Most Earth-surface calcites precipitate out of isotopic equilibrium

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Oxygen-isotope thermometry played a critical role in the rise of modern geochemistry and remains extensively used in (bio-)geoscience. Its theoretical foundations rest on the assumption that $^{18}$O/$^{16}$O partitioning among water and carbonate minerals primarily reflects thermodynamic equilibrium. However, after decades of research, there is no consensus on the true equilibrium $^{18}$O/$^{16}$O fractionation between calcite and water ($^{18}$α$_{cc/w}$). Here, we constrain the equilibrium relations linking temperature, $^{18}$α$_{cc/w}$, and clumped isotopes ($\Delta_{47}$) based on the composition of extremely slow-growing calcites from Devils Hole and Laghetto Basso (Corchia Cave). Equilibrium $^{18}$α$_{cc/w}$ values are systematically ~1.5‰ greater than those in biogenic and synthetic calcite traditionally considered to approach oxygen-isotope equilibrium. We further demonstrate that subtle disequilibria also affect $\Delta_{47}$ in biogenic calcite. These observations provide evidence that most Earth-surface calcites fail to achieve isotopic equilibrium, highlighting the need to improve our quantitative understanding of non-equilibrium isotope fractionation effects instead of relying on phenomenological calibrations.

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Here, we extend the isotopic equilibrium baseline to low temperatures based on another instance of extremely slow-growing calcite, originating from an unusual karstic environment. We find that this equilibrium baseline displays a slope (i.e., temperature sensitivity) indistinguishable from that for faster-growing calcite, with a constant oxygen-18 enrichment of ~1.5‰.

We also compare the clumped-isotope ($\Delta_{47}$) compositions of these two slow-growing calcites to that of biogenic calcite produced by bivalves and foraminifera, and also observe subtle but resolvable $\Delta_{47}$ differences between “equilibrium” and biogenic calcite. We conclude that most calcites precipitating at the surface of the Earth fail to achieve complete isotopic equilibrium.

Results

Slow-growing calcite from Laghetto Basso. The subaqueous calcite coating found at the bottom of Laghetto Basso, a small lake in Corchia Cave (Italy), provides an apparently continuous paleoclimate record of the last 960 ka\(^{30}\). In situ observations of pH and temperature spanning more than 10 years, along with numerous isotopic and elemental analyses of water samples (ref. \(^{31}\) and Supplementary Table 1), demonstrate that modern pool water is thermally and chemically stable, with pH = 8.2 ± 0.1 and $T = 7.9 ± 0.2 ^\circ$C (1 SD). Drip counting conducted over several hours in May 2017 suggests that the lake received 50–60 L per day during this period. Based on an estimated lake volume of 20 m\(^3\), water residence time is expected to be on the order of 1 year, much longer than the ~33 h required for 99% isotopic equilibration between DIC and water. What’s more, in contrast to most karstic environments of paleoclimatic interest, dripwater must percolate through the Corchia Cave system for long durations on the order of years to decades before reaching Laghetto Basso\(^{31}\). As a result, the subaqueous calcite precipitates from a solution which is already very close to chemical and isotopic equilibrium with host rocks and the local cave atmosphere\(^{30,31}\).

Laghetto Basso calcite shares many other similarities with Devils Hole mammillary calcite, making it very likely that it was also precipitated in isotopic equilibrium. Both sites are characterized by low values of calcite saturation indices (0.18 ≤ $\Omega$ ≤ 0.30), very slow growth rates (≤ 0.8 µm/day), similar surface textures and crystal fabrics, and comparable solution ratios of [DIC]/[Ca\(^{2+}\)] and [Mg\(^{2+}\)]/[Ca\(^{2+}\)]\(^{31,32}\). In the context of the present study, the most significant difference between the two sites is the higher pH in Laghetto Basso (8.2 versus 7.4 at Devils Hole). Although pH is expected to influence $^{18}$O/$^{16}$O fractionation between water and rapidly-precipitating calcite, this effect decreases with slower crystallization rates\(^{27,33}\), and becomes negligible (≤ 0.05‰) at the very slow growth rates considered here.

Oxygen-18 equilibrium. The oxygen isotope compositions of Devils Hole and Laghetto Basso waters are known from earlier studies, with respective $\delta^{18}$O,WSMOW values of −13.54 ± 0.05‰\(^{16}\) and −7.39 ± 0.09‰ (refs. 20,34, Supplementary Table 1). We sampled calcite from the outer surface of coatings from both sites and measured their carbon and oxygen stable-isotope compositions (Table 1). Both samples yield calcite/water oxygen-18 fractionation factors ($\alpha_{cc}$) which are 1.5% greater than predicted by the experimental calibration of Kim and O’Neil\(^{15}\) (Fig. 1), defining an equilibrium baseline (Eq. (1), with crystalization temperature $T$ in kelvin) whose slope is indistinguishable from that of the synthetic precipitates:

$$10^3 \ln(\alpha_{cc}) = 17.57 \times 10^3 / T - 29.13$$

The regression uncertainties are best expressed by reformulating the above equation so that regression errors in its slope and...
Clumped-isotope disequilibrium in biogenic calcites. As a complementary characterization of isotopic equilibrium, we also measured the clumped-isotope composition ($\Delta_{47}$) of these two calcite samples (Table 1). Clumped isotopes describe statistical anomalies in the abundance of isotopologues with multiple rare isotopes, such as $^{13}$C$^{18}$O$^{16}$O$_2$–$^{16}$O$_2$. In the same way that carbonate $^{18}$O values potentially record equilibrium oxygen-isotope fractionation factors between the mineral and aqueous phases, $\Delta_{47}$ values of calcite are expected to reflect temperature-dependent isotopic equilibrium constants within the mineral phase$^{37}$, providing a complementary but independent isotopic thermometer.

The Devils Hole–Laghetto Basso calibration for equilibrium values of $\Delta_{47}$ in calcite at Earth-surface temperatures (Fig. 2a) is described by the following equation:

$$\Delta_{47} = 46.0 \times 10^3 / T^2 + 0.1423$$  \hspace{1cm} (3)

Again, reformulating Eq. (3) so that regression errors in its slope and intercept values are independent yields:

$$\Delta_{47} = A \times 10^3 \left(1 / T^2 - 1 / T_0^2\right) + B$$

$$A = 46.0 \pm 2.8 \text{ (1 SE)}$$
$$B = 0.6786 \pm 0.0029 \text{ (1 SE)}$$

$$T_0 = 292.9 \text{ K}$$  \hspace{1cm} (4)

The slope of this regression is statistically indistinguishable from those obtained by several recent $\Delta_{47}$ calibration studies$^{28,38–40}$. However, precise comparisons between clumped-isotope measurements performed in different laboratories remain challenging due to several methodological issues$^{41,42}$. For instance, earlier $\Delta_{47}$ measurements of Devils Hole calcite$^{43,44}$ are not directly comparable to the values reported here because they are anchored to CO$_2$ standards instead of the carbonate standards used in our study. To circumvent this problem, we compare our equilibrium observations to the clumped-isotope compositions of planktonic and benthic foraminifera collected from marine sediment core-tops$^{45}$ and of modern calcitic bivalves from environments with minimal seasonal variability, all of which were analyzed in a single laboratory, following identical analytical procedures, using the same set of carbonate standards, within a limited time frame (10 months).

Laghetto Basso calcite yields a slightly lower $\Delta_{47}$ value than the biogenic samples formed at similar temperatures, but this difference arguably remains within analytical uncertainties. By contrast, the clumped-isotope composition of Devils Hole calcite plots $17 \pm 5 \text{ ppm (1SE)}$ below the extrapolated foraminifer regression line, and $27 \pm 8 \text{ ppm}$ below the bivalve line. It is notable that Devils Hole calcite precipitates from waters with a significantly lower pH than most biogenic carbonates. For example, several foraminiferal species are known to actively elevate pH at calcification sites by at least 0.5 units above typical seawater pH values of 8.2$^{46–48}$. However, pH is only expected to influence $\Delta_{47}$ in fast-growing carbonates$^{33,44,49,50}$. Thus, if the biogenic carbonates analyzed here had achieved clumped-isotope equilibrium, they should not display large $\Delta_{47}$ departures from the DVH-LGB baseline regardless of pH.

One possible interpretation of these results is that biogenic samples formed at low temperatures achieve quasi-equilibrium clumped-isotope compositions, but warmer samples do not.

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**Table 1 Crystallization conditions and stable-isotope compositions of water and calcite from Devils Hole and Laghetto Basso**

| Sample | Devils Hole | Laghetto Basso |
|--------|-------------|---------------|
| pH     | 7.4         | 8.2           |
| Ionic strength | $10.5 \times 10^{-3}$ | $5.2 \times 10^{-3}$ |
| Growth rate (mol m$^{-2}$ s$^{-1}$) | $1.8 \times 10^{-10}$ | $3.1 \times 10^{-10}$ |
| Temperature ($°C \pm 1$ SD) | $33.7 \pm 0.2$ | $7.9 \pm 0.2$ |
| Water $^{18}$O (‰ ± 1SE) | $-13.54 \pm 0.05$ | $-7.39 \pm 0.09$ |
| Calcite $^{18}$O (‰ ± 1SE) | $-1.95 \pm 0.01$ | $0.02 \pm 0.02$ |
| Calcite $^{18}$O (‰ ± 1SE) | $-15.83 \pm 0.04$ | $-4.48 \pm 0.03$ |
| $1000 \ln(^{18}$O/$^{16}$O) (‰ ± 1SE) | $28.13 \pm 0.06$ | $33.8 \pm 0.10$ |
| $\Delta_{47}$ (‰ ± 1SE) | $0.6309 \pm 0.0041$ | $0.7247 \pm 0.0040$ |

Because of low supersaturation conditions and extremely slow growth rates, the composition of these two natural samples is very likely to record equilibrium values of $^{18}$O/$^{16}$O and $\Delta_{47}$.

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**Fig. 1** Equilibrium $^{18}$O/$^{16}$O fractionation between calcite and water ($^{18}\alpha_{cc/w}$) as a function of crystallization temperature (T). The equilibrium baseline defined by slow-growing calcites from Devils Hole and Laghetto Basso (colored confidence region, Eq. (2)) is indistinguishable from the theoretical prediction of Watkins et al.$^{27}$ (dashed line), which is quasi-identical to the original prediction by Coplen$^{16}$.

Intercept values are independent:

$$10^3 \ln(^{18}\alpha_{cc/w}) = A \times 10^3 \left(1 / T - 1 / T_0\right) + B$$

$$A = 17.57 \pm 0.43 \text{ (1 SE)}$$
$$B = 29.89 \pm 0.06 \text{ (1 SE)}$$

$$T_0 = 297.7 \text{ K}$$  \hspace{1cm} (2)

The temperature sensitivity of Eq. (1) is 0.20‰ per K at 20 °C, which is similar to that of equilibrium oxygen-18 fractionation between dissolved (bi)carbonate ions ($\text{CO}_3^{2-}$, $\text{HCO}_3^-$) and water (0.19‰ and 0.21‰ per K, respectively$^{35}$). Our findings are thus consistent with the hypothesis that the kinetic components of $^{18}\alpha_{cc/w}$ vary primarily with pH, crystallization rate, and/or solution saturation, but remain relatively insensitive to temperature (at least within the range of typical Earth-surface conditions), as postulated in several theoretical models$^{37,39}$.

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One possible interpretation of these results is that biogenic samples formed at low temperatures achieve quasi-equilibrium clumped-isotope compositions, but warmer samples do not.
between slow-growing inorganic calcite and the biogenic samples is
and temperature uncertainties indicates that the observed difference
covariance based on conservative estimates of analytical errors
Comparison of the 95% confidence intervals. Solid regression lines take into account analytical
errors in \( \Delta^{18}O \) and \( \Delta^{14}C \) values of foraminifera and bivalves are
from the equilibrium baseline. More generally, despite statistically
different regression slopes (Fig. 2b), an analysis of
regression slope, the \( \Delta^{18}O \) values for foraminifera and bivalves are
intercept values for slow-growing and biogenic calcite. Both of the biogenic
regression lines differ significantly (\( p \leq 10^{-3} \)) from the equilibrium baseline
defined by slow-growing calcites. Colored bell-shaped curves represent the
probability distributions of regression slopes.

Alternatively, if we assume that clumped isotopes in biogenic
samples and in slow-growing calcites are characterized by the same
regression slope, the \( \Delta^{18}O \) values for foraminifera and bivalves are
respectively 11 ± 3 and 17 ± 5 ppm (1SE) higher than predicted
from the equilibrium baseline. More generally, despite statistically
indistinguishable regression slopes (Fig. 2b), an analysis of
covariance based on conservative estimates of analytical errors
and temperature uncertainties indicates that the observed difference
between slow-growing inorganic calcite and the biogenic samples is
statistically significant (\( p \leq 10^{-3} \)). Contrary to the case of oxygen
isotopes, these differences are not much larger than the current
precision limits on \( \Delta^{18}O \) measurements, particularly when taking
inter-laboratory discrepancies into account.

Our ability to jointly define equilibrium values for the two
independent isotopic thermometers opens up interesting new
possibilities. For instance, combining \( \Delta^{18}O \) and \( \Delta^{14}C \) observations
clearly exposes large isotopic differences between our biogenic
carbonates and the slow-growing calcites, without requiring any
assumptions on their crystallization temperatures (Fig. 3). We
anticipate that this kind of combined observations will be most
useful in studies such as those of diagenetic carbonates, where
temperatures remain poorly constrained but where the oxygen-
isotope composition of parent waters may be estimated from
independent methods (e.g., fluid inclusions\( ^{51} \)).

Discussion
Our findings demonstrate that mammillar calcite from Devils
Hole is not an anomalous outlier, but rather that natural calcites
formed at crystallization rates much slower than those achieved
so far in laboratory experiments are systematically enriched in
oxygen-18 relative to carbonates precipitating more rapidly from
equilibrated DIC solutions. This observation offers support to
theoretical models in which oxygen-18 fractionation between DIC
and calcite (\( \Delta^{18}O_{cc/DIC} \)) varies between an equilibrium limit and a
kinetic limit, respectively corresponding to low versus high values
of crystallization rate, saturation index, and ionic strength\( ^{23,27,29} \).
The fact that the slope of the equilibrium regression line in Fig. 1
is indistinguishable from that of Kim and O’Neil\( ^{15} \) or from that of
equilibrium fractionation between dissolved (bi)carbonate ions
and water\( ^{35} \) implies that both the equilibrium limit and the
kinetic limit of \( \Delta^{18}O_{cc/DIC} \) do not vary strongly with temperature.

An important prediction of these theoretical models is that
virtually all biogenic and most inorganic calcites precipitating at
the surface of the Earth crystallize too rapidly to achieve DIC-
calcite equilibrium. This conclusion is not invalidated by the fact
that some rapidly-precipitating inorganic carbonates such as
speleothems\( ^{18} \) or travertines\( ^{32} \) often display higher \( \delta^{18}O \) values
than predicted by Kim and O’Neil\( ^{15} \), because this observation
may be simply explained by isotopic disequilibrium between DIC
and water due to Rayleigh fractionation of the DIC pool under
conditions of rapid \( CO_2 \) degassing\( ^{35} \). Carbonates formed close to
isotopic equilibrium are only expected to be found in

**Fig. 2** Three calibrations of clumped isotopes in carbonates (\( \Delta^{18}O \)) as a function of crystallization temperature \( T \). a Observed relations between \( \Delta^{18}O \)
and \( T \) in slow-growing calcite from Devils Hole and Laghetto Basso, in modern calcitic bivalves (Supplementary Table 2), and in foraminifera from sedimentary core-tops (data from Peral et al.\( ^{45} \)), all of which were analyzed in the same laboratory over a short period of time. Error bars represent 95% confidence intervals. Solid regression lines take into account analytical errors in \( \Delta^{18}O \) as well as uncertainties on crystallization temperature. b Comparison of the 95% confidence regions of regression slopes and 20 °C intercept values for slow-growing and biogenic calcite. Both of the biogenic regression lines differ significantly (\( p \leq 10^{-3} \)) from the equilibrium baseline defined by slow-growing calcites. Colored bell-shaped curves represent the probability distributions of regression slopes.

**Fig. 3** Equilibrium versus biogenic calcites in \( \Delta^{18}O_{cc/w} \) - \( \Delta^{14}C \) space. Combining the \( \Delta^{18}O_{cc/w} \) and \( \Delta^{14}C \) thermometers requires some constraints
on water \( \delta^{18}O \) values, but makes it possible to test whether carbonates precipitated in isotopic equilibrium even if crystallization temperature is unknown. Dashed line corresponds to the equilibrium baseline defined by Eqs. (1) and (3). Error bars represent 95% confidence intervals.
environments with very low supersaturation states, such as for example recrystallized carbonates from deep-sea sediments\textsuperscript{25}, or carbonates associated with low-temperature hydrothermal alteration of young oceanic crust\textsuperscript{35}. Deeper away from the surface, isotopic equilibrium might be the rule rather than the exception for diagenetic or metamorphic carbonates formed at significantly warmer temperatures, where isolate exchange reaction rates are much faster.

The biogenic carbonates analyzed here yield Δ\textsubscript{47} values 5–20 ppm higher than equilibrium, and it appears possible that the magnitude of clumped-isotope disequilibrium decreases at low calcification temperatures. It should be noted that "oxygen-18 equilibrium", referring to oxygen-isotope exchange between water and mineral phases, and "clumped-isotope equilibrium", referring to the internal distribution of isotopes within the mineral phase, are logically independent, i.e., neither implies the other, because ultimately they reflect partial or complete isotopic exchanges occurring in transitional phases such as amorphous calcite or crystal-surface phases\textsuperscript{25,26,28,44}. By contrast, achieving oxygen-18 equilibrium between water and calcite requires establishing a series of intermediate equilibria: between water and DIC, then between DIC and calcite, either directly or through the intermediate phases mentioned above. Each of these exchange steps may fail to achieve equilibrium, which potentially manifests in very different ways. For example, rapid CO\textsubscript{2} degassing of DIC solutions is associated with kinetic isotope fractionation effects which strongly affect both \( δ^{18}O \) and \( Δ_{47} \), contrary to the disequilibrium observations reported here which only weakly affect the latter.

Our findings provide robust new evidence that the majority of calcites precipitated at the surface of the Earth achieve neither oxygen-18 nor clumped-isotope equilibrium, probably because most of them precipitate rapidly from supersaturated solutions. In most cases, kinetic components of \( 18\text{O/16}\text{O}_{\text{DIC}} \) typically decrease carbonate \( δ^{18}O \) values by 1–2‰, even in "well-behaved" biogenic carbonates where \( 18\text{O/16}\text{O}_{\text{DIC}} \) varies primarily with temperature. As noted by Watkins et al.\textsuperscript{27}, oxygen-isotope thermometry works reasonably well in spite of these strong kinetic effects because many types of natural carbonates precipitate under limited ranges of pH and growth rates. However, the observation that non-equilibrium oxygen-18 effects in coccolithophores have varied drastically at geologic time scales\textsuperscript{25,27} offers a cautionary tale regarding the long-term applicability of modern calibrations for biogenic carbonates. Moving beyond phenomenological characterization of oxygen-isotope and \( Δ_{47} \) thermometry calls for substantial improvements in our ability to model isotopic fluxes and fractionations in the water/DIC/carbonate system. In our view, the use of non-classical isotopic tracers, such as clumped isotopes and oxygen-17 anomalies (\( Δ^{17}O \)), offers appealing new opportunities to test and improve these models.

**Methods**

**Inorganic calcite samples.** Holocene Devils Hole calcite (sample DVH) was collected from the outer surface of sample DH2C-8, which was previously described by Winograd et al.\textsuperscript{58} and Cople\textsuperscript{16}. After a 15-min ultrasonic bath treatment with reagent-grade methanol, we abraded the surface of DH2C-8 to a maximum depth of 100 μm using a programmable micro-mill at its slowest setting. Laghetto Basso calcite (sample LGB) was collected from the top of core CD3-12, located a few centimeters away from core CD3, which was described by Drysdale et al.\textsuperscript{38}. Each half of CD3-12 was ultrasonically cleaned in deionized water to remove loose particles from the active growth surface, then air-dried at ambient temperature. Calcite was abraded from 15 discrete 1-cm\textsuperscript{2} regions of its outer surface using a Dremel hand tool fitted with a diamond burr and a magnification lens. The depth of abrasion was estimated to be no more than 100 μm. Both DVH and LGB powders were then rinsed in methanol and dried at room temperature.

**Bivalve samples.** Three specimens of Antarctic scallop species Adamussium colbecki were collected at a water depth of 15 m near the Dumont d’Urville Antarctic Station in January 2007 (66.685°S, 140.008°E). Seawater contained by the ROSAME network (Réseau d’Observation Sub-Antarctique et Antarctique du niveau de la MÉ), remains stable annually (mean T = −1.8 °C) except for a summer warming peak around −0.5 °C between January and March\textsuperscript{59}. Seawater \( δ^{18}O_{\text{SMOW}} \) value, estimated from the Global Seawater Oxygen-18 Database of Schmidt et al.\textsuperscript{61}, is −0.26 ± 0.006%\textsubscript{o}.

Five live specimens of the deep-sea oyster species Neopycnodonte cochlear were collected in March 2010 from the Lacaze-Duthiers canyon (42.533°N, 3.453°E, Mediterranean Sea) at a depth of 270 m, about 20 km east of the coast. Mean annual temperature remains constant at 13.5 ± 0.1 °C\textsuperscript{59}. Local \( δ^{18}O_{\text{SMOW}} \) values vary seasonally between 0.23 and 0.93‰, with an average of 0.70‰ (M. Sebilo, pers. comm.).

Four live Saccostrea cucullata oysters from the warm shallow waters of the Kenyan coast (Tiwi Beach, 4.239°S, 39.604°E) were collected in September 2005. Local seawater temperatures vary annually from 25.1 to 28.5 °C (T = 26.8 ± 0.9 °C). Anomalous local temperature remains constant at 13.5 ± 0.1 °C\textsuperscript{59}. Local \( δ^{18}O_{\text{SMOW}} \) values are indistinguishable from zero, the null hypothesis that the two data sets follow the same relationship between \( Δ^{17}O \) and \( T \) cannot be excluded.

**Foraminiferal samples.** Peral et al.\textsuperscript{45} analyzed Late Holocene foraminifera collected from 13 marine sediment core-tops, comprising 9 planktonic and 2 benthic species. Calcification temperatures were estimated based on the gridded seawater \( δ^{18}O \) model of LeGrande and Schmidt\textsuperscript{62}, assuming the oxygen-18 fractionation law of Kim and O’Neil\textsuperscript{15}. Note that the observed differences between the slow-growing inorganic calcites and the foraminifera only increase if calcification temperatures were derived instead from the oxygen-18 fractionation law of Shackleton\textsuperscript{63}.

**Traditional stable-isotope analyses.** Traditional stable-isotope analyses (\( δ^{13}C \), \( δ^{18}O \)) of samples DVH and LGB were performed using a MultiCarb system coupled to an Isoprime 100 mass spectrometer in dual-inlet mode. International carbonate standards NBS 19 (\( δ^{18}O_{\text{VPDB}} = +1.95‰ \)) and NBS 18 (\( δ^{13}C_{\text{VPDB}} = +0.13‰ \)) were analyzed along with DVH and LGB. All samples and standards were analyzed six times, with each replicate analysis requiring about 150 μg of carbonate. Sample \( δ^{13}C \) and \( δ^{18}O \) values were computed directly from ion current ratios 45/44 and 46/44 using the IUPAC recommended oxygen-17 correction parameters of Brand et al.\textsuperscript{64}. As recommended by Cople\textsuperscript{16}, final \( δ^{18}O_{\text{VPDB}} \) values are scaled to the nominal oxygen isotope compositions of NBS 19 and NBS 18. The overall external reproducibility (standard deviation) of these measurements were 0.02‰ for \( δ^{18}O_{\text{VPDB}} \) and 0.04‰ for \( δ^{13}C_{\text{VPDB}} \).

**Clumped-isotope analyses.** Clumped isotope measurements were performed according to previously described protocols\textsuperscript{41,44}. Carbonate samples were converted to CO\textsubscript{2} by phosphoric acid reaction at 90 °C. After cryogenic removal of free CO\textsubscript{2} and humect, CO\textsubscript{2} was bubbled through a purification column packed with Porapak Q and held at −20 °C, then quantitatively recollected by cryogenic trapping and transferred into an Isoprime 100 dual-inlet mass spectrometer equipped with six Faraday collectors (m/\textit{z} 44–49). Pressure-dependent background current corrections were measured independently for each sample. Background-corrected ion current ratios were converted to \( δ^{18}O \) and \( δ^{13}C \) as described by Daéron et al.\textsuperscript{41}, using the IUPAC oxygen-17 correction parameters\textsuperscript{44}. The raw \( Δ_47 \) values were converted to the "absolute" \( Δ_47 \) reference frame defined by the "ETH" carbonate standards\textsuperscript{65}. The overall external reproducibility (standard deviation) of \( Δ_{47} \) measurements for carbonate standards and samples is 15 ppm. Average \( Δ_{47} \) values are based on 22 replicate analyses (each) for samples DVH and LGB, 20 replicates for N. cochlear, 17 for S. cucullata, and 12 for A. colbecki. Full analytical errors are derived from the external reproducibility of carbonate standards (\( N = 151 \)) and samples (\( N = 93 \)) within each analytical session, and conservatively account for the uncertainties in raw \( Δ_{47} \) measurements as well as those associated with the conversion to the "absolute" \( Δ_{47} \) reference frame.

**Statistical methods.** Relationships between \( Δ_47 \) and crystallization temperature are modeled using weighted orthogonal distance regressions of the form \( Δ_{47} = A/T^2 + B \) in order to account for errors in both variables. In all three regressions, root mean square weighted deviation (RMSWD) values are smaller than one, implying that analytical and observational errors are sufficient to explain the scatter in mean deviations.

Analysis of covariance (ANCOVA) was performed by first computing the probability for the null hypothesis that two independent regression lines have identical slopes. If the two slopes are statistically indistinguishable (at a given confidence level), observations from both data sets are jointly fit to a new model with two parallel lines. If the distance between these two lines is statistically indistinguishable from zero, the null hypothesis that the two data sets follow the same relationship between \( Δ_47 \) and \( T \) cannot be excluded.
51. Dassié, E. P. et al. A newly designed analytical line to examine fluid inclusion isotopic compositions in a variety of carbonate samples. Geochim. Geophys. Geosyst. 19, https://doi.org/10.1002/2017GC007289 (2018).

52. Kele, S. et al. Temperature dependence of oxygen- and clumped isotope fractionation in carbonates: a study of travertines and tufas in the 6–9 °C temperature range. Geochim. Cosmochim. Acta 168, 172–192 (2015).

53. Hendy, C. H. The isotopic geochemistry of speleothems—I. The calculation of the effects of different modes of formation on the isotopic composition of speleothems and their applicability as palaeoclimatic indicators. Geochim. Cosmochim. Acta 35, 801–824 (1971).

54. Fattal, M. S. & DePaolo, D. J. Ca isotopes in carbonate sediment and pore fluid from ODP Site 807A: the Ca$^{2+}/$aq–calcite equilibrium fractionation factor and calcite recrystallization rates in Pleistocene sediments. Geochim. Cosmochim. Acta 71, 2524–2540 (2007).

55. Coogan, L. A., Daïron, M. & Gillis, K. M. Seafloor weathering and the oxygen isotope ratio in seawater: insight from whole-rock δD and carbonate δ18O and δD$_{\text{fl}}$ from the Troodos ophiolite. Earth Planet. Sci. Lett. 508, 41–50 (2019).

56. Guo, W. Carbonate Clumped Isotope Thermometry: Application to Carbonaceous Chondrites and Effects of Kinetic Isotope Fractionation. PhD dissertation, California Institute of Technology (2009).

57. Stoll, H. M. Limited range of interspecific vital effects in coccolith stable isotopic records during the Paleocene–Eocene thermal maximum. Palaeoecography 20, 1 (2005).

58. Winograd, I. J. et al. Devil’s Hole, Nevada, δD$^{18}$O record extended to the mid-Holocene. Quat. Res. 66, 202–212 (2006).

59. Lartaud, F. et al. Experimental growth pattern calibration of Antarctic scallop shell (Aequipecten opercularis) δD and δ18O isotope fractionation in seawater and the benthonic foramifera genus Uvigerina: isotopic changes in the ocean during the last glacial. Colloq. Int. CNRS 219, 203–210 (1974).

60. Schmidt, G. A., Bigg, G. R. & Rohling, E. J. Global Seawater Oxygen-18 mass spectrometry (IUPAC Technical Report). Pure Appl. Chem. 82, 1719–1733 (2010).

61. Coplen, T. B. More uncertainty than necessary. Palaeoecography 11, 369–370 (1996).

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Author contributions

M.D. initiated the project aiming to determine baseline equilibrium Δ$_{\text{fl}}$ values based on natural slow-growing calcite. He set up the clumped-isotope facility at LSCE and oversaw the quality of all clumped-isotope measurements; performed most of the clumped-isotope analyses of DVH and LGB; designed the statistical analysis and figures presented here; wrote the present report with primary contributions from R.N.D., D.B. and T.B.C., and additional contributions from other co-authors. R.N.D. conducted preliminary work that originally recognized that Laghetto Basso calcite could be used to constrain oxygen-isotope equilibrium fractionation at low temperatures; helped conceive the research; compiled the water chemistry data from Laghetto Basso; prepared and subsampled the outer surface of CD3-12. M.P. selected sedimentary core-tops; picked, identified, and cleaned foraminifera; performed foraminiferal clumped-isotope analyses; compiled sea-water composition estimates. D.H. selected and subsampled bivalve specimens; performed bivalve clumped-isotope analyses. D.B. co-developed the LSCE clumped-isotope facility and sampled the outer surface of DHCl2-8; performed some of the clumped-isotope analyses of DVH and LGB. T.B.C. provided sample DHCl2-8. F.L. helped select bivalve specimens; collected seawater samples from Lacaze-Duthiers canyon; compiled seawater composition estimates for A. colbecki. G.Z. oversaw the geochemical monitoring of Laghetto Basso and collected core CD3-12.

Additional information

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