 0.014	0.629	0.710	0.628	0.009	0.004

Table 1. Stable isotope compositions ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and Δ_{47}) of cultured coccoliths for each individual culture residue. The $\Delta^{13}\text{C}$ corresponds to the “ $\delta^{13}\text{C}_{\text{carb}}$ - $\delta^{13}\text{C}_{\text{DIC}}$ ” offset (the carbon isotopic composition of the coccolith and that of the dissolved inorganic carbon in the medium, respectively), and $\Delta^{18}\text{O}$ corresponds to “ $\delta^{18}\text{O}_{\text{carb}}$ - $\delta^{18}\text{O}_{\text{medium}}$ ” (the oxygen isotopic composition of the coccolith and that of the medium, respectively). Coccolith Δ_{47} values are reported in the absolute Carbon Dioxide Equilibrated Scale (defined by Dennis et al., 2011) for acid digestion at 25 °C and 90 °C ($\Delta_{47\text{CDES25}}$ and $\Delta_{47\text{CDES90}}$ respectively). The $\pm 0.014\text{‰}$ uncertainty applied on each single Δ_{47} value corresponds to the 1SD long-term external reproducibility obtained at IPGP on replicate measurements of homogeneous carbonate reference materials (with $n > 300$). “Av. $\Delta_{47\text{CDES}}$ ” values correspond to the mean of Δ_{47} measurements for a given species at a given temperature. The variability of the Δ_{47} values are reported as: “ $\pm 1\text{SD}$ ” = one standard deviation of the mean; “ $\pm 1\text{SE}$ ” = one standard error of the mean (i.e., $1\text{SD} / \sqrt{n}$; that is the uncertainty considered in most of previous Δ_{47} calibrations studies).

Species	Slope	Intercept	r^2
<i>E. huxleyi</i>	0.0311 (± 0.0139)	0.3415 (± 0.1662)	0.88
<i>C. braarudii</i>	0.0332 (± 0.0255)	0.3262 (± 0.3080)	0.77
<i>C. leptoporus</i>	0.0310 (± 0.0243)	0.3495 (± 0.2913)	0.78

Table 2. Parameters (slopes and intercepts) of the three $\Delta_{47} - 1/T^2$ calibrations obtained for the three species. Mentioned uncertainties correspond to the 95% confidence intervals.

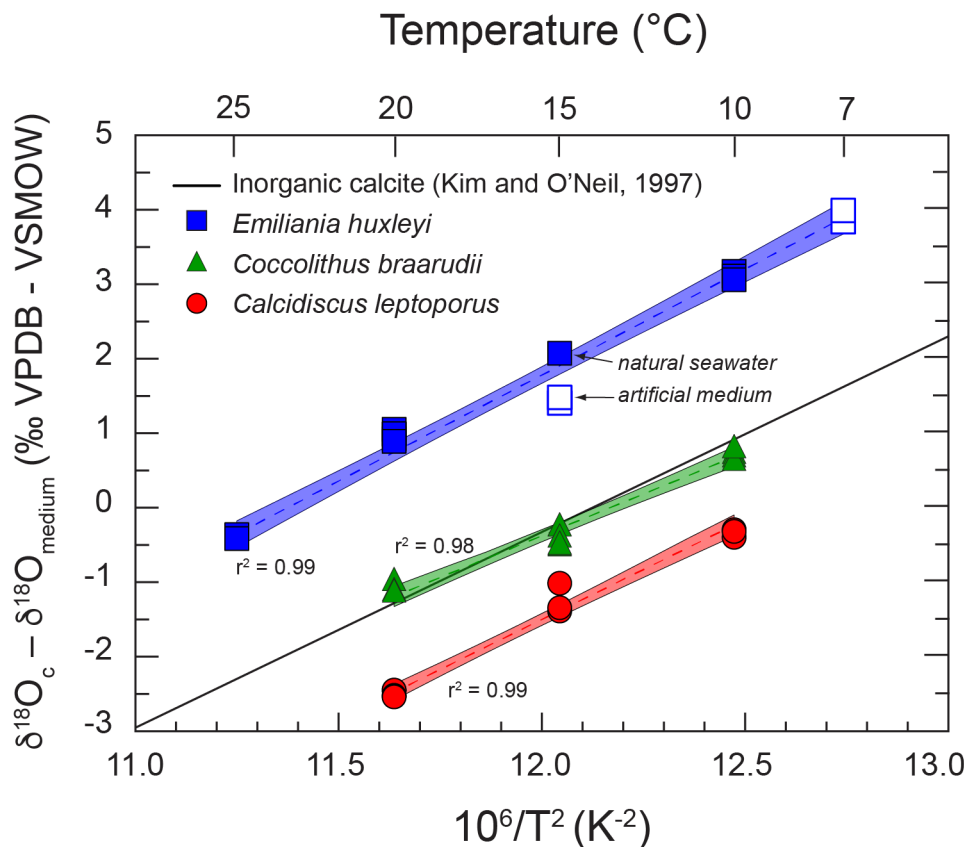


Figure 1. Relationship between the oxygen isotope composition of coccolith calcite expressed as the offset $\delta^{18}\text{O}_{\text{carb}} - \delta^{18}\text{O}_{\text{medium}}$ with growth temperature. Each single datapoint represents one stable isotope analysis of one given culture residue. Growth temperatures are given in $^{\circ}\text{C}$ (top x axis) or using the $10^6/T^2$ notation (bottom x axis). The black line represents the composition of inorganic calcite by Kim and O’Neil (1997). The three cultured species are distributed on either side on the inorganic reference with *Coccolithus braarudii* calcifying at near inorganic condition (in line with previous reports; Ziveri et al., 2003; Rickaby et al., 2010; Candelier et al., 2013; Hermoso et al., 2014; Stevenson et al., 2014). In contrast, *Emiliana huxleyi* and *Calcidiscus leptoporus* are isotopically distinct from the inorganic calcite of Kim and O’Neil (1997) with the assignment of the former to an “isotopically heavy group” and the later to an “isotopically light group”. The correlations between $\delta^{18}\text{O}_{\text{carb}} - \delta^{18}\text{O}_{\text{medium}}$ offsets and $1/T^2$ are linear fits (dashed lines) plotted with their respective 95% confidence intervals (colored envelop areas).

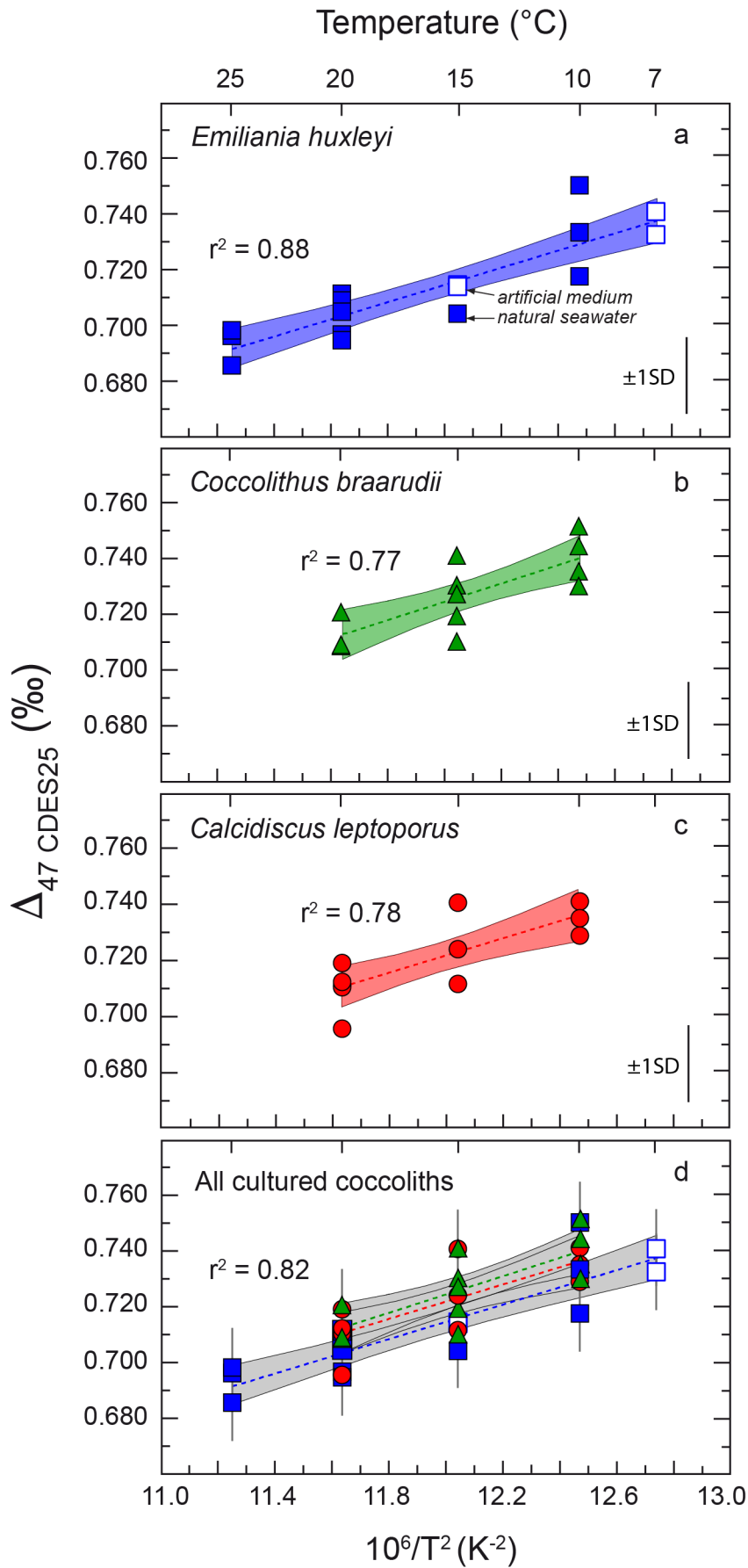


Figure 2. Relationship between the carbonate clumped isotope composition of coccolith calcite and calcification temperature. All the data are reported in the CDES reference frame for acid digestion performed at 25 °C ($\Delta_{47\text{CDES}_{25}}$ in Table 1). Growth temperatures are given in °C (top x axis) or using

the $10^6/T^2$ notation (bottom x axis). Each datapoint represents one Δ_{47} measurement of one individual culture residue. The three studied species are plotted in distinct panels: (a) *Emiliana huxleyi*, (b) *Coccolithus braarudii* and (c) *Calcidiscus leptoporus*. Panel (d) shows the three species combined. For all the panels, the reported uncertainties on Δ_{47} data correspond to the long-term external reproducibility of $\pm 0.014\%$ (1SD) obtained at IPGP on homogeneous carbonate reference materials and uncertainties on growth temperatures (± 0.2 °C) are smaller than the symbol size. For each investigated species, the regression lines (dashed lines) are plotted with their 95% confidence intervals (colored or grey areas).

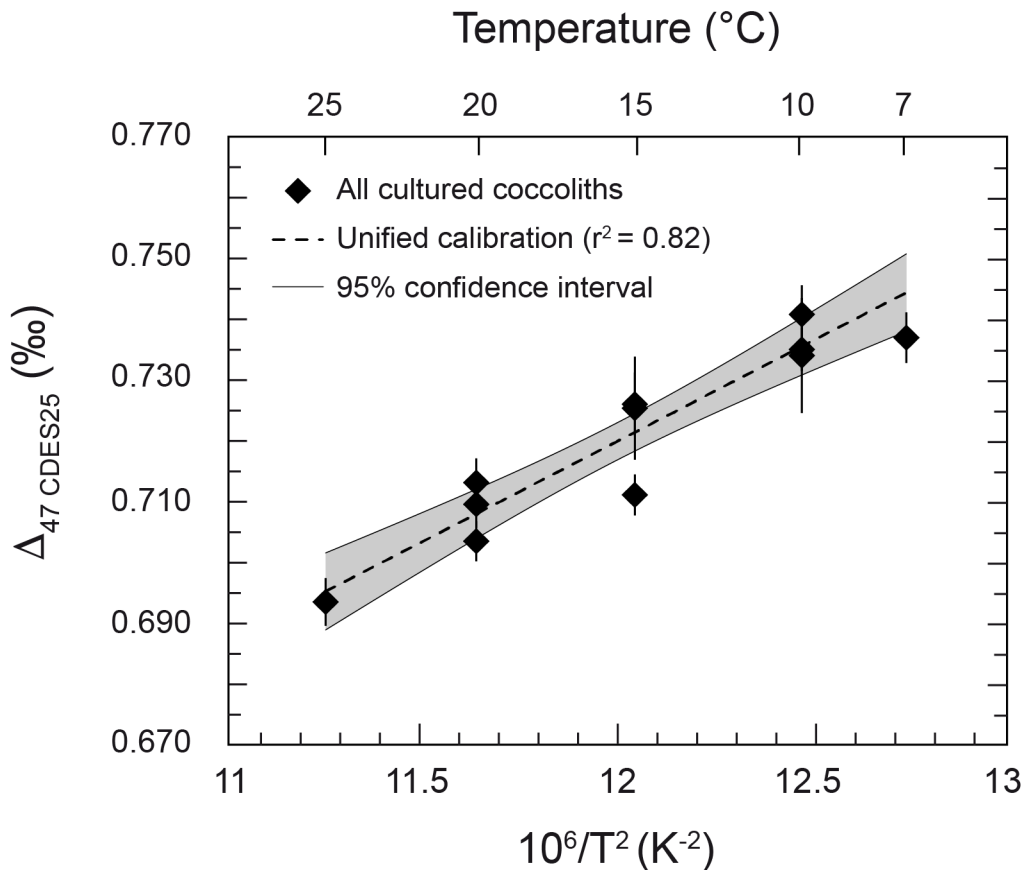


Figure 3. Unified (coccolith-integrated) correlation between $\Delta_{47\text{CDES25}}$ values and temperature. Each datapoint represents the average of all Δ_{47} data obtained for a given species at a given temperature. The error bars are reported as $\pm 1\text{SE}$ for averaged Δ_{47} data, following common practice. The linear fit ($r^2 = 0.82$) of the calibration line (dashed line) is plotted with its 95% confidence interval (grey area). The equation of the calibration is given in Eq. 2 in text.

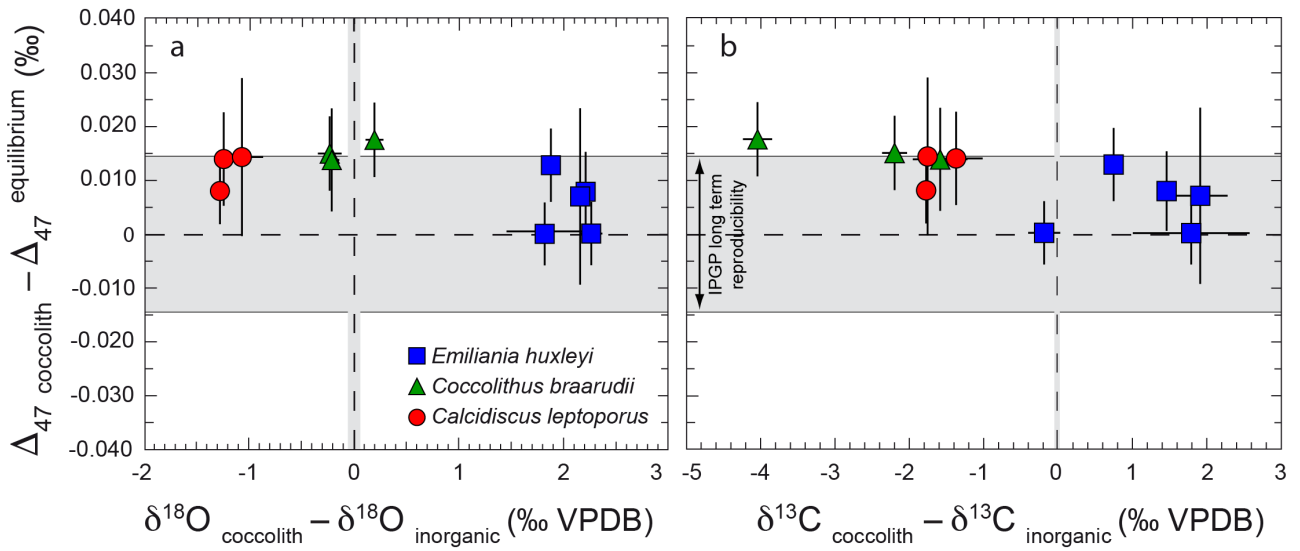


Figure 4. Relationship between the vital effect in clumped (y -axis) and traditional carbon oxygen isotope values (x axes) expressed as the deviation of coccolith Δ_{47} from equilibrium/inorganic conditions for the three cultures coccolith species. Theoretical equilibrium Δ_{47} values ($\Delta_{47}^{\text{equilibrium}}$) were calculated using the theoretical Δ_{63} abundance of $^{13}\text{C}^{18}\text{O}^{16}\text{O}_2$ isotopologues inside the calcite lattice derived from ab-initio calculations (Schauble et al., 2006) and accounting for a Δ^* fractionation factor of + 0.268‰ (see text). Equilibrium $\delta^{18}\text{O}$ values are from Kim and O’Neil (1997). Equilibrium $\delta^{13}\text{C}$ values were obtained by adding 1‰ to the isotopic composition of the DIC, as determined by Romanek et al. (1992). In both panels, each datapoint represents the average of isotopic (Δ_{47} , $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) measurements ($n = 2$ to 5) for a given species and a given temperature reported with their respective one standard deviation of the mean. The grey shaded areas represent our 1SD long-term external reproducibilities of replicated measurements on homogeneous standard materials for Δ_{47} , $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ ($\pm 0.014\text{‰}$, $\pm 0.05\text{‰}$ and $\pm 0.02\text{‰}$, respectively). We observe that strong species-specific vital effects imprint the oxygen (panel a) and carbon (panel b) isotopic compositions for the three species, whereas this isotopic phenomenon is not apparent in Δ_{47} values.

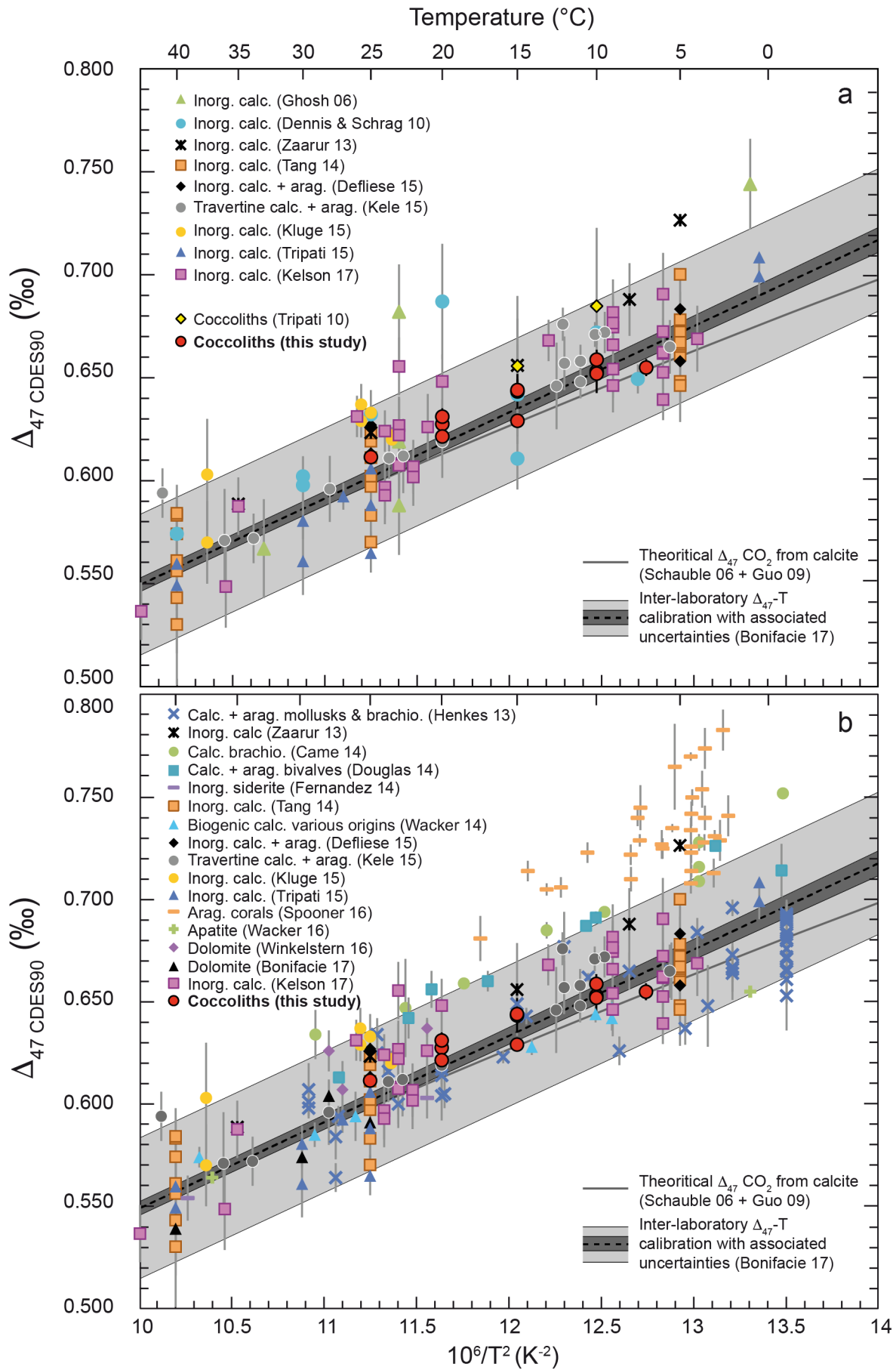


Figure 5. Comparison of the data from the present study with previously published Δ_{47} calibration data for either inorganic calcite (panel a) or a larger dataset of biogenic carbonates or inorganic minerals with other mineralogies than calcite (panel b). All Δ_{47} data correspond to averaged values

with uncertainties reported as 1 SE. All Δ_{47} data refer to CO_2 released after digestion at 90 °C. All data obtained from acid digestion at other temperatures were converted into the $\Delta_{47}^{\text{CDES90}}$, applying the following corrections: $\Delta^*_{25-90} = -0.082\text{‰}$; $\Delta^*_{70-90} = -0.020\text{‰}$ and $\Delta^*_{100-90} = +0.019\text{‰}$ (after Defliese et al., 2015). All Δ_{47} data are also reported relative to the absolute CDES reference frame. Data are either reported as given in the publication when *directly* projected into CDES (that is when standards of equilibrated CO_2 with absolute Δ_{47} values known from theoretical calculations – or for some studies carbonate standards with absolute Δ_{47} values previously scaled with equilibrated CO_2 gases – and run interspersed with unknown samples were used to construct the absolute CDES frame) or *indirectly* (retrospectively) projected using the Tertiary Transfer Functions TTF given in Table 4 of Dennis et al., (2011). Because the choice of Tertiary Transfer Functions to retrospectively transfer data into the absolute CDES frame is a source of unquantifiable errors (particularly when there is insufficient information about standards that were co-analyzed with the calibration samples), panel (b) only shows Δ_{47} data that were *directly* projected into the CDES in order to filter from possible bias. In both panels, the grey curve represents the equilibrium Δ_{47} reference obtained by adding the fractionation factor $\Delta^* = +0.268\text{‰}$ to the Δ_{63} theoretical predictions from Schauble et al. (2006) for calcite mineral. The dashed black line represents the inter-laboratory composite calibration of Bonifacie et al. (2017) plotted with its 95% confidence envelop (dark grey area). The pale grey area represents the 95% confidence envelop for a temperature prediction for a single Δ_{47} measurement. All data plotted in this Figure were corrected from ^{17}O interferences with the parameters from Santrock et al. (1986) (although not explicitly specified in most cases), with the exception of the recent data from Kelson et al. (2017) correcting ^{17}O interferences with the parameters from Brand et al. (2010). Note that our coccolith Δ_{47} data are not significantly changed if reprocessed with ^{17}O correction values from Brand et al. (2010).

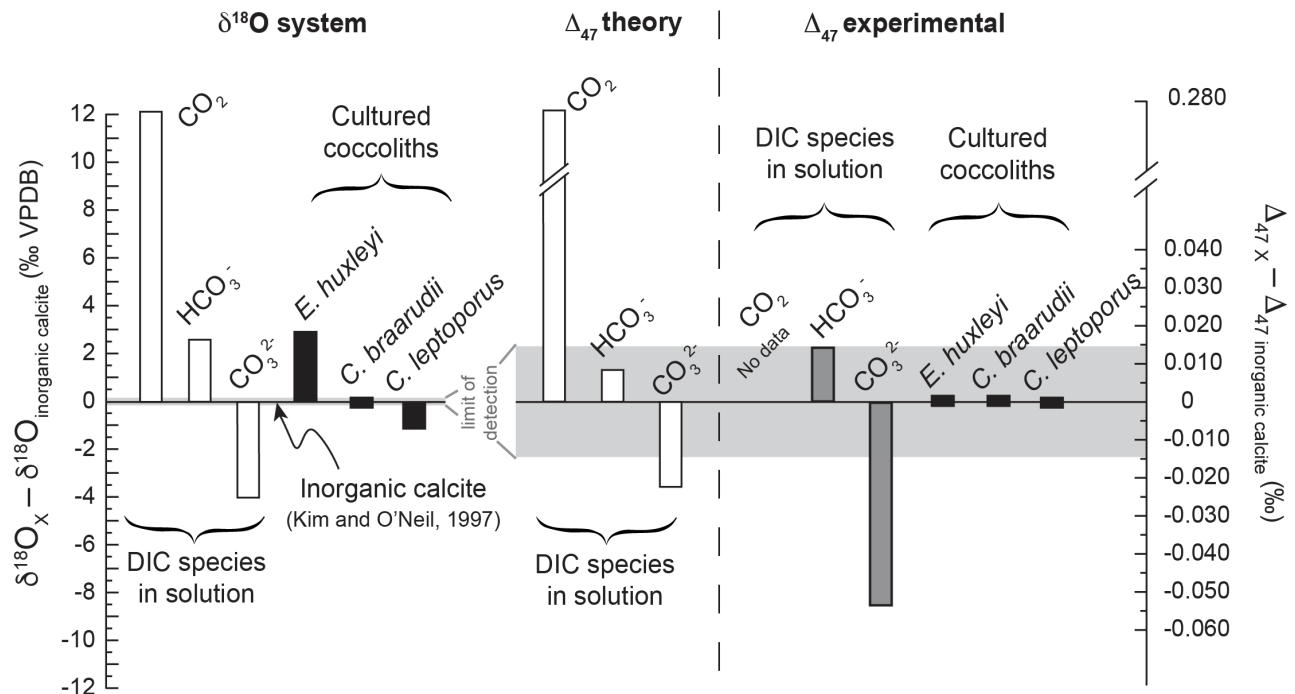


Figure 6. Biogeochemical insights from the comparison between coccolith, equilibrium and DIC isotopic compositions (Δ_{47} and $\delta^{18}\text{O}$). All the data presented were adjusted to a temperature of 25 °C. The $\delta^{18}\text{O}$ value for inorganic calcite is from Kim and O'Neil (1997). $\delta^{18}\text{O}$ value for CO_2 , HCO_3^- and CO_3^{2-} (white histograms) are from Beck et al. (2005). The Δ_{47} value for inorganic calcite is derived using the theoretical Δ_{63} abundance of $^{13}\text{C}^{18}\text{O}^{16}\text{O}_2$ isotopologues into calcite lattice out of

ab initio calculations to which the fractionation factor $\Delta^* = + 0.268\text{‰}$ is added (experimentally determined by Guo et al., 2009 and transferred here into the absolute CDES reference frame). The white and grey histograms depict the compositions of the DIC species, whereas black histograms correspond to measured coccolith compositions in the present study. The grey zones represent the analytical precision for the oxygen and Δ_{47} measurements ($\pm 0.05\text{‰}$ and $\pm 0.014\text{‰}$, respectively). The Δ_{47} theoretical values of DIC species are calculated from Wang et al. (2004) for CO_2 [note that the CO_2 values is for gaseous CO_2] and are from Hill et al. (2014) for HCO_3^- and CO_3^{2-} (white histograms). Δ_{47} experimental values are from Tripathi et al. (2015) for HCO_3^- and CO_3^{2-} (grey histograms).