Surface temperature is a fundamental parameter of Earth’s climate. Its evolution through time is commonly reconstructed using the oxygen isotope and the clumped isotope compositions of carbonate archives. However, reaction kinetics involved in the precipitation of carbonates can introduce inaccuracies in the derived temperatures. Here, we show that dual clumped isotope analyses, i.e., simultaneous $\Delta_{47}$ and $\Delta_{48}$ measurements on the single carbonate phase, can identify the origin and quantify the extent of these kinetic biases. Our results verify theoretical predictions and evidence that the isotopic disequilibrium commonly observed in speleothems and scleractinian coral skeletons is inherited from the dissolved inorganic carbon pool of their parent solutions. Further, we show that dual clumped isotope thermometry can achieve reliable palaeotemperature reconstructions, devoid of kinetic bias. Analysis of a belemnite rostrum implies that it precipitated near isotopic equilibrium and confirms the warmer-than-present temperatures during the Early Cretaceous at southern high latitudes.
Equilibrium both calcite and aragonite minerals, as no systemic difference in rate temperature estimates26,27. Precise determination of carbonate composition of these (palaeo) remains of marine calcifiers and terrestrial carbonates has served as one of the most widely used palaeotemperature proxies1-3. However, to reconstruct carbonate growth temperatures using the oxygen isotope thermometer, the oxygen isotope composition of water from which carbonates precipitated. Under thermodynamic equilibrium, the clumped isotope composition of a carbonate depends only on its crystallisation temperature. However, in addition to temperature and fluid δ18O, kinetic effects occurring during carbonate (bio)mineralisation can influence the isotopic composition of the carbonates. Departures from oxygen isotope and clumped isotope equilibrium due to kinetic effects pose considerable challenges in obtaining accurate palaeotemperature estimates. Such kinetic departures are present in most Earth-surface carbonates, most notably speleothems2,6-9, brachiopod shells10-13, and coral skeletons14-20. If unaccounted for, these kinetic biases could lead to significant over- or underestimation of the carbonate formation temperatures6,7,12,16,18,21. Despite numerous efforts, it remains difficult to identify and correct for these kinetic effects, especially in extinct calcifiers without modern analogues22-24.

The Δ48 value is a measure of the excess abundance of the 18O–12C–18O isotopologue in a given pool of CO2 molecules relative to the stochastic distribution25. Theoretical studies suggest that simultaneous measurements of Δ48 and Δ47 in CO2 evolved from phosphoric acid digestion of carbonates, referred to here as dual clumped isotope thermometry, can identify kinetic effects involved in carbonate formation26,27. Moreover, this method holds the potential to correct for kinetic biases and derive accurate temperature estimates26,27. Precise determination of carbonate Δ48 values has not been possible until recent advances in mass spectrometry, due to the very low abundance of 18O–12C–18O, the main isotopologue contributing to the Δ48 signal in carbonate minerals25.

Here we report simultaneous high precision Δ47 and Δ48 measurements on representative carbonate samples of different origins and demonstrate the potential of dual clumped isotope thermometry in quantitative palaeotemperature reconstruction by comparing these experimental observations with independent theoretical predictions.

Results

The temperature dependence of equilibrium Δ47 and Δ48 values. Before examining the potential kinetic isotope effects in our carbonate samples, we constrained the equilibrium Δ47 vs Δ48 relationship for carbonates by integrating theoretical estimations with experimental measurements (Fig. 1). First, we derived empirical equilibrium Δ47 (CDES90) vs temperature and Δ48 (CDES90) vs temperature relationships by combining the theoretically estimated equilibrium temperature dependence of carbonate Δ43 and Δ44 values28 with our experimentally determined acid fractionation factors (0.194‰ and 0.138‰, respectively, see “Methods”). We chose to use the theoretical calcite calibration for both calcite and aragonite minerals, as no systemic difference in equilibrium Δ47 values between the two has been observed in the majority of studies29. Secondly, we analysed the clumped isotope composition of a vein calcite from Devils Hole (DHC2-8) and used it to anchor our empirically derived equilibrium Δ47 (CDES90) vs temperature and Δ48 (CDES90) vs temperature relationships. The Devils Hole carbonate is believed to have precipitated extremely slowly (0.1–0.8 μm year−1) under stable environmental conditions at 33.7(±0.8) °C, and thus its isotopic composition has been postulated to represent thermodynamic equilibrium30-32. Specifically, we calculated the differences between the Δ47 (CDES90) and Δ48 (CDES90) Values of DHC2-8 and the corresponding values predicted by our empirical relationships at 33.7 °C. We then added these differences (0.010‰ for Δ47 (CDES90) and −0.021‰ for Δ48 (CDES90)) to the empirical relationships, leading to the final equilibrium relationships (0–40 °C):

Δ47 (CDES90) = 0.3642 – 2.914 × 10^2 / T + 1.800 × 10^5 / T^2 – 2.657 × 10^7 / T^3 + 1.493 × 10^9 / T^4,

Δ48 (CDES90) = 0.1742 – 5.897 × 10 / T + 1.252 × 10^4 / T^2 + 5.039 × 10^6 / T^3 – 5.631 × 10^8 / T^4,

where T is in K. Lastly, the combination of Eqs. (1) and (2) leads to our estimated equilibrium Δ47 (CDES90) vs Δ48 (CDES90) relationship for carbonates (Fig. 1):

Δ47 (CDES90) = −0.4771 + 9.102 × Δ48 (CDES90) − 31.709 × Δ48 (CDES90) + 65.561 × Δ48 (CDES90)^3 − 54.560 × Δ48 (CDES90)^4.

To check whether Eqs. (1–3) indeed represent thermodynamic equilibrium, especially at temperatures below 33.7 °C, we...
analysed two additional carbonate samples which are thought to have precipitated slowly from their parent solutions and thus may exhibit equilibrium $\Delta_{47}$ and $\Delta_{48}$ signatures: a cave pool carbonate (Obi 87-1) and a cryogenic cave carbonate (MSK 2b), formed at 4 (±1.5) °C and 0 °C, respectively (see “Methods”). In particular, the $\delta^{13}$C and $\delta^{18}$O values of the cryogenic carbonate sample suggest crystallisation over the final stage of the freezing process during which isotopic equilibrium is closely approached33,34. Furthermore, our estimated temperature dependence of carbonate formation provides the most substantial evidence so far that most of these carbonates did not form under isotopic equilibrium (Fig. 2, Supplementary Fig. 2). Kinetic departures from isotopic equilibrium 32,39,40, kinetics associated with the (de)hydration/(de)hydroxylation reactions are predicted to introduce large disequilibrium signatures in the carbonate crystal lattice37,38.

Carbonates formed in kinetic environments. The clumped isotope composition and the corresponding uncertainties of the carbonate standards and samples analysed in this study are listed in Tables 1 and 2, respectively. In addition to the carbonates that have formed in or close to thermodynamic equilibrium (i.e. the Devils Hole carbonate, the cave pool carbonate, and the cave cryogenic carbonate; see above), we analysed representative carbonates from a variety of kinetic environments (see “Methods”), including a synthetic speleothem, a natural stalagmite (Spannagel Cave, Austria), a warm-water coral (Porites lutea), a cold-water coral (Desmophyllum pertusum), a modern brachiopod (Magellania venosa), and a fossil belemnite (Blemnopsis sp.).

| Table 1 Long-term $\Delta_{47}$ (CDES90) and $\Delta_{48}$ (CDES90) values of carbonate reference materials. |
|---------------------------------------------------------------|
| **Standard** | **Replicates** | **$\Delta_{47}$ (CDES90) (%)** | **2 SE (1 SD)** | **$\Delta_{48}$ (CDES90) (%)** | **2 SE (1 SD)** |
| ETH 1 | 78 | 0.212 | 0.002 (0.010) | 0.142 | 0.008 (0.036) |
| ETH 2 | 71 | 0.212 | 0.003 (0.011) | 0.138 | 0.007 (0.029) |
| ETH 3 | 74 | 0.615 | 0.002 (0.010) | 0.299 | 0.010 (0.042) |

This table considers data measured during the April-August 2019 measurement period combined with those reported in Fiebig et al.25. All replicate analyses from the April-August 2019 measurement period are presented in Supplementary Data 1. The average $\Delta_{47}$ (CDES90) and $\Delta_{48}$ (CDES90) values for the total number (149) of ETH 1 and ETH 2 replicates are 0.212(±0.002)‰ and 0.140(±0.005)‰, respectively. SE, standard error; SD, standard deviation.

| Table 2 Clumped isotope composition ($\Delta_{47}$ (CDES90) and $\Delta_{48}$ (CDES90)) of the samples. |
|---------------------------------------------------------------|
| **Sample (type)** | **Replicates** | **$\Delta_{47}$ (CDES90) (%)** | **2 SE** | **$\Delta_{48}$ (CDES90) (%)** | **2 SE** |
| DHC2-8 (vein calcite) | 5 | 0.575 | 0.006 | 0.226 | 0.023 |
| Obi 87-1 (pool carbonate) | 7 | 0.660 | 0.007 | 0.267 | 0.024 |
| MSK 2b (cryogenic cave carbonate) | 8 | 0.674 | 0.005 | 0.271 | 0.029 |
| MHD1 (synthetic speleothem) | 6 | 0.547 | 0.008 | 0.254 | 0.027 |
| SPA121-02 (stalagmite) | 6 | 0.644 | 0.009 | 0.290 | 0.025 |
| PC1_2005 (warm-water coral) | 10 | 0.626 | 0.005 | 0.136 | 0.022 |
| JR (cold-water coral) | 10 | 0.719 | 0.005 | 0.189 | 0.030 |
| Mv413b (brachiopod) | 9 | 0.675 | 0.004 | 0.250 | 0.015 |
| 66-4-65 (belemnite) | 9 | 0.610 | 0.003 | 0.251 | 0.021 |

This table considers data measured during the April-August 2019, September-December 2019, and January-March 2020 measurement periods. All replicate analyses are presented in Supplementary Data 2-4. The $\delta^{18}$O and $\delta^{13}$C values of the samples are reported in Supplementary Table 1.

Carbonates formed in kinetic environments. The clumped isotope composition and the corresponding uncertainties of the carbonate standards and samples analysed in this study are listed in Tables 1 and 2, respectively. In addition to the carbonates that have formed in or close to thermodynamic equilibrium (i.e. the Devils Hole carbonate, the cave pool carbonate, and the cave cryogenic carbonate; see above), we analysed representative carbonates from a variety of kinetic environments (see “Methods”), including a synthetic speleothem, a natural stalagmite (Spannagel Cave, Austria), a warm-water coral (Porites lutea), a cold-water coral (Desmophyllum pertusum), a modern brachiopod (Magellania venosa), and a fossil belemnite (Blemnopsis sp.).

The mean clumped isotope values of our analysed samples range from 0.547‰ to 0.719‰ for $\Delta_{47}$ (CDES90) values, and from 0.136‰ to 0.290‰ for $\Delta_{48}$ (CDES90) values (Fig. 2a, Table 2, Supplementary Fig. 2). Except for the belemnite sample, which plots within 2 SE (95% confidence interval) of the equilibrium $\Delta_{47}$ (CDES90) vs $\Delta_{48}$ (CDES90) line (Eq. (3)), all the other investigated carbonate samples show significant offsets from the expected clumped isotope equilibrium (Fig. 2, Supplementary Fig. 2). Both the synthetic speleothem and the stalagmite plot below the $\Delta_{47}$ (CDES90) vs $\Delta_{48}$ (CDES90) equilibrium line, with offsets of $-0.036\%$ and $-0.030\%$ in the $\Delta_{47}$ (CDES90) values and $+0.024\%$ and $+0.018\%$ in the $\Delta_{48}$ (CDES90) values, respectively. In contrast, the two modern corals and the brachiopod plot above the equilibrium line, with offsets ranging from $+0.038\%$ to $+0.069\%$ in $\Delta_{47}$ (CDES90) and from $-0.004\%$ to $-0.095\%$ in $\Delta_{48}$ (CDES90).

Discussion

If clumped isotope equilibrium was attained in all samples, their $\Delta_{47}$ (CDES90) and $\Delta_{48}$ (CDES90) values should correlate, due to the exclusive temperature dependence of clumped isotope equilibrium. The fact that the $\Delta_{47}$ (CDES90) and $\Delta_{48}$ (CDES90) values of our investigated samples do not follow such a simple trend provides the most substantial evidence so far that most of these carbonates did not form under isotopic equilibrium (Fig. 2a, Supplementary Fig. 2). Kinetic departures from isotopic equilibrium in the carbonate-water system generally arise from two processes: the slow conversion between dissolved carbon dioxide ($CO_2$ (aq)) and bicarbonate ($HCO_3^-$) through (de)hydration/(de)hydroxylation reactions (Eqs. (4) and (5), respectively)36 and the incorporation of dissolved inorganic carbon species (DIC) into the carbonate crystal lattice37,38.

$$CO_2(aq) + H_2O \leftrightarrow H^+ + HCO_3^- \quad (4)$$

$$CO_2(aq) + OH^- \leftrightarrow HCO_3^- \quad (5)$$

While DIC incorporation seems to introduce only negligible departures from clumped isotope equilibrium32,39,40, kinetics associated with the (de)hydration/(de)hydroxylation reactions are predicted to introduce large disequilibrium signatures in the $\Delta_{63}$ and $\Delta_{64}$ values of the DIC26,27. These disequilibrium $\Delta_{63}$ and $\Delta_{64}$ values of the DIC are expected to be directly transcribed to the $\Delta_{47}$ and $\Delta_{48}$ values of the carbonates when carbonates precipitate quickly from the solution.
Fig. 2 The nature and extent of kinetic isotope effects in representative carbonates. a The isotopic compositions of the stalagmite, synthetic speleothem, cold-water coral, warm-water coral, and the brachiopod deviate significantly from their respective equilibrium compositions (coloured rectangles on the equilibrium line), while the belemnite is indistinguishable from the equilibrium line. b The cold- and warm-water corals show positive Δ47 (CDES90) offsets and negative Δ48 (CDES90) offsets from the thermodynamic equilibrium, which are consistent with kinetic effects derived from CO2 absorption (purple arrow). In comparison, the stalagmite and the synthetic speleothem show negative Δ47 (CDES90) offsets and positive Δ48 (CDES90) offsets that are consistent with kinetic effects derived from CO2 degassing (pink arrow). Disequilibrium offsets were calculated relative to the equilibrium Δ47 (CDES90) and Δ48 (CDES90) values expected at the corresponding carbonate growth temperatures (Eqs. (1–3)). All error bars depict 2 standard errors (95% confidence interval35).

Specifically, under open-system conditions, the evolution of the clumped isotope composition of the DIC follows nonlinear trajectories in Δ47 vs Δ48 space26. For example, for an aqueous solution starting from isotopic equilibrium, the carbonate clumped isotope compositions will increasingly deviate from equilibrium during CO2 degassing and CO2 absorption, i.e., when dehydration/dehydroxylation reactions dominate over the hydration/hydroxylation reactions, and vice versa (Eqs. 4 and 5). However, as the forward reaction progresses and the product concentration increases, the backward reaction will become more and more significant until the forward and backward reaction rates become identical and the isotopic equilibrium is attained again (Fig. 3a)26,27. The exact pattern and extent of these disequilibrium effects during CO2 absorption and CO2 degassing depend on several parameters26,27, such as solution temperature, pH, the isotopic compositions of the gaseous CO2 and the solution, and for calcifying organisms, carbonic anhydrase activity that facilitates the interconversion between dissolved carbon dioxide and bicarbonate (Fig. 3a).

Most carbonates in our study exhibit systematic departures from the clumped isotope equilibrium line (Eqs. (1–3)) that are consistent with the model predictions26,27, supporting that CO2 absorption and CO2 degassing reactions are the primary drivers for the observed departures from isotopic equilibrium (Fig. 3a). During scleractinian coral biomineralisation, carbonic dioxide derived from metabolic respiration is transformed to bicarbonate via hydration/hydroxylation reactions (Eqs. (4) and (5)) in the calcifying fluid and then subsequently to carbonate ion (CO32−) from which the solid carbonate skeleton precipitates14,26. In the early stage of CO2 absorption, the Δ47 values of coral skeletons are predicted to show a positive departure from the expected equilibrium values while their Δ48 values show a negative offset (Fig. 3a, Supplementary Data 5). Such a pattern is observed in both the warm-water and cold-water corals in our study (Fig. 2).

Moreover, the observed offsets from the respective equilibrium Δ47 and Δ48 values co-vary and plot close to the model predictions where the CO2 absorption kinetics dominate (Fig. 3a, see “Methods”). Similar to the two scleractinian corals, the isotopic composition of the fast-growing brachiopod (Magellania venosa) plots above the Δ47 vs Δ48 equilibrium line (Fig. 2a), showing a positive offset in Δ47 but only a minimal negative offset in Δ48 (Fig. 2b). The direction of such departures from equilibrium is consistent with the disequilibrium initiated by hydration/hydroxylation reactions26, supporting earlier suggestions that hydration and hydroxylation reactions are the dominant causes of the observed clumped isotope disequilibrium in modern brachiopods12.

Speleothem precipitation, unlike biomineralisation, is induced by the degassing of CO2 from aqueous solutions. The removal of CO2 from the very thin solution film on the surface of a speleothem, i.e., the transformation of bicarbonate to dissolved carbon dioxide via dehydroxylation reactions (backward reactions in Eqs. (4) and (5)), increases the saturation state of calcium carbonate and leads to carbonate precipitation6,27,41,42. In the early stages of CO2 degassing, carbonate Δ47 values are expected to show a negative offset and Δ48 values to show a positive offset from equilibrium27 (Fig. 3a, Supplementary Data 5). This pattern is observed in the isotopic compositions of both the synthetic speleothem and the stalagmite samples, which plot below the clumped isotope equilibrium line (Fig. 2). Moreover, the Δ47 and Δ48 offsets observed in the synthetic speleothem quantitatively agree with the model prediction that explicitly simulates this carbonate formation27 (Fig. 3a, see “Methods”).

The agreements between the measured carbonate clumped isotope compositions and our model simulations suggest that dual clumped isotope analysis can be used to identify the dominant reactions involved in carbonate (bio)mineralisation.
Furthermore, when combined with numerical modelling, coupled $\Delta_{47}$ and $\Delta_{48}$ analysis shall allow a more accurate reconstruction of the carbonate formation environment such as temperature, pH, and carbonic anhydrase activity\textsuperscript{26,27}. In particular, disequilibrium offsets observed in the two scleractinian corals and the natural and synthetic speleothems fall close to the initial linear segment of the model-predicted departure from isotopic equilibrium, whose slope is relatively insensitive to temperature, pH, and carbonic anhydrase activity\textsuperscript{26} (Fig. 3a). This opens up a unique opportunity to accurately reconstruct carbonate formation temperatures even if the measured $\Delta_{47}$ and $\Delta_{48}$ values were affected by kinetic effects\textsuperscript{26,27}. Under the conditions specific to the formation of our samples (see “Methods”), the model predicts approximately linear correlations between disequilibrium $\Delta_{47}$ and $\Delta_{48}$ values in carbonates formed during the early stages of CO$_2$ degassing and CO$_2$ absorption, with slopes of $-1.0$ and $-0.6$, respectively (Fig. 3a, Supplementary Data 5). Projecting the measured carbonate isotope composition to the equilibrium $\Delta_{47}$ and $\Delta_{48}$ line (Eq. (3)) along these predicted linear kinetic trajectories, shall yield their actual formation temperatures, unbiased by kinetic effects\textsuperscript{26,27}. Specifically, this leads to temperature estimates of $34\pm1^\circ$C, $2\pm1^\circ$C, $1\pm1^\circ$C, and $3\pm1^\circ$C for the synthetic speleothem, the stalagmite, the cold-water coral, and the warm-water coral, respectively (Fig. 3b). These temperature estimates are indistinguishable within $2\$SE from the actual formation temperatures of these carbonates, i.e., $30.7\pm0.3^\circ$C, $0.0\pm2.0^\circ$C, $7.2\pm1.0^\circ$C, and $29.3\pm1.0^\circ$C, respectively. In contrast, estimates based on the measured $\Delta_{47}$ data alone would result in inaccurate and unreasonable temperatures (Fig. 3b). For example, the synthetic speleothem, the observed disequilibrium $\Delta_{47}$ effect of $-0.36\%0$ is similar to that observed in some natural systems\textsuperscript{43}, and would yield a temperature that is $10$–$18^\circ$C too warm compared to its actual formation temperature. Similarly, $\Delta_{47}$ thermometry would overestimate the formation temperatures of the stalagmite sample by $4$–$14^\circ$C, while the $\Delta_{47}$-derived temperatures of the warm-water and the cold-water corals would be lower than their actual growth temperatures by $11$–$17^\circ$C and $18$–$22^\circ$C, respectively.

For the modern brachiopod specimen, however, even after correction using a model-predicted kinetic slope of $-0.6$ for CO$_2$ absorption, one would still underestimate its growth temperature by $4$–$13^\circ$C. Compared to scleractinian coral calcification, the mechanism of brachiopod calcification is less explored\textsuperscript{12,13}. The current model simulations of CO$_2$ absorption were designed based on the mechanism of coral calcification and thus may not have captured the primary sources of isotopic disequilibrium in brachiopods. More studies on specimens of known growth temperatures are required to evaluate the full potential that dual clumped isotope thermometry holds for accurate palaeotemperature reconstructions. However, if it were demonstrated that isotopic compositions of most biogenic carbonates and speleothems follow the early, linear trajectory of departure from equilibrium, accurate palaeotemperature reconstructions would become possible on...
well-preserved samples even if they are affected by kinetic effects.

The dual clumped isotope analysis also makes it possible to evaluate the extent of isotopic disequilibrium in fossil carbonates, even if the carbonate’s growth temperature and the isotopic composition of its parent water are not known. This, for the first time, offers the opportunity to investigate if the isotopic composition of extinct calcifiers was affected by kinetic effects. The isotopic composition of the Cretaceous belemnite sample falls within 2 SE of the \( \Delta_{47} \) vs \( \Delta_{46} \) equilibrium line (Fig. 2a), suggesting that it precipitated indistinguishable from thermodynamic equilibrium at a temperature of 20.5(±1.9) °C. This temperature estimate supports southern high-latitude warmth during the Early Cretaceous44,45.

More generally, our finding that the belemnite did but the brachiopod did not form in isotopic equilibrium has significant implications for the palaeotemperature estimates derived from the oxygen isotope compositions of these two archives. Multiple studies have reported colder \( \delta^{18}O \)-derived belemnite palaeotemperatures compared to coeval brachiopod-based temperature estimates, when using the same \( \delta^{18}O \) vs temperature relationship for both taxa and assuming a constant oxygen isotope composition1,3,46,47. To reconcile this discrepancy, it was proposed that belemnites migrated to colder waters47,48. However, based on the most current assessment of belemnite palaeobiology49, both long-distance and vertical migration to significantly colder waters is unlikely for these animals. Belemnites presumably lived in the upper 200 m of the water column and were mostly restricted to continental shelves. Our results thus support the apparent warm temperature estimates derived from brachiopod \( \delta^{18}O \) reflect the kinetic bias in their isotopic compositions12.

We have demonstrated that the dual clumped isotope method, i.e., the simultaneous measurement of carbonate \( \Delta_{47} \) and \( \Delta_{48} \), in a single phase, makes it possible to identify carbonates that did not precipitate in thermodynamic equilibrium from their parent water. The comparison of the measured isotopic compositions with theoretical predictions enabled us to pinpoint the dominant kinetic processes responsible for the isotopic disequilibrium and to reconstruct carbonate formation temperatures accurately. Our results highlight the potential that dual clumped isotope thermometry holds for accurate palaeoclimate reconstructions and the identification of the kinetic fractionation processes dominant in carbonate (bio)mineralisation.

Cave pool carbonate: A 3.5-cm thick subaqueous calcite sample (Obi 87-i) was collected in 2008 from a perennial pool (Silbersee) in Rasslsystem cave, which is part of the Silbersee Gléi (46.3146N, 14.5480E), a series of caves in the Southern Alps of Austria, located at approximately 1100 m above sea level. The Obi Caves are hypogenic in origin51, i.e., they were not connected to the surface and hence had a very stable microclimate until discovered during mining activities in the 1870s. The Silbersee pool, located in the inner part of Rasslsystem cave, has a surface area of 7 × 4 m and is 1.5 m deep. The water temperature between 1998 and 2002 was 5.4(±0.1) °C, closely corresponding to the long-term mean annual air temperature outside the cave at this elevation52. The sample analysed in this study is a 4 mm wide subsample retrieved from 2.7 cm above the bottom of Obi 87-i, and is estimated to be 1500 years old based on the U-Th dating of a lower layer in Obi 87 (a layer estimated to have formed about 3800 years before present at 1.5 cm above the base of Obi 87) and assuming a constant calcite growth rate of 5.3 μm year⁻¹ (unpublished data, C. Spötl). Although the water temperature about 1500 years before present is not precisely known, it likely corresponded to the mean annual air temperature outside the cave at that time in a similar way as the modern pool temperature does. Various temperature proxy data for the Alps suggest that the mean annual air temperature fluctuated by up to ±1.5 °C in the last two millennia before the industrial revolution53. Considering the ca 1.5 °C warming in the Alps during the past century, we estimate the water temperature of Silbersee pool from which Obi 87-i was sampled ca 1500 years ago to be 4.0(±1.5) °C.

The subaqueous calcite sample Obi 87-i can precipitate in oxygen isotope equilibrium with water54. Prior to isotope analyses, Obi 87-i was cleaned in de-ionised water in an ultrasonic bath, crushed and homogenised using an agate mortar and pestle, and dried in a vacuum oven at 30 °C. The calcite sample was scratched off the glass plate after the experiment was completed and corresponded to the first 5 cm of flow, i.e., the first 24 s of CaCO₃ precipitation.

Stalagmite: A calcite sample (SPA121-02) was retrieved from a stalagmite in Spannagel Cave in the Austrian Alps (47.0803 N, 11.6717 E), an extensive cave system with the main entrance at 2523 m above sea level. SPA121-02 is a 4-mm-thick stalagmite with a thin layer that records a long growth history from MIS 7 to MIS 54. The calcite sample was retrieved from a stalagmite in the axial part of the stalagmite SPA121 using a diamond-coated band saw. The slice was then cleaned in an ultrasonic bath in de-ionised water, dried, and crushed and homogenised with an agate mortar and pestle without rehydration.

Cold-water coral: A scleractinian, zoanthellate coral Desmophyllum pertusum (formerly known as Lophelia pertusa) (JR) was collected alive at Trænaudaget, Norwegian Sea (66.97333 N, 11.10883 E) at a water depth of 300 m during cruise POS325-356E. The annual mean seawater temperature at the collection site is 2 ± 1 °C. Within a hand-buoydrill, a calcite tube was cut from the axis of the colony, and the septa were removed, i.e., only the theca walls were sampled. The sample was cleaned in an ultrasonic bath using de-ionised water and dried in a vacuum oven at 30 °C before being crushed and homogenised using an agate mortar and pestle.

Warm-water coral: A scleractinian, zoanthellate coral Porites lutea (PCL-2005) was collected at the Rashoddo Atoll, Maldives (4.293776 N, 72.977115 E) at a water depth of 1 m. For isotopic analysis, a 0.25 cm thick section was cut from the coral core. Based on sclerochronological analysis, this section corresponded to the growth in the year 2005 when the annual mean temperature at the collection site was 28.5(±1) °C. The mean annual temperature at this location was 29.6(±0.6) °C for the last 10 years. To remove material that may have been thermally altered when the section was initially cut from the colony, the surface 0.5 mm was scraped away. Then, the section was cleaned in an ultrasonic bath using de-ionised water and
dried in a vacuum oven at 30 °C before being crushsed and homogenised using an agate mortar and pestle.

Mouse brain tissue: A terebratulid brachiopod Magellania venosa (Mt143-b) was collected from Punta Gruesa, Chile (42.409833 S, 72.423333 W) from 20 m below sea level. The annual mean temperature at the collection location is 11.4 °C (±1.7 °C)45. Magellania venosa is one of the fastest-growing modern brachiopods, with growth rates ranging from 3.8 mm year⁻¹ (adult) to 17.3 mm year⁻¹ (juvenile)46. For this study, we sampled a 2 × 3 cm area in the middle part of the ventral valve. First, we abraded the primary layer of the shell using a slow-speed hand-drill and a diamond drill bit, cleaned the shell in an ultrasonic bath with de-ionised water, dried it in a vacuum oven at 30 °C, and finally homogenised the material using an agate mortar and pestle. The anterior part of the same specimen showed the largest offset from equilibrium in δ47 values in a previous study42.

Cretaceous belemnite: A belemnite Belemnopsis sp. (66–6.65) was retrieved from DSDP Site 511 at the Falkland Plateau (51.004676 S, 46.971667 W). The investigated rostrum solidum shows excellent preservation based on cathodoluminescence, and trace element analyses42,46. Burial temperatures remained below 100 °C for this core, which makes the solid-state alteration of the clumped isotope composition of this sample unlikely61,62. The same sample in this study was analysed for its δ47 values, together with other belemnites, to reconstruct Early Cretaceous southern high latitude palaeotemperatures41.

Mass spectrometry. We performed the CO2 chloride isotope analyses of sample carbonates on a Thermo Scientific 253 Plus gas source isotope ratio mass spectrometer during April–March 2020, in three measurement sessions (April–August 2019, September–December 2019, and January–March 2020), following the method of Fiebig et al.25. Samples were measured in 5–10 replicates. Each replicate analysis consists of 13 acquisitions (10 cycles of reference and samples comparisons in each acquisition and 2 s integration time during each cycle). The raw clumped isotope values (indicated by subscript Δ) were calculated using the [Brand]/IUPAC isotopic parameters29,63. A correction for scale compression: The intercepts for the 1000 °C and the 25 °C non-linearity corrected (CDES90) values of the carbonate reference materials have a term δ47 (raw) vs δ47 (CDES90) = 0.018 ± 0.002 ‰. These analyses further argue that there is no contamination on the carbonate to the CDES90. All measured values can be found in Supplementary Data 1–4.

Acid fractionation factors. To be able to compare the experimentally measured clumped isotope compositions of a carbonate, i.e., the δ47 (CDES90) and the presumably uncontaminated equilibrated CO2 gas to check for potential contamination in the analyte. All δ47 (raw) values of the carbonates remain below 100 °C for this core, which makes the solid-state alteration of the clumped isotope composition of this sample unlikely61,62. The same sample in this study was analysed for its δ47 values, together with other belemnites, to reconstruct Early Cretaceous southern high latitude palaeotemperatures41.

Numerical modelling. We used numerical models to simulate the evolution of the isotopic composition of the DIC during (1) CO2 absorption, i.e., the key process involved in coral calcification26, and (2) the laboratory carbonate precipitation of the synthetic speleothem27 (Supplementary Data 5).

(1) CO2 absorption simulations were constructed using the IsoDIC model to mimic the internal calcification environment of scleractinian corals26. Specifically, the modelled calcification environment consisted of an aqueous solution ([DIC] = 2 mM, δ13C-DIC = −0.8, and pH = 8.8 for cold-water corals and pH = 8.5 for warm-water corals), which was exposed to a CO2-containing atmosphere (pCO2 = 1100 ppm2 and Δ44/SO2 = −15‰). The temperature of the modelled calcification environment corresponded to the mean growth temperatures of the cold- and warm-water corals, i.e., 7.2 °C and 28.9 °C, respectively. The catalytic enhancement of the inter-conversion between CO2 (aq) and HCO3⁻ by carbonic anhydrase decreases with increasing the rate constants of CO2 (aq) (dehydration reactions26. The initial oxygen and clumped isotope compositions of both the DIC and air CO2 were assumed to be in isotopic equilibrium with the water (δ18O/H2O = 0‰ SMOW) at the above described temperatures.

(2) To model the isotopic composition of the synthetic speleothem, simulations were conducted using the IsoDIC model based on the laboratory experiment26 (T = 30.7 °C, pCO2 = 1007 ppm, water film thickness of 100 μm, Δ44/SO2 = −44.7‰, Δ44/SO2 = −10.6‰ VPDB, Δ44/SO2 = −6.0‰).
δ18O H2O = −9‰ VSMOW, see above as well and yielded an initial solution composition of pH = 6.3, [DIC] = 181 mM, [Ca2+] = 4.9 mM, δ13C DIC = −31.2‰, and δ18O DIC = −5.9‰ VPDB, which are close to the experimentally determined values (pH = 6.34, [DIC] = 182 mM, [Ca2+] = 5 mM, δ13C DIC = −31.9±1.3‰, and δ18O DIC = −8.69±0.11‰ VPDB, see above).

Data availability
The analytical and model data supporting the findings of this study are available within the article, its Supplementary Information, and Supplementary Data files. All data files are additionally deposited at: [https://doi.org/10.5281/zenodo.3784963](https://doi.org/10.5281/zenodo.3784963)

Code availability
The figures were generated using code written in R, available at: [https://doi.org/10.5281/zenodo.3784963](https://doi.org/10.5281/zenodo.3784963)

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**Author contributions**

D.B., W.G., and J.F. planned the study, interpreted the data, and wrote the manuscript with contributions from all authors. D.B., N.L., K.M., E.K., and J.F. performed the clumped isotope analyses. W.G. conducted the numerical modelling. T.B.C., E.G., M.H., D.H., G.D.P., J.R., D.S., and C.S. collected and provided the samples.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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