$^{13}$C–$^{18}$O isotope signatures and ‘clumped isotope’ thermometry in foraminifera and coccoliths

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Abstract

Accurate constraints on past ocean temperatures and compositions are critical for documenting climate change and resolving its causes. Most proxies for temperature are not thermodynamically based, appear to be subject to biological processes, require regional calibrations, and/or are influenced by fluid composition. As a result, their interpretation becomes uncertain when they are applied in settings not necessarily resembling those in which they were empirically calibrated. Independent proxies for past temperature could provide an important means of testing and/or expanding on existing reconstructions. Here we report measurements of abundances of stable isotopologues of calcitic and aragonitic benthic and planktic foraminifera and coccoliths, relate those abundances to independently estimated growth temperatures, and discuss the possible scope of equilibrium and kinetic isotope effects. The proportions of $^{13}$C–$^{18}$O bonds in these samples exhibits a temperature dependence that is generally similar to that previously been reported for inorganic calcite and other biologically precipitated carbonate-containing minerals (apatite from fish, reptile, and mammal teeth; calcitic brachiopods and molluscs; aragonitic coral and mollusks). Most species that exhibit non-equilibrium $^{18}$O/$^{16}$O ($\delta^{18}$O) and $^{13}$C/$^{12}$C ($\delta^{13}$C) ratios are characterized by $^{13}$C–$^{18}$O bond abundances that are similar to inorganic calcite and are generally indistinguishable from apparent equilibrium, with possible exceptions among benthic foraminiferal samples from the Arctic Ocean where temperatures are near-freezing. Observed isotope ratios in biogenic carbonates can be explained if carbonate minerals generally preserve a state of ordering that reflects the extent of isotopic equilibration of the dissolved inorganic carbon species.

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1. INTRODUCTION

Records of past ocean conditions are essential to understanding the evolution of the Earth. The $\delta^{18}$O of carbonates (Epstein et al., 1951; Shackleton and Opydyke, 1973; Erez and Luz, 1982), metal/calcium ratios in foraminifera (Boyle and Hester, 1982; Delaney et al., 1995; Rosenthal et al., 1997; Lea et al., 1999, 2000; Elderfield and Ganssen, 2000; Lear et al., 2000; Lea, 2003; Sadekov et al., 2008), and the molecular structures of organic biomarkers (Müller et al., 1996; Volkman, 2000; Schouten et al., 2002) can be used to give quantitative estimates of temperature and water composition. If accurately interpreted, sedimentary records of these and other proxies can be used to examine temperatures, global ice volume, water salinity, and carbonate chemistry (Emiliani, 1955; Shackleton, 1974; Keigwin, 1980, 1982; Lynch-Stieglitz et al., 1999; Lear et al., 2000; Tripati et al., 2001, 2003, 2005, 2006, 2009; Tripati and Zachos, 2002; Tripati and Elderfield, 2004, 2005; Elderfield et al., 2010).

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However, the sensitivity of inorganic ($\delta^{18}O$, Mg/Ca) and organic (the alkenone unsaturated index U$_{37}^{29}$ and the tetraether index of arachaeal lipids with 86 carbon atoms TEX$_{86}$) temperature proxies to biological, ecological, and non-temperature environmental factors can introduce systematic differences, errors, or ambiguities in climate reconstructions (e.g., Suchs et al., 2000; Lipp et al., 2008). This is of importance because discrepancies between proxies (MARGO Project Members, 2009) have led to uncertainties in our understanding of past climate, including attempts to assess climate sensitivity to greenhouse gas forcing. Almost all temperature proxies are subject to “vital effects” (i.e., differences between the chemical properties of biologically precipitated and inorganic minerals) and/or fluid composition (Shackleton and Opdyke, 1973; Bemis et al., 1998; Turich et al., 2007) (e.g., Figs. 1 and 2). For example, both $\delta^{18}O$ and element/Ca ratios in foraminifera and many other biogenic carbonates need to be used with a species-specific fractionation factor (or apparent partition coefficient) in order to estimate temperature (e.g., Bemis et al., 1998; Tripati et al., 2003; Elderfield et al., 2006). Most existing temperature proxies must be referenced to fluid composition: use of $\delta^{18}O$ or Mg/Ca ratios in foraminifera as a proxy for temperature requires knowledge of seawater $\delta^{18}O$ or Mg/Ca, respectively. For example, there is a significant correlation between Mg/Ca and salinity in both culture and core-top samples (Nuernberg et al., 1996; Lea et al., 1999; Arbuszewski et al., 2008; Ferguson et al., 2008; Kiskürek et al., 2008; Mathien-Blard and Bassinot, 2009). The TEX$_{86}$ molecular temperature proxy is significantly correlated with seawater nitrate levels (Turich et al., 2007). These and other factors contribute to uncertainties in the interpretation of past conditions, particularly for extinct taxa, for which one cannot establish robust empirical calibrations of the proxy.

Here we present a global calibration dataset that demonstrates the applicability of a new thermodynamically-based temperature proxy, the ‘clumped isotope’ thermometer, to foraminifera and coccoliths. We also use these new data to discuss the origins of vital effects (or non-equilibrium isotopic fractionations) in the $\delta^{18}O$ of foraminifera and coccoliths. Carbonate clumped isotope thermometry in foraminifera and coccoliths has the potential to advance our understanding of past oceanographic conditions because theoretically it could circumvent many of the issues associated with other proxies (i.e., sensitivity to water composition). This approach is based on the principle that ordering, or ‘clumping’, of heavy isotopes into bonds with each other in molecules is temperature-dependent due to an internal isotope exchange reaction in carbonate minerals (Ghosh et al., 2006a; Schauble et al., 2006; Eiler, 2007).

2. BACKGROUND

2.1. Carbonate clumped isotope thermometry

In carbonates, rare heavy isotopes (i.e., $^{13}C$ and $^{18}O$) occur in a pool of abundant light isotopes. The proportion of $^{13}C$ and $^{18}O$ that are bound to each other within the carbonate mineral lattice is predicted to be temperature-dependent based on principles of statistical thermodynamics (Schauble et al., 2006). The equilibrium constant for the following reaction (Schauble et al., 2006) forms the theoretical basis for the carbonate ‘clumped isotope’ thermometer:

$$\text{Ca}^{12}\text{C}^{18}\text{O}^{16}\text{O}_{2} + \text{Ca}^{13}\text{C}^{16}\text{O}_{3} \rightleftharpoons \text{Ca}^{11}\text{C}^{18}\text{O}^{16}\text{O}_{2} + \text{Ca}^{12}\text{C}^{16}\text{O}_{3}$$

(Reaction 1)

The temperature-dependent ‘clumping’ of heavy isotopes into bonds with each other is driven by subtle vibrational energy differences between isotopologues. Each isotopologue has
unique vibrational properties which therefore impact their thermodynamic stability, with the species on the right-side of Reaction 1 favored at lower temperatures, promoting the clumping of heavy isotopes into bonds with each other (Schauble et al., 2006). In an equilibrium precipitate, if the equilibrium constant for Reaction 1 is known and the abundance of all four isotopic species is measured, the temperature can be calculated independent of the isotopic composition of the water in which the carbonate grew.

In practice, clumped isotope thermometry is based on analyses of $^{13}C$-$^{18}O$ in CO$_2$ produced by acid digestion of carbonates. Fractionation during acid digestion is a predictable and apparently reproducible function of digestion temperature (Ghosh et al., 2006a; Guo et al., 2009). The abundance of the doubly substituted isotopologue $^{13}C$-$^{18}O$ is reported using the variable $D_{47}$, defined as the enrichment, in per mil ($\delta$), of $^{13}C$-$^{18}O$ above the amount expected for a random distribution of isotopes among all CO$_2$ isotopologues (Eiler and Schauble, 2004). Measurements have been made to calibrate $D_{47}$ in inorganic calcite precipitated at known temperatures and biogenic carbonates including fish otoliths, mollusks, brachiopods, teeth, micritic carbonates in lake, and coral (Ghosh et al., 2006a, 2007; Came et al., 2007; Eagle et al., 2010; Huntington et al., 2010). These published calibration data are shown in Fig. 3.

A recently published calibration of synthetic calcite (Dennis and Schrag, 2010; not shown) differs from the trend reported by Ghosh et al. (2006a) at low temperatures, with a shallower slope and higher intercept than previously reported. The source of this discrepancy is not clear. It might reflect any combination of analytical and/or experimental artifacts in either or both of these studies. Further experimental work will be needed to resolve this issue for the purposes of our discussion, we compare our results to the inorganic calcite calibration from Ghosh et al. (2006a) as their calibration samples were measured on the same instrument as the ones used for this study.

The biogenic carbonates examined in previous studies generally adhere to the inorganic calcite calibration, with a sensitivity of $\approx0.004–0.005$ per mil/°C. The observed exceptions to this trend (Fig. 3) are measurements of aragonite from two different types of marine organisms: Red Sea coral (Ghosh et al., 2006a) and fish otoliths (Ghosh et al., 2007). The Red Sea coral data exhibit a wide range of variability in $D_{47}$ (a range of $\approx0.10–0.12$ per mil) for a relatively small (seasonal) range of calcification temperatures ($\approx6$ °C; defined in the original publication by the instrumental record for the region, and also by Sr/Ca-based temperature estimates). Most of the samples from this individual coral are enriched in $^{13}C$-$^{18}O$ bonds by 0.02–0.04 per mil relative to inorganic calcite. This data set also possibly exhibits a higher temperature sensitivity relative to most biogenic carbonates and inorganic calcite. In contrast, the otolith dataset exhibits a similar temperature sensitivity to the inorganic calcite calibration, but lower absolute values.

The origin of these two types of systematic deviations is unknown. They may indicate the occurrence of non-equilibrium biological fractionations in some calcifying organisms, record analytical artifacts, and/or reflect uncertainties in estimating the temperature of calcification for the analyzed materials.

Fig. 2. $\delta^{18}O$ and $\delta^{13}C$ ratios of different species of foraminifera and coccoliths measured in this study compared to values predicted from an inorganic calibration. Inorganic values represent carbonate precipitation in apparent equilibrium. (A) Relationship between deviations from inorganic carbonate values of $\delta^{18}O$ and $\delta^{13}C$ for mixed-layer dwelling planktic foraminifera and bulk carbonate. (B) Same as (A) but for thermocline-dwelling planktic foraminifera. (C) Same as (A) and (B) but for benthic foraminifera.
Since this thermometer was developed, it has been applied to reconstruct temperature in several contexts. In addition to constraining temperature, measurements have been paired with carbonate \( \delta^{18}O \) to determine ambient water \( \delta^{18}O \). Published applications thus far include (a) estimating continental temperatures using paleosol carbonates to study the tectonic history of the Bolivian Altiplano and other regions (Ghosh et al., 2006b; Quade et al., 2007; Huntington et al., 2010), (b) constraining variations in terrestrial temperature using speleothems (Affek et al., 2008), (c) reconstructing Pennsylvanian and Silurian temperature and seawater \( \delta^{18}O \) using well-preserved molluscs and brachiopods, to look at the long-term influence of CO\(_2\) and cosmic rays on climate (Came et al., 2007), (d) using the precipitation temperature of carbonates in primitive meteorites to study their alteration history (Guo and Eiler, 2007), (e) reconstructing the body temperature of extinct taxa (Eagle et al., 2010), (f) assessing the susceptibility of \( \Delta_{47} \) to diagenetic resetting (Came et al., 2007; Dennis and Schrag, 2010; Eagle et al., 2010).

### 2.2. Controls on the oxygen isotope composition of foraminifera and coccoliths

The \( \delta^{18}O \) values of planktic and benthic foraminifera, and coccoliths, depend on both temperature and seawater \( \delta^{18}O \), which itself depends on global ice volume and salinity. Therefore interpretations of carbonate \( \delta^{18}O \) are ambiguous unless one can call on independent constraints on either temperature or seawater \( \delta^{18}O \). In addition, although a few species appear to precipitate their tests in apparent isotopic equilibrium with the seawater from which they grew, disequilibrium fractionations of unknown origin (i.e., vital effects) are common (Shackleton and Opydyke, 1973; Bemis et al., 1998). The \( \delta^{18}O \) values of some modern foraminiferal taxa deviate from apparent equilibrium (i.e., values predicted using inorganic calibrations) by up to \( 3-4/_{oo} \) (e.g., Fig. 2; Fig. 1 of Electronic Appendix 1), and it is possible that the magnitude of this disequilibrium may potentially vary over time. A similar range of non-equilibrium fractionations has been reported in coccoliths (Ziveri et al., 2003). It has been shown that the \( \delta^{13}C \) of inorganic calcite (McCrea, 1950; Udistowski and Hoefs, 1993; Beck et al., 2005), foraminiferal calcite (Spero et al., 1997; Bemis et al., 1998), and other biogenic carbonates (Rollion-Bard et al., 2003) may also vary as a function of carbonate \( \delta^{18}O \), particularly for biogenic carbonates precipitated by extant taxa, for which one cannot establish robust empirical calibrations of the fractionation with respect to water.

### 3. METHODS

#### 3.1. Samples

For this study, we calibrated the thermometer with measurements of 17 species of foraminifera and coccoliths that are independently known to have grown at temperatures ranging from \(-0.9\) to \(29.2 ^\circ C\), including both calcitic and aragonitic taxa. Of the 41 calibration samples we examined, 24 were of planktic foraminifera, 11 of benthic foraminifera, 4 of bulk carbonate in core-top sediments, and 2 of cultured coccoliths. Counting replicates of 13 samples, 61 separate analyses (i.e., extractions of CO\(_2\) from carbonate) were made in total. This represents the first detailed survey of the clumped isotope composition of multiple marine species from sediment core-tops, and the first measurements of cultured carbonate-secreting organisms. All samples are monospecific except where noted (i.e., for bulk carbonate samples or for mixed species of benthic foraminifera). Samples ranged from 4 to 12 mg in size, with 200–500 individuals for each planktic foraminiferal sample and between 100 and 200 individuals for each benthic foraminiferal sample. Planktic foraminifera were picked from the >250 \( \mu \)m size fraction and benthic foraminifera were picked from the >212 \( \mu \)m size fraction. Samples of Arctic benthic foraminifera were provided by author H.B.

Table 1 of Electronic Appendix 2 contains a description of sample and locality information. Tables 2 and 3 of Electronic Appendix 2 list constraints on calcification temperature and other hydrographic parameters (water \( \delta^{18}O \), the \( \delta^{13}C \) of dissolved inorganic carbon), respectively. The latter table also shows estimates of the saturation state of bottom waters and pH.
for localities where samples of benthic foraminifera were taken, with carbonate system parameters calculated from hydrographic data in the GLODAP database (Sabine et al., 2005) using constants from the Best Practices Handbook (Dickson et al., 2007).

Monospecific cultures of Emiliania huxleyi and Coccolithus pelagicus were grown by author P.H. in 0.1 μm filtered and aged-seawater, enriched with nutrients, vitamins and trace elements. Coccolithophores were grown for at least three consecutive batch cultures (~20 generations for E. huxleyi) at the desired temperature before inoculating final cultures in three 21 acid-cleaned bottles. Coccolithophores were maintained under a 14/10 h light/dark cycle, and the growth rates were monitored to ensure that harvesting was undertaken during the exponential growth phase. Samples were harvested onto 0.2 μm polycarbonate membrane filters under vacuum, rinsed with Aristar grade ethanol, taken into suspension in ethanol and transferred to 10 ml centrifuge tubes.

3.2. Sample pre-treatment

To remove contaminants, we developed a protocol that uses a cold dilute buffered solution of hydrogen peroxide to remove organic matter and adhering particles, which is modified from the cleaning protocols designed for measuring metal/calcium ratios in foraminifera (Boyle and Keigwin, 1982). All foraminiferal and coccolith samples were dried overnight in an oven at 50–70 °C. Most samples were cleaned to remove secondary carbonates and organic matter (Table 4 of Electronic Appendix 2). Foraminiferal samples were crushed between two glass plates, and then sonicated in distilled water for 1 min and rinsed (repeated 5×), sonicated in methanol and rinsed (repeated 5×), and then sonicated in distilled water again and rinsed (repeated 3×). Samples with high organic contents were cleaned to remove organic matter by reaction for 15–60 min (depending on the organic content) with a cold dilute solution of 1% H2O2. Samples were then centrifuged, decanted, and rinsed four times with distilled water, twice in methanol, and then twice again in distilled water. Samples were then dried again in a 50–70 °C oven overnight. Treatment of carbonate standards with this protocol did not affect their value.

3.3. Sample analyses

As described elsewhere (Ghosh et al., 2006a; Eiler, 2007; Huntington et al., 2009), between 4 and 12 mg of sample was reacted with phosphoric acid at 25 °C (with the exception of some samples from localities MD97-2138, V24-109, and OP 806A which were reacted at 90 °C) and the product CO2 purified using cryogenic separation and gas chromatography. The sample gas was analyzed for masses 44–49 AMU on a Thermo 253 gas source isotope ratio mass spectrometer, for 8–16 acquisitions of 10 cycles each, with an integration time of 8 s per cycle (Ghosh et al., 2006a). The total integration time was 640–1280 s (for both sample gas and working gas) and total analysis time (including peak centering, background measurement, and pressure balancing) ranged from between 4 and 8 h. Measurements were made to yield a 16-V signal for mass 44, with peak centering, background measurement, and pressure balancing before each acquisition. These conditions were selected to ensure stable ion currents and minimize time-dependent fractionation of sample and reference gas reservoirs.

Data for this study was collected between April 2007 and January 2009. Each day we analyzed a heated gas composed of CO2 with a stochastic distribution of isotopes between isotopologues. Gases with different bulk δ18O and δ13C ratios in quartz breakseals were heated to 1000 °C for 2 h and then quenched at room temperature. These standard gases (or heated gases) were then purified and analyzed using the same protocol as sample gases, and used for the calculation of sample Δ47 values, where

\[\Delta_{47} = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \text{ and } R_{47} \text{ refers to the 47/44 ratio of the analyzed CO}_2 \] (Eiler and Schauble, 2004).

Several of the samples were analyzed blind by authors R.E. and N.T. (i.e., without knowledge of their origin or relationship to the present study). No systematic differences were observed between these analyses and the remaining samples (Table 4 of Electronic Appendix 2). These analyses were performed in the lab of author J.E.

We used published acid digestion fractionation factors for determining carbonate δ13C ratios. The value used for calculating calcite 18O/16O ratios from analyzed CO2 is 1.01025 for samples digested at 25 °C (Das Sharma et al., 2002; Guo et al., 2009) and 1.00821 for samples digested at 90 °C (Swart et al., 1991). Aragonitic samples reported in this paper were digested at 25 °C and the acid digestion fractionation factor used for calculations is 1.01034 (i.e., 1000 ln z = 10.44; Sharma and Clayton, 1965).

For calculations of equilibrium 18O/16O ratios in calcite (and to calculate δ18O-calcification temperatures), we used a published relationship (Kim and O’Neil, 1997) to describe calcite−water fractionation:

\[1000 \ln \frac{R_{\text{calcite-H}_2O}}{R_{\text{atmosphere}}} = \frac{8.01 \times 10^{-3} \times T^0}{T} \]

and another expression for aragonite (Kim et al., 2007):

\[1000 \ln \frac{R_{\text{aragonite-H}_2O}}{R_{\text{atmosphere}}} = \frac{1.78 \times 10^{-3} \times T}{T} - 31.14 \]

where T is temperature in K. For these calculations, temperatures are determined by applying the published inorganic calibration equation of Ghosh et al. (2006a,b), and water δ18O is from a published database (LeGrande and Schmidt, 2006). Calculations of equilibrium 13C/12C ratios in calcite and aragonite used the published relationships determined by Romanek et al. (1992), δ13C ratios for dissolved inorganic carbon shown in Table 3 of Electronic Appendix 2.

The presence of organics can yield significant isobaric interferences with CO2 isotopologues that can impact both accuracy and precision, resulting in an apparent temperature bias and/or larger internal errors. We monitored masses 48 and 49 to screen for isobaric interferences from the presence of these compounds. Data for samples that were measured but are excluded from the discussion are listed in Table 5 of Electronic Appendix 2.

3.4. Precision and accuracy

3.4.1. Precision

We report mean sample Δ47 values and the standard error of each sample mean in Table 4 of Electronic Appendix 2.
Error propagation factored in uncertainty in measurement of the $\Delta_{47}$ of CO$_2$ and uncertainty in heated gas normalization (Huntington et al., 2009). Several of the measurements of foraminifera and coccoliths made in this study required a substantial increase in counting times and numbers of replicated samples, relative to past clumped isotope studies, in order to achieve precisions appropriate for paleoceanographic application. Most of these data are relatively precise (SE of 0.005–0.011/°e) and have approximately half the uncertainty typical of such data (0.011–0.025/°e) (Huntington et al., 2009).

A histogram of the standard errors for each sample is shown in Fig. 2 of Electronic Appendix 1, with separate distributions shown for samples that were cleaned to remove organic matter and those samples that were analyzed uncleaned. The average standard error of sample means for uncleaned samples is 0.019 ± 0.014/°e. In contrast, the average standard error of sample means for cleaned samples are about 1/2–1/3 this value (0.008 ± 0.003/°e). In some cases, the standard error for each sample mean was reduced to ~0.003/°e, corresponding to temperature uncertainties of ±0.5–0.8 °C (1 SE). Low errors similar to these should be routinely achievable through the measurement of large samples, many replicate analyses, and sample pre-treatment to remove organic contaminants that can introduce isobars within the relevant mass range.

### 3.4.2. Accuracy

We measured multiple standards repeatedly over this period, typically every 4–10 analyses, in order to determine accuracy and monitor long-term external precision. These standards included NBS-19 which yielded a mean $\Delta_{47}$ of 0.350 ± 0.008/°e (1 SE), mean $\delta^{18}O_{V-PDB}$ of −2.193 ± 0.031/°e, and mean $\delta^{13}C_{V-PDB}$ of 1.845 ± 0.099/°e. These stable isotope ratios are statistically indistinguishable at the 95% confidence level to those previously reported (Coplen, 1995; Ghosh et al., 2006a).

### 4. RESULTS AND DISCUSSION

#### 4.1. Relationship between temperature and $\Delta_{47}$

Theoretical calculations predict that in carbonate minerals the enrichment in $^{13}C$ versus $^{18}O$ bonds relative to a stochastic distribution will decrease with increasing temperature (Schauble et al., 2006; Guo et al., 2009). The relationship between the $\Delta_{47}$ of CO$_2$ produced during acid digestion of carbonate minerals and temperature has been determined using measurements of inorganic calcite (Ghosh et al., 2006a), aragonitic fish otoliths (Ghosh et al., 2007), and apatite (Eagle et al., 2010). Ghosh et al. (2006a) reported the following $\Delta_{47}$–temperature relationship for inorganic calcite: $\Delta_{47} = \frac{4092.1T - 0.02}{T}$ (with $T$ in K). Similar slopes have been reported for otoliths (Ghosh et al., 2007) and biologically precipitated apatite (Eagle et al., 2010); the intercept for otoliths was reported to be significantly lower.

For calibration samples measured in this study (Table 2 of Electronic Appendix 2), calcification temperatures were estimated from cruise reports and the Levitus Ocean Atlas (Levitus et al., 1994). These calcification temperatures are occasionally referred to here as ‘Levitus temperatures’. Fig. 4A vs. C shows that the $\Delta_{47}$ of carbon dioxide produced from acid digestion of the calibration samples measured in this study exhibits a negative correlation with the independently known Levitus temperatures. An alternative means of estimating calcification temperatures for modern samples is based on combining measurements of carbonate and water $\delta^{18}O$ (Table 2 of Electronic Appendix 2). Water $\delta^{18}O$ can be estimated for modern samples using a published database (LeGrande and Schmidt, 2006). For these calculations, we used the inorganic fractionation factors described in Section 3.3.

Fig. 4D and F show $\Delta_{47}$ values of samples measured in this study compared to $\delta^{18}O$-based calcification temperatures. Systematic errors can be introduced into temperature calculations that use this method simply due to the presence of non-equilibrium isotope fractionation (i.e., vital effects) for oxygen isotopes in foraminifera and coccoliths (Bemis et al., 1998; Ziveri et al., 2003). As a result, we use the first method for estimating calcification temperatures in the remainder of this paper except where noted. However, the use of $\delta^{18}O$-based calcification temperatures to estimate the actual growth temperatures of our calibration samples does not significantly impact the results for all samples with the exception of the thermocline-dwelling planktonic (Fig. 4B vs. E), and does not affect our conclusions (for example, results in Table 6 of Electronic Appendix 2 show that the slope and standard error of a regression through the foraminifera and coccolith data calculated using $\delta^{18}O$-calcification temperatures is 0.05051 ± 0.00124, compared to 0.05037 ± 0.00119 calculated using Levitus temperatures).

All but two of the measured cultured and core-top samples are within one standard error of the inorganic calcite data (Fig. 4). A sample of the thermocline-dwelling planktonic foraminifera Globorotalia tumida is offset by about 0.04/°e. The other three samples of G. tumida that were analyzed do also systematically deviate by lesser amounts from the inorganic calcite calibration (brown squares in Fig. 4B and E). The second sample is of the epifaunal foraminiferal species Planulina wuellerstorfi with growth temperatures of −0.8 ± 0.2 °C, and is also offset to lower $\Delta_{47}$ values (by 0.04/°e) relative to the inorganic calcite data, although the other two samples of P. wuellerstorfi that have been analyzed (that calcified at warmer temperatures) do not deviate from the inorganic dataset (green triangles in Fig. 4C and F). The 95% confidence interval for the $\Delta_{47}$ values of these two samples overlap with the 95% confidence interval of the inorganic calcite calibration.

These two samples belong to two populations of data that are notable because both populations systematically deviate from the inorganic calcite data (and the other biogenic carbonate data shown in Fig. 5). The largest systematic offset between the inorganic calcite line and our data is observed in thermocline-dwelling planktonic foraminifera, which generally have lower $\Delta_{47}$ values than the remainder of the samples (Figs. 4C and 5B). Estimated growth temperatures are more uncertain for these samples than for others surveyed in this study, due to the presence of strong vertical gradients in seawater temperature for the environmental niche inhabited by these organisms. If we assume the
inorganic calcite calibration applies to these samples, then it suggests that growth temperature may have been underestimated using the Levitus database (Fig. 4B) and may be more accurately estimated using $\delta^{18}O$-calcification
temperatures (Fig. 4E). We assume that inaccuracies in estimating growth temperature for thermocline-dwelling planktics accounts for the large range of values observed in the $\Delta_{47}$ residuals calculated using the Levitus database (i.e., the vertical range observed in Fig. 6B and C).

All of the benthic foraminiferal samples from the Arctic Ocean also are offset to heavier values relative to the inorganic calcite line (Fig. 4). One sample is of the benthic foraminifera *P. wuellerstorfi*, as described above. A second sample of the infaunal species *Oridorsalis umbonatus* is offset by 0.02‰ in the same direction (purple triangles in Fig. 4E and F) relative to inorganic calcite. Other samples of *P. wuellerstorfi* and *O. umbonatus* analyzed that calcified at warmer temperatures do not exhibit a systematic offset from the inorganic dataset. The offset observed in these Arctic benthic foraminifera does not appear to be due to a carbonate ion effect (Fig. 7A). We discuss the possible significance of these observed offsets in Section 4.3.2.

4.1.1. Calibration of the carbonate ‘clumped isotope’ thermometer

Table 6 of Electronic Appendix 2 compiles the slopes and intercepts for $\Delta_{47}$–temperature relationships calculated using different populations of data taken from this study and other publications (Ghosh et al., 2006a,b, 2007; Came et al., 2007; Eagle et al., 2010; Huntington et al., 2010). We applied weighted least-squares linear regression analysis to the data and used the regression model of York (1969) (described in the appendix of Huntington et al., 2009). Each calibration sample has an uncertainty associated with both the determined $\Delta_{47}$ value and the estimated growth temperature. This regression model assumes that the errors in both variables are uncorrelated, with a symmetrical solution that yields the same regression and errors if the variables are switched.

The values for slopes and intercepts, and their 95% confidence bands, are shown in Fig. 8. A new calibration through the inorganic calcite data is $\Delta_{47} = 0.05870 \pm 0.013_{-0.138}^{+0.01} \times T - 0.014$, similar to the calibration reported by Ghosh et al. (2006a). Fig. 4 shows that the confidence bands for the inorganic calcite calibration line would overlap with the 95% confidence bands for all the samples (the plotted error bars are 1 SE). A weighted least-squares linear regression of any of the other populations (of data for biogenic carbonates or through all of the data) yields a slope and intercept that is shallower but indistinguishable from the value determined for inorganic calcite (Figs. 5 and 8). Fig. 8 shows that the slopes and intercepts of all the populations are statistically equivalent at the 95% confidence level. The slope of the equilibrium line therefore may be shallower than the Ghosh et al. (2006a) inorganic calibration and be better approximated by the more precisely determined slope of the regression through all of the inorganic and biogenic data (Table 6 of Electronic Appendix 2), which is closer to theoretical predictions (Schauble et al., 2006; Guo et al., 2009).

These observations indicate that $^{13}C$–$^{18}O$ bond abundance in most or all of these biogenic carbonates are statistically indistinguishable from inorganic calcite that is assumed to have precipitated in equilibrium. Although the data as a group conform to the slope predicted by the
regression through the inorganic calcite data, a potential source of uncertainty stems from the small range of growth temperatures among samples of any one species. Therefore any attempt to assess whether each species individually conforms to the same overall trend as the group is subject to a large uncertainty. We also note that the regression through data for benthic foraminifera (Fig. 8) has a lower slope and higher intercept than the values for the inorganic calcite regression due to the low $\delta^{47}$ values observed in the two samples from the Arctic Ocean (with temperatures of $-0.8^\circ$C), although these systematic deviations do not appear to be taxon specific, as the samples of *O. umbonatus* and *P. wuellerstorfi* from warmer settings do not exhibit similarly large departures from the inorganic calcite calibration (or the remaining population of biogenic data).

### 4.2. Relationship between seawater composition and $\Delta_{47}$

Seawater composition appears to have a negligible influence on $\Delta_{47}$. In this respect, at least, the clumped isotope thermometer may be simpler to interpret than the $\delta^{18}$O, Mg/Ca and TEX$_{86}$ thermometers. The relationship between measured stable isotope compositions and seawater chemistry is shown in Figs. 7 and 9. The $\delta^{13}$C of dissolved inorganic carbon and the $\delta^{18}$O of water (Table 3 of Electronic Appendix 2) are both uncorrelated with the deviations in $\Delta_{47}$ from apparent equilibrium (Fig. 9). These observations are consistent with predictions that bond order should be independent of the $\delta^{18}$O of water and the $\delta^{13}$C of dissolved inorganic carbon (Schauble et al., 2006). Although the dataset is small, no evidence for a carbonate ion effect is apparent in the $\Delta_{47}$ data for benthic foraminifera (Fig. 7). A relationship is observed for $\delta^{13}$C in these data, and has been reported for foraminiferal $\delta^{18}$O in previous work (Spero et al., 1997). The observations of a carbonate ion effect on carbonate $\delta^{18}$O and $\delta^{13}$C in inorganic precipitation experiments is hypothesized to reflect the difference in $^{18}$O/$^{16}$O and $^{13}$C/$^{12}$C ratios in HCO$_3^-$ and CO$_3^{2-}$ ions, and the pH-dependence of speciation for dissolved inorganic carbon (e.g., Zeebe, 2007). Similar abundances of $^{13}$C–$^{18}$O

Fig. 6. Comparison of $\Delta_{47}$ and $\delta^{18}$O residuals (measured minus predicted from an inorganic calibration) to each other. To estimate residuals, $\Delta_{47}$ data were compared to the new inorganic calibration derived in this study listed in Table 6 of Electronic Appendix 2 (which is similar to the Ghosh et al. (2006a) calibration). $\delta^{18}$O data for calcite samples were compared to values calculated using the inorganic calibration of Kim and O’Neil (1997); data for aragonite samples were compared to values calculated using the calibration of Kim et al. (2007). Temperature estimates used for calculated inorganic values are from Levitus et al. (1994) with the exception of (C) (estimates use $\delta^{18}$O-based calcification temperatures). With the exception of the data shown in (B) and (C), the slopes of linear regressions through all of the data in each figure panel do not significantly differ from zero at the 95% confidence level; no significant species-specific trends are observed. (A) Comparison of $\Delta_{47}$ residuals to $\delta^{18}$O residuals for different species of mixed-layer dwelling planktic foraminifera and bulk carbonate. (B) Same as (A) but for thermocline-dwelling planktics. The slope of a linear regression through these data is $0.026 \pm 0.010$ (1 SE) and is significantly non-zero ($p = 0.05$). We attribute this systematic deviation to uncertainties in estimating growth temperatures for thermocline-dwellers. (C) Same as (B) but uses $\delta^{18}$O-based calcification temperatures. (D) Same as (A) and (B) but for benthic foraminifera.
bonds (relative to a random distribution) have been predicted for both HCO$_3^-$ and CO$_3^{2-}$ ions (Schauble et al., 2006; Guo, 2008; Guo et al., 2008), although there are no observational constraints available. At 25 °C, the equilibrium constant for the ‘clumping’ reaction in calcite (Reaction 1) is predicted to be 1.000410, while the equilibrium constant for the analogous isotope exchange reaction between HCO$_3^-$ isotopologues is estimated to be 1.000427. The theoretically predicted ~0.02‰ difference in $^{13}$C-$^{18}$O bond abundance between dissolved HCO$_3^-$ and CO$_3^{2-}$ ions is subtle (Guo, 2008; Guo et al., 2008). The similarity in $^{13}$C-$^{18}$O bond excess for carbonate and bicarbonate ions implies there may be a weak and possibly pH effect on clumping in marine carbonates given the range of pH observed within foraminifera and other organisms (Erez et al., 2008).

### 4.2.1. Calculated seawater $^{18}$O values

In Fig. 10 we show a comparison of observed (i.e., measured) seawater $^{18}$O (x-axis; LeGrande and Schmidt, 2006) to estimated water $^{18}$O values for the foraminifera and coccolith samples (y-axis), with average species-specific differences reported in Table 7 of Electronic Appendix 2. The calculated water $^{18}$O values use measurements of carbonate $^{13}$C, measurements of $\Delta_{47}$ converted to temperature using the new regression through inorganic calcite data, and published relationships for the temperature-dependent oxygen isotope fractionation between inorganic calcium carbonate (Kim and O’Neil, 1997; Kim et al., 2007). The difference between these estimated values and measured water $^{18}$O (LeGrande and Schmidt, 2006) reflects uncertainties in the measured stable isotope values and in the temperature calibrations for $^{18}$O and $\Delta_{47}$ (Table 7 of Electronic Appendix 2).

Given the general conformity of the $\Delta_{47}$ data to the inorganic carbonate calibration (Fig. 4), these deviations could potentially be used to assess if $^{18}$O ratios in certain taxa are subject to significant non-equilibrium fractionations. Most of the data are within 2‰ of the 1:1 line shown in Fig. 10, consistent with evidence for species-specific offsets of 0–2‰ which have been reported for several of the taxa (Bemis et al., 1998). If the species-specific offsets (Table 7 of Electronic Appendix 2) can be attributed solely to ‘vital effects’ and are precisely determined, then the results indicate that the magnitude of disequilibrium fractionation for G. ruber and P. obliquiloculata < G. sacculifer and G. bulloides < benthic foraminifera, and for G. menardii < G. hirsuta < G. tumida < G. truncatulinoides. The small sample sizes, however, mean that this analysis is not statistically significant for most of the species, with the possible exceptions of G. ruber (n = 6) and G. sacculifer (n = 7) which appear to have shell $^{18}$O values that are close to equilibrium with seawater. The same approach could potentially be applied to larger datasets comprised of paired $^{18}$O and $\Delta_{47}$ measurements of core-top foraminifera samples as a method of constraining the magnitude of vital effects in different species.

### 4.3. The significance of $\Delta_{47}$ observations for the origin of vital effects in $^{18}$O and $^{13}$C

All of the samples we have measured with precisely constrained growth temperatures (i.e., mixed-layer planktic...
foraminifera, coccoliths, bulk carbonate, benthic foraminifera excepting two samples of benthic foraminifera from the Arctic Ocean) do not exhibit large deviations from the inorganic calibration (diifferences are <0.02; Fig. 5). Given the taxonomic breadth represented by these samples, these results suggest that foraminifera and coccoliths generally grow at equilibrium with respect to their proportions of $^{13}$C–$^{18}$O bonds, despite significant 'vital effects' of up to 1–2$\%$ expressed in $\delta ^{18}$O (Fig. 6) and $\delta ^{13}$C (Fig. 11). In almost all of the samples, there is no evidence for species-specific offsets from inorganic calcite in $\Delta_{47}$, and deviations of $\Delta_{47}$ and $\delta ^{18}$O from inorganic values are generally not correlated with each other (Fig. 6). Fig. 11 shows that the same is true for $\Delta_{47}$ and $\delta ^{13}$C. This result provides a new constraint on the origin of vital effects, and therefore on the mechanism of biomineralization, in foraminifera and coccoliths. While additional experimental and theoretical studies are needed to fully understand the significance of this result, we discuss some possible explanations of the following observations: (1) values of $\Delta_{47}$ that are statistically indistinguishable from inorganic calcite (the Arctic benthic foraminifera that are the exception to this trend is discussed in more detail in Section 4.3.2), (2) enriched/depleted $\delta ^{18}$O and $\delta ^{13}$C relative to inorganic calcite, and (3) small departures in $\Delta_{47}$ from inorganic calcite that are statistically uncorrelated with variations in $\delta ^{18}$O.

One model that has been proposed is that oxygen and carbon isotope disequilibrium in carbonate minerals may

Fig. 8. Slope and intercepts (mean and 95% confidence interval) for $\Delta_{47}$—temperature relationships determined using different populations of calibration samples listed in Table 6 of Electronic Appendix 2. The large horizontal bar corresponds to the 95% confidence interval for the inorganic calcite relationship as determined in this study, and overlaps with all of the other regressions. The thin horizontal bar corresponds to the 95% confidence interval for a regression through all data, and overlaps with almost all of the other regressions.
arise if carbonate precipitation is from a mixture of dissolved inorganic carbon species that are at local isotopic equilibrium, and not just from carbonate ions (Zeebe, 1999, 2007). The speciation of dissolved inorganic carbon is pH-dependent. Several studies have determined that the \( \delta^{18}O \) and \( \delta^{13}C \) of the individual species are isotopically distinct at a given temperature (Usdowski and Hoefs, 1993; Zeebe, 1999, 2007; Beck et al., 2005). As pH varies, the isotopic composition of the DIC pool is predicted to change. The different equilibrium fractionations between dissolved inorganic carbon species and water, for example, results in a pH-dependence for the bulk \( \delta^{18}O \) of the dissolved inorganic carbon pool (e.g., Zeebe, 2007).

It is known that carbonate-precipitating organisms can maintain differences in pH between internal and external environments (e.g., across membranes) (Spero et al., 1997; Adkins et al., 2003; Erez, 2003), and in foraminifera, invaginated seawater vacuoles exhibit a range of pH values from 6 to 9 (Erez et al., 2008). It may be that these organisms calcify from a mixture of dissolved inorganic carbon species, or from an internal dissolved inorganic carbon reservoir that differs in pH and/or isotopic composition from seawater but maintains local equilibrium. Metabolic CO\(_2\) can also contribute to the dissolved inorganic carbon pool of foraminifera and coccoliths (Spero et al., 1997; Adkins et al., 2003; Erez, 2003). The pattern of isotopic fractionation we report here is consistent with this “pH hypothesis,” given the pH-dependence of dissolved inorganic carbon speciation, coupled with the relatively large difference in equilibrium \( 18O/16O \) ratios (and \( 13C/12C \) ratios) for HCO\(_3^-\) and CO\(_2^-\) (Zeebe, 1999, 2007, 2009; Beck et al., 2005) and the theoretically predicted pattern of \( 13C-18O \) bond abundance for dissolved HCO\(_3^-\) and CO\(_2^-\) (Guo, 2008; Guo et al., 2008).

Another possibility is that vital effects or isotopic disequilibrium in \( \delta^{18}O \) and \( \delta^{13}C \) reflect kinetic isotope effects during the hydration and hydroxylation of CO\(_2\) (which would drive \( ^{18}O \) depletion and \( ^{13}C \) depletion), perhaps combined with
metabolic changes in the $^{13}$C of the dissolved inorganic carbon pool that derive from photosynthesis and/or respiration (McConnaughey, 1989). If the rates of reaction for various isotopically normal, singly and doubly substituted isotopologues of CO$_2$ differ, then a kinetic origin for the vital effect could produce non-equilibrium clumped isotope values in calcium carbonate, and might result in observations of correlated deviations from inorganic calcite in $^d^{18}$O, $^d^{13}$C, and $^d_4$. No such patterns are systematically observed in our data set (Figs. 6 and 11). The 'kinetic' hypothesis for the origin of vital effects in foraminifera and coccoliths can only be consistent with our observations if the kinetically controlled oxygen and carbon isotope fractionations lead to proportions of $^{13}$C–$^{18}$O bonds that are typically within $\pm 0.01\%$ of thermodynamic equilibrium across a range of temperatures, which is possible but has not yet been demonstrated.

Several observations argue against the 'kinetic' hypothesis for the origin of vital effects in foraminifera and coccoliths (excluding the Arctic benthic foraminifera). Counterexamples of kinetically controlled precipitation mechanisms that lead to large departures from equilibrium $\Delta_{17}$ values of product carbonate are known (Ghosh et al., 2006a; Affek et al., 2008; Guo, 2008). It is not clear how kinetic models would account for enrichments in $^d^{18}$O relative to equilibrium, as observed in some foraminiferal taxa (Fig. 6) and other biogenic carbonates (e.g., McConnaughey, 1989). In addition, kinetic isotope effects during the hydration and hydroxylation of CO$_2$ are unlikely to be important for organisms in which carbonic anhydrase is present, as this enzyme catalyzes equilibrium between carbonate species and water. Different forms of carbonic anhydrase have been detected in a number of organisms but its direct role in the calcification process is uncertain (e.g., coccoliths, corals, molluscs, eggshells; Gay and Mueller, 1973; Miyamoto et al., 1996; Ziveri et al., 2003). An important caveat is that despite its occurrence in many systems, equilibration of the dissolved inorganic carbon system and associated isotopic equilibrium in biologically precipitated minerals is only likely to occur if (1) carbonic anhydrase specifically occurs at the site of uptake/production, (2) it is present at the site of calcifying reactions, and (3) is working efficiently. The observation, however, that $\Delta_{17}$ residuals for almost all samples are apparently normally distributed (see discussion in Section 4.5) is also consistent with the hypothesis that kinetic isotope effects are not discernable in most of the biogenic data. Future theoretical calculations and experiments are necessary, however, to quantify isotopic fractionations of doubly substituted isotopologues associated with different reactions (i.e., hydroxylation and hydration reactions;
protonation of carbonate ion and deprotonation of bicarbonate ion) accurately.

4.3.1. Mechanisms for recording solution pH in solid phase

The “pH hypothesis” requires that the HCO\textsubscript{3}/CO\textsubscript{3}\textsuperscript{2−}/CO\textsubscript{2} ratio of ambient dissolved inorganic carbon to be preserved in the solid phase, although a mechanism for recording solution pH in calcium carbonate has not yet been elucidated (Zeebe, 1999, 2007; Adkins et al., 2003; Watson, 2004). Although both bicarbonate and carbonate ions may be present at the site of calcification, surface complexation modelling of the carbonate–water interface indicates that the mineral surface preferentially sorbs carbonate ion relative to bicarbonate (Van Cappellen et al., 1993; Kim et al., 2006). One possible explanation is that the ratio of chemisorbed carbonate and bicarbonate at the mineral surface in the site of calcification is proportional to the solution carbonate/bicarbonate ratio. If precipitation is sufficiently rapid (relative to the timescale for equilibration of dissolved inorganic carbon species), when deprotonation of bicarbonate occurs there is not time for the newly formed carbonate ion to isotonically re-equilibrate with the solution, resulting in apparent vital effects in δ\textsuperscript{18}O and δ\textsuperscript{13}C, and subtle effects that may or may not be detectable for Δ\textsubscript{47}. If precipitation is slow, there may be sufficient time for newly formed carbonate ions to isotopically re-equilibrate with water and dissolved inorganic carbon, so that δ\textsuperscript{18}O, δ\textsuperscript{13}C, and Δ\textsubscript{47} signatures are all close to equilibrium. Observations supporting this explanation are discussed in Section 4.3.2.

Alternately, biologically precipitated carbonates may be quantitatively precipitated from a pool of HCO\textsubscript{3}/CO\textsubscript{3}\textsuperscript{2−} and CO\textsubscript{2}, and therefore the pH of the original solution (which sets the proportions of these dissolved inorganic carbon species) is recorded in the solid phase (Zeebe, 1999, 2007). The reservoir being quantitatively precipitated need not be an entire seawater vacuole or seawater-like calcifying space, although this is possible. Instead, it should be sufficient to quantitatively precipitate HCO\textsubscript{3} and CO\textsubscript{3}\textsuperscript{2−} ions in the solution boundary layer, or at the site of calcification itself, as long as there is local isotopic equilibrium.

A third possibility is that if there is a relationship between calcification rate (which is related to carbonate ion concentration) and the occurrence of lattice defects, the amount of HCO\textsubscript{3} directly incorporated in the calcite structure may function to maintain local charge balance (Duffy et al., 2005). This idea is consistent with the recent observation using nuclear magnetic resonance spectroscopy that
HCO$_3^-$ is present in synthetic and natural biogenic and abiotic calcite, with significant structural accommodation associated with the substitution of HCO$_3^-$ for CO$_3^{2-}$ groups (Feng et al., 2006).

4.3.2. Data for samples from near-freezing temperatures: possible kinetic effects?

Data for the two samples of benthic foraminifera from $-8^\circ$ to $-0^\circ$ C do exhibit deviations of up to $-0.02^\%$ to $-0.04^\%$ from the calibration for inorganic calcite (Fig. 4C and F). It is possible that (1) these offsets simply reflect measurement error, (2) that CO$_3$ produced during acid digestion of these samples partially re-equilibrated during sample preparation, or (3) that the samples still retained contaminating phases (e.g., coccoliths). It is also possible that these departures in $\Delta_{47}$ reflect non-equilibrium isotope effects due to the slow kinetics associated with the hydration and hydroxylation of aqueous CO$_2$ (McConnaughey, 1989). Another kinetic mechanism proposed for depletion in $\delta^{13}$O and $\delta^{14}$C in carbonate minerals relative to equilibrium is diffusion; however, it is unlikely that the pattern reflects isotopic discrimination during diffusion of carbonate species to the growing crystal surface (Turner, 1982; Grossman, 1984) because it should drive enrichment in $\Delta_{47}$ (Eiler and Schauble, 2004).

These observations could indicate that it is the relative balance of the timescale for equilibration relative to the rate of precipitation that governs whether a kinetic isotope effect is expressed in $\Delta_{47}$ (and also in $\delta^{13}$O and $\delta^{14}$C). Given that the timescales for dissolved inorganic carbon equilibration are longer at lower temperatures (Zeebe and Wolf-Gladrow, 2001), it is possible that the observed departures for the two low-temperature benthic foraminiferal samples reflect a kinetic isotope effect. Constraints on growth rate, however, are not available to evaluate whether the rate of carbonate precipitation by these organisms is sufficiently rapid relative to the timescale for equilibration of dissolved inorganic carbon species.

However, it is interesting to note that similarly large deviations from inorganic calcite are not reported in deep-sea corals that have grown at low temperatures (Fig. 3) (Ghosh et al., 2006a). This difference may reflect the extremely slow growth rates of deep-sea coral, and/or the efficient activity of carbonic anhydrase. Growth rates for deep-sea coral of 0.04–2 mm/year have been reported for _Desmophyllum cristagalli_ and _Caryophyllia profunda_, and higher rates of up to 6–8 mm/year reconstructed for _Enallopsammia rostrata_ and _Lophelia_ sp. (Wilson, 1979; Bell and Smith, 1999; Risk et al., 2002; Adkins et al., 2004).

If this explanation for large ($0.02$–$0.04^\%$) deviations from the inorganic calcite calibration is true, then we would predict that detailed measurements may show that the slow-growing deep-sea coral _D. cristagalli_ is closest to equilibrium, whereas the faster growing taxa _Lophelia_ sp. and _E. rostrata_ exhibit larger deviations from equilibrium. Other taxa from cold-water environments with rapid growth rates (i.e., molluscs) might also show much larger deviations from equilibrium than the data previously reported. Finally, we would predict that $\Delta_{47}$ and $\delta^{18}$O deviations from equilibrium should not be observed in organisms with carbonic anhydrase present at the site of calcification and working efficiently, or in organisms that do not have carbonic anhydrase present at the site of mineral formation but mineralize extremely slowly. The converse should be observed for other calcifying organisms that mineralize rapidly and do not have carbonic anhydrase present at the site of calcification or working efficiently.

4.3.3. Uncertainties in the inorganic calcite calibration and implications

It is possible that the observed differences between the biogenic data and the published inorganic calcite data at low temperatures reflect uncertainties in the inorganic calcite calibration. The $\Delta_{47}$ value for the sample grown at near-freezing temperatures is poorly constrained (Ghosh et al., 2006a). The inorganic calcite calibration is based on replicate measurements of synthetic materials precipitated at 23, 33, and 50 °C, and a single analysis of a synthetic sample that was grown at 1 °C that had a measurement uncertainty of 0.016 $\%$. The samples at 23, 33, and 50 °C have carbonate $\delta^{18}$O values that are lower than predicted by the Kim and O’Neill (1997) equation by $-1.07^\%$, $-0.39^\%$, and $-0.04^\%$, respectively. Uncertainties in the temperature of precipitation contribute an error in the calculation of equilibrium values of 0.25 $\%$, 0.50 $\%$, and 0.25 $\%$. This inorganic calcite sample precipitated at cold temperatures also had a measured $\delta^{13}$C value that was 0.54 ± 0.05 $\%$ lighter than the equilibrium value.

It is possible that the $\delta^{18}$O and $\Delta_{47}$ ratios reported for this synthetic sample (and possibly the 23 °C sample) may reflect a kinetic isotope effect that drives $\delta^{18}$O to low values and $\Delta_{47}$ to higher values, relative to equilibrium. If true, then the $\Delta_{47}$ values of the Arctic Ocean benthic foraminiferal samples may be a closer determination of equilibrium calcite $\Delta_{47}$ values at low temperatures, and the $\Delta_{47}$ values previously reported for deep-sea coral (Ghosh et al., 2006a) could in fact reflect a kinetic isotope effect. One kinetic process that would result such an effect in $\delta^{18}$O (depletion relative to equilibrium) and $\Delta_{47}$ (enrichment relative to equilibrium) is diffusion (Eiler and Schauble, 2004). It is interesting to note that the Red Sea coral reported in Ghosh et al. (2006a) exhibits a similar pattern of apparent isotopic disequilibrium. Careful studies of kinetic isotope effects from growth experiments are necessary to fully explore the significance of these observations in biogenic carbonates.

4.4. Mineral structure effects

We observe no discernable mineralogic differences in $\Delta_{47}$ between aragonitic and calcitic biogenic carbonates (Figs. 12 and 13). $\Delta_{47}$ analyses of other aragonitic biogenic carbonates, such as deep-sea coral and mollusks, also resemble the inorganic calcite calibration (Ghosh et al., 2006a; Came et al., 2007; Eiler, 2007; Huntington et al., 2010). These observations are consistent with _ab initio_ calculations that predict $\Delta_{47}$ differences between carbonate minerals with different mineral structures should be small (less than 0.02 $\%$ between aragonite and calcite at 25 °C) (Schauble et al., 2006; Eagle et al., 2010). These similarities
may arise if the different carbonate minerals preserve a state of ordering that reflects isotopic equilibration of the dissolved inorganic carbon species, as proposed above, rather than of carbonate ions within the solid itself. The observed slopes through both the inorganic and biogenic data are similar but slightly steeper than the theoretical values (Schauble et al., 2006; Guo et al., 2009) (Fig. 8). The $0.001–0.002$‰/$^\circ$C discrepancy is potentially attributable to uncertainties in the theoretical calculations.

4.5. Utility for paleoclimatic and paleoceanographic studies

If the isotope systematics (including $\Delta_{47}$ values) of foraminifera and coccoliths generally conform to our observations, then the application of carbonate clumped isotope thermometry of the biogenic carbonates could significantly improve our ability to reconstruct past temperatures and seawater composition. The observations reported above suggest that nearly all of the biogenic samples that have been studied are indistinguishable from inorganic calcite and therefore likely precipitated in apparent equilibrium. These observations imply that by using this proxy, we are essentially exchanging unknown sources of error of an empirically calibrated proxy with known errors from clumped isotopes that we can interrogate statistically. The standard error of sample means for cleaned samples (Fig. 2 of Electronic Appendix 1) are indistinguishable from a normal distribution at the 95% confidence level (Shapiro–Wilk test; $p = 0.54$). The difference between the measured $\Delta_{47}$ values from these biogenic carbonates and the values predicted from the inorganic calcite calibration (or the calibration through all of the data) are also indistinguishable from a normal distribution at the 95% confidence level (Fig. 14). This distribution is what we would predict if both the measurement errors and the residuals are simply due to random errors in counting statistics with a Poisson distribution.

On the basis of these observations, we suggest that the calibration derived from all inorganic and biogenic carbonate data with precise temperature constraints should be used for paleothermometry (Table 6 of Electronic Appendix 2). This calibration is $\Delta_{47} = -0.022\Delta_{\text{cal}} - 0.009 + 0.0627\Delta_{\text{cal}} + 0.0115$. The average difference between temperatures predicted using this calibration (or the inorganic

![Fig. 12. Comparison of measured $\Delta_{47}$ values to $\Delta_{47}$ residuals (measured minus predicted from an inorganic calibration) for data from foraminifera, coccoliths, and bulk carbonate.](image)

![Fig. 13. Comparison of $\Delta_{47}$–temperature relationships for samples with different mineralogy to theoretical calculations (Guo et al., 2009). The regressions for different populations of data have a steeper slope and shallower intercept to the modelled values. (A) Data for aragonitic and calcitic samples (mean and 1 SE for $y$-values and mean and range for $x$-values shown) compared to regressions through inorganic calcite data, all data, and modelled calcite and aragonite relationships. (B) Comparison of modelled and observed regression lines for calcite. (C) Comparison of modelled and observed regression lines for aragonite.](image)
calcite calibration) and independently estimated calcification temperature is \( \sim 0.7 \, ^\circ C \pm 2.5 \, ^\circ C \) (1σ), similar to what has been reported for calibrations of other temperature proxies including \( \delta^{18}O \) (Bemis et al., 1998) and Mg/Ca (Anand et al., 2003; Elderfield et al., 2006).

In addition to allowing us to accurately reconstruct temperature, the development of \( \Delta_{47} \) records for sites where there are existing proxy data would help leverage the interpretation of other proxies. We suggest that clumped isotopes can potentially be applied to assess whether differences between existing proxy reconstructions reflects environmental factors (i.e., fluid composition, seasonality and depth of production) or other factors. Proxy “carrier” phases (e.g., alkenones vs. planktic foraminifera) may have different depths and seasons of maximum abundance. As a result, reconstructions for the same site may not reflect conditions during the same time of year or at the same water depths (e.g., Mix, 2006). Different carrier phases also do not have the same susceptibility to secondary processes such as lateral transport and water column dissolution (e.g., Benthien and Mueller, 2000). One direct application of clumped isotope thermometry could be to assess whether apparent mismatches in sea surface temperature reconstructions from alkenones and foraminiferal Mg/Ca data for the Last Glacial Maximum at specific sites (MARGO Project Members, 2009) arise due to some of these processes.

**5. CONCLUSIONS**

A challenge for all forms of carbonate clumped isotope thermometry is the need for very large and clean samples, long analytical durations, and/or extensive replication in order to reduce the errors of \( \Delta_{47} \) measurements to the level necessary for precise temperature determinations. Our analyses of such samples suggest that clumped isotope thermometry can yield accurate and precise determinations of past calcification temperatures when applied to foraminifera and coccoliths. The results of this detailed survey of multiple taxa implies that “vital effects” in \( \Delta_{47} \) may be unlikely in extinct species (in contrast to \( \delta^{18}O \) and \( \delta^{13}C \)), although additional studies on the systematics governing “clumping” in carbonates are necessary. In this study, we observe that the \( \Delta_{47} \) of primary carbonate precipitated by foraminifera and coccoliths and other biogenic carbonates is generally indistinguishable from inorganic precipitates. Clumping of heavy isotopes of carbon and oxygen into bonds with each other is independent of the \( \delta^{18}O \) of the water in which the mineral precipitated, and the \( \delta^{13}C \) of dissolved inorganic carbon. No evidence for a relationship between pH and \( ^{13}C-^{18}O \) bond order is discernable in the limited dataset presented, and no mineralogic differences are observed. In the species we have examined, the mechanism(s) responsible for significant non-equilibrium fractionation in \( \delta^{18}O \) do not bias \( \Delta_{47} \), with a possible exception temperatures a few cases where \( \Delta_{47} \) values deviate by \( 0.02-0.04^{\circ} \) relative to inorganic calcite and other biogenic carbonates.

These data constrain the upper magnitude of \( \Delta_{47} \) fractionation in models of stable isotope vital effects that rely on equilibrium or alternative kinetic mechanisms. Our findings suggest that in foraminifera and coccoliths, dissolved inorganic carbon at the mineral–solution interface...
is generally indistinguishable from local isotopic equilibrium, and appears to be consistent with the hypothesis that solution pH may be recorded in the solid phase (Zeebe, 1999, 2007; Usdowski and Hoefs, 1993). This may occur if dissolved inorganic carbon is quantitatively precipitated from reservoirs such as seawater vacuoles or at the site of calcification, if CO$_2^-$ and HCO$_3^-$ groups chemisorbed to the mineral surface are present in proportion to their abundance in the adjacent reservoir of dissolved inorganic carbon, or if HCO$_3^-$ incorporation in lattice maintains local charge balance to compensate for lattice defects. The balance of the timescale for equilibrium of dissolved inorganic carbon species relative to the rate of precipitation could govern whether a kinetic isotope effect is expressed in ‘clumping’. More work is needed to investigate the controls on $\Delta_{47}$ in biogenic carbonates precipitated in environments where the timescale for equilibrium of the dissolved inorganic carbon system is slow relative to typical carbonate precipitation rates, we note that similar patterns in $\Delta_{47}$, $\delta^{18}O$ and $\delta^{13}C$ (relative to inorganic calcite) have been observed in molluscs, brachiopods, deep-sea corals, and bioapatite in teeth from mammals, reptiles, and fish (Ghosh et al., 2006a; Eiler, 2007; Eagle et al., 2010). As a result, we suggest that the same processes likely govern isotopic fractionation in these other carbonate-precipitating organisms. If true, this may limit the applicability of the carbonate ‘clumped isotope’ thermometer in some settings.

**AUTHOR CONTRIBUTION STATEMENT**

A.T. designed the project, prepared and measured most of the samples, analyzed the results, interpreted the data, and wrote and revised the manuscript. R.E. prepared and measured samples, provided input to the analysis of the data, and drafted some of the figures. N.T. prepared and measured samples. H.B. provided some of the samples used in this study. P.H. cultured the coccoliths that were used in this study. J.E. trained A.T. in $\Delta_{47}$ analysis and interpretation. All co-authors contributed to discussions of this work.

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**APPENDIX A. SUPPLEMENTARY DATA**


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