13C–18O bonds in carbonate minerals: A new kind of paleothermometer

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Abstract

The abundance of the doubly substituted CO₂ isotopologue, 13C18O16O, in CO₂ produced by phosphoric acid digestion of synthetic, inorganic calcite and natural, biogenic aragonite is proportional to the concentration of 13C–18O bonds in reactant carbonate, and the concentration of these bonds is a function of the temperature of carbonate growth. This proportionality can be described between 1 and 50 °C by the function: $D_{47} = 0.0592 \cdot 10^6 \cdot T - 0.02$, where $D_{47}$ is the enrichment, in per mil, of 13C18O16O in CO₂ relative to the amount expected for a stochastic (random) distribution of isotopes among all CO₂ isotopologues, and $T$ is the temperature in Kelvin. This relationship can be used for a new kind of carbonate paleothermometry, where the temperature-dependent property of interest is the state of ordering of 13C and 18O in the carbonate lattice (i.e., bound together vs. separated into different CO₃²⁻ units), and not the bulk δ¹⁸O or δ¹³C values. Current analytical methods limit precision of this thermometer to ca. ± 2 °C, 1σ. A key feature of this thermometer is that it is thermodynamically based, like the traditional carbonate–water paleothermometer, and so is suitable for interpolation and even modest extrapolation, yet is rigorously independent of the δ¹⁸O of water and δ¹³C of DIC from which carbonate grew. Thus, this technique can be applied to parts of the geological record where the stable isotope compositions of waters are unknown. Moreover, simultaneous determinations of $D_{47}$ and δ¹³C for carbonates will constrain the δ¹⁸O of water from which they grew.

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1. Introduction

Oxygen isotope exchange equilibria between carbonate minerals and water form the basis of the oldest and most widely used type of geochemical paleothermometer (Urey, 1947; McCrea, 1950; Epstein et al., 1953; Emiliani, 1955, 1966a,b). The carbonate–water thermometer is a landmark of both paleoclimate research and isotope geochemistry, but suffers from one simple but important weakness: the oxygen isotope compositions of both carbonates and the waters from which they grew must be known to determine temperature. Carbonates are a widespread and often well-preserved part of the geological record, but only rarely do we have direct and independent evidence for the oxygen isotope compositions of ancient waters.

Various approaches have been taken for resolving or circumventing this difficulty. For example, it is possible to estimate the δ¹³C of the ancient ocean by modeling δ¹³C gradients in marine sediment pore-waters (Schrag et al., 1996, 2002; Adkins et al., 2002), based on oxygen isotope compositions of benthic foraminifera (Shackleton, 1967), or based on reconstructed sea-level changes and the estimated δ¹³C of glacial ice (Dansgaard and Tauber, 1969). These approaches are generally only useful for study of Pleistocene marine records—an important but small subset of all the potential uses of carbonate paleothermometry. Similarly, there are several marine paleothermometers based on speciation of planktonic organisms, relative abundances of alkenones, or the Mg/Ca or Sr/Ca ratios of corals, foraminifera, and other carbonate-producing organisms. These thermometers can also be used to precisely re-construct Pleistocene marine temperature, but are unsuitable for extrapolation in temperature, apply only
to the ocean, and are of uncertain value in the deep geological past.

We present the principles, calibration data and an illustrative application of a new paleothermometer based on ‘clumping’ of $^{13}$C and $^{18}$O in the carbonate mineral lattice into bonds with each other—that is, we examine not only the $^{13}$C/$^{12}$C and $^{18}$O/$^{16}$O ratios of carbonates, but also the fraction of $^{13}$C and $^{18}$O atoms that are joined together into the same carbonate ion group ($^{13}$C$^{18}$O$^{16}$O$^2$–). This thermometer is based on a thermodynamically controlled stable isotope exchange equilibrium among components of the carbonate crystal lattice. Because it involves a homogeneous equilibrium (reaction among components of a single phase), it rigorously constrains the temperature of carbonate growth based on the isotopic composition of the water from which it grew or other phases with which it co-exists.

1.1. A paleothermometer based on ordering of $^{13}$C and $^{18}$O in carbonate minerals

Carbonate minerals contain 20 different isotopologues, or isotopic variants, of the carbonate ion group (Table 1). The most abundant of these, $^{12}$C$^{16}$O$^2$– ($\approx$98.2%) contains no rare isotopes. The next three most abundant, $^{13}$C$^{16}$O$^2$– ($\approx$1.1%), $^{12}$C$^{18}$O$^{16}$O$^2$– ($\approx$0.6%) and $^{12}$C$^{17}$O$^{16}$O$^2$– ($\approx$0.11%) are singly substituted (i.e., contain one rare isotope). Collectively, these four isotopologues constitute almost all ($\approx$99.99%) of the carbonate ions in natural carbonate minerals, and effectively control their bulk $^{13}$C, $^{17}$O and $^{18}$O values. However, most of the isotopic diversity—16 different isotopologues in all—is contained in the doubly, triply and quadruply substituted carbonate ion units that make up the remaining $\approx$100 ppm. Each of these multiply substituted isotopologues has unique vibrational properties, and therefore they must differ from one another in thermodynamic stability (among other things).

In a carbonate crystal at thermodynamic equilibrium, the relative abundances of the various carbonate ion isotopologues must conform to equilibrium constants for reactions such as:

$$^{13}$C$^{16}$O$^2$– + $^{12}$C$^{18}$O$^{16}$O$^2$– = $^{13}$C$^{18}$O$^{16}$O$^2$– + $^{12}$C$^{16}$O$^2$–.

(Reaction 1)

There are many independent reactions of this type, but we focus only on this one because it involves the most abundant (and therefore most easily measured) doubly substituted isotopologue ($^{13}$C$^{18}$O$^{16}$O$^2$–).

Urey (1947), Bigeleisen and Mayer (1947), and Wang et al. (2004) examine the thermodynamics of reactions analogous to Reaction 1 involving isotopologues of simple molecular gases. They show that equilibrium constants for such reactions are temperature dependent and generally promote ‘clumping’ of heavy isotopes into bonds with each other (increasing the proportions of multiply substituted isotopologues) as temperature decreases. If Reaction 1 follows similar principles, its equilibrium constant should be near 1 at very high temperatures and increase (driving the reaction to the right) with decreasing temperature. Thus, in thermodynamically equilibrated carbonates, the equilibrium constant for Reaction 1 can serve as the basis of a geothermometer, provided that the temperature dependence of this reaction is known and the abundances of all the reactant and product isotopic species can be measured.

Reaction 1 can be thought of as analogous to order/disorder exchange reactions among cation sites in pyroxenes.
and feldspars (e.g., Myers et al., 1998). For this reason, we
describe the thermometer based on Reaction 1 as the
$^{13}$C–$^{18}$O order/disorder carbonate thermometer. A less
precise, but less ungainly term we also use here is the ‘car-
bonate clumped-isotope thermometer’. The important fea-
ture of this thermometer is that it involves a homogeneous
equilibrium (that is, a reaction among components of one
phase, rather than between two or more phases), and there-
fore rigorously constrains temperature without knowing
the isotopic composition of a second phase.

We are aware of no way one could directly measure abundances of $^{13}$C$^{18}$O$^{16}$O$_2$– ionic groups in carbonate
minerals with sufficient precision to be useful for paleother-
ometry. They make up only ca. 60 ppm of natural car-
bonates (Table 1), and we show below that they must be
analyzed with relative precision of ca. 10$^{-5}$. It seems
unlikely that any spectrometric method could meet these
requirements. However, Eiler and Schauble (2004) and Af-
beck and Eiler (2005), recently showed that it is possible to
analyze $^{13}$C$^{18}$O$^{16}$O in CO$_2$ at natural abundances and with
the necessary precision. We show here that the abundance
of $^{13}$C$^{18}$O$^{16}$O in CO$_2$ produced by phosphoric acid so-
ni cation of carbonate minerals is proportional to the abun-
dance of $^{13}$C$^{18}$O$^{16}$O$_2$– ionic groups in those minerals
themselves. Thus, combination of the mass-spectrometric
methods of Eiler and Schauble (2004) and Affek and Eiler
(2006) with long-established methods of phosphoric acid
digestion of carbonates can constrain the equilibrium con-
stant for Reaction 1, and therefore the growth temperature,
in a sample of solid carbonate.

2. Samples and methods

2.1. Samples

2.1.1. Natural and synthetic calcite standards

We studied one inter-laboratory calcite standard (NBS-
19, distributed by the IAEA) and three intra-laboratory
calcite standards, ‘MARJ-1’, ‘MZ carbonate’ and ‘Sigma-
carb’. Two of the standards (NBS-19 and MARJ-1) were
purified from Italian Carrara marbles that were metamor-
phosed to upper-greenschist facies during the mid-Tertiary
(Friedman et al., 1982; Molli et al., 2000; Leiss and Molli,
2003; Ghosh et al., 2005). MAR-J1 studied here has a grain
size of less than 250 µm and a texture and chemical and O
and C isotope composition similar to NBS-19 (Ghosh
et al., 2005). The MZ carbonate standard was obtained
from MERCK (http://chemdat.merkco.de/) and the Sig-
a-carb standard was obtained from Sigma–Aldrich chemi-
cal supply (http://www.sigmaalrich.com/). Both of these
carbonates were produced by passing carbon dioxide
through a slurry of calcium oxide and water, producing a
very fine precipitate of calcite (the industrial term for this
reaction is the ‘carbonation process’). The typical grain size
of these carbonate powders is 40–50 µ, based on measure-
ment under a binocular microscope. It is unimportant for
our purposes whether the carbonation process promotes
oxygen isotope exchange equilibrium between carbonate
and water; it is important only that the carbonate precipi-
tates are chemically pure and isotopically homogeneous
when sampled in mg-sized aliquots, and thus provide a use-
ful basis for establishing the precision of our analyses.

2.1.2. Equatorial surface coral

We examined a sample of Porites surface coral collect-
ed from the west shore of Sumatra in the equatorial
Indian Ocean. This specimen, Mm97Bc, was obtained
from K. Sieh and was collected from the crest of a living
coral head in 1 m water depth at Memong Island (98.54
E; 0.035 N) (Natawidjaja, 2003, 2004). We estimate the
mean growth temperature of this coral to be
29.3 ± 2°C, based on instrumental records from this re-
region, which is characterized by a weak seasonality and
little spatial variability over hundreds of km (Abram
et al., 2003). Additional information about this sample
can be found in (Natawidjaja, 2003). This aragonitic cor-
al slab (1–2 cm) was sampled using a file, yielding
≈100 µm powder.

2.1.3. Deep sea coral

We examined two specimens of D. dianthus (a deep sea
coral also previously referred to as D. cristagalli). Sample
47407, described in Adkins et al. (2003), was collected at
549 m water depth in the Southern Pacific ocean (54.49 S,
129.48 W) and grew at an estimated average temperature
of 5.5 ± 1.0 °C, based on instrument records from similar
depth and location. Sample 47413 was collected at 420 m
water depth off the south shore of New Zealand (50.38 S,
167.38 E) and grew at an estimated average temperature
of 8.0 ± 0.5 °C, also based on local instrument records.
These aragonitic corals were sampled by breaking off frag-
ments of their fragile septa, followed by crushing to a mean
grain size of 100 µm. Both samples are from the Smithso-
nian collections.

2.1.4. Calcites grown inorganically at known temperature

We grew calcite by removing CO$_2$ from aqueous solu-
tions of sodium bicarbonate and calcium chloride or from
calcite-saturated solutions, using a method similar to that
described by Kim and O’Neil (1997). Two different vari-
ants of this method were employed:

(1) Sample HA1 was prepared in the following way: first,
CO$_2$ (99.96%, Air Liquide) was bubbled through de-ionized water at room temperature for
1 h. Next, NaHCO$_3$ (AR, Malllickrodt) was added
to the acidified water in quantities required to pro-
duce a 5 mM solution (ΣCO$_3^{2−}$). After complete
dissolution of NaHCO$_3$, CaCl$_2$ (99.8%, Fisher
scientific) was added to the solution, in amounts
required to make an equimolar NaHCO$_3$:CaCl$_2$
solution, and the solution was stirred to complete
dissolution. CO$_2$ was then removed from this
solution using methods described below, forcing
precipitation of calcite. The yield of calcite from this experiment was low, and so we modified our procedure for the remaining experiments.

(2) The rest of the samples were prepared by first bubbling CO\textsubscript{2} into a stirred suspension of CaCO\textsubscript{3} (99% pure obtained from Sigma–Aldrich chemical supply, approximately 0.5 g in 800 ml de-ionized water) for 1–2 h. The un-dissolved CaCO\textsubscript{3} was removed by gravitational filtering through a grade-2 Whatman filter. The solution was then purged of CO\textsubscript{2} as described below, forcing precipitation of calcite. Solutions prepared in this way yielded far more calcite per unit time, perhaps because partly dissolved nuclei of starting calcite remained in suspension, providing templates for calcite precipitation.

Regardless of the way the solution was prepared, once ready, approximately 200 ml was poured into an Erlenmeyer flask that was sealed with a rubber stopper equipped with inlet and outlet BEV-A-LINE tubing. This was placed in a controlled-temperature water bath and allowed to thermally equilibrate for 1 h. The temperature in the water bath was monitored throughout our experiment using a mercury thermometer. The temperature stability of the chilled bath was ± 0.2 °C, that of the room temperature bath was ± 1 °C, and that of heated water bath was ± 2 °C. After thermally equilibrating, N\textsubscript{2} gas (99.96%, Air Liquide) was bubbled first through a similar Erlenmeyer flask filled with the same de-ionized water used to make our solution (to minimize isotopic evolution of the solution due to evaporation into the N\textsubscript{2} gas stream) and then through the solution itself through a Pasteur pipette dipped in the solution and attached to the rubber stopper sealing the Erlenmeyer flask. The N\textsubscript{2} flow rate was approximately 10 ml/min for samples HA1 through HA7 and HA12 and approximately 50 ml/min for sample HA9. Carbon dioxide dissolved in the solution partitioned into the N\textsubscript{2} and was removed from the system, promoting super saturation and slow precipitation of calcium carbonates (Fig. 1, panel 1). The bubbling rate of N\textsubscript{2} was controlled by a regulator attached to the gas cylinder and was monitored by counting the number of bubbles per 30 s (10 ml/min was equivalent to about 20 bubbles to 30 s).

Precipitation experiments were performed at room temperatures (23 ± 1 °C), in an ice–water bath temperature (1 ± 0.2 °C), and in heated water baths (at 33 ± 2 and 50 ± 2 °C). The time required for the first appearance of visible precipitate depended on the composition of the solu-

Fig. 1. Schematic illustration of apparatus used in this study. Panel 1: System of vessels and purge gases used in the synthesis of calcite from bicarbonate solutions. See text for explanation. Panel 2: vacuum and carrier-gas apparatus used for phosphoric acid digestion of carbonate and clean-up of product CO\textsubscript{2}. Powdered carbonate sample and phosphoric acid are placed in separate arms of the reaction vessel, evacuated, placed in a constant-temperature bath, and then the vessel is tipped to mix acid with sample. Product CO\textsubscript{2} is cryogenically purified of water and other trace gases, condensed into a small glass vessel, and transferred to the carrier-gas system for further purification. There, CO\textsubscript{2} is entrained in a He stream flowing at 2 ml/min, passed through a 32 m long 530 μm ID Supelco gas-chromatography column held at −10 °C and re-collected in a glass trap immersed in liquid nitrogen. Finally, the re-collected CO\textsubscript{2} is returned to the vacuum system and cryogenically separated from He purge gas prior to mass spectrometric analysis.
tion, temperature, and bubbling rate, and was typically about 1 day. It took at least one day and a maximum of five days to generate sufficient material (~10 mg CaCO₃) for both X-ray diffraction and isotopic analysis. Upon completion of each experiment, the solid carbonate precipitated on the walls and at the bottom of the vessel was removed using a rubber spatula, filtered by injecting the suspension through a Whatman GF/C filter paper and then air dried for at least 48 h prior to storage for isotopic analysis.

The mineralogy of every precipitate was identified by X-ray diffraction analysis and some of the precipitates were examined by optical microscopy. Further details about each sample are summarized in Table 5.

An aliquot (15–10 ml) of the supernatant was stored in an air-tight polypropylene container for δ¹⁸O analysis using a GasBench II water-equilibration system attached to a Thermo Finnegan Delta Plus located at University of California Irvine, with analytical precision of ± 0.1‰. The differences in δ¹⁸O between calcites and waters (see Table 5 and the Results and Discussion, below) fall broadly within the range previously observed for inorganic calcite synthesis experiments such as the ones we performed (O’Neil et al., 1969; Kim and O’Neil, 1997), but do not agree exactly (the average disagreement in the difference (δ¹⁸Ocarbonate – δ¹⁸Owater) is 0.3‰). This disagreement could reflect any combination of: temperature variations during experiments; failure to maintain a constant isotopic composition of solution during experiments or between sampling and final analyses; and analytical errors in determinations of the δ¹⁸O of water and carbonate. All of these factors potentially apply both to our experiments and those to which we compare our results. Variations and errors in temperature influence the accuracy of our calibration of Reaction 1, but the other factors should not (see Fig. 4 in the Section 3, below). We do not believe we can independently determine which combination of these errors in our experiments and those of Kim and O’Neil (1997), explains this discrepancy between the two studies. However, its magnitude translates into a discrepancy in apparent temperature of only 2 °C. This is comparable to the precision in temperature corresponding to our best analytical precision in Δ⁴⁷, and thus any error in our experiments implied by this comparison seems unlikely to introduce a significant additional error in the thermometer based on Reaction 1.

2.2. Phosphoric acid digestion of carbonates

We extracted CO₂ from carbonates by reaction with anhydrous phosphoric acid, following the methods of McCrea (1950) and Swart et al. (1991). Fig. 1 (panel 2) illustrates the glass vacuum apparatus used for this purpose. This apparatus uses McCrea-type reaction vessels and conventional vacuum cryogenic procedures to trap product CO₂ and separate it from trace water. We imagine that more sophisticated, automated devices should also be appropriate for analyses such as those we describe, although large samples (ca. 5 mg) are preferred to generate the intense mass–47 ion beams needed for precise isotopic analyses, and not all such systems can easily accommodate such large samples. The details of our phosphoric acid digestion procedure are as follows:

- Each reaction vessel is loaded with ca. 5 mg of sample and 10 ml of ~103% phosphoric acid (density 1.90 g/ml) and evacuated to a baseline pressure of ~4 × 10⁻³ mbar for more than 2 h.
- Each reaction vessel is then immersed a NESLAB water bath, held at a temperature of 25 °C (unless otherwise noted), and allowed to thermally equilibrate for 1 h prior to tipping the acid reservoir to spill over the sample powder, starting the acid digestion reaction.
- Unless otherwise noted, the reaction is allowed to proceed for at least 12 h and usually ca. 16 h (over night).
- Product CO₂ is then cryogenically collected into a glass trap immersed in liquid nitrogen, and then released by warming the trap to ~77.8 °C (by immersing the trap in a dry ice + ethanol slurry), leaving any trace water frozen in the trap.
- Product CO₂ is then cryogenically collected into a small (~1 cc) evacuated glass sample vessel.

2.3. Purification of analyte CO₂

Multiply substituted isotopologues make up a small fraction (10’s of ppm at most) of CO₂ having natural stable isotope abundances, and so accurate analysis requires a virtual absence of isobaric interferences from contaminant gases (Eiler and Schauble, 2004). The most important of these contaminants are hydrocarbons and halocarbons (Eiler and Schauble, 2004). These are most easily removed by gas-chromatography, and can be monitored in all samples by simultaneous analysis of masses 47, 48 and 49. These contaminants typically contribute nearly equally to all three of these masses, producing distinctive and highly correlated relationships between relatively small 47 excess (tenth’s of per mil) and proportionately greater excesses in 48 (several per mil) and 49 (tens of percent; Eiler and Schauble, 2004). Therefore, each CO₂ sample analyzed in this study was entrained in a He stream flowing at 2 ml/min and passed through a 30 m long 530 μm ID Supelco gas-chromatography column packed with porous divinyl benzene polymer held at −10 °C, and re-collected in a glass trap immersed in liquid nitrogen. The gas-chromatography column was held in an oven (Hewlett Packard instruments Model description: Perfect fit Model no: G1530A), modified so that it could be purged with the boil-off gas from a tank of liquid nitrogen. See Fig. 1, panel 2 for further details. For these conditions and our typical sample size (ca. 50 μmol), elution times are 1 h with collection efficiency of >95%. Small variations in collection efficiency within this range appear not to be associated with isotopic fraction-
et al. (2004). Briefly, the samples were measured 3–10 times, such that the standard for these ion intensities and analytical durations. Most & is typically 0.03

Finally, the re-collected CO₂ was then cryogenically figured to simultaneously collect ion beams corresponding to $\text{M/Z} = 44$, 45 and 46 (read through $3 \times 10^8$ to $1 \times 10^{11} \Omega$ resistors), as well as 47, 48 and 49 (read through $10^{12} \Omega$ resistors). All measurements were made in dual inlet mode, and with a typical source pressure sufficient to maintain the mass-44 ion beam at a current of 160 nA. Each analysis involves 10 cycles of sample-standard comparison and each cycle involves 8 s integration of sample and standard ion beams. Analyses were standardized by comparison with an intra-laboratory reference gas whose bulk composition had been previously calibrated against CO₂ produced by phosphoric acid digestion of NBS-19, and whose abundance of mass-47 isotopologues was established by comparison with CO₂ that had been heated to 1000 °C to achieve the stochastic distribution. These heated gas standards were prepared to have bulk stable isotope compositions similar to those of unknowns, in order to minimize the potential errors associated with mass spectrometric nonlinearities (which are observable when samples and standards differ by more than ca. 20–30‰ in any given isotope ratio). See Eiler and Schauble (2004) for further details regarding protocols for standardizing measurements of mass 47 CO₂.

Abundances of mass-47 CO₂ are reported using the variable $\Delta_{47}^D$, defined as in Eiler and Schauble (2004) and Wang et al. (2004). Briefly, the $\Delta_{47}^D$ value is the difference in per mil between the measured 47/44 ratio of the sample and the 47/44 ratio expected for that sample if its stable carbon and oxygen isotopes were randomly distributed among all isotopologues—a case described as the stochastic distribution. External precision of individual measurements of $\Delta_{47}^D$ is typically 0.03‰, consistent with counting-statistics limits for these ion intensities and analytical durations. Most samples were measured 3–10 times, such that the standard error of their $\Delta_{47}^D$ values is in the range 0.01–0.02‰.

Finally, we define here the variable $\Delta_{47}^{13C}\text{O}_{16O_2}$. By analogy with $\Delta_{47}^D$ for CO₂, $\Delta_{47}^{13C}\text{O}_{16O_2}$ equals the deviation, in per mil, of the abundance of $^{13C}\text{O}_{16O_2}$ in a carbonate crystal from the abundance expected for the stochastic distribution of all stable isotopes in that crystal. To first order, $\Delta_{47}^{13C}\text{O}_{16O_2}$ equals $k_{eq}^D$ (the equilibrium constant for Reaction 1), $-1 \times 1000$.

3. Results

3.1. External precision of CO₂ extracted at 25 °C from carbonate standards

Fig. 2 plots all analyses of the NBS-19, MAR-J1, MZ carbonate and Sigma-carb standards made between January, 2004 and April, 2005. Each data point represents the average of between 1 and 10 analyses of the gas from a single acid extraction experiment; the error bar is ± 1σ (the standard error for that group of analyses—the appropriate statistic to evaluate the reproducibility of separate acid extraction experiments).

Carbon dioxide extracted from NBS-19 was analyzed eight times where each gas was analyzed 4 or more times (so that the standard error for each sample is expected to be ca. 0.01‰). These yield an average and standard devi-
ation for their $\Delta_{47}$ values of 0.341 ± 0.034 (Table 2). One of these eight samples (the $\Delta_{47}$ value of 0.27, measured on 9/29/2004) is a $2\sigma$ outlier to the rest of the group; the remaining 7 have an average and standard deviation of 0.352 ± 0.019—the expected reproducibility based on counting statistics alone. There is no obvious reason why the measurement on 9/29/2004 is an outlier to this population, although our sample purification procedures have improved through time and we suspect data generated before 11/2004 are more prone to unidentified contaminants than those generated after. Three samples of CO2 from MAR-J1 were analyzed 4 or more times each, and yielded an average and standard deviation for $\Delta_{47}$ of 0.341 ± 0.019 (Table 2). NBS-19 and MAR-J1 are both Italian marbles, and so we find it unsurprising that their $\Delta_{47}$ values are indistinguishable from each other. Table 2 and Fig. 2 also present analyses of CO2 samples extracted from these materials where each gas was analyzed only 1, 2 or 3 times each. These data are similar to the measurements summarized above, but scatter more widely (ca. ± 0.03‰ or 1σ), as expected for their poorer counting statistics. Curiously, the mean $\Delta_{47}$ values for CO2 analyzed fewer than 4 times are slightly lower than those analyzed four or more times for two of three standards for which such a comparison can be made (NBS-19 and MAR-J1, but not MZ carbonate). We suspect that this reflects a difference in standardization and/or trace contamination between relatively early generated data (prior to 6/2004, most of which were analyzed 1–3 times and were less carefully cleaned) and more recent data (most of which are analyzed 4 or more times and were more carefully cleaned).

Eleven samples of CO2 from Sigma-carb standard were measured where each gas was analyzed 4 or more times. These yield an average and standard deviation for $\Delta_{47}$ of 0.551 ± 0.019. This reproducibility is poorer than expected from counting statistics alone. There is no obvious reason why the measurements are comparable to counting statistics (0.01–0.02 ‰) over their 4 months interval. We suspect this is evidence that external precision over short time periods (weeks) is systematically better: When data are grouped by week, this grouping of data over short time periods is also evident in Fig. 2, where one can see that measurements of Sigma-carb standard drift by ca. 0.01–0.03 ‰ over several-week timescales rather than being randomly distributed over their 4 months interval. We suspect this is evidence that the external precisions of our acid extraction measurements are comparable to counting statistics (0.01–0.02 ‰) over short time periods, but that some aspect of our measurements, such as mass spectrometer calibration and/or the temperature or cleanliness of acid extraction, drifts subtly from week to week. Finally, only one measurement of CO2 from MZ standard involved 4 or more analyses of product CO2. However, the group of all samples (most of which were analyzed only 1–3 times) over a 3 month period

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yields an average and standard deviation for $\Delta_{47}$ of 0.64 ± 0.02; the precision of these data is indistinguishable from the expected limits from counting statistics (±0.02–0.03 for these data).

These data indicate that long-term external precision in analyses of $\Delta_{47}$ for CO$_2$ produced from acid digestion of carbonates is similar to counting statistics (±0.01–0.02) over short time periods (days to weeks), but can degrade to twice the counting statistics limit (±0.03%) over periods of months. One implication of this result is that the precision for $\Delta_{47}$ measurements of unknowns should be maximized by normalization to a carbonate standard measured within the same week under the same conditions.

3.2. Effects of varying the temperature of phosphoric acid digestion

In the absence of any analytical fractionation, the $\Delta_{47}$ value of CO$_2$ produced by carbonate acid digestion should equal the $\Delta_{47}(\text{CO}_2)$ value of reactant carbonate (this is easily shown by sampling statistics; see Table 1). However, reaction of carbonate with phosphoric acid releases only 2/3 of the carbonate oxygen as CO$_2$; the remainder remains in solution. This reaction is accompanied by an oxygen isotope fractionation, yielding CO$_2$ that is ca. 10% higher in $\delta^{18}$O than reactant carbonate. The exact magnitude of this fractionation varies with reaction temperature and differs among the various carbonate minerals (Sharma and Clayton, 1965; Swart et al., 1991; Kim and O’Neil, 1997).

The physical cause of this fractionation is unclear. It might reflect a temperature-dependent exchange equilibrium between extracted CO$_2$ and residual O in solution, in which case the $\Delta_{47}$ value of product CO$_2$ should equal the equilibrium value for gaseous CO$_2$ at the temperature of extraction (Wang et al., 2004; Eiler and Schauble, 2004) and no information regarding ordering of $^{13}$C and $^{18}$O in the carbonate mineral lattice should be preserved. It might instead reflect a kinetic isotope effect acting on the C–O bond; in this case we expect that $\varepsilon_{\text{CO}_2}$ should differ for $^{13}$C and $^{12}$C carbonate ions, and $\Delta_{47}$ of extracted CO$_2$ should be proportional but not equal to $\Delta_{13C^{18O}}$/$\Delta_{12C^{16O}}$. We expect that in this case any offset between $\Delta_{13C^{18O}}$ and $\Delta_{47}$ should vary with extraction temperature, because $\varepsilon_{\text{CO}_2}$ varies with temperature. If the physical cause is a kinetic isotope effect acting on metal-O bonds, we expect that there should be little or no sensitivity to $^{13}$C–$^{12}$C substitution, and $\Delta_{47}$ of product CO$_2$ should equal or closely approach $\Delta_{13C^{18O}}$/$\Delta_{12C^{16O}}$.

We extracted CO$_2$ from Sigma-carb standard at 25, 50 and 80 °C and from NBS-19 at 25, 35 and 45 °C. Table 3 and Fig. 3 summarize results of these experiments. The NBS-19 data discussed here were generated very early in this study and lacked the careful purification, standardization and replication characteristic of other data in this paper; they cannot be directly compared with NBS-19 data in Table 2 and Fig. 2. However, they do provide useful constraints on the temperature effect on acid digestion fractionations and so are included here. The $\delta^{18}$O value of product CO$_2$ decreases with increasing reaction temperature, with an average slope of −0.028%/°C per °C (Table 3; this slope is similar to that found by Swart et al., 1991). Also as expected, the $\delta^{13}$C value of product CO$_2$ is similar at all reaction temperatures. The $\Delta_{47}$ value of product CO$_2$ decreases with increasing reaction temperature with an overall slope of −0.0016%/°C per °C. The results of these experiments are inconsistent with an exchange equilibrium between extracted CO$_2$ and residual oxygen because the $\Delta_{47}$ value of product CO$_2$ is far from the internal equilibrium for CO$_2$ gas at the temperatures of extraction (which varies from $\Delta_{47}$ of 0.93%/°C at 25 °C to 0.64%/°C at 80 °C; Wang et al., 2004; Eiler and Schauble, 2004). Thus, these results suggest that the $\Delta_{47}$ value of CO$_2$ produced by acid digestion of carbonate has some simple proportionality to the $\Delta_{13C^{18O}}$ value of that carbonate, and that this proportionality is only weakly dependent on reaction temperature.

Table 3
Stable isotope compositions of CO$_2$ released from NBS-19 and Sigma carbonate reacted at three different temperature with 103% phosphoric acid

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\delta^{13}$C$_{\text{PDB}}$</th>
<th>$\delta^{18}$O$_{\text{SMOW}}$</th>
<th>Temperature of reaction (°C)</th>
<th>$\Delta_{47}$</th>
<th>Standard error of $\Delta_{47}$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBS-19</td>
<td>1.95</td>
<td>39.00</td>
<td>25</td>
<td>0.28</td>
<td>0.032</td>
</tr>
<tr>
<td>NBS-19</td>
<td>2.01</td>
<td>38.61</td>
<td>35</td>
<td>0.19</td>
<td>0.035</td>
</tr>
<tr>
<td>NBS-19</td>
<td>1.97</td>
<td>38.29</td>
<td>45</td>
<td>0.21</td>
<td>0.038</td>
</tr>
<tr>
<td>NBS-19</td>
<td>1.96</td>
<td>38.09</td>
<td>45</td>
<td>0.28</td>
<td>0.036</td>
</tr>
<tr>
<td>Sigma carb</td>
<td>−42.31</td>
<td>20.76</td>
<td>25</td>
<td>0.54</td>
<td>0.028</td>
</tr>
<tr>
<td>Sigma carb</td>
<td>−42.28</td>
<td>19.81</td>
<td>50</td>
<td>0.52</td>
<td>0.034</td>
</tr>
<tr>
<td>Sigma carb</td>
<td>−42.20</td>
<td>19.22</td>
<td>80</td>
<td>0.47</td>
<td>0.026</td>
</tr>
</tbody>
</table>
3.3. Analyses of carbonates subjected to high-temperature re-crystallization

Aliquots of MZ and Sigma-carb standards and an aragonitic deep sea coral (47413) were re-crystallized by loading them into a sealed Pt capsule and heating to 1100 °C for 48 h in a TZM (Tungsten Zirconium Molybdenum alloy) cold-seal pressure vessel under 1000 bars of pressure. Experimental charges were quenched in air at the end of each experiment. The recovered calcite crystals were examined under the binocular and petrographic microscopes and showed evidence of pervasive re-crystallization and coarsening. This high-temperature re-crystallization procedure should drive Reaction 1 toward a stochastic distribution. Thus, if acid digestion produces no difference between \( \delta^{13}C_{18O16O2} \) and \( \delta^{13}C_{47} \), we should find that CO\(_2\) extracted from these materials has a \( \delta^{47} \) value of 0. If we find some other result, it could indicate a fractionation associated with acid digestion (as was suggested in the last section by the dependence of \( \delta^{47} \) values on extraction temperature).

Table 4 and Fig. 4 summarize the results of analyses of CO\(_2\) produced by acid digestion of these materials. We find they yield CO\(_2\) with \( \delta^{47} \) values of 0.14 ± 0.03 (for re-crystallized Sigma-carb standard), 0.22 ± 0.03 (for re-crystallized MZ standard), and 0.25 ± 0.02 (for deep sea coral 47413). These data suggest that CO\(_2\) produced by phosphoric acid digestion at 25 °C is subtly higher in \( \delta^{47} \) than the \( \delta^{13}C_{18O16O2} \) value reactant carbonate. The range of results for these re-crystallized materials is greater than expected by analytical precision alone, suggesting either that our heating experiments failed to entirely equilibrate the starting materials at high temperatures.

Table 4

<table>
<thead>
<tr>
<th>Sample</th>
<th>Before re-crystallization</th>
<th>After re-crystallization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \delta^{13}C_{CPDB} )</td>
<td>( \delta^{13}C_{SMOW} )</td>
</tr>
<tr>
<td>47413 aragonite coral</td>
<td>0.74 ± 0.012</td>
<td>−6.51</td>
</tr>
<tr>
<td>MZ carbonate</td>
<td>0.64 ± 0.024</td>
<td>−13.15</td>
</tr>
<tr>
<td>Sigma carb</td>
<td>0.55 ± 0.025</td>
<td>−42.31</td>
</tr>
</tbody>
</table>
temperatures or that these samples experienced different degrees of re-equilibration during quenching. It is noteworthy that the $\Delta_{47}$ values of CO$_2$ extracted from these materials after re-crystallization is positively correlated with the $\Delta_{47}$ values of CO$_2$ extracted from them before re-crystallization (Table 4 and Fig. 4). This observation supports the interpretation that our heating protocol failed to fully equilibrate them during high-temperature re-crystallization (e.g., perhaps coarse grains retain a core that did not undergo re-crystallization and re-setting), and, by extension, that Reaction 1 is highly resistant to resetting in the absence of recrystallization. Therefore, the lowest $\Delta_{47}$ value measured in CO$_2$ released from re-crystallized Sigma-carb (0.14‰) likely represents the maximum fractionation accompanying acid digestion.

Despite these ambiguities, both the dependence of $\Delta_{47}$ of CO$_2$ on acid digestion temperature and the positive $\Delta_{47}$ values observed in CO$_2$ from high-temperature re-crystallized carbonate support the interpretation that acid digestion involves an isotopic fractionation of $\Delta_{47}$, and that fractionation must be controlled if one is to achieve precise results for unknown samples (as one must do when analyzing $\delta^{18}O$ of carbonates by phosphoric acid digestion). Fortunately, in the case of $\Delta_{47}$, the temperature effect on the acid-digestion fractionation is subtle (0.0016‰ per °C), and so only need be controlled to within ±10–15 °C to keep errors smaller than the standard errors of our most precise measurements. Further work will be needed to establish the exact amplitude of the acid digestion fractionation and whether or not the same fractionation applies to dolomites, magnesites, siderites and other non-CaCO$_3$ carbonates. For our present purposes, we restrict ourselves to analysis of calcite and aragonite at constant temperatures of acid digestion, and discuss variability in $\Delta_{47}$ of evolved CO$_2$ rather than attempting to correct such data back to inferred values of $\Delta_{10C02}^{PDB}$.

### 3.4. Calcite precipitated from aqueous solution at known, controlled temperatures

We examined the influence of carbonate growth temperature on the $\Delta_{47}$ value of CO$_2$ extracted from carbonate by analyzing CO$_2$ extracted from calcites grown in the laboratory at known, controlled temperatures from aqueous solutions (see Section 2.1, above, for a description of the methods used to synthesize these calcites). Table 5 summarizes the results of these analyses, and Figs. 4 and 5 plot the $\Delta_{47}$ values of CO$_2$ extracted from these calcites vs. $10^6/T^2$, where $T$ is the measured growth temperature, in Kelvin. We also plot in Fig. 4 the value of $\Delta_{10C02}^{PDB}$ predicted by Schaub and Eiler (2004); for calcite that is in equilibrium with respect to Reaction 1, and the results of analyses of carbonates re-crystallized at 1100 °C. The data for calcites grown from aqueous solution show a correlation between the temperature of calcite precipitation and the $\Delta_{47}$ value carbon dioxide extracted from that calcite. A least-square

<table>
<thead>
<tr>
<th>Sample details</th>
<th>Temperature (°C)</th>
<th>$\delta^{18}O$ water (SMOW)</th>
<th>$\delta^{13}C_{PDB}$</th>
<th>$\delta^{18}O_{PDB}$</th>
<th>$\Delta_{47}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcite HA7</td>
<td>23 ± 1</td>
<td>−7.88</td>
<td>−23.72</td>
<td>−23.75</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−23.75</td>
<td>−23.75</td>
<td>−23.75</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−23.75</td>
<td>−23.75</td>
<td>−23.75</td>
<td>0.58</td>
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<td>−23.75</td>
<td>−23.75</td>
<td>−23.75</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−23.75</td>
<td>−23.75</td>
<td>−23.75</td>
<td>0.58</td>
</tr>
<tr>
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<td>−23.74</td>
<td>−23.74</td>
<td>−23.74</td>
<td>0.58</td>
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<tr>
<td>Standard error</td>
<td></td>
<td>0.01</td>
<td>0.01</td>
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</table>

(continued on next page)
Table 5 (continued)

<table>
<thead>
<tr>
<th>Sample details</th>
<th>Growth temperature (°C)</th>
<th>δ18O water</th>
<th>δ13C_PDB</th>
<th>δ18O_PDB</th>
<th>Δ47</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcite HA4</td>
<td>50 ± 2</td>
<td>-7.45</td>
<td>-26.38</td>
<td>-14.67</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-26.38</td>
<td>-14.71</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-26.38</td>
<td>-14.71</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-26.38</td>
<td>-14.70</td>
<td>0.58</td>
</tr>
<tr>
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<td></td>
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<td>-26.38</td>
<td>-14.70</td>
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<td>-26.38</td>
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<tr>
<td></td>
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<td>-26.38</td>
<td>-14.70</td>
<td>0.52</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>-26.38</td>
<td>-14.70</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>Standard error</td>
<td></td>
<td>0.004</td>
<td>0.015</td>
<td>0.014</td>
<td></td>
</tr>
</tbody>
</table>

Deep sea corals

47407    5.5 ± 1.0  0.3 ± 0.2  -2.51  0.95  0.76
   (Adkins et al., 2003)
Average       -2.51  0.95    0.74
Standard error 0.002  0.004  0.019

47413    8.0 ± 0.5  —     -6.52  -0.04    0.77
   (Adkins et al., 2003)
Average       -6.52  -0.04  0.74
Standard error 0.003  0.003  0.012

Surface coral

Indonesian Surface Coral
Mm97-Bc (~1960) 29.3 ± 2  —  -1.54  -5.27  0.63 ± 0.034

Fig. 5. Values of Δ47 of CO2 extracted from calcites grown from aqueous solution (reproduced from Fig. 4) and of deep-sea and surface corals, plotted vs. 10°/T2, where T is the known growth temperature in Kelvin. Note that both surface and deep-sea aragonitic corals generally conform to the trend defined by inorganic calcites. Deep-sea coral 47413 lies slightly (0.02%) above this trend, perhaps suggesting a subtle ‘vital effect’ (this aliquot of this sample exhibits extreme vital effects on its δ13C and δ18O values; Table 5).

linear fit to these data yields the relationship (with correlation coefficient r = 0.94):

$$\Delta_{47} = 0.0592 \cdot 10^6 \cdot T^{-2} - 0.02$$

Note that this equation relates the Δ47 value of carbon dioxide produced by phosphoric acid digestion of carbonate to the growth temperature of that carbonate. This is the simplest way to derive a paleotemperature equation directly from our data, but keep in mind that it reflects a combination of the temperature dependence of Reaction 1 and a ≤ 0.14‰ enrichment in Δ47 relative to Δ(D18O/18O) caused by acid digestion at 25 °C. Note also that this relationship might not be suitable for extrapolation to temperatures greater than 50 °C because we know from analysis of high-temperature re-crystallized calcites that the trend could pass through a Δ47 value as high as 0.14‰ when 10°/T2 approaches 0. Nevertheless, this equation is suitable for interpolation and we suggest that it should be used for paleothermometry in the temperature range 1–50 °C until data become available for higher temperature experimental carbonates. The temperature sensitivity of this equation, combined with the best analytical precision for Δ47 that we have achieved on homogenized, high-purity calcite (ca. ±0.01‰), implies that Reaction 1 can be used to perform carbonate paleothermometry at near-earth-surface temperatures with precision of ca. ±2 °C.

3.5. Analysis of natural corals grown at known, approximately constant temperatures

We find that our sample of *Porites* coral collected from the Sumatran surface ocean yields CO2 having a Δ47 value of 0.63‰. When plotted in Fig. 5 at this coral’s inferred growth temperature (29.3 ± 2 °C), this result is consistent with the temperature dependence we determined for synthetic calcite. Similarly, our analyses of two samples of the deep-sea coral, *D. dianthus*, yield Δ47 values of 0.74‰ (sample 47407, grown at 5 ± 1 °C) and 0.75‰ (sample 47413, grown at 8 ± 0.5 °C). These results are generally consistent with our inorganic calcite calibration (Fig. 5), although coral 47413 lies above this calibration by slightly more (0.02) than analytical uncertainty. All three of these corals are aragonitic, rich in organic matter, and, exhibit ‘vital effects’ in their δ18O and δ13C values (subtle in the case of Sumatran *Porites*; variable and severe in the case of deep-sea *D. dianthus*). The fact that all three samples yield results broadly consistent with our inorganic calcite calibration curve indicates several important things about the analysis of Δ47 values in natural carbonates:

- First, aragonite and calcite appear to exhibit the same relationship between carbonate growth temperature and the Δ47 value of CO2 produced by phosphoric acid extraction.
- Second, vital effects that influence δ18O and δ13C of biologically mediated carbonate appear not to strongly influence Δ47 values of CO2 extracted from that carbon-
ate. This is consistent with models for the vital effect, which assume that carbonate deposition occurs in local isotopic equilibrium (Bohm et al., 2000; Adkins et al., 2003). These models describe the vital effect by way of reservoir effects on the sampled C and O pools, not through kinetic fractionations. The equilibrium constant for Reaction 1 is independent of bulk isotopic composition, and so should not be sensitive to such reservoir effects. Note, however, that the aliquot of sample 47/13 analyzed here has the greatest vital effect on its 813C and 818O values (Table 5; see also Adkins et al., 2003), and is the one sample to fall slightly above the inorganic calibration line. Thus, it is possible that vital effects produce a small increase in $A_{47}$ relative to that predicted for inorganic calcite. Nevertheless, it is clear from Fig. 5 that this is a second-order perturbation on the general conformance of biogenic aragonite to our inorganic calcite calibration line.

- Finally, organic contaminants are potentially problematic for measurements of $A_{47}$, and so it is significant that these measurements yielded an easily interpreted result despite the large concentration of organic matter in the analyte corals. This result suggests that our CO$_2$ purification procedures are successful at removing whatever volatile organic contaminants might be produced by acid digestion of natural corals.

3.6. Speculations and initial evidence regarding the kinetics of 13C–18O ‘clumping’

All geothermometers are meaningful only if one has a clear understanding of the geological environments in which they reach equilibrium and the effects of subsequent processes on re-setting that equilibrium. It seems likely to us, based on the known rapid kinetics of carbonate acid-base chemistry in aqueous solution (Zeebe et al., 1999) and previous experience with the kinetics of the carbonate–water oxygen isotope thermometer (Spero et al., 1997), that carbonate precipitation from water or re-crystallization in the presence of water often achieves or closely approaches local equilibrium with respect to Reaction 1 at any temperature $\geq 0^\circ$C (perhaps lower in the case of carbonates grown from brines or other low-temperature solutions). This inference is supported by our observation that carbonates grown in the temperature range 1–50 °C, both inorganically and biologically, are enriched in 13C–18O bonds by ca. 0.5–0.7\%/°C, as expected by theory (Fig. 5). Note, however, that some processes, such as air–sea exchange and biological carbonate precipitation, can involve rapid, selective transport of dissolved inorganic carbon species, and consequential departures from local equilibrium with respect to oxygen isotope exchange reactions (Zeebe et al., 1999). We suspect such aqueous environments might also generate non-equilibrium abundances of 13C–18O bonds in dissolved inorganic carbon species, and thus in the carbonate minerals grown from such species.

The preservation of growth temperatures through later geological history raises a separate and more complicated set of questions: First, if carbonate grows at low temperature and is then heated without re-crystallization, at what temperatures and after what times will it re-equilibrate to take on the new, higher-temperature distribution? This question must be addressed before we can know how deeply buried a carbonate must be before it ceases to faithfully record its original precipitation temperature. Second, if a carbonate grows or re-crystallizes at high temperature and then cools, what will be the ‘blocking temperature’ at which Reaction 1 stops continuously re-equilibrating? This question must be answered before we can apply the 13C–18O carbonate thermometer to high-temperature metamorphic rocks.

These and related questions cannot be answered without studying the systematics of $A_{47}$ measurements of CO$_2$ extracted from carbonates having high-temperature histories, and calibration of the 13C–18O order/disorder carbonate thermometer above 50 °C. Such work is beyond the scope of this study, although we can comment on several relevant observations found here. First, our attempts to re-crystallize calcite and aragonite at 1100 °C were only partially successful, based on the fact that differences in 13C–18O ordering among starting materials were not completely erased (Fig. 4 and Table 5). This suggests that Reaction 1 is surprisingly refractory to re-equilibration at high temperatures. Second, NBS-19 and MAR-J1 are calcites from Italian marbles that were metamorphosed to upper-greenschist facies during the mid-Tertiary and must have spent several (perhaps even tens of) million years in the shallow crust at temperatures below 200 °C (Molli et al., 2000). Carbon dioxide extracted from them has $A_{47}$ values of 0.34–0.35 relative to blocking temperature at 1100 °C. If the dashed curve linking our high- and low-temperature experimental data in Fig. 4 is correct, these values imply an apparent temperatures near ca. 200 °C. These data suggest that the ‘blocking temperature’ of the 13C–18O clumping reaction is significantly higher than earth-surface temperatures, even in rocks that have set for millions of years at lower temperatures. These data are not yet sufficient for a quantitative analysis of the kinetics of Reaction 1, but point toward it being suitably refractory for preserving paleotemperatures over geological time scales, so long as re-crystallization has not occurred.

3.7. The seasonal cycle in 13C–18O ordering in Porites coral from the northern Red Sea

In this section, we discuss an application of the 13C–18O order/disorder carbonate paleothermometer to a recent Porites coral from the northern Red Sea. This thermometer has an ideal precision of ± 2 °C, 1σ, and the sample we examine is believed to have grown over only a ca. 6–10 °C temperature range (for reasons discussed below). Therefore, it should be possible for us to detect seasonality in growth temperature of this coral, but only if our methods can consistently generate data on natural materials
with precision of $\pm 0.01$–$0.02^\circ/oo$. This study is principally a test of the ‘practical’ limits to precision of our data. Serendipitously, these data also provide suggestive evidence for a vital effect or other artifact that produces a non-equilibrium $^{13}$C-$^{18}$O ordering in at least some corals under some conditions (perhaps confirming our suggestion, above, regarding deep-sea coral 47413; see Fig. 5).

The northernmost Red Sea experiences unusually large seasonal variations in sea-surface temperature (SST) between winter minima averaging 21.2 $^\circ$C and summer maxima averaging 27.6 $^\circ$C (Rayner et al., 1996), but varying from place to place and year to year. Winter minima average 21.2 $^\circ$C whereas summer maxima average 27.6 $^\circ$C. Super-imposed on this seasonality is an inter-annual to decadal variability of ca. 1–2 $^\circ$C. Note also that near-surface water temperatures in shallow coastal waters can experience local excursions outside the range observed by regional instrument records.

The Ras Muhammed Peninsula is surrounded by a narrow fringing shallow-water reef, including coral colonies growing at a water depth of $\sim 5$ m. Sample BR1-1 of *Porites lutea* was sampled in July, 1995 at Beacon rock (27°50.9’N, 34°18.6’E) located on the south side of the Ras Muhammed Peninsula, within the boundaries of Ras Muhammed National Park and near the southern tip of the Sinai Peninsula (Egypt). A core was collected from a hemispherical coral colony using an underwater pneumatic drill. The core was drilled vertically, parallel to the major axis of coral growth.

An X-ray radiograph of BR1-1 reveals density bands that we interpret as a seasonal growth pattern. We initially sampled this coral at 1-mm increments (measured perpendicular to the growth banding) using a low-speed drill. Splits of the drilled powders were reacted with phosphoric acid at 90 $^\circ$C in a modified auto carbonate device and the purified CO$_2$ analyzed for $^{13}$C and $^{18}$O on a gas source stable isotope ratio mass spectrometer located at the stable isotope laboratory in Department of Earth and Planetary Sciences, Harvard University, Cambridge, Massachusetts. Additional splits of the same drilled powders were analyzed for Sr/Ca ratio by isotope dilution on an ICP-AES at Harvard. Both of these sets of measurements used techniques that have been previously described (Billups et al., 2004) and will be detailed further in a later publication. The results of these measurements are listed in Table 6 and plotted in Fig. 6. All three variables exhibit a seasonal cycle; maximum $^{18}$O values and Sr/Ca ratios and minimum $^{13}$C values correspond to cold winter temperatures (mid-February) and the converses correspond to warmer summer temperatures. The strong seasonality in geochemical variables also corresponds to the density-banding pattern observed in the X-ray radiograph (low density corresponds to summer; high density corresponds to winter).

The arid climate in the northern Red Sea results in large and seasonally varying evaporative enrichments in the $^{18}$O of surface water, which prohibit straightforward application of the conventional carbonate-water oxygen isotope exchange thermometer to the $^{18}$O record in Fig. 6 (see Felis et al., 2000). If we adopt Felis et al.’s (2000) empirical calibration of the $^{18}$O-carbonate thermometer in this region, we conclude that our sample (which varies in Sr/Ca variation of from 9.08 and $9.62 \times 10^{-3}$) we find that it grew at temperatures between 28.5 and 19.5 $^\circ$C. Both records imply summer temperature maxima of ca. 26–28 $^\circ$C, similar to instrument records for the northern Red Sea (Genin et al., 1995), but winter minima of 17–20 $^\circ$C, several degrees colder than the average for those records. These data suggest that $\Delta_A$ of CO$_2$ extracted from BR1-1 should vary by ca. 0.05$^\circ/oo$, between a summer minimum of ca. 0.63 and a winter maximum of ca. 0.68.

We re-sampled this coral core by cutting a slab parallel to the drilled transect shown in Fig. 7 and slowly rubbing it against an abrasive tool and recovering the powder this produced. This was done instead of drilling in order to minimize the possibility of disturbing the $^{13}$C-$^{18}$O ‘clumping’ in carbonate by frictional heating. Our average sample spacing is 1–1.5 mm. The average growth rate of the coral varies between $\sim 10$ and 20 mm/year; the interval we sampled corresponds to a summer with two Sr/Ca minima (temperature maxima) separated by a brief, low-amplitude Sr/Ca maximum (at $\sim 65$ mm sampling distance), a broad, strong winter Sr/Ca maximum (temperature minimum) at $\sim 75$ mm, a broad, strong summer Sr/Ca minimum at $\sim 85$ mm, and a fall rise in Sr/Ca terminating at $\sim 90$ mm.

Recovered powders were reacted with phosphoric acid at 25 $^\circ$C and their product CO$_2$ analyzed for $^{13}$C, $^{18}$O and $\Delta_A$, as for previous measurements described in this paper (Table 6). All extracted CO$_2$ samples were analyzed five times, so the expected, average external precision in $\Delta_A$ for each sample should be ca. $\pm 0.013$. We assume that the acid digestion fractionation and the $\Delta_A$ temperature calibration for *Porites lutea* is the same as that for inorganic calcite (as appears to be true for Sumatrarn *Porites* coral and approximately true for deep-sea *D. dianthus* coral; Fig. 5). However, note that Red Sea corals have atypical calibrations of their Sr/Ca and $^{18}$O paleothermometers (Felis et al., 2000, 2004), and so we should perhaps be alert to the possibility of an unusual temperature sensitivity in the clumped isotope thermometer.

Table 5 lists the $^{18}$O and $^{13}$C of BR1-1 coral (corrected for acid fractionation) and the $\Delta_A$ value of CO$_2$ extracted from that same coral (not corrected for acid fractionation; i.e., these data can be directly compared with the experimental data in Figs. 4 and 5). The variations in $^{18}$O and $^{13}$C we observe are consistent with those previously analyzed on samples drilled from the same coral head (Fig. 6). Because the growth bands in this coral are curved
and vary in thickness, we can only roughly correlate our samples with those previously analyzed for Sr/Ca. Our re-sampled interval lies closest to the band between 70 and 90 mm on the distance scale used in Fig. 6, but with an uncertainty on the order of several mm. Therefore, we used δ13C values measured in both sets of samples (Figs. 6B and 7, inset) to correlate measurements of Δ47 in one sample suite with measurements of Sr/Ca ratio in the other.

Fig. 7 compares the Sr/Ca ratio and Δ47 stratigraphy, after spatially aligning the two data sets as described above. These Sr/Ca data are a sub-set of those shown in Fig. 6. Curves are fit through both data sets using an exponential smoothing technique (http://www.cas.lancs.ac.uk/glossary_v1.1/td.html#smooth) contained within the SigmaPlot® software package (www.systat.com/products/SigmaPlot/). Values of Δ47 exhibit spatial variations consistent with a quasi-sinusoidal curve having a period of ~20 mm—similar to the seasonal cycles in Sr/Ca, δ18O and δ13C. Thus, the first-order test we make in this section—attempting to retrieve a coherent seasonal cycle from a low-amplitude record—appears to have succeeded.

The Sr/Ca curve exhibits three minima over the interval sampled for Δ47: one at ~85, one at ~68 mm and one at ~62 mm. These latter two minima are separated by a low-amplitude maximum that likely reflects a summer or fall temperature anomaly rather than a winter. All of these
minima correspond to minima in $D_{47}$. Similarly, both the winter maximum in Sr/Ca ratio at $/C_{24}$ 75 mm and the fall rise in Sr/Ca at $/C_{90}$ correspond to two maxima in $D_{47}$ (we did not measure the $D_{47}$ value of carbonate corresponding to the low-amplitude maximum in Sr/Ca ratio at $/C_{65}$ mm) (Fig. 7B). Thus, variations in Sr/Ca ratio and $D_{47}$ value are in phase with one another and have the same sign, as expected. However, the amplitude of $D_{47}$ variations is greater than expected, perhaps reflecting a vital effect acting during winter growth in this coral.

The three minima sampled by our traverse have average $D_{47}$ values of between 0.64 and 0.65 ($\pm 0.01$), corresponding to apparent temperatures of between 26.5 and 24.5 ($\pm 2$) °C, respectively. These are consistent with summer maximum temperatures in the northern Red Sea, both as observed in instrument records and as inferred from interpretations of the $\delta^{18}O$ and Sr/Ca variations of Red Sea corals (Felis et al., 2000, 2004). Similarly, the late fall samples near 90 mm have an average $D_{47}$ of 0.70 ± 0.02, corresponding to an apparent temperature of 15 ± 4. This average is lower than expected based on Sr/Ca and $\delta^{18}O$ records, but overlaps at the 1σ level the independently estimated winter temperature minimum. We have not bracketed this value with data for coral grown the following spring, so it is possible that we did not sample its maximum $D_{47}$ value. Nevertheless, these data that do exist are consistent with the expected temperature variations. In these, respects, the carbonate clumped isotope thermometer appears to have successfully retrieved the known sea surface temperature history of the northern Red Sea, as recorded in BRI-1.

However, there is one noteworthy complication to our data set for BRI-1: The winter maximum $D_{47}$ value observed near the sampling distance of 75 mm averages 0.74 ± 0.01, corresponding to an apparent temperature of 8 ± 2 °C—far lower than expected (Fig. 7B; expected $D_{47}$ values are shown by a dashed curve). It seems unlikely to us that this discrepancy reflects analytical error because five closely spaced samples precisely define this high $D_{47}$ value. It also seems unlikely that this discrepancy reflects true, lower-than expected winter temperatures at which BRI-1 grew, simply based on the weight of evidence from Sr/Ca and $\delta^{18}O$ thermometry (although both are based on empirical calibrations and might not have captured locally extreme temperatures experienced by the shallow-water sample we have studied). The only remaining possibility that occurs to us is that the temperature calibration for the carbonate clumped isotope thermometer in this sample of *Porites lutea* is more temperature sensitive than in inorganic calcite due to a vital effect. This is unexpected both because of the success of our application of the inorganic calibration to Sumatran *Porites* and deep-sea *D. dianthus* corals and because the inorganic calibration yielded acceptable temperatures for the summer, spring and fall parts of the BRI-1 record. Nevertheless, we hypothesize that the winter band in this sample grew under conditions that promoted an unknown vital effect and drove up the $D_{47}$ value to values ca. 0.04 ± 0.01% higher than the thermodynamic equilibrium at its growth temperature. We speculate that this vital effect might be associated with slower than average growth during winter months, although there is insufficient information as yet to speculate on its physical cause.
pect that a vital effect should lead to a correlation between $A_{47}$ and $\delta^{13}C$, which is not observed. We suggest the most useful next step towards advancing these problems will be detailed study of cultured corals and other carbonate-precipitating organisms.

### 4. Summary and future directions

Ordering of $^{13}C$ and $^{18}O$ into bonds with each other within the crystal lattice of carbonate minerals is a temperature-dependent phenomenon that can be characterized by analysis of $^{13}C^{18}O$ in CO$_2$ produced by phosphoric acid digestion. Phosphoric acid digestion appears to produce a small (≤0.14‰) enrichment in $^{13}C^{18}O$ relative to proportions of $^{13}C^{16}O_2$–carbonate ions in reactant carbonate minerals, but this effect depends only weakly on acid reaction temperature (0.0016‰ per °C) and so is relatively easily controlled.

Analyses of natural and synthetic carbonates grown at known temperatures demonstrate that ‘clumping’ of $^{13}C$ and $^{18}O$ isotopes in calcite and aragonite is subtle at earth-surface temperatures (ca. 0.7‰ enrichments in $^{13}C^{18}O$ bonds compared to their expected abundance in a material with randomly distributed stable isotopes). Moreover, the temperature dependence of this effect is subtle (the range is ca. 0.75–0.50‰ between 1 and 50 °C, respectively). Nevertheless, proportions of $^{13}C^{18}O$ bonds in carbonates can be measured consistently with external precision as good as 0.01–0.02‰, even in complex natural materials, and so this thermometer can constrain growth temperature with precision as good as ±2 °C. This uncertainty is too large for some problems (e.g., Pleistocene tropical SST variations, which are thought to be a few degrees or less) but sufficient for resolving many problems in paleoclimate research, meteoritics and the thermal history of soils and shallow crustal rocks.

The most important feature of the carbonate clumped isotope thermometer is that it is based on a homogeneous equilibrium, and thus it is rigorously defined based only on measurements of the isotopic content of carbonate minerals. That is, it is independent of the isotopic composition of water from which carbonate grew, or of any other phase with which carbonate might have co-existed. For this reason, it does not suffer from the ‘ice volume problem’ and analogous problems with carbonate–water exchange thermometers, which are rigorously defined only when the composition of carbonates and waters from which they grew are independently known. Also, the clumped isotope thermometer is based on a thermodynamic exchange equilibrium, and so is suitable for interpolation and applications to past times and diverse settings, unlike various empirical phylogenetic or morphological thermometers.

Analyses of biogenic aragonite from deep-sea and surface corals grown at known temperatures generally conform to the inorganic calibration of the carbonate clumped isotope thermometer; this includes samples of $D. dianthus$ corals that exhibit pronounced ‘vital effects’ in their $\delta^{13}C$ and $\delta^{18}O$ values. Thus, there does not generally appear to be a ‘vital’ effect in biological materials. However, the $D. dianthus$ specimens showing the greatest ‘vital effect’ releases CO$_2$ that is higher in $A_{47}$ than expected by our inorganic calibration (by just more than analytical precision); similarly, seasonal variability in $A_{47}$ in CO$_2$ from Red Sea $Porites$ coral suggests winter growth may be associated with an anomalous enrichment in $^{13}C^{18}O$ ‘clumping’. We are unsure what physical processes might be responsible for such an effect, and suggest future studies of cultured organisms grown under controlled conditions will be required to fully understand it.

Analysis of carbonates that have undergone high-temperature re-crystallization followed by slow cooling suggests the blocking temperature of the carbonate clumped isotope thermometer (i.e., the temperature at which it ceas-
es to continuously re-equilibrate over geological timescales) is on the order of 200 °C. Thus, is seems likely that the sub-surface environments amenable to study by this approach will be those reached during diagenesis, catagenesis, burial metamorphism, anochimemorphism, some types of ore formation, meteoric aqueous alteration and similar environments in which water is abundant and temperatures are in the range 0–200 °C.

Data presented here calibrate the $^{13}$C–$^{18}$O order/disorder carbonate thermometer for inorganic calcite between 1 and 50 °C, and document its applicability to biogenic aragonite. However, several additional studies are needed to fully flesh-out this thermometer's calibration. High-temperature experiments, perhaps using hydrothermal and/or piston-cylinder apparatus, are needed to define the temperature sensitivity of $^{13}$C–$^{18}$O bond formation above 50 °C. Experiments on carbonates other than calcite and aragonite are needed at all temperatures; calibration data for dolomite will be particularly useful for studies of ancient carbonate sediments. Additional constraints on the kinetics of $^{13}$C–$^{18}$O ordering during diagenesis, low-grade metamorphism and cooling of high-temperature rocks will be necessary before the carbonate clumped-isotope thermometer can be confidently applied to these important upper-crustal environments.

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