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Key Points:
- *Thoracosphaera heimii* precipitates calcite with near-equilibrium $\delta^{18}O$ values
- Very dilute batch cultures agree with near-monospecific core top assemblages
- $\delta^{18}O$ of *T. heimii* allows reconstructing intermediate photic zone temperature

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Calibration of stable isotope composition of *Thoracosphaera heimii* (dinoflagellate) calcite for reconstructing paleotemperatures in the intermediate photic zone

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Abstract In this study we investigate the temperature dependence of oxygen isotope ratios preserved in calcite formed by the dinoflagellate *Thoracosphaera heimii*, focusing primarily on the development of a geological proxy. Geochemical analysis of the calcite shells produced by this species represents a valuable proxy for reconstructing environmental conditions in the intermediate photic zone. Calibration is based on isotopic analysis from culture experiments performed in very dilute batch conditions, as well as from near-monospecific *T. heimii* assemblages separated from core top sediments. Results are similar for both approaches and indicate that *T. heimii* shells have oxygen isotope compositions close to equilibrium values predicted for inorganic calcite precipitation. This calibration of the isotopic composition of dinoflagellate calcite indicates that monospecific assemblages of *T. heimii* can be used to unravel paleotemperatures in the intermediate photic zone by applying isotopic transfer functions for equilibrium calcite. In culture, however, a $\delta^{18}O$ offset of $-1‰$ is observed at temperatures $<17°C$, which falls below the natural temperature range of this species. Culture analyses also reveal a relationship between temperature and carbon isotope composition of calcite. The mechanisms behind this relationship remain to be explored, but their identification may provide a better understanding of carbon isotope systematics from both biogeochemical and geological perspectives. Comparison of the oxygen isotope composition of *T. heimii* shells with that of shallower dwelling organisms, such as the coccolithophores, represents a valuable proxy for determining temperature gradients within the photic zone and may enable reconstruction of the evolution of the depth of the thermocline.

1. Introduction

Generating reliable sea surface temperatures (SSTs) is a principle aim for paleoceanographers and is fundamental for successful modeling of climate sensitivity in the past, present, and future. This has motivated considerable effort in the development of paleotemperature proxies, yet uncertainties remain on the fidelity of SST estimates. Most of the studies aiming to derive paleotemperatures are based on planktic foraminifera oxygen isotope composition ($\delta^{18}O$) because their tests can be easily isolated from sediments by hand-picking.

Calcareous phytoplankton algae, such as coccolithophores and calcareous dinoflagellates, thrive in the photic zone, and hence, calcification takes place close to the interface between the atmosphere and the ocean. The geochemistry of their shells may hence represent a valuable addition to the paleoceanography toolbox to augment data from foraminifera enabling more precise reconstruction of the physico-chemistry of the photic zone. The increasing interest in phytoplankton geochemistry has been made possible by recent development and application of sedimentological techniques enabling the isolation and geochemical analysis of their micron-sized calcified shells [*Minoletti et al.*, 2001; *Stoll and Ziveri*, 2002; *Minoletti et al.*, 2004; *Minoletti et al.*, 2005; *Stoll*, 2005; *Zonneveld et al.*, 2007; *Hermoso et al.*, 2009; *Minoletti et al.*, 2009; *Bolton et al.*, 2012; *Rousselle et al.*, 2013].

Calcareous dinoflagellates mainly thrive in warm, low-latitude waters often characterized by low nutrient levels [*Zonneveld et al.*, 2000; *Kohn and Zonneveld*, 2010]. In situ measurements have shown that maximum abundance of living cells is found in the photic zone below the mixed layer although it has to be acknowledged that this observation comes from very dilute cell counts [*Karwath et al.*, 2000; *Vink et al.*, 2000; *Wendler et al.*, 2002a, 2002b; *Vink*, 2004; *Kohn and Zonneveld*, 2010]. This putative deeper habitat with respect to that of the coccolithophores owes to their intolerance to high-energy water motion [*Thomas and Gibson*, 1990].

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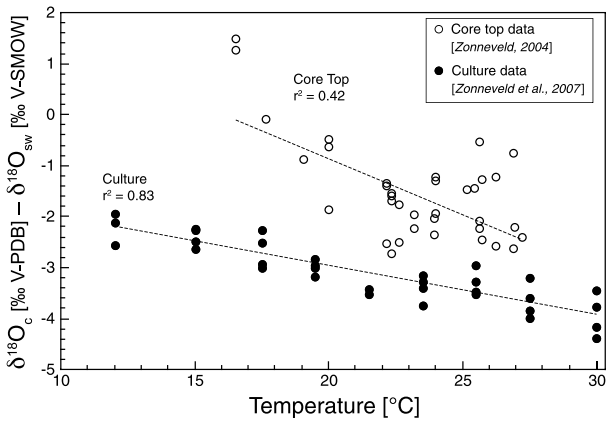


Figure 1. Previously reported magnitude of oxygen isotope fractionation estimated by the $\delta - \delta$ notation for *T. heimii* calcite from culture (black circles) and core tops (open circles). Data are from *Zonneveld et al.* [2007] and *Zonneveld* [2004], respectively. There is a good overall relationship between temperature and oxygen isotope composition of cultured calcispheres, with replicates comprised within a mean 1‰ array for the same temperature. The relatively negative $\delta^{18}O$ values indicate a vital effect toward isotopically light values. Hence, these data indicate that *T. heimii* calcite can be tentatively assigned to the "light group" (sensu *Dudley et al.* [1986]). Conversely, in the core top study, no correlation is found between temperature and $\delta^{18}O$, with significantly more positive $\delta^{18}O$ values with respect to the culture data.

Another contrasting feature with the coccolithophores is the mode of biomineralization of *T. heimii* dinoflagellates with a combined external and internal precipitation of calcite, as originally described based on morphological observations of living cells [*Tangen et al.*, 1982]. This feature has been subsequently supported by geochemical evidence [*Gussone et al.*, 2010; *Van de Waal et al.*, 2013]. Morphological and molecular investigations indicate that *T. heimii*-like morphologies and genes are present since the Upper Cretaceous [*Hildebrand-Habel et al.*, 1999; *Gottschling et al.*, 2008]. This long lasting record indicates that monospecific *T. heimii* analyses may enable a long-term investigation of the thermal evolution of the intermediate photic zone.

To date, the comprehensive culture study of *Zonneveld et al.* [2007] is the only one that has measured ^{18}O fractionation (approximated from the difference between $\delta^{18}O_c$ and $\delta^{18}O_{medium}$) in *T. heimii* calcite under a wide range of growth temperatures (Figure 1). The authors observed a strong linear correlation ($r^2 = 0.83$) between temperature and oxygen isotope composition of *T. heimii* calcite. Compared to inorganic prediction [*Kim and O'Neil*, 1997], *T. heimii* calcite was measured with significantly negative $\delta^{18}O_c$, with a mean offset of -3‰. In sediments, despite the careful methodological approach presented by *Zonneveld* [2004], no convincing correlation could be found between temperature and $\delta^{18}O$ of *T. heimii* in Atlantic Ocean core tops ($r^2 = 0.42$) (Figure 1). Values of $\delta^{18}O_c$ obtained from separated fractions were also significantly higher (~1.5‰) than culture residues. Hence, the inconsistency between culture and core top data makes the achieved calibration hardly transferable to the geological record [*Kohn and Zonneveld*, 2010]. Recent reappraisals of the magnitude of the vital effect, i.e., the isotopic offset between biominerals and inorganic calcite precipitated from the same fluid, in "geologically relevant" coccoliths have highlighted the influence of the culture method on the isotopic composition of calcite (see review by *Hermoso* [2014]). Discrepancies in the magnitude of vital effects between studies are the likely consequence of culture artifacts—primarily due to a reservoir effect affecting the pool of dissolved inorganic carbon (DIC) [*Hermoso et al.*, 2014]. Indeed, when cultured at very low cell density, coccolith calcite has been reported closer to equilibrium and matches the core top data [*Candelier et al.*, 2013].

In this study, we attempt an integrated culture and core top calibration using state-of-the-art methodologies to calibrate stable isotope composition for *T. heimii* with a geological (Cenozoic) perspective. We have carried out very dilute batch cultures in the laboratory to minimize possible culture artifacts and applied a microseparation technique to produce near-monospecific (>90%) *T. heimii* fractions that is based on a cascade of micro-filtering steps [*Minoletti et al.*, 2009]. Beyond a simple empirical calibration and discussion of the discrepancies with previous studies, here we attempt a mechanistic understanding of the vital effect in *T. heimii* affecting the oxygen isotope composition, and we discuss the applicability of the paleotemperature proxy based on monospecific fractions of *T. heimii*. In addition to oxygen isotopes, we present carbon isotope data indicating that temperature might exert a second-order control on $\delta^{13}C$ calcite in *T. heimii*.

2. Material and Methods

2.1. Culture Setup

Studies based on laboratory cultures of calcifying organisms have provided calibration equations that enable translation of $\delta^{18}O_c$ into temperature estimates for a wide range of planktic calcifiers [*Dudley et al.*, 1986;

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Bemis et al., 1998; *Ziveri et al.*, 2003; *Candelier et al.*, 2013]. There are many methodological issues, spanning from purely inorganic to physiological aspects that can contribute to altering the isotopic values in laboratory cultures [*Hinga et al.*, 1994; *Langer et al.,* 2006; *Barry et al.*, 2010; *Candelier et al.*, 2013; *Hermoso et al.*, 2014]. This point is well illustrated by the fact that in several cases, significantly different isotopic compositions and fractionation factors have been documented in different studies on the same species (see synthesis in *Stoll and Ziveri* [2004] and *Hermoso et al.* [2014]). As most of these artifacts originate from a drift of the carbonate system ([DIC] and pH) in the culture medium, it has been recommended to keep biomass low during batch culture experiments, implying harvesting the bioassay at very dilute cell concentration [*Barry et al.*, 2010].

With these potential artifacts in mind, we conducted dilute batch cultures of a monoclonal strain of *Thoracosphaera heimii* (RCC 1511 from the Roscoff Culture Collection: http://www.roscoff-culture-collection.org) originated from Japanese coastal waters. This strain was grown at seven temperatures between 13 and 27°C. The batch cultures were undertaken in 2.7 L Nalgene polycarbonate flasks, without a headspace. The culture medium consisted of aged English Channel seawater, with nutrient, trace metal, and chelator enrichments corresponding to K/6 [*Keller et al.*, 1987] and vitamins to that of the f/2 recipe [*Guillard*, 1975]. Prior to sterilization, media were bubbled with atmospheric air for 24 h. pH was adjusted to 8.2 by addition of 0.2 M NaOH, and sterilization of the culture medium was achieved by filtration through a 0.22 μm pore size Stericup filter device. Cultures were illuminated by using daylight fluorescent bulbs at a light intensity of ~150 μmol photons m^{-2} s^{-1} with a 14/10 light/dark photoperiod.

Prior to the start of the experiment, cultures were gradually acclimated (0.5°C/day) to the target temperature and maintained for at least 10 generations at the target temperature. The initial cell density of *T. heimii* in the experiments was ~50 cells per mL. Aliquots of the culture medium were sampled at the beginning and end of the experiments, filtered through a 0.22 μm disc-syringe filter and kept at 4°C for subsequent measurement of $\delta^{18}O_{sw}$ of the culture medium. Bubbling of the culture media at 15°C with ambient air was undertaken to ensure that the DIC level and $\delta^{13}C$ of DIC were in equilibrium with the atmosphere [*Zeebe et al.*, 1999]). By applying the same procedure, *Hermoso et al.* [2014] measured carbon isotope composition of the DIC in the culturing medium close to 0‰.

The culture flasks were gently shaken twice a day to allow redistribution of the cells in the flask. Cell counts were undertaken using a Sedgewick-Rafter cell containing a subsample of 1 mL of medium and observed under an inverted microscope. Cultures were harvested when cell density reached between 6000 and 15,000 cells per mL. At this stage, population growth was still at the early stage of the exponential phase and therefore under nutrient- and DIC-replete conditions. For each temperature, cultures were implemented in duplicate. At 15°C, two additional flasks were left grown until the population reached late exponential phase in order to assess the effect of the cell density and consequent chemical drift of medium composition on isotope composition in *T. heimii* calcite.

2.2. Core Top Samples

2.2.1. Location of Samples Sites

Core top samples were selected from two oceanographic settings. Seven sites were recovered from intermediate latitudes at ~30°S in the Southern Atlantic and Indian oceans and between 20°N and 40°N in the Northern Atlantic and one from the Northern Somali Basin at 4°N (Table 1). Except for the Somali site, all sites are located within or at the edge of oceanic gyres. The sampling interval was always the topmost few centimeters of each core. The relatively recent age of sedimentary particles enables core top calibration with environmental parameters assumed to be close to present day conditions.

2.2.2. Retrieving Environmental Parameters (T, $\delta^{18}O_{sw}$) for the Calibration

Calcification of *T. heimii* takes place in the intermediate photic zone, typically at or just below the base of the mixed layer [*Karwath et al.*, 2000; *Vink et al.*, 2000; *Wendler et al.*, 2002a, 2002b; *Vink*, 2004; *Kohn and Zonneveld*, 2010]. To assess the environmental parameters of this water mass, we first evaluated the depth of the mixed layer, the base of which was defined using seawater potential density [*Levitus and de Boyer Montégut*, 1994; *de Boyer Montégut*, 2004]. Values of mean annual temperature and $\delta^{18}O_{sw}$ for the Intermediate Photic zone were then integrated over a 30 m thick water mass below the base of the mixed layer, on average between 50 and 80 m (Table 1). Temperature and $\delta^{18}O_{sw}$ were retrieved from the World Ocean Atlas 2009 [*Locarnini et al.*, 2010] and from the global 1° × 1° gridded compilation of *LeGrande and Schmidt* [2006]. Temperature values are comprised between 15 and 27°C, which is a similar range to that of the culture experiments (13–27°C).

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Table 1. Location and Oceanographic Parameters of the Studied Sites[a,b]

Site/station	Latitude	Longitude	Water Depth (m)	Sampling Interval (cm)	Fraction	Sedimentation Rate (cm/kyr)	Reference	Mixed Layer Depth (m)	$\delta^{18}O_{T.\ heimii}$ (‰ V-PDB)	$\delta^{18}O_{T.\ heimii}$ (‰ V-PDB)	IPZ Temperature (°C)	IPZ $\delta^{18}Osw$ (‰ V-SMOW)	IPZ $[CO_3^{2-}]$ (μmol kg^{-1})
VM16-206	23°N 33	46°W 48	3733	1–3	<10 μm	1	*Cléroux et al.* [2013]	57	−0.99	−3.31	23.7	1.36	250
MD09-06	30°S 79	48°E 26	4192	0–1	10–20 μm	N/A	N/A	26	−1.29	−3.67	20.4	0.50	222
VM30-76	33°N 60	31°W 48	3567	0–1	10–20 μm	4	*Mix and Ruddiman* [1985]	56	−0.59	−2.54	18.5	1.02	219
VM17-165	32°N 75	41°W 90	3924	12–13	<10 μm	2	*Vogelsang et al.* [2001]	63	−0.02	−2.14	19.4	1.04	212
MD82-449	29°S 37	35°W 39	3028	0–1.5	10–20 μm	1	*Barash and Lavrov* [1977]	24	−0.45	−2.03	20.7	0.87	224
MD104-24	4°N 35	49°E 26	2576	0–1	10–20 μm	20	*Klöcker et al.* [2005]	32	−2.17	−1.31	25.6	0.49	226
SU90-03	40°N 50	32°W 05	2475	2.5–3.5	<10 μm	4	*Chapman et al.* [2000]	76	−0.51	−3.04	16.6	0.82	184
SU90-08	43°N 35	30°W 41	3080	0–1	<10 μm	5	*Grousset et al.* [1993]	128	0.27	−2.68	15.2	0.70	178

[a]IPZ stands for Intermediate Photic Zone.
[b]All values are mean annual averages.

In gyre oceanographic regime, production of *T. heimii* is almost constant throughout the year [*Zonneveld*, 2004; *Kohn et al.*, 2011]. In this study, we used mean annual temperature and $\delta^{18}O_{sw}$ to represent estimates of seawater physico-chemistry. Conversely, the offshore Somali MD104-24 site corresponds to warm waters with a pronounced upwelling-driven seasonality related to a monsoon regime [*Fischer et al.*, 1996; *Peeters et al.*, 2002; *Schott et al.*, 2009]. In this region, the production of *T. heimii* is higher at the end of the SW monsoon (August to September) and at the beginning of the inter-monsoon (October to November), periods that correspond to the lowest and highest annual temperatures, respectively [*Wendler et al.*, 2002a]. As most *T. heimii* shells were formed at either extreme of the temperature range, the mean annual temperature was assumed to integrate this variability. The seasonal variability of the $\delta^{18}O_{sw}$ is not known at this site.

2.2.3. Microseparation Protocol

Shells of *T. heimii* were physically isolated from sediments according to the protocol of *Minoletti et al.* [2001, 2009] based on cascade microfiltering steps. Approximately 1–2 g of bulk sample was suspended in deionized water, previously adjusted to pH 8 by addition of ammonia to minimize calcite dissolution. Gentle stirring allowed complete disaggregation of the sediment. The suspension was first filtered through 315 and 160 μm sieves and then through 40 and 20 μm nylon nets to concentrate foraminifera and their fragments. Finally, the suspension was filtered onto 10 μm pore size polycarbonate membranes (Millipore). Near-monospecific *T. heimii* assemblages were thus gathered from the 10 to 20 μm interval. We chose a purity threshold of 90%, below which fractions were regarded as not sufficiently purified. For those samples in which *T. heimii* assemblages were not pure, we applied a subsequent purification step based on strong ultrasonic treatment and selective breakage of particles by strong ultrasonic supply [*Minoletti et al.*, 2009].

After separation, smear slides were prepared for each fraction using the technique described by *Koch and Young* [2007]. Relative abundances were estimated by counting at least 300 particles under cross-polarized light using a Zeiss Axioscope 40 microscope equipped with a 63× Plan-Neofluar objective.

Foraminiferal shells and fragments are the most abundant calcite particle in all fractions coarser than 20 μm. The fraction <10 μm contains coccoliths, such as *Coccolithus pelagicus*, *Calcidiscus leptoporus*, *Gephyrocapsa* spp., and *Emiliania huxleyi* and their fragments. Between these two size ranges, the 10–20 μm fraction is composed mainly of *T. heimii* shells, with other rare calcareous components, such as foraminiferal fragments and rare lopadoliths of the coccolithophore *Scyphosphaera apsteinii* (Figures 2a and 2b). In samples MD09-06 and VM30-76, the proportion of *T. heimii* shells was lower than our target purity (<90%), and foraminiferal fragments may contaminate the isotopic signal. In these cases, an additional purification method based short runs (60 s) of strong ultrasonic treatment [*Minoletti*

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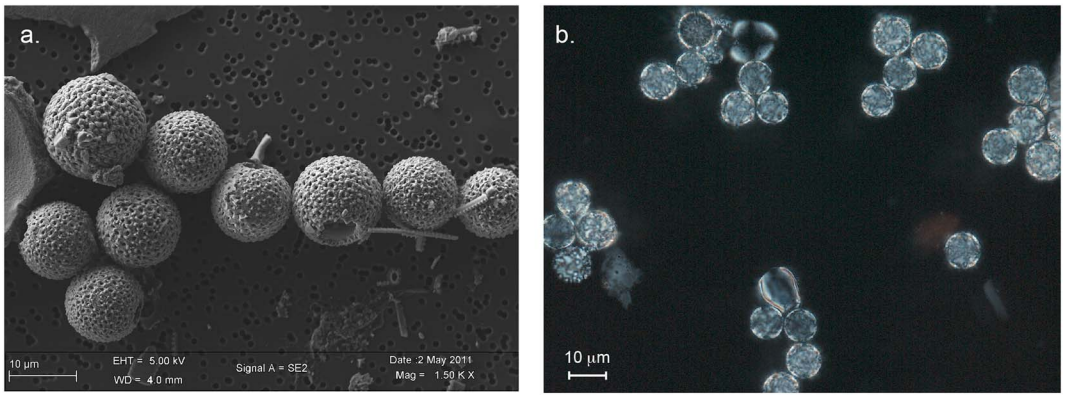


Figure 2. (a) SEM image and (b) smear-slide micrograph of a sedimentary *T. heimii* assemblage obtained from core top sample MD82-449 (Southern Atlantic Ocean). This near-monospecific *T. heimii* assemblage was obtained in the 10–20 µm fraction by the microfiltering protocol described in *Minoletti et al.* [2009].

et al., 2009] was applied, and it was possible to induce fragmentation of *T. heimii* shells while retaining foraminiferal fragments in their original size spectrum. *Thoracosphaera* fragments were subsequently collected in a <10 µm fraction from the original 10–20 µm fractions exposed to strong ultrasonic waves.

2.3. Stable Isotope Measurements

Carbonate stable isotopes were analyzed from about 80 µg of culture residue or near-specific *T. heimii* assemblages from sediments on a VG Isoprime mass spectrometer. Samples were first cleaned using 10% hydrogen peroxide (H_2O_2) to remove the organic matter, rinsed three times, and dried at 60°C. In the instrument they were reacted with purified phosphoric acid (H_3PO_4) at 90°C. Calibration to V-PDB standard via NBS-19 was undertaken daily using the Marceau, a Carrara marble standard. Reproducibility of replicated standards was usually better than 0.05‰ for $\delta^{13}C_c$ and 0.1‰ for $\delta^{18}O_c$. Carbonate data are expressed against the V-PDB standard for both oxygen and carbon isotopes (Tables 1 and 2).

Water sample measurements were made from the CO_2 in the headspace equilibrated with a 0.5 mL aliquot in Exetainer tubes using Gas Bench II coupled to a Delta V Advantage mass spectrometer. Reproducibility of replicated standards was usually better than 0.2‰ for $\delta^{18}O_{sw}$. The oxygen isotope composition of water samples is expressed against V-SMOW standard (Table 1).

As $\delta^{13}C$ of the DIC (predominantly HCO_3^-) changes with temperature [*Mook et al.*, 1974], we assessed the carbon isotope composition of each medium for a given temperature using the equation from *Lynch-Stieglitz et al.* [1995], and assuming a $\delta^{13}C$ of the DIC at 0% at 15°C [*Hermoso et al.*, 2014]. Data are given in Table 2.

Table 2. Temperature, Specific Growth Rate, and Isotopic Data From the Culture Experiment[a]

Code	Temperature (°C)	µ (day^{-1})	$\delta^{18}O_{T.\ heimii}$ (‰ V-PDB)	$\delta^{13}C_{T.\ heimii}$ (‰ V-PDB)	$\delta^{18}O_{medium}$ (‰ V-SMOW)	$\delta^{13}C_{DIC}$ (‰ V-PDB)
13A	13	0.23	−0.25	−6.36	0.67	−1.20
13B	13	0.24	−0.01	−6.03	0.67	−1.20
15A	15	0.26	−0.16	−6.62	0.69	−1.38
15B	15	0.28	−0.32	−6.83	0.69	−1.38
17A	17	0.29	−0.12	−5.79	0.65	−1.56
17B	17	0.34	−0.37	−6.14	0.65	−1.56
20A	20	0.38	−1.24	−4.23	0.69	−1.84
20B	20	0.38	−1.14	−4.49	0.69	−1.84
23A	23	0.52	−1.60	−4.32	0.70	−2.12
23B	23	0.53	−1.72	−4.63	0.70	−2.12
25A	25	0.50	−2.16	−3.92	0.68	−2.30
25B	25	0.48	−2.25	−4.07	0.82	−2.30
27A	27	0.46	−2.03	−3.81	0.67	−2.48
27B	27	0.46	−1.94	−3.83	0.67	−2.48

[a]Isotope compositions of culture residues and $\delta^{18}O$ of the culturing medium were measured for all temperatures. Carbon isotope compositions of the DIC for each temperature derived from the measurement at 15°C with $\delta^{13}C$ of DIC = 0‰ [*Hermoso et al.*, 2014]. For each other temperature, we used the equation given by *Lynch-Stieglitz et al.* [1995] to account for the temperature dependence on $\delta^{13}C$ of DIC.

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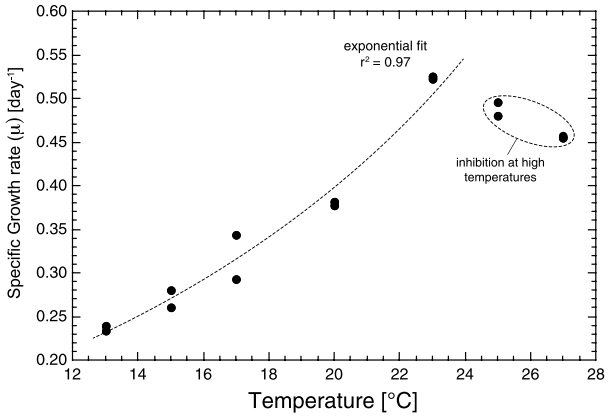


Figure 3. Specific growth rate of cultured *T. heimii* (strain RCC 1511) with respect to temperature. Growth rates exponentially increase up to an ecological optimum at ~24°C, confirming the affinity for relatively warm waters of this species, although highest temperatures seem to inhibit cell division.

2.4. Expression of Results

In the present study, the composition of *T. heimii* calcite is expressed for both isotope systems by using the "$\delta - \delta$" notation, i.e., $\delta^{18}O_c - \delta^{18}O_{sw}$ (V-PDB − V-SMOW) and $\delta^{13}C_c - \delta^{13}C_{DIC}$ (V-PDB − V-PDB). Such isotopic values do not correspond to fractionation coefficients but represent convenient estimates routinely used in paleoceanography to normalize microfossil geochemistry and allows comparison between regions with different seawater chemistries. Comparison with an inorganic reference is achieved via the offset of *T. heimii* calcite with the equilibrium calcite calculated after the equation of *Kim and O'Neil* [1997] approximated to quadratic expression by *Bemis et al.* [1998] for oxygen and after the equation of *Romanek et al.* [1992] for carbon.

Two corrections were applied to the intercept of the inorganic calcite $\delta^{18}O$ equation (see *Candelier et al.* [2013]). The effect of pH on ^{18}O apparent fractionation consists of a $\delta^{18}O$ decrease of 1.42‰ for an increase of 1 pH unit [*Spero et al.*, 1997; *Zeebe*, 2001; *Watkins et al.*, 2014]. As the work by *Kim and O'Neil* [1997] was based on experiments conducted at pH 7.8 and our *T. heimii* were grown at pH 8.2, a correction of −0.568 was applied. In addition, we reverse the $d^{18}Osw$ conversion from V-PDB to V-SMOW scale by subtracting 0.27‰ [*Hut*, 1987] from the intercept. This adjustment is necessary to present all data on the same scale, in the present case in V-SMOW, as is common practice in the calcareous phytoplankton literature. The resulting inorganic reference equation is as follows:

$$\delta^{18}O_{c\ [‰\ V-PDB]} - \delta^{18}O_{sw\ [‰\ V-SMOW]} = -0.211 \times T_{[°C]} + 3.410 - 0.568 - 0.27) \quad (1)$$

3. Results

3.1. Algal Growth and Medium Drift

Specific growth rate (μ) increased from 13 to 23°C, with an exponential trend from 0.24 to 0.52 day^{-1} ($r^2 = 0.97$) (Figure 3). Growth rates diminished at 25 and 27°C, possibly indicating an ecological optimum at 24°C for this strain and applied experimental setup (light and nutrient levels). Final cell concentrations at the end of the experiments were between 4000 and 16,000 cells per mL. Even the highest cell density corresponds to very little utilization of the DIC resource in the culture medium. A conservative estimate of 5% DIC drawdown is obtained by considering ~2000 pg C per cell [*Young and Ziveri*, 2000]. This represents significantly lower yields compared to the end of the culture study of *Zonneveld et al.* [2007]. In our experiments, the typical drift in pH measured at the end of the experiments was less than 0.1 unit owing to our very dilute cell concentration strategy. This stability of the growth medium testifies for the stable composition of the carbonate system that the cells were subjected to throughout the experiments, with similar conditions at the beginning and the end of the experiments.

3.2. Oxygen Isotope Values of Culture Residues

Growth temperature strongly influences the oxygen isotope composition of *T. heimii* calcite. Over the 13 to 27°C interval, the range in $\delta^{18}O_c$ was ~2.2‰ with isotopic ratios linearly decreasing with temperature, as expected from equilibrium prediction (Figure 4). The replicates were in good agreement with a mean standard deviation on the order of 0.10‰, indicating that obtained magnitudes of fractionation estimated from $\delta_c - \delta_{sw}$ values are reproducible and show a very strong linear relationship with temperature ($r^2 = 0.91$). The equation linking oxygen isotope composition and temperature is:

$$\delta^{18}O_{c\ [‰\ V-PDB]} - \delta^{18}O_{sw\ [‰\ V-SMOW]} = -0.17 \times T_{[°C]} + 1.52\ (r^2 = 0.91) \quad (2)$$

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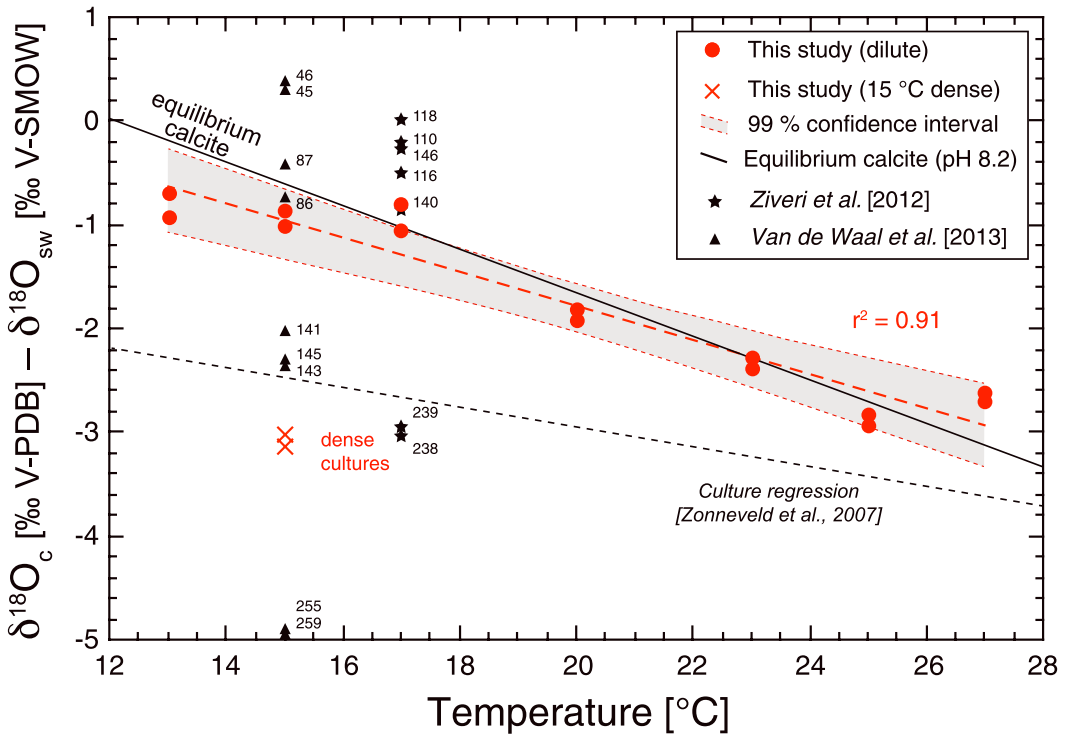


Figure 4. Oxygen isotope composition of *T. heimii* calcite (expressed as $\delta^{18}O_{T.heimii\ [‰\ V\text{-}PDB]} - \delta^{18}O_{sw\ [‰\ V\text{-}SMOW]}$) versus temperature in laboratory culture experiments. Data from dilute bioassays are represented by red circles. High cell density 15°C batch cultures (2‰ isotopically lighter than the very dilute 15°C assay) are indicated by red crosses. Overall, the data show a very good linear regression coefficient ($r^2 = 0.91$). The equilibrium calcite line was calculated after *Kim and O'Neil* [1997] and adjusted for pH 8.2 (see equation (1) in text). At temperatures greater than 15°C, the agreement with the equilibrium reference is remarkably good, while at lower temperatures, $\delta^{18}O$ values are offset by about 1‰ toward negative values. The culture regression of *Zonneveld et al.* [2007] is indicated with the dashed line; other culture data sets are indicated with black stars [*Ziveri et al.*, 2012] and black triangles [*Van de Waal et al.*, 2013]. Numbers next to symbols correspond to the carbonate ion concentrations in the medium expressed in $\mu mol\ kg_{sw}^{-1}$. For comparison, our cultures were grown at $[CO_3^{2-}]$ of ~250 $\mu mol\ kg_{sw}^{-1}$.

The root mean square error (RMSE) of the distribution ($n = 14$) indicates a residual of about 1.6°C indicating the good accuracy of the culture calibration. A −2‰ offset was recorded between the early and late exponential phase data points at 15°C (Figure 4). Taken at face value, this offset would represent an unrealistic ~12°C discrepancy. This result illustrates that performing very dilute cultures in batch is a prerequisite for measuring meaningful ^{18}O fractionation factors. It is worth noting that the oxygen isotope measurements obtained for temperatures lower than 17°C depart from the inorganic reference. Disregarding the two lowest temperatures, also corresponding to minimum growth rate of *T. heimii*, our culture calibration is parallel and indistinguishable from the inorganic reference (Figure 4):

$$\delta^{18}O_{c\ [‰\ V-PDB]} - \delta^{18}O_{sw\ [‰\ V-SMOW]} = -0.19 \times T_{[°C]} + 2.09 \quad (r^2 = 0.86) \tag{3}$$

The RMSE corresponds to the "internal" reliability of the calibration, in the sense that it is based on the distribution of measurements and their degree of scattering with respect to the linear regression line. The same approach can be used to assess an "external" uncertainty of $\delta^{18}O$ composition in *T. heimii* compared to the inorganic calcite (equation (1)). The RMSE using predicted values that derive from equation (1) is 3.0°C ($n = 14$). Not surprisingly, the lowest temperatures (13 and 15°C) correspond to maximal residuum of the population (Figure 4). Disregarding these coldest temperature, RMSE in the range of 17–27°C is significantly reduced and quantified to 2.3°C ($n = 10$). These contrasting vital effects for oxygen isotopes on both sides of the 17°C cutoff temperature challenge the univocal assignment of *T. heimii* to an isotopically "light group" [*Zonneveld et al.*, 2007; *Ziveri et al.*, 2012; *Van de Waal et al.*, 2013]. However, our data support a relatively light isotopic composition at 15°C, as previously documented at this temperature [*Hermoso et al.*, 2014].

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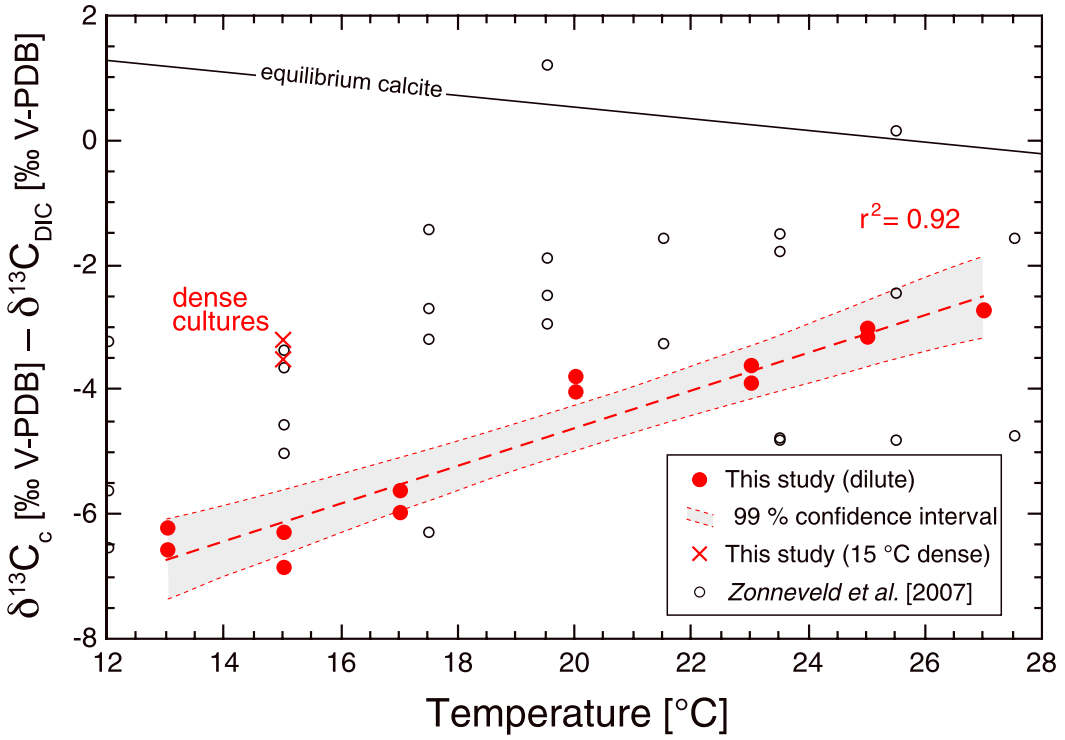


Figure 5. Carbon isotope composition of cultured *T. heimii* calcite (expressed as $\delta^{13}C_{T.heimii\ [‰\ V\text{-}PDB]} - \delta^{13}C_{DIC\ [‰\ V\text{-}PDB]}$) versus temperature. Key for symbols in inset. High cell density 15°C batch cultures are isotopically 2‰ heavier than the very dilute 15°C assay. The data show a good linear regression coefficient ($r^2 = 0.86$). Carbon isotope composition of equilibrium calcite is calculated from $\delta^{13}C_{DIC}$ (Table 2) by applying the equation of *Romanek et al.* [1992].

3.3. Carbon Isotope Values of Culture Residues

The $\delta^{13}C_c - \delta^{13}C_{DIC}$ values of *T. heimii* are linearly correlated with temperature ($r^2 = 0.92$) (Figure 5). Because temperature and $\delta^{18}O_c$ are also correlated, there was a strong relationship between carbon and oxygen values ($r^2 = 0.86$). We measured very negative $\delta^{13}C_c$ for *T. heimii* shells that fall between the composition of equilibrium calcite (calculated after *Romanek et al.* [1992]) and that of aqueous CO_2 assumed to be the substrate assimilated by the cell (around −10.2‰) calculated after *Lynch-Stieglitz et al.* [1995].

3.4. Oxygen Isotope Values of Microseparated *T. heimii* Fractions

For *T. heimii* fractions, $\delta^{18}O_c - \delta^{18}O_{sw}$ values range from −0.43‰ at 15.2°C for North Atlantic site SU90-08 to −2.66‰ at 25.6°C for site MD104-24 (Offshore Somalia) and are negatively correlated to temperature (Figure 6). The equation linking oxygen isotope fractionation to temperature in the core top approach is:

$$\delta^{18}O_{c\ [‰\ V-PDB]} - \delta^{18}O_{sw\ [‰\ V-SMOW]} = -0.19 \times T_{[°C]} + 2.16 \quad (r^2 = 0.82) \tag{4}$$

Core top fractions are in very good agreement with culture data over the entire investigated temperature range, i.e., including the lowest temperature at 15.2°C. When plotted on the same graph, the samples from *Zonneveld* [2004] that where of comparable purity to the present study (>90%) confirm this regression line while less pure *T. heimii* fractions exhibit scattered and overall, more positive $\delta^{18}O_c$ values (Figure 6).

3.5. Carbon Isotope Values of Microseparated *T. heimii* Fractions

The $\delta^{13}C_c$ values of *T. heimii* fractions exhibit relatively light values, ranging from −1.31‰ to −3.67‰ (Table 1). This observation is in agreement with data from cultured *T. heimii* shells (Figure 5) and with evidence from the geological record [*Friedrich and Meier*, 2003; *Kohn et al.*, 2011]. As relatively easily done for the oxygen isotope system (see section 2.2.2.), retrieving oceanic $\delta^{13}C$ of DIC relevant for *T. heimii* found in core tops has been found to be challenging. First, the coverage and resolution of available databases for

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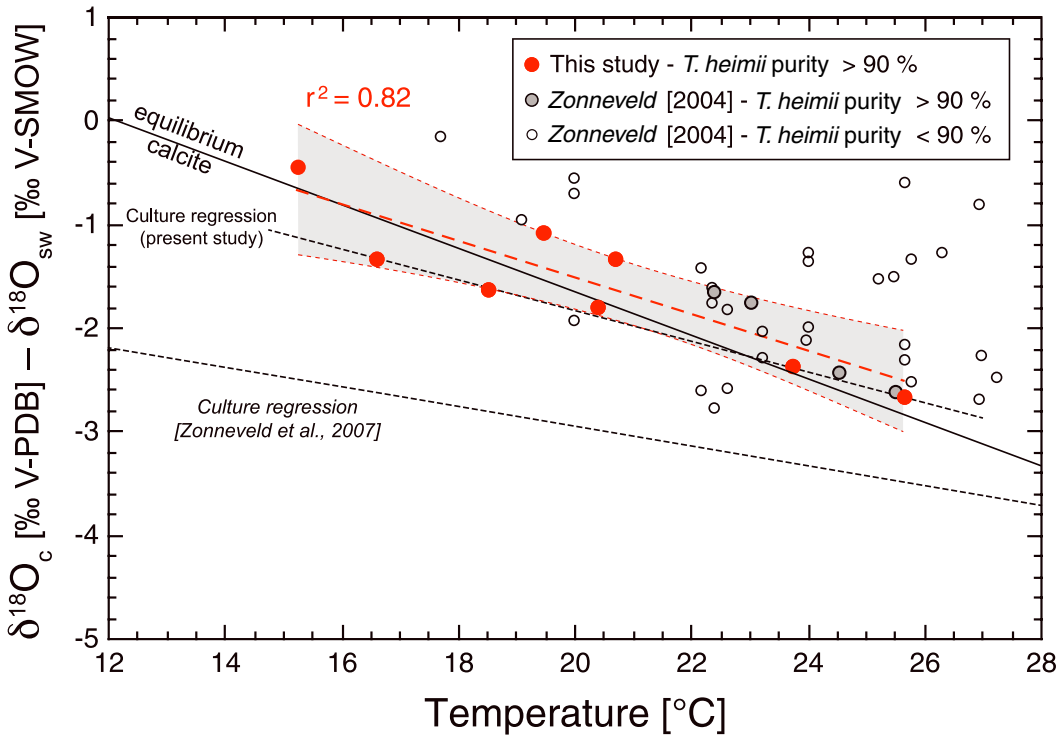


Figure 6. Oxygen isotope composition of *T. heimii* calcite (expressed as $\delta^{18}O_{T.heimii\ [‰\ V\text{-}PDB]} - \delta^{18}O_{sw\ [‰\ V\text{-}SMOW]}$) versus temperature in core tops. *Thoracosphaera* fractions from *Zonneveld* [2004] are shown by gray circles (purity >90%) and open circles (purity <90%). The oxygen isotope composition of *T. heimii* microseparated fractions is highly correlated with temperature of the intermediate photic zone ($r^2 = 0.82$—linear regression is depicted by a thick red dashed line) and close to that of equilibrium calcite. The 99% confidence interval indicated by the gray shaded area shows a ±0.5 dispersion of the points with respect to the overall trend. The core top calibration equation has a slope and intersect that are indistinguishable from those of culture experiment (this study). The only appreciable difference between our culture and core top calibration relies on the lowest temperature data points. The offset from equilibrium toward negative $\delta^{18}O$ values <17°C is not seen in core top data.

present-day carbon isotope of DIC in seawater are rather scarce [*Gruber et al.*, 1999]. Second, *T. heimii* shells laid down on the seafloor were produced before the infusion of isotopically light carbon into the surface waters (as referred to as the Suess effect). Last, *T. heimii* thrives in the intermediate photic zone layer while available data correspond to surface waters. As a result, data from present-day waters do not accurately account for $\delta^{13}C_{DIC}$ values to which *T. heimii* from our microseparated fractions were exposed to.

These methodological and conceptual issues prevent us to express carbon isotope fractionation using the "$\delta^{13}C_c - \delta^{13}C_{DIC}$" notation. Consequently, taking *T. heimii* $\delta^{13}C_c$ as a proxy for carbon isotope fractionation in core tops can only be ascertained if all aforementioned parameters are not themselves temperature dependent. Estimates of paleo-$\delta^{13}C_{DIC}$ that would reflect the composition of *T. heimii*'s depth habitat could be made using the carbon isotope signature of the foraminifera *Globorotalia menardii* or *Neogloboquadrina dutertrei* extracted from the same core top sediments. Species-specific coefficient allowing conversion of foraminiferal $\delta^{13}C_c$ into $\delta^{13}C_{DIC}$, as determined by *Spero et al.* [2003] might be applied to constrain $\delta^{13}C_c$ of the intermediate photic zone. Unfortunately, we did not have access to the coarse fraction of our core top sediments, nor found previously-published $\delta^{13}C$ of foraminifera for these sites. With all these problems in mind, we refrained from interpreting sedimentary $\delta^{13}C_c$ values of *T. heimii*. Nevertheless, raw isotopic data are presented in Table 1.

4. Discussion

4.1. Oxygen Isotope Composition: "Light" Versus "Equilibrium" Group?

Our culture data indicate that *T. heimii* precipitates calcite in near-equilibrium conditions (Figure 4). The main source of DIC assimilation in *T. heimii* has been demonstrated to be CO_2 [*Van de Waal et al.*, 2013]. The fact

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that $\delta^{18}O_c$ was not shifted toward more positive values indicates that the very heavy oxygen isotope composition of CO_2 (~ +7‰ with respect to HCO_3^- [*Zeebe*, 1999]) is not preserved in calcite produced by *T. heimii*. This implies total re-equilibration of the oxygen isotope system (H_2O/CO_2-HCO_3^--CO_3^{2-}). In solution, total re-equilibration is attained within approximately 12 h at 15°C [*Zeebe and Wolf-Gladrow*, 2001].

The oxygen isotope system of fast growing coccolithophores such as *E. huxleyi* is not completely re-equilibrated at time of calcification and their calcite therefore reflects to some extent the isotopic composition of the isotopically heavy CO_2 source [*Hermoso et al.*, 2014]. This thermodynamic feature typifies the so-called "heavy group" [*Dudley et al.*, 1986]. The near equilibrium $\delta^{18}O_c$ of *T. heimii* might be explained by the proven presence of carbonic anhydrase inside the cell and in the boundary layer [*Van de Waal et al.*, 2013], which speeds up the re-equilibration between DIC and H_2O [*Uchikawa and Zeebe*, 2012]. Additionally, a long residence time of the intracellular DIC pool between assimilation and calcification could account for equilibrium composition, as for the coccolithophore *Coccolithus pelagicus* [*Hermoso et al.*, 2014], which, like *T. heimii*, has a relatively slow growth rate.

At low temperatures (13 and 15°C), offsets from equilibrium of about −1‰ were recorded in *T. heimii* calcite, as observed by *Hermoso et al.* [2014]. Over this temperature range, *T. heimii* can hence be assigned to the isotopically "light group", like the coccolithophore *Calcidiscus leptoporus*. The change in oxygen isotope values at the lowest temperatures corresponds to the ecological lower limit for this species in terms of temperature tolerance (Figure 3). Lower growth rate would leave more time for thermodynamic re-equilibration of the DIC with H_2O, but this process is also slower at low temperatures, so an explanation based on kinetics of the oxygen isotope system can be ruled out.

From a chemical point of view, $\delta^{18}O_c$ lower than equilibrium can result from a higher relative proportion of CO_3^{2-} compared to HCO_3^- at the site of calcification (relative to the ratio of the external fluid) or from kinetic effect [*Watkins et al.*, 2014]. This change in the DIC system would be accompanied by increased pH. We hypothesize that a less active carbon concentrating mechanism and/or less intense calcification at low temperature could account for higher pH. With a lowered net CO_2 flux, as evidenced by limited growth rate at low temperature, calcification would occur in a less acidic environment. Under this assumption, calcite grown at lower temperatures would reflect the higher pH and concomitant higher abundance of CO_3^{2-} with respect to HCO_3^- and therefore would have lower oxygen isotope values. An alternative hypothesis is that the re-equilibration time is not achieved completely below 17°C due to the reduced activity of carbonic anhydrase at low temperature. Further biogeochemical work is required to test these hypotheses, especially to characterize passive diffusion of CO_2 versus HCO_3^- uptake in *T. heimii* and more broadly the carbon concentrating mechanisms (or CCMs) under a range of temperatures.

4.2. Comparison With Previous Culture Studies

4.2.1. Implications of the Culture Technique for the Magnitude of the Vital Effect

The culture strategy (dilute versus dense) has a strong effect on the isotopic composition of *T. heimii* calcite. This point is well illustrated by the batch cultures at 15°C that reached the late exponential phase with cell division being hampered by unfavorable living conditions. Low $\delta^{18}O_c$ are unlikely to be the consequence of changed $\delta^{18}O$ of the medium during growth, as initial and final measurements were within analytical error. Rather, we explain this drift by an altered chemistry of the medium with substantial elevation of pH due to the consumption of CO_2. This "culture artifact" may hence correspond to the combination of reduced [CO_{2aq}] and high pH, at which enzymatic activity, including those involved in the CCMs, is ineffective. Other environmental parameters could be involved in such large shifts toward negative $\delta^{18}O_c$ values, including the effect of pH [*Zeebe*, 1999, 2001] and potentially the influence of reduced light and nutrient levels.

4.2.2. The Pioneering Work of *Zonneveld et al.* [2007]

Zonneveld et al. [2007] performed an extensive culture campaign investigating ^{18}O fractionation in *T. heimii* under a wide range of temperatures (Figure 1). They documented the evolution of carbonate chemistry during growth, which can be used to better interpret the oxygen isotope compositions of *T. heimii* calcispheres produced in the laboratory. The authors calculated a linear equation for ^{18}O fractionation according to temperature from their culture examination of two strains of *T. heimii*. They grew *T. heimii* using an initial pH of 8.0, which is 0.2 pH unit higher than in our experimental condition.

In the culture study of *Zonneveld et al.* [2007], the pH drifts were significant, by +0.4 pH unit. This drift is similar to that we observed in our late exponential assays. As cell density and hence calcification, follow an

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exponential curve, the results are more representative of the final conditions rather than the controlled initial conditions in a batch culture. Combining the duration of the batch experiments, the volume of culture medium, the biomass harvested and the drift in pH, this would suggest that the culture assays presented by *Zonneveld et al.* [2007] were not carried out at very low cell concentration, although it is claimed that the cultures were still in the exponential growth phase at the end of the experiments.

Carbon isotope ratios measured on *T. heimii* cultured by *Zonneveld et al.* [2007] and our dense 15°C batches provide compelling evidence for a culture artifact with very high $\delta^{13}C_c$ having been reported. With a fairly high degree of DIC (CO_2) utilization during the course of experiments, $\delta^{13}C$ of the medium is more strongly affected by ^{12}C fixation into organic matter and calcite is formed from a DIC pool with high $\delta^{13}C_{DIC}$ values [*Hinga et al.*, 1994; *Hermoso et al.*, 2014].

Taken together, these observations indicate that the non-equilibrium $\delta^{18}O_c$ of *T. heimii* cultured by *Zonneveld et al.* [2007] are likely to have been induced by a reservoir effect, as was the case in our dense 15°C cultures. *Candelier et al.* [2013] reevaluated the magnitude of ^{18}O vital effect for the coccolithophore *Calcidiscus leptoporus* reported in previous studies by undertaking very dilute batch cultures. The earlier work by *Dudley et al.* [1986] reported $\delta^{18}O_c$ ~1.5‰ lighter than the data of *Candelier et al.* [2013]. It therefore appears that both coccolithophores and calcareous dinoflagellates are similarly affected by the culture artifact induced by high cell concentrations. This similarity between groups is also best explained by a reservoir effect with $[CO_{2aq}]$ becoming limiting with growth, while we acknowledge that there are many other parameters of the growth medium that may simultaneously drift (total alkalinity, $[Ca^{2+}]$, [DIC], $[CO_3^{2-}]$, and pH).

4.2.3. Perturbation Experiments of *Ziveri et al.* [2012] and *Van de Waal et al.* [2013]

Among the species investigated by *Ziveri et al.* [2012], *T. heimii* records the highest dependence of $\delta^{18}O$ on the carbonate ion concentration. Over a range between 50 and 250 $\mu mol\ kg_{sw}^{-1}$ of CO_3^{2-} in the culture medium, the change in $\delta^{18}O_c$ was as high as 5‰ for a given temperature (Figure 4). With less CO_3^{2-} in the external medium, oxygen isotopes are shifted toward positive $\delta^{18}O$ values, whereas with more carbonate ion availability and therefore relatively higher saturation state with respect to calcite, the isotopic composition of *T. heimii* calcite was close to that of CO_3^{2-}. In coccolithophores such as *C. leptoporus*, this effect can be induced by relatively high pH at the site of calcification in the coccolith vesicle and may explain the ~ −1.5‰ offset from equilibrium calcite in $\delta^{18}O$ values [*Ziveri et al.*, 2012; *Candelier et al.*, 2013].

The concept of a pH effect on oxygen isotope composition was originally introduced to describe a purely inorganic phenomenon [*McCrea*, 1950] and later used to describe observations in planktic foraminifera [*Bemis et al.*, 1998; *Zeebe*, 1999]. Due to the distinct $\delta^{18}O$ values between the DIC species in seawater, there is a great effect via the relative proportion of DIC species on $\delta^{18}O$ of calcite (see 2.4.). To investigate this pH effect, *Ziveri et al.* [2012] bubbled CO_2 in the culture medium. Similarly, with an oceanic acidification perspective, *Van de Waal et al.* [2013] used the same technique to reach target CO_3^{2-} concentrations. As a result, the isotopic composition of calcite is influenced by the relative proportion of CO_3^{2-} with respect to $CO_2 + HCO_3^-$, but also by the total amount of DIC available for the cell. These absolute and relative changes of CO_2 explain why the response in oxygen isotope values in manipulated coccoliths is much greater than observed for the sole pH effect measured on foraminifera [*Spero et al.*, 1997]. By using an alternative method by which target CO_3^{2-} concentrations were obtained through addition of strong acid or base, meaning the total amount of DIC remained unchanged, *Ziveri et al.* [2012] recorded a pH effect (referred as to a carbonate ion effect in their study) on coccolithophore species similar to that previously reported for foraminifera [*Spero et al.*, 1997; *Zeebe*, 1999] yet lower than that measured on *T. heimii*.

From these perturbation experiments, it is thus difficult to establish a direct link between CO_3^{2-} concentrations, pH, and $\delta^{18}O$ change with temperature, as more than one parameter was modified. Our experiments were not intended to examine the effect of the status of the carbonate system on $\delta^{18}O$, but rather to investigate the temperature record in conditions as close as possible to the natural environment, i.e., keeping all other parameters constant by using very dilute batch cultures.

4.3. Reconciled Culture and Core Top Data

4.3.1. Temperature and $\delta^{18}O$ of Dinoflagellate Calcite

There is a good concordance between culture and core top data in our study (Figures 4 and 6). In both data sets, strong linear correlation coefficients demonstrate the foremost control of temperature on oxygen isotope composition in *T. heimii* calcite. This concordance in the slopes and intersects of the equations

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(equations (2)–(4)) means that our calibration is transferable to natural environments and that $\delta^{18}O$ of *T. heimii* calcite may be used as a robust paleothermometer for assessing seawater temperatures of the intermediate photic zone.

From our culture and core top studies, it is clear that calcification of *T. heimii* is made in near isotopic equilibrium with ambient seawater for the oxygen isotopes. This absence of apparent vital effect potentially indicates a relatively low degree of biological control on calcification in *T. heimii* for this isotopic system. This geochemical observation may be related to the suggested partially external mode of biomineralization in *T. heimii* [*Tangen et al.*, 1982; *Inouye and Pienaar*, 1983; *Gussone et al.*, 2010].

Below 17°C, $\delta^{18}O$ values of the calcite of cultured *T. heimii* are slightly offset from equilibrium (~ −1‰), but this departure was not recorded in core top data. Indeed, at 15.2°C, which is the mean annual temperature of site SU90-08, *T. heimii* exhibited equilibrium $\delta^{18}O$ values. This slight discrepancy would suggest that the calcite of cultured *T. heimii* is isotopically shifted due to a process that is not operating in the natural environment. Further biogeochemical work would be useful to confirm the existence of this differential vital effect and to understand its origin. Examining core tops where calcification took place in temperatures colder than 15°C would also help reconcile datasets and confirm whether *T. heimii* belongs to the isotopically "light group" at low temperature. Nevertheless, *T. heimii* mainly thrives in low-latitude waters characterized by temperature corresponding to the range where its $\delta^{18}O$ composition is not offset [*Karwath et al.*, 2000; *Kohn and Zonneveld*, 2010]. Considering the long-term cooling of the Cenozoic, it could be inferred that temperatures of *T. heimii* habitat in the pelagic environment have never been lower than 15°C. As such, the possible limitation of our calibration may have no effect on the applicability of paleotemperature reconstruction with a geological perspective.

In their attempt to reconstruct seawater temperatures over the last 45 ka, *Kohn et al.* [2011] measured $\delta^{18}O_c$ of *T. heimii* and two planktic foraminifera, *Globigerinoides ruber* and *Globigerina bulloides*, from the tropical North Atlantic Ocean. Oxygen isotope values of *T. heimii* shells from the Late Holocene fell between −0.5‰ and −1‰. The authors calculated seawater temperatures using the equation of *Zonneveld* [2004] (Figure 1) and found unrealistically low temperatures. As an alternative, they assumed that calcite precipitation of calcareous dinoflagellates occurred in isotopic equilibrium with ambient water, using the raw equation of *Kim and O'Neil* [1997]. The results appeared to be more consistent with what was expected for the thermal status of the upper water column of this interval, especially when compared to foraminiferal data.

Yet in the study by *Kohn et al.* [2011], the $\delta^{18}O$ of *T. heimii* were measured ~0.5‰ heavier than *G. ruber* (var. pink), a symbiotic foraminifera that spends its entire life cycle in the mixed layer [*Deuser and Ross*, 1989; *Farmer et al.*, 2007; *Pearson*, 2012]. Reconstructing similar temperatures from *T. heimii* and *G. ruber* is still a contentious result, as these organisms calcify in distinct water masses. Instead, the recorded oxygen isotope offset between both taxa that calcify in equilibrium with seawater supports relatively deep habitat for *T. heimii*. At the sites of *Kohn et al.* [2011], the present-day temperature difference between mixed layer and intermediate photic zone waters is ~3°C. Applying our calibration to *T. heimii* $\delta^{18}O$ would reconcile the data. Indeed, this alternative correction of $\delta^{18}O$ values in *T. heimii* would lead to a decrease of ~2°C in isotopic temperature estimates relative to *G. ruber* (var. pink). This figure would make *T. heimii*-derived temperatures compatible with a calcification depth deeper than *G. ruber*. Overall, this downcore study case of *T. heimii* $\delta^{18}O$ confirms the validity of our calibration for the geological record.

4.3.2. A New Proxy for Thermal Gradients Within the Photic Zone

Recent methodological advances, like that used in the present study, now allow microseparation of coccoliths and calcareous dinoflagellate shells in sediments. These sedimentological techniques enable near-monospecific geochemical measurements on these nannofossils and hence examination of the vital effect in the geological record [e.g., *Bolton and Stoll*, 2013]. This effort contributes to the ongoing development of new proxies for evaluating accurate temperatures of the uppermost water column. Coccoliths and some surface-dwelling foraminifera species, such as *G. ruber*, are ideal for deriving the temperature of the mixed layer, corresponding to SSTs. In addition, there is a promising opportunity to decipher parameters other than temperature by undertaking cross-species comparison of $\delta^{18}O$ of coccolith taxa [*Rickaby et al.*, 2010; *Hermoso et al.*, 2014]. Due to its deeper habitat, the calibration of *T. heimii* geochemistry is of primary importance since it enables characterization of the intermediate part of the photic zone, i.e., the upper part of the thermocline. Hence, measuring paired coccolith and dinoflagellate calcite isotopes might be used to

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constrain the thermal structure of the photic zone. Comparison of isotopic signals in calcite of the coccolithophore *Coccolithus pelagicus* before its poleward migration [*Bown*, 1998; *Sato et al.*, 2004] with those of *T. heimii* would be of particular help for reconstructing thermal gradients within the photic zone and for capturing long-term variability in the thermocline depth. The $\delta^{18}O_{sw}$ variability within the photic zone is small and usually lower than analytical errors of isotope measurements (±0.1‰); hence, differences in isotopic signals may reflect a temperature signal, after a suitable correction accounting for the vital effect is achieved.

4.3.3. A Temperature Component Driving $\delta^{13}C$ of *T. heimii* Calcispheres

There is a positive correlation between carbon isotope composition of *T. heimii* and temperature in our batch cultures (Figure 5). In our cultures, $\delta^{13}C$ of aqueous CO_2 was constant around −10.2‰, whereas the $\delta^{13}C$ of DIC, predominantly consisting of HCO_3^-, and hence that of inorganic calcite slightly decreases (Figure 5) [*Romanek et al.*, 1992; *Lynch-Stieglitz et al.*, 1995]. Under the safe assumption that *T. heimii* assimilates CO_2 [*van de Waal et al.*, 2013], variation of carbon isotope composition in calcite cannot be explained by the decrease in the isotopic composition of the carbon substrate assimilated by the cell and subsequently used for calcification. Increase in $\delta^{13}C_c$ of *T. heimii* may not correspond to a change in the magnitude of $^{12}C/^{13}C$ fractionation. With elevation in temperature and accompanying higher carbon fixation in organic matter [*van de Waal et al.*, 2013], we hypothesize that the DIC pool used for calcification becomes heavier due to more intense Rayleigh fractionation linked to RUbisCO fractionation that favors ^{12}C-carbon. Hence, we hypothesize that the linear relationship between temperature and $\delta^{13}C_c$ observed in our cultures may also be driven by a modulation of the Rayleigh fractionation of the carbon isotope system with higher ^{12}C partitioning into organic matter at high temperature [*van de Waal et al.*, 2013]. This biogeochemical feature represents a commonality with the carbon isotope system in symbiot-bearing foraminifera [*Bemis et al.*, 2000].

Overall, covariation in $\delta^{18}O/\delta^{13}C$ signals in the geological record should not always be regarded as a diagenetic feature [*Marshall*, 1992], as this work demonstrates that they may convey a pristine biogeochemical signal. Further investigation of the coupling between the two isotopic systems (oxygen and carbon) should be conducted. Notably, a full mechanistic understanding of stable isotope fractionation in calcite-producing dinoflagellates would also require separate analyses of the internal versus external calcite layers. It would be particularly interesting to determine how the inner layer that is precipitated intracellularly (hence presumably with higher coupling between the photosynthetic and calcification carbon pools) compares isotopically with the outer layer that is supposed to be under a lesser degree of biological control.

5. Conclusions

This study represents a reconciled culture and core top calibration of the temperature record on $\delta^{18}O$ of the calcareous dinoflagellate *T. heimii*. Previous studies have produced conflicting data between culture results and the natural environment. The combination of two methodologies implemented here, very dilute batch cultures and microseparation of extremely enriched (>90%) *T. heimii* assemblages from core top sediments, enabled this successful calibration. Differences with previous studies may originate from a drift in the composition of the growth medium and insufficiently purified sedimentary material, respectively.

Our work demonstrates the near-equilibrium composition of *T. heimii* calcite for oxygen isotopes. In our culture data, however, there seems to be a mismatch with lower (1‰) $\delta^{18}O$ with respect to equilibrium below 17°C, which is not seen in core top data. The cause of this discrepancy remains elusive, although reduced DIC assimilation and consecutive reduced growth rates may explain this change of the vital effect with higher pH values at the site of calcification. As the occurrence of this species at such low temperatures is rare, this discrepancy does not represent a pitfall for our calibration, although this offset should be kept in mind if *T. heimii* are extracted from temperate regions. Downcore investigation of stable isotopes from *T. heimii* microfractions represents a promising means to reconstruct the evolution of temperature of the intermediate photic zone. When combined with the shallower dwelling coccolithophores, interspecies $\delta^{18}O$ may help unraveling the thermal structure of the mixed layer.

The very negative $\delta^{13}C$ values in calcite found in this study confirm previous culture and geological evidence. This geochemical feature contrasts with observations made in coccolithophores in which a relatively high degree of coupling between calcification and calcification lead to more positive $\delta^{13}C$. Furthermore, as both carbon and oxygen isotopes seem to be under a common temperature control, this may help understanding and interpreting covariation between $\delta^{13}C$ and $\delta^{18}O$ in the geological record.

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